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Multifactorial Etiology of Gastric Cancer

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Abstract

The prevalence of gastric cancer is associated with several factors including geographical location, diet, and genetic background of the host. However, it is evident that infection with *Helicobacter pylori* (*H. pylori*) is crucial for the development of the disease. Virulence of the bacteria is also important in modulating the risk of the disease. After infection, *H. pylori* gains access to the gastric mucosa and triggers the production of cytokines that promote recruitment of inflammatory cells, probably involved in tissue damage. Once the infection is established, a cascade of inflammatory steps associated with changes in the gastric epithelia that may lead to cancer is triggered. *H. pylori*-induced gastritis and *H. pylori*-associated gastric cancer have been the focus of extensive research aiming to discover the underlying mechanisms of gastric tissue damage. This research has led to the association of host genetic components with the risk of the disease. Among these is the presence of single nucleotide polymorphisms (SNPs) in several genes, including cytokine genes, which are able to differentially modulate the production of inflammatory cytokines and then modulate the risk of gastric cancer. Interestingly, the frequency of some of these SNPs is different among populations and may serve as a predictive factor for gastric cancer risk within that specific population. However, the role played by other genetic modifications should not be minimized. Methylation of gene promoters has been recognized as a major mechanism of gene expression regulation without changing the primary structure of the DNA. Most DNA methylation occurs in cytosine residues in CpG dinucleotide, but it can also be found in other DNA bases. DNA methyltransferases add methyl groups to the CpG dinucleotide, and when this methylation level is too high, the gene expression is turned off. In *H. pylori* infection as well as in gastric cancer, hypermethylation of promoters of genes involved in cell cycle control, metabolism of essential nutrients, and production of inflammatory mediators, among others, has been described. Interestingly, DNA changes like SNPs or mutations can create CpG sites in sequences where transcription factors normally sit, affecting transcription.

In this chapter, we review the literature about the role of SNPs and methylation on *H. pylori* infection and gastric cancer, with big emphasis to the *H. pylori* role in the development of the disease due to the strong association between both.

Keywords

Helicobacter pylori; Gastric cancer; Single nucleotide polymorphisms; Methylation

1. Introduction

In 2008, close to one million new cases of gastric cancer (7.8% of the total cases) were estimated, with 736,000 deaths (9.7% of the total) due to the disease in the same period, making gastric cancer the second leading cause of cancer-related deaths worldwide (1). However, the incidence and mortality of gastric cancer around the world varies significantly according to the geographical location. The incidence in Asia and Eastern Europe is more than 20 cases per 100,000 individuals, contrasting with incidence rates lower than 10 cases per 100,000 individuals in North America, New Zealand, and Oceania (2, 3). The contrast in the survival rate of stomach cancer is significant as well. Japan, North America, and Western Europe have the highest survival rates (52, 21, and 27%, respectively) compared

with only 6% survival in the sub-Saharan regions (2). Parkin et al. suggested that the incidence of stomach cancer is higher in men than in women in most of these regions (2). In the United States, it is estimated that approximately 13,000 men and 8,000 women were diagnosed with gastric cancer in 2010 (4); more than 10,000 of them are expected to have died as a direct result of the neoplasia.

Several classifications of gastric cancer have been proposed over the years, based on different aspects including histopathology, clinical aspects, and endoscopic characteristics (5–9). However, the most widely followed classification is the one by Laurén (8), which, after few later updates, classifies cancer into intestinal and diffuse types, according to structural characteristics of the tumors. In general, the diffuse type seems to be diagnosed at earlier stages, more frequent in women than in men, and to be associated with specific blood types and associated to pangastritis without atrophy (10, 11). In contrast, the intestinal type of gastric cancer is more associated with gastritis in the corpus that leads to atrophy and intestinal metaplasia, dysplasia, and finally cancer (see below) (10). In addition, the intestinal type seems to be more common in men and diagnosed at later ages (11, 12). The observed decline in gastric cancer globally seems to be associated to a reduction in the incidence of the intestinal type, while there is an increase of the diffuse-type gastric cancer (13, 14).

2. Risk Factors: *Helicobacter pylori* Is Fundamental for Gastric Cancer Development

Infection with *Helicobacter pylori* (*H. pylori*) is considered essential for the development of gastric cancer, such that *H. pylori* has been classified as a type I carcinogen by the International Agency for Research in Cancer (IARC) (15). It is estimated that nearly half of the world's population is infected with this bacterium; however, most people are asymptomatic, and approximately 1–3% develop cancer (16–18). This infection induces an inflammatory response that increases the infiltration of lymphocytes, macrophages, and plasma cells into the gastric mucosa. Neutrophils can also be found when acute inflammation is present. When the inflammatory response is not accompanied by loss of gastric glands (atrophy), it is referred to as non-atrophic gastritis (NAG), according to the updated Sydney classification (19). NAG lesions are associated with the development of duodenal ulcer, especially if it is localized to the gastric antrum (20). A small percentage of patients with NAG progress to multifocal atrophic gastritis (MAG). MAG is characterized by the loss of gastric glands and the appearance of fibrotic tissue (21). This disease can later progress to MAG with intestinal metaplasia (MAG-IM), in which cells of the gastric epithelium are replaced by intestinal absorptive and goblet cells (for a graphical view of the lesions, please see refs. (21, 22)). MAG-IM is considered to be a true preneoplastic lesion leading to the development of dysplasia, with abnormal nuclear morphology and abnormal tissue architecture. It is estimated that up to 85% of patients with dysplasia and a high degree of atypical features progress to invasive carcinomas (23). Even though some of these lesions may regress to the previous, less malignant states, the rate of progression is higher than the rate of regression (24).

It is widely accepted that gastric cancer is the result of the above-described cascade of histological events leading from normal epithelia to cancer. However, the molecular and cellular events controlling the transition from one step to the next are not yet fully understood. Inflammation is a common finding in cancer (25). The inflammatory process is mediated by pro- and anti-inflammatory cytokines, the levels of which are controlled, among other things, by changes in the primary sequence of the DNA sequence. Several single nucleotide polymorphisms (SNPs) in genes encoding cytokines involved in the inflammatory process have been associated with risk of gastric cancer among several

populations (26–29). Our work with premalignant inflammatory stages in African-American and Caucasian individuals from the southern region of the United States has suggested that many of these SNPs associations observed in gastric cancer are also present in the premalignant stages and that there is a significant difference in the frequency of these SNPs between the two ethnic groups (22, 30). These findings are important because it would help to identify people at increased risk of developing cancer at an earlier stage, allowing for better intervention strategies and remediation of the possible mucosal damage already inflicted by the inflammatory reaction.

3. Biology of *H. pylori* Infection

H. pylori is a gram-negative bacterium that infects humans early in life (31). Infection occurs at earlier ages in developing countries than in more advanced areas (31, 32) and is related to socioeconomic status (33, 34). Upon infection, *H. pylori* gains access to the mucosa overlying the gastric epithelia, delivering several pathogenic factors. Vacuolating cytotoxin (VacA) can be secreted as a soluble protein (35) but can also be found attached to the membrane of the bacteria (36). VacA is responsible for the formation of large intracellular vacuoles in mammalian cells (37) and for opening channels on the membrane of the gastric epithelial cells (38), allowing molecules like urea to enter the gastric lumen (39). Once in the gastric lumen, the urea is broken down into ammonia and carbon dioxide by the *H. pylori* urease, a metalloenzyme that uses nickel as a cofactor (40). The importance of *H. pylori* urease is evidenced by the fact that *urease* (–) *H. pylori* strains are unable to colonize the stomachs of several animal models (41, 42). In addition, *H. pylori* urease accounts for up to 10% of the total protein produced by the bacteria (43). The ammonia generated by the breakdown of urea can, by itself, neutralize the gastric acid (44), thus helping the bacteria survive and causing damage to the gastric epithelia (45).

The cytotoxin-associated antigen (CagA) is injected into the membranes of the gastric cells by a type IV secretion system (46, 47). Once inserted into the host's cell membrane, CagA is activated by phosphorylation at the carboxy-terminal end of the protein by c-Src/Lyn kinases (48). This phosphorylation occurs at the tyrosine residues of the EPIYA motifs (protein domains formed by glutamic acid, proline, isoleucine, tyrosine, and alanine residues) (48, 49). Phosphorylation-activated CagA recruits the cytoplasmic SRC homology 2 domain-containing tyrosine phosphatase (SHP-2 tyrosine phosphatase) to the membrane and deregulates the phosphatase domain (49). Tyrosine phosphorylation of the CagA protein and its subsequent binding to the SHP-2 phosphatase are essential for the induction of the cellular changes associated with CagA since *H. pylori* harboring *cagA* genes without the EPIYA motifs are able to translocate the protein into the cell membranes, but once there, it is not phosphorylated nor able to induce any cellular changes (48, 49). Activated and SHP-2 associated CagA triggers a cascade of phosphorylation events (50) that lead to changes in the cell shape (46, 48). Interestingly, the promoter activity of the *H. pylori cagA* gene was found to be increased in the presence of NaCl in a dose-dependent manner (48, 51). Furthermore, the levels of the CagA protein were higher in *H. pylori* grown in higher salt concentrations and resulted in increased interaction with gastric epithelial cells and increased phenotypic changes associated with CagA (51, 52). CagA is also responsible for the induction of inflammatory responses, including interleukin (IL) 8 released by gastric epithelia, which serves as a chemotactic factor for inflammatory cells (53–56). Once recruited to the gastric mucosa, the inflammatory cells mount a response essentially mediated by lymphocyte-derived cytokines which, if not controlled, can promote tissue damage.

Another important factor produced by *H. pylori* is the arginase enzyme which is encoded by the *rocF* gene (57–59). This enzyme is found in many other organisms (60) and is involved

in the generation of urea and ornithine, the latter being the primary source for the production of polyamines (61). In *Leishmania*, the generation of polyamines is essential for the survival of the parasite, such that arginase gene knockout parasites are unable to survive in culture media unless supplemented with polyamines (62). In *H. pylori*, the enzyme seems to be critical for survival of the *H. pylori* in acidic environments, but the lack of the gene does not affect colonization of mouse stomach (57). Mendz and Hazell (63) have shown that *H. pylori* lacks some of the enzymes required for the synthesis of L-arginine (L-Arg) and depends on L-Arg generated by the host. We and others have shown that *H. pylori* arginase can inhibit functions of both macrophages and T cells, making the bacterium able to control both acquired and innate immune responses to the infection (64, 65).

4. Immune Dysfunction Caused by *H. pylori* Products in T Cells

H. pylori antigens, including urease and *H. pylori* DNA, can impair T-cell function (66, 67). It has been shown that lysates of *H. pylori* reduce the proliferation of both peripheral blood lymphocytes (PBL) and Jurkat cells (68). This effect is not limited to T cells but also includes human monocytic cell lines and even human gastric cell lines (67, 69). Several studies have been designed to identify the factors involved in the modulation of the immune response to *H. pylori*. VacA and CagA antigens have been associated with virulence in *H. pylori* and are believed to be the mediators of T-cell dysfunction. Paziak-Domanska et al. (70) have shown that crude extracts of CagA+VacA- *H. pylori* G27 leads to downregulation of PHA-induced proliferation of T cells. This effect was not observed when crude extracts of the isogenic CagA-VacA+ was used. On the other hand, *H. pylori* CagA and VacA are responsible for the downregulation of the proliferation of gastric cell lines (71, 72), an effect not mediated by apoptosis (73). Interestingly, the dysfunction of T cells observed in *H. pylori* infection is also seen in gastric cancer (74, 75).

We have shown that *H. pylori* arginase contributes to the depletion of L-Arg in culture media, leading to the downregulation of the CD3 ξ molecule, essential for activation of T cells (64). Some studies have shown that in gastric cancer there is a reduced expression of CD3 ξ in T cells in local lymph nodes (76). Whether this happens in response to the infection with *H. pylori*, or if the virulence of the bacteria is differentially associated with this event, is still to be determined.

5. Cytokine Production in Response to *H. pylori* Infection

It has been shown that mice deficient in B and T cells (*RAG-1*^{-/-}), or mice deficient in T cells alone (*TCR β* ^{-/-}), do not develop gastritis when infected with *Helicobacter felis* (77). This supports the idea that the immune response is essential for the development of gastritis after infection with *Helicobacter*. In addition, interferon response factor element 1-deficient mice (*IRF-1*^{-/-}), which do not produce IFN γ , also fail to develop gastritis after infection with *H. pylori*. Mohammadi et al. (78) and Nedrud et al. (79) clearly demonstrated that C57Bl/6 mice infected with *H. felis* develop aggressive gastritis due to a strong Th1 response. In contrast, Balb/c mice that have a preferential Th2 response developed a protective immune response. Therefore, the type of cytokine response is closely associated with the pathological outcome of the infection.

In humans, most reports agree that a Th1 response is elicited both *in vitro* and *in vivo* after *H. pylori* exposure, while a Th2 response is absent or negligible. Increased levels of IFN γ in the mucosa of patients infected with *H. pylori* were observed both *in situ* and after purifying the epithelia-infiltrating lymphocytes. No production of IL4 or IL5 could be detected (80, 81). Cytokines like IL8 and IL6 have also been reported to be increased; however, the increase of IL8 appears to be independent of the presence of *H. pylori* and may be more of a response to the inflammatory process initiated by the infection (82). In contrast, increased

expression of IL6 within the gastric mucosa is largely associated with the presence of the bacteria, such that its levels significantly decrease after clearance of the infection (82).

6. *H. pylori* Infection, Arginine, Arginase, and Immunity

In humans, the metabolism of the amino acid L-Arg has been associated with regulation of the immune responses (83). In patients with trauma, liver transplantation, and some tumors, an increase production of arginase I has been linked to significantly decreased responses of T cells (84, 85). The lack of L-Arg induces a low expression of CD3 ζ , which has been associated with reduction in T-cell proliferation and decreased production of cytokines (85–87). However, it is possible to suggest that in addition to the CD3 ζ molecule, other mechanisms might be important in the induction of this altered state of the T lymphocytes. Recent data has shown that one of the mechanisms associated with L-Arg reduction includes an increased expression of the cationic amino acid transporter (CAT-2B) in murine myeloid-derived suppressor cells (MDSC, characterized as macrophages in mice), which increase the uptake of L-Arg by CAT-2B (86). This mechanism is not present in human MDSC, which are characterized as polymorphonuclear neutrophils (PMN). In contrast, human MDSC release arginase I into the microenvironment, where it depletes L-Arg and induces T-cell dysfunction by impairing all the described functions of T cells (88, 89). Interestingly, the same phenomenon has been described in the placenta of pregnant women, suggesting some role of L-Arg metabolism in the tolerance of the fetus (90).

The enzyme arginase (EC 3.5.3.1) is one of the enzymes involved in the metabolism of L-Arg, producing L-ornithine and urea, the first needed for the synthesis of polyamines required for cell proliferation (91, 92). Additionally, nitric oxide synthase (EC 1.14.13.39) metabolizes L-Arg into citrulline and nitric oxide, an innate mechanism involved in cytotoxic cellular responses mediated by macrophages (93, 94).

L-Arg metabolism by arginase is emerging as an important regulator of T-cell responses in human diseases, including infectious diseases and cancer (95–101). Our work with *H. pylori* has shown that the presence of an arginase enzyme, previously characterized in the bacteria (102), is responsible for reducing the expression of the CD3 ξ molecule in Jurkat T cells and primary T lymphocytes cultured in the presence of *H. pylori* (64). In addition, *H. pylori* arginase plays an important role in reducing the levels of nitric oxide production by macrophages, an event that may be associated to increased survival of the bacteria (65). On the other hand, *H. pylori* infection also induces the expression of macrophage arginase II, which reduces intracellular availability of arginine with subsequent reduction of nitric oxide responses (103, 104). In fact, the critical role of arginase II in *H. pylori* infection has been shown in arginase knockout mice (*arg2*^{-/-}) (105). This study suggested that arginase II affects the degree of cellular immunity against *H. pylori*, by reducing the levels of Th1/Th17 cytokines, including IFN γ , IL17a, and IL12p40 (105). The latter has been shown also in macrophages in the intestinal muscularis (jejunum and ileum) of mice infected with *Helicobacter hepaticus* (106). Even though these macrophages did not show any signs of infection by the *Helicobacter*, those obtained from infected mice had significantly reduced induction of inflammatory cytokines than those obtained from uninfected, after being stimulated *in vitro* with LPS and IFN γ (106). These results indicate that even if the *Helicobacter* never encounters cells of the immune system, soluble factors released by the bacteria, or by the inflamed gastric epithelia, may influence the immune response associated with gastric damage. The possibility about *H. pylori* being able to invade the gastric mucosa and interact directly with cells of the immune system is still controversial, but there are some research showing actual *in vivo* phagocytosis of *H. pylori* at the gastric level (107–109). This controversy is far from being solved, but some *in vitro* assays suggest that, even if ingested, *H. pylori* is able to delay the intracellular killing, at least by macrophages (110–

113). If this is a phenomenon that actually happens *in vivo*, it may lead to intracellular *H. pylori* replication, as shown *in vitro* (114), and explain the persistence of the infection, which in turns may lead to antibiotic resistance, selecting more aggressive strains able to induce stronger inflammatory responses associated to mucosal damage.

7. Single Nucleotide Polymorphisms and the Regulation of Cytokine Genes

Under normal circumstances, modulation of the immune response is achieved at several levels, including gene expression. SNPs are, in general, biallelic variations of one nucleotide occurring throughout the genome. SNPs can cause alteration of the primary structure of a protein. If, on the other hand, the allelic variation does not involve changes in the amino acid sequence, it is referred to as synonymous or conservative. SNPs in noncoding regions may be involved in splicing or in the formation of transcription factor-binding regions. Several SNPs in cytokine genes have been associated with the regulation of cytokine levels. For example, one haplotype on the *IL10* gene (*IL10-1082A/IL10-819T/IL10-592A*) has been associated with reduced levels of IL10 production (115). On the other hand, *IL1B-511T/T* and the presence of allele 2 of the IL1 receptor antagonist gene (*IL1RN*2*) is associated with increased levels of IL1 β production in the mucosa (116). This has been associated with gastric inflammation and intestinal metaplasia (116, 117). Pociot et al. (118) have also shown that a C to T change at *IL1B+3954* position is associated with increased secretion of IL1 β from monocytes after stimulation with LPS. Other studies (119, 120) using transiently transfected cells have shown that allele A at position -308 of the *TNFA* gene (*TNF*2*) is associated with increased levels of TNF- α , suggesting a role for this SNP in inflammatory and infectious processes. The proinflammatory IL6 is responsible for inducing fever after injection of IL1 in animals (121). The levels of IL6 are also controlled by genetic mechanisms. An SNP at position -174 (G > C change) has been associated with differential production of IL6 with increased activity of promoters containing G (121). After stimulation with LPS, PBMCs obtained from healthy individuals with *IL6-174GG* or *IL1-174GC* genotypes produced significantly higher amounts of IL6 in response to LPS than individuals with the *IL6-174CC* (121). Furthermore, haplotype analysis of the *IL6* promoter has suggested that the IL6 expression is controlled by the interaction of at least four polymorphisms in the *IL6* promoter (122). The clinical importance of genetically controlled levels of cytokines has been demonstrated in transplantation (123, 124), autoimmune diseases (125), and infectious diseases (126, 127).

8. Cancer Health Disparities, Ethnicity, and SNPs

The incidence of most cancers is higher in African-Americans than in Caucasians (128). Many factors may be playing a significant role in these disparities. According to the National Institutes of Health, cancer health disparities are defined as “all adverse differences in cancer incidence, cancer prevalence, cancer death, cancer survivor-ship, and burden of cancer or related health conditions that exist among specific population groups in the United States” (129). Even though the socioeconomic status is highlighted as one of the major factors leading to lack of appropriate health care, it is possible to suggest that ethnic differences are also playing some role in defining such disparities. Recent reports have shown that the composition of genetic blocks between African-Americans and Caucasians is different, with more heterogeneity observed in the African-American group (130, 131). This could be associated to a differential genetic background that makes one individual more susceptible to suffer specific diseases, including cancer (132). These differences may include differential transcription of regulatory genes, increased transcription of genes that promote inflammation and reduced transcription of those that are anti-inflammatory. Because gene transcription may be affected by the presence of SNPs at the promoter level, these may potentially be used as determinants of risk of disease in specific ethnic groups. An example

of differential SNPs distribution between ethnic groups and its possible association with disease is given by the gene of the multidrug transporter (*MDR1*), which mediates the transport of many types of drugs including anticancer drugs (133, 134). The frequency of one SNP in exon 26 of the *MDR1* gene (a C > T change at position 3435) has been found to be differentially associated with African-Americans, Caucasians, and Asian populations (135), as well as with differential expression of the MDR1 protein and with plasma levels of several drugs (136–142). Additional work has shown that such SNP is in linkage disequilibrium with two other nearby SNPs forming haplotype blocks differentially associated with three ethnic groups in Asia (143). Regarding to inflammatory mediators, several cytokine SNPs have been associated with the development of gastric cancer. A seminal work by El-Omar et al. (26) associated a transition from C to T at position –511 of the *IL1B* gene (*ILB-511C>T*) with gastric cancer in European populations. This finding has been later confirmed by other groups (28, 29, 144, 145), even though racial and ethnicity factors have been associated with differential gastric cancer risk in various populations worldwide. However, most studies agree that the presence of allele *IL1B-511T* increases the risk of intestinal-type and noncardia gastric cancer in Caucasian but not in Asian populations, a fact that has been validated by several meta-analyses (146–149). Interestingly, this SNP has also been linked to increased secretion of IL1 β (116, 117); this, in turn, is associated with reduction of gastric acid secretion (150), promoting the colonization by *H. pylori*. *IL1B-511* is in near complete linkage disequilibrium with another SNP at position –31 (*IL1B-31*) (26), and its capacity to modulate *IL1B* gene transcription is modified depending on the presence of other nearby SNPs (151). This strongly suggests that SNP's association with disease needs to be studied not only individually but also as haplotypes.

The biological activity of the IL1 β is regulated by the presence of a natural antagonist, the interleukin 1 receptor antagonist (IL1ra), which is encoded by the *IL1RN* gene (152). Allele 2 of a variable number of tandem repeats (VNTR) on intron 2 of the *IL1RN* gene (*IL2RN*2*) has been associated with reduced levels of IL1ra (153, 154) and with the increased risk of several types of cancer, including gastric cancer (26–28).

Another cytokine important in the initiation and maintenance of immune responses is tumor necrosis factor alpha (TNF- α), in which SNPs have been associated with gastric cancer as well as other types of cancer. The presence of allele A at position –308 of the *TNFA* gene (*TNF-308A*) has been associated with an increased risk of gastric cancer and non-small cell lung carcinoma (28, 29, 155). In addition to the effect of the *TNF-308* SNP, *TNF-857T* has been linked to the development of gastric intestinal metaplasia (156) and gastric B-cell lymphoma (157).

Interleukin 8 (IL8) is a member of the CXC chemokine family and functions as a chemoattractant for neutrophils (53–56). Its role in gastric cancer is suggested by the high levels of mRNA and IL8 protein in gastric cancer cell lines (82, 158, 159). Gastric cancer patients carrying the *IL8-251A* allele or the haplotype of *IL8 AGT/AGC* (–251/+396/+781) had a two- and fourfold increased risk of developing adenocarcinoma of gastric cardia, respectively (160). Interestingly, the *IL8-251AA* genotype increases the risk of gastric cancer only in individuals infected with *H. pylori* CagA+ strains (161).

IL10 is an anti-inflammatory cytokine (162). The level of IL10, as mentioned before, is associated with the presence of specific SNPs and haplotypes in its promoter region, and these, in turn, have been associated with differential risk of gastric cancer and other malignancies (29, 115, 163–165).

Recent genome-wide association studies (GWAS) using Japanese and Korean populations found that two SNPs in the prostate stem cell antigen gene (*PSCA*) were significantly associated with diffuse-type gastric cancer (166). A later study showed that one of those SNPs, rs2976392, is associated with a significant increase risk of both gastric cancer types, intestinal and diffuse, in a Chinese population (167). These results were further confirmed by a more recent GWAS in a Chinese population, which, in addition to finding that the same two SNPs in the *PSCA* gene were associated with noncardia gastric cancer, also found that risk of gastric cardia cancer was associated with two SNPs, rs22742223 and rs3765524, that create missense mutations in the region 10q23 encoding the phospholipase C ϵ 1 (*PLCE1*) (168).

Our work with African-American and Caucasian individuals from Louisiana has identified SNPs, alone or arranged in haplotypes, in several cytokine genes differentially associated with more severe forms of gastritis (22, 30). Interestingly, African-Americans have higher frequency of proinflammatory SNPs and haplotypes in both *IL1B* and *IL10* genes (22, 30), present higher incidence of more aggressive forms of the disease (22, 30), and are infected more frequently with aggressive *H. pylori* strains (30). Taken together, and considering that these inflammatory stages may lead to gastric malignancy, our results may help explain in part why African-Americans have increased risk of developing gastric cancer than Caucasian individuals.

In summary, the balance of the pro- and anti-inflammatory responses to an offending agent (*H. pylori*) appears to play a central role in gastric mucosal damage and repair. Defects on the type of the response elicited, or in their balance, result in an abnormal environment that can be detrimental for the host and favor the development of malignancy. However, the interplay between the virulence of the bacteria and the genetic background of the host is crucial in determining the fate of the inflammation initiated by the *H. pylori* infection.

9. DNA Methylation, *H. pylori* Infection, and Gastric Cancer

DNA methylation has been described as one important way of gene regulation that occurs normally in imprinted genes, X-chromosome inactivation, and silencing of tumor suppressor genes, among other situations (169). Most DNA methylation events occur in CpG dinucleotides and very especially in those located in gene promoters (170). Even though the mechanisms of this regulation are not totally understood, many clues point to either a direct interference (by the methyl groups) of the binding of transcription factors or by the formation of protein complexes by the recruitment of methyl-binding proteins, which ultimately inhibit transcription (171–176).

DNA methylation is carried out by several DNA methyltransferase (DNMT) enzymes, DNMT1, DNMT2, and DNMT3 (comprising 3A, 3B and 3L) involved in *de novo* and maintenance methylation of hemi- and unmethylated DNA sequences (177, 178). Interestingly, there is an increased expression of DNMT proteins in gastric cancer tissues, as compared to tissues with normal histology. A recent study has found an SNP at position –448 of the *DNMT3A* gene (*DNMT3A-448A*) highly associated with risk of gastric cancer in a Chinese population (179). The presence of *DNMT3A-448A* increases more than twofold the activity of the promoter, and homozygous carriers of this SNP (*DNMT3A-448AA*) have more than sixfold increased risk of gastric cancer when compared with *GG* carriers (179).

Hypermethylation of gene promoters has been described in gastric tissues, and this process seems to be directly associated with the inactivation of specific genes in gastric cancer samples (180, 181). However, as different population may have differences in their genetic contents, differences in the methylation patterns may also vary. In a sample from Colombia,

South America, when comparing two populations with different risks of gastric cancer, hypermethylation of the *RPRM* gene was associated with the disease and with infection with virulent *H. pylori* strains (*cagA*+/*vacA s1m1*+) in the high-risk population (182), as compared with an area of low risk for gastric cancer. *H. pylori* virulence seem to be also associated with differential methylation on enzymes involved in the pathway that generates *S*-adenosylmethionine, the universal donor of methyl groups in humans (183). In a study from Brazil, it was found that infection with virulent strains of *H. pylori* is associated with a polymorphism in the methylenetetrahydrofolate reductase gene (*MTHFR*) enzyme (*MTHFR C677T*) in patients 60 years old or older (184). In addition, hypermethylation of cell cycle controlling genes (E-cadherin and *CDKN2A*) have been reported in patients infected with *H. pylori* (184–188). Regarding to E-cadherin (encoded by the gene *CDH1*), the hypermethylation of this gene seems to be directly related to the infection with *H. pylori* since its eradication by antibiotic treatment lead to a significant reduction of the methylation level (188). This type of inactivation of this gene adds to the importance of *CDH1* in the process of the progression of malignancy associated with gastric cancer. One study using New Zealand families have found that a G to T mutation in the sequence of exon 7 leads to an aberrant product and is associated with familial gastric cancer (189).

Many factors may influence the degree of methylation on one specific genomic region. One of those factors is the presence of SNPs that either create CpG sites at the promoter levels, maybe modifying the binding of proteins involved in the transcription machinery, or increase the binding of transcription factor that promotes the increased transcription of the gene. One example of the latter is the effect that C to T change at position –511 in the *IL1B* gene (*IL1B-511T*) has on the methylation of CpG islands of several genes, including *TWIST1* and *CYPB1* (190). It was noted that gastric cancer patients with the allele *IL1B-511T* had significantly increased methylation on genes like *TWIST1*, *CAGNA1G*, *GRIN2B*, *CYPB1*, and *CRABP1*, when compared to individuals with the allele *IL1B-511C* (190). One possible explanation of these results may be the association between the levels of IL1 β at the gastric mucosa and the *IL1B* gene polymorphisms. It has been shown that individuals with the *IL1BTT* genotype have significantly higher IL1 β at the gastric level than those with the genotype *IL1BCC* genotype (117). This cytokine has a plethora of effects, and among them are both the increased expression of *DNMT1* and the increased activity of the enzyme, which, as discussed before, is involved in the transfer of *S*-adenosylmethionine to cytosine residues in CpG sites (183).

10. Other Risk Factors Associated with Gastric Cancer

It is very clear that environmental factors are involved in the development of gastric cancer. Studies have shown that the risk of gastric cancer changes if people move to geographical areas with different gastric cancer risk, either increasing or decreasing, according to the risk of the new area of settlement (191, 192). Several factors have been associated with the development of gastric cancer, including environmental, microbial, and genetic factors (33, 193). It has been shown that fruits and vegetables reduce the risk of gastric cancer, without regard to the anatomical position or histological type of cancer (194). A large study involving more than 10,000 individuals reported that those with the very low to none intake of fruits and vegetables had a relative risk (RR) of developing gastric cancer of 5.5 (95% CI 1.7–18.3), compared to those with a high intake of these foods (195). In addition, a study with more than 12,000 individuals from seven countries reported a reduced risk for gastric cancer in individuals with high consumption of fruits, even though no effect was associated with vegetable consumption (196). Such studies have helped identify the specific micronutrients that are involved in preventing this malignancy. Despite conflicting results (197–199), it is commonly found that beta-carotene intake is inversely associated with the

risk of gastric cancer (200–203), while the consumption of salted meats seem to increase the risk of the disease (204, 205).

11. Models of *H. pylori*-Induced Gastric Inflammation

The role of the immune system in response to infection with *Helicobacter* strains has been extensively studied using animal models. These models have helped to clarify the process of the infection, the preneoplastic steps, and the severity and different outcomes of the neoplasia. Mongolian gerbils have been used as *H. pylori* infection models because they develop a disease very similar to human gastritis (206). However, phenotypic studies in gerbils are limited because of the lack of many gerbil cell-specific reagents. Mice infected with *H. felis* have also been used to study the human gastritis. Furthermore, *H. pylori* strains have been adapted to infect mice and are preferred because the *Helicobacter* infection seems to be highly host specific (77, 207, 208).

In humans, it has been shown that T cells isolated from the antral mucosa of patients with active gastritis or duodenal ulcer disease, associated with *H. pylori* infection, are preferentially producing Th1 cytokine (77, 80, 209–211). Other studies have shown that CD4⁺ T-cell clones isolated from the gastric mucosa of these patients proliferate in response to specific *H. pylori* antigens, including CagA, VacA, and urease, thus showing antigen specificity (209, 210). This enhanced proliferation is related to the cytokine response which appears to be associated with the presence of CagA and VacA (73). Patients infected with *H. pylori* strains expressing these two proteins show an activation of nuclear transcription factors AP-1 and NFkB and several tyrosine kinases including MAP kinases. All of these factors participate in the transactivation of proinflammatory cytokine genes (212–214).

Even though most of the research on *H. pylori*-induced gastritis has focused on T cells, other cells involved in the inflammatory reaction including the gastric epithelium, polymorphonuclear cells, and macrophage/dendritic cells play an important role in the response to *H. pylori* infection. Gastric epithelial cells can produce IL6 and IL10 upon contact with *H. pylori* (215). Furthermore, they express B7.1 and B7.2 costimulatory molecules, suggesting they could play an important role as antigen-presenting cells (216). Initial reports about the role of macrophage/dendritic cells suggested that *H. pylori* severely impairs phagocytosis and antigen processing in these cells, a mechanism that may be dependent on the presence of the CagA protein (217). Furthermore, urease from *H. pylori* can degrade urea which is needed to produce CO₂ and NH₃, effectively blocking the bactericidal function of peroxynitrite, a metabolite derived from nitric oxide (218). Thus, it is possible that the detrimental effects of *H. pylori* on macrophages could lead to the T-cell dysfunction observed in chronic infections.

12. Concluding Remarks

Even though many advances in the understanding of gastric cancer have been made, the disease is still one of the malignancies with the highest incidence and mortality rates worldwide. The identification of *H. pylori* as a crucial player in the pathology of gastric cancer was a pivotal step in the understanding and control of the disease. However, it is important to fully understand the inflammatory response initiated by the infection in order to fully block the cascade of events that lead to gastric cancer. This pathogen–host interaction is one of the highest hierarchies since *H. pylori* has evolved mechanisms to hijack the immune responses of the host and make its survival easier. Even though the environment, the host's genetic background, diet, and gender, among other factors, add to the *H. pylori*-associated risk of gastric cancer, making the disease very complex and difficult to understand and devise strategies to prevent, cure, and/or better treat patients diagnose with the disease, our efforts should converge in finding the commonalities of the disease: what is

common among individuals who become infected with *H. pylori* and also among the different strains of the bacteria able to colonize and induce inflammation in humans. Genetic and epigenetic markers of the infection and of the damage induced by it are necessary tools to devise strategies aiming at limiting the degree of inflammation and to restore the homeostasis of the gastric environment. These markers will probably show differences among populations and related to *H. pylori* virulence, but our actual capacity to fully sequence the human genome will, for sure, identify those common DNA sequences and transcripts able to modify the risk not only of being infected with the bacteria but also of developing gastric cancer.

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