REVIEW

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Evolutionary History of the *Helicobacter pylori* Genome: Implications for Gastric Carcinogenesis

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The genome of the bacterium *Helicobacter pylori* has evolved over the millennia since its migration out of Africa along with its human host approximately 60,000 years ago. Human migrations, after thousands of years of permanent settlement in those lands, resulted in seven prototypes of genetic populations of *H. pylori* with distinct geographical distributions. In all continents, present day isolates of *H. pylori* have molecular markers that reflect population migrations. The colonization of the Americas as well as the slave trade introduced European and African strains to the New World. The relationship between *H. pylori* genome and gastric cancer rates is linked to the presence of the *cagA* gene, but the knowledge on this subject is incomplete because other genes may be involved in certain populations. A new situation for *Homo sapiens* is the absence of *H. pylori* colonization in certain, mostly affluent, populations, apparently brought about by improved home sanitation and widespread use of antibiotics during the last decades. The disappearance of *H. pylori* from the human microbiota may be linked to emerging epidemics of esophageal adenocarcinoma, some allergic diseases such as asthma and some autoimmune disorders. **(Gut Liver 2012;6:21-28)**

Key Words: *Helicobacter pylori*; Genome; Gastric carcinogenesis

INTRODUCTION

Helicobacter pylori is a Gram-negative spiral bacterium that colonizes the gastric mucosa of more than half of the world population and is the main recognized cause of gastritis, peptic ulcers, gastric adenocarcinoma and some types of gastric lymphoma.[1-4] It has been estimated that human organisms consist of 10^{13} eukaryotic cells and 10^{14} microbial cells.[5] *H. pylori* may be considered prominent member of the human microbiota and has accompanied constantly *Homo sapiens* in its complex migration history.[6] *H. pylori* is usually acquired during childhood and is believed to be transmitted within families. The bacterium is well adapted to survive in the gastric mucus for decades, and most infected persons remain asymptomatic.

One of the characteristics of *H. pylori* is a great genetic diversity, and it is known that different strains may interact differently with their human host influencing the clinical outcome. The extensive genomic diversity among *H. pylori* isolates results from a high mutation rate and a frequent exchange of genetic material during infections with multiple *H. pylori* strains.[7-11] *H. pylori* strains from different geographic areas show clear phylogeographic differentiation, and studies of the genetic variants serve as markers of human migrations. In addition, the worldwide geographic distribution of the different *H. pylori* populations seems to correlate the clinical outcomes. This review examines the role of *H. pylori* genomic variants in the biology and outcomes of the infection.

CARCINOGENIC POTENTIAL

Gastric cancer is the second leading cause of cancer-related death in the world.[12] Based on epidemiologic evidence, the International Agency for Research on Cancer concluded in 1994 that the infection with *H. pylori* was a class I carcinogen for humans.[13] It has been estimated that 5.5% of the total cases of cancer worldwide and more than 60% of gastric cancer cases are caused by this bacterial infection.[14] Approximately one half of the gastric cancer cases occurs in East Asia, and the countries with the highest incidence rates are Korea, Mongolia, Japan, and China.[15]

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H. pylori infection is a necessary but not a sufficient factor in gastric carcinogenesis. The hypothetical causal link between *H. pylori* infection and gastric cancer was challenged by Holcombe,[16] making reference to several countries in Africa where the infection was practically universal but the gastric cancer rates were very low. He called this phenomenon the "African enigma." Similar trends have been observed in other populations around the world, and since gastric cancer is considered a multifactorial disease, factors such as diet and co-infections have been offered as potential explanations for the enigma.[17-19]

Independent from other factors that may modulate the risk of acquiring gastric cancer, the genotype of the infecting *H. pylori* strain is a determining factor. The carcinogenic effects of *H. pylori* infection have been linked to its virulence factors, mainly the cag pathogenicity island (*cag* PAI) and the vacuolating cytotoxin gene A (*vacA*).[2,20,21] The *cag* PAI is a ~40 kb locus that contains 27 to 31 genes, hypothesized to have been acquired horizontally and integrated into the glutamate racemase gene.[22] Present day isolates of *H. pylori* may or may not contain the *cag* PAI. The cytotoxin-associated gene A (*cagA*)[23] is the most investigated gene of the *cag* PAI and the main recognized virulence factor. It encodes CagA, an oncoprotein that is injected into mammalian cells, undergoes phosphorylation by host cell kinases, and affects cytoskeletal and tissue structure, as well as cell proliferation.[24,25] Several of the genes in the *cag* PAI encode for a type IV secretion system[26] used for the injection of CagA into host cells. Infection with *cagA*-positive *H. pylori* strains is associated with high risk of peptic ulcers and gastric carcinoma.[20,21,25] In East Asian countries, virtually all of the *H. pylori* isolates are *cagA*-positive, whereas in Western countries approximately 60% to 70% of isolates are *cagA*-positive.[27] Mixed infections with *cagA*-positive and *cagA*-negative strains have been reported.[28]

CagA is known for the variability in its C-terminal region, which includes a Glu-Pro-Ile-Tyr-Ala (EPIYA) sequence that is present in variable numbers in CagA from different strains. On the basis of sequences flanking the EPIYA motifs, four distinct segments have been described: EPIYA-A, -B, -C and -D. EPIYA-A and EPIYA-B are in almost all CagA isolates. EPIYA-C is characteristic of CagA from *H. pylori* from Europe, North America, and Australia (thus named "Western CagA"). EPIYA-D is specific to CagA produced by *H. pylori* circulating in East Asian countries (termed "East Asian CagA"). Most CagA isolates contain three EPIYA segments (ABC or ABD), but the number can vary from one to seven.[27] Studies have shown greater phosphorylation and oncogenic potential in East Asian CagA (due to the presence of the EPIYA-D segment) compared to Western CagA.[29,30] Also, among Western CagA species, greater phosphorylation and oncogenic potential has been observed in those with a greater number of EPIYA-C motifs.[27,31,32] Therefore, distinct CagA isoforms may contribute to the differences in gastric cancer rates between East Asian and Western countries.

Unlike the *cag* PAI, the gene *vacA* is present in virtually all *H. pylori* strains examined.[33,34] It encodes VacA, a protein that may damage epithelial cells by inducing the formation of vacuoles. Strains vary considerably in production of cytotoxin activity, primarily due to variations in *vacA* gene structure. The regions of greatest diversity are localized near the 5' end of *vacA* (signal sequence, showing allele types s1a, s1b, s1c, or s2) and in the mid-region of *vacA* (alleles m1 or m2). *H. pylori* strains that possess *vacA* s1m1 genotypes are associated with increase in gastric epithelial cell injury and greater risk of peptic ulcer and gastric cancer compared with *vacA* s2m2 strains.[35,36] *vacA* s1a predominates in Northern Europe; s1b in the Iberian peninsula and in Latin America; s1c is only found in South Eastern Asia.[37] The relationship between *vacA* s1m1 alleles and gastric cancer is consistent with the distribution of vacA genotypes throughout the world. In regions where the prevalence rate gastric cancer is high, such as Colombia and Japan, most *H. pylori* strains contain type s1m1 alleles. Strains with *vacA* s2 alleles are more

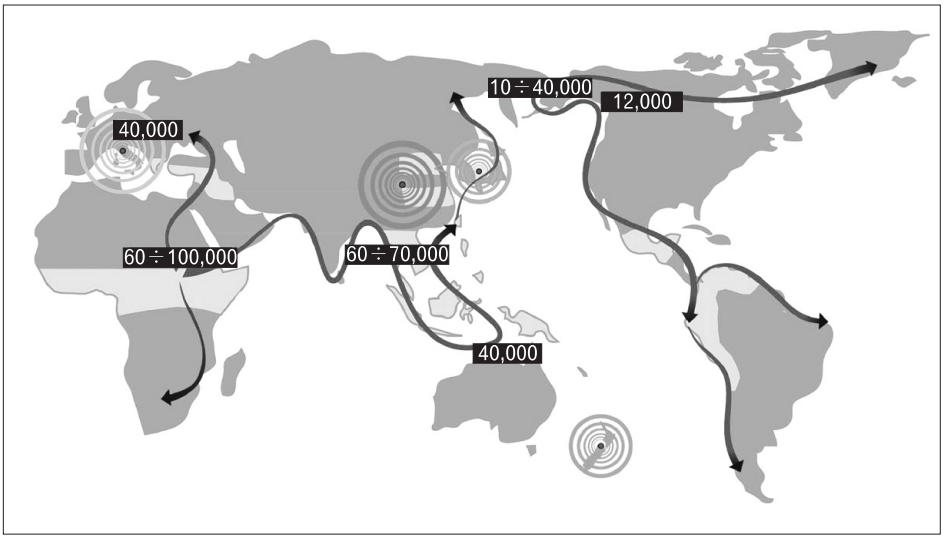

Fig. 1. World map indicating human migrations (arrows) and time range in years since the migrations happened. *H. pylori* accompanied man during the migrations and the four major *H. pylori* populations (as known in 1999: Europe, Northern Asia, Southern Asia, and New Zealand) are represented by concentric circles of different colors. Light green areas indicate the locations where development of agriculture and animal breeding started, resulting in expansion of human populations (From Covacci A, et al. Science 1999;284:1328-1333, with permission).[26]

frequent in regions of the world with low rates of gastric cancer, such as Australia and North America. A third vacA polymorphic site, the intermediate (i) region was more recently identified, with variants i1 and i2. Strains type i1 were associated with higher risk of gastric adenocarcinoma.[38]

OUT OF AFRICA

Increasing evidence supports the hypothesis that *H. pylori* was already established in human stomachs at least 100,000 years ago, before the human migration out of Africa about 60,000 years ago.[26] Fig. 1, from Covacci *et al*.,[26] displays the ancient human migrations and the approximate time of each migration wave. It has been hypothesized that after migration out of Africa, humans reached Asia by a southern coastal route, through India and then into South East Asia and Australasia.[39] That route extended along the region known as Sundaland (including the Malay peninsula, Sumatra, Java, and Borneo), that was part of the Asian mainland as a result of low sea levels during the last ice age.[40] Also due to the low sea levels, Australia, New Guinea, and Tasmania were connected in a continent called Sahul, separated from Sundaland by a few narrow deep-sea channels.[41] The modern humans settled Europe about 30,000 to 40,000 years ago, probably entering via two routes: from Turkey along the Danube corridor into Eastern Europe, and along the Mediterranean coast. Finally, *Homo sapiens* and *H. pylori* crossed the Bering Strait together approximately 12,000 years ago and then continued their migration to the Americas.

During the last decade, genotyping of *H. pylori* strains obtained from multiple human populations around the world has demonstrated that the genetic diversity of this bacterium reflects human migrations and subsequent geographic and ethnic separation between human groups. Analyses of genetic diversity of *H. pylori* are more frequently carried out by comparing sequences of housekeeping or virulence associated genes. Some authors have suggested that genotyping of *H. pylori* may be superior to human genetic markers to distinguish geographically related populations.[42] Analysis of multilocus sequence typing (MLST) of housekeeping genes has shown to be a robust and consistent test to study ancestry and evolution of *H. pylori* populations. Based MLST of seven housekeeping genes (*atpA, efp, mutY, ppa, trpC, ureI, yphC*) and one virulence-associated gene *(vacA)* of 370 strains isolated from 27 human populations, Falush *et al*.[43] identified four main clusters of *H. pylori* populations with distinct geographical distribution identified as: 1) hpEurope, 2) hpAfrica1, 3) hpAfrica2, and 4) hpEastAsia. Further analysis split hpEastAsia into hspAmerind, hspEAsia and hspMaori subpopulations. hpAfrica1 was subdivided into hspWAfrica and hspSAfrica.[43] More recent studies identified other main clusters: hpNEAfrica, hpAsia2, and hpSahul (Table 1).[6,41]

Table 1. Genetic Ancestral Prototype Populations of *Helicobacter pylori*[6,41,43,46,48]

H. pylori population	Geographic distribution
hpEurope	Europe, Middle East, India, and Iran
hpAfrica1	
hspWAfrica	Western Africa
hspSAfrica	South Africa
hpAfrica2	South Africa
hpNEAfrica	Ethiopia, Somalia, Sudan, northern Nigeria
hpEastAsia	
hspEAsia	East Asians
hspMaori	Taiwan Aboriginals, Melanesians, Polynesians
hspAmerind	Native Americans
hpAsia2	Northern India, Bangladesh, Thailand, Malaysia
hpSahul	Australia Aboriginals and Papua New Guineans

EVOLUTION OF ASIAN *H. pylori* STRAINS

It could be speculated that the *H. pylori* genome from the ancient Asian strains were less carcinogenic than the present more carcinogenic Asian and European strains. This change may be reflected in the elevated gastric cancer rates observed in Korea and Japan, where the *H. pylori* strains possess CagA with EPIYA-D motifs, linked to higher phosphorylation and higher cancer risk. Although the genotype of the ancient Asian strains is not known, it is generally assumed that that their virulence and carcinogenic potential was very low.[44] Censini *et al*.[22] linked the hypothetical emergence of virulent variants from less virulent variants to the acquisition into the *H. pylori* genome of the *cag* PAI. Present day evidence suggests that the secular evolution of the ancient Asian strains differed in Asia and the Americas. In both continents the *cag* PAI was inserted into the *H. pylori* genome, but the CagA protein in East Asian strains has an EPIYA-D motif and the Western version has instead one or more EPIYA-C motifs. Recent evidence supports a possible evolutionary pathway for the EPIYA-D segment by sequence rearrangement within the *cagA* gene.[45] This work, by Furuta *et al*.,[45] supports that the left half of the EPIYA-D segment characteristic of East Asian CagA was derived from Western-type EPIYA, and suggests that Amerind-type EPIYA could have been the intermediate, through rearrangements of specific sequences within the gene.

The value of *H. pylori* genome as a marker of human migrations has been well illustrated in studies carried out in Ladakh, an isolated location in the trans-Himalayan region of Northern India.[42] Two separate groups with independent social and religious characteristics inhabit Ladakh: Buddhists, primarily of Mongoloid Tibetan descent, and Muslims who migrated from Pakistan in the 14th century. The classical human genetic markers were insufficient to distinguish the two Ladakh communities. But *H. pylori* sequence analyses clearly showed the differences

between Buddhist and Muslim groups.[42] In India, a study using MLST of seven housekeeping genes found that most *H. pylori* isolates share significant homology with hpEurope strains, and the *cag* PAI revealed European ancestry as well.[46] A recent study of *H. pylori* strains from Cambodia,[47] analyzing the same seven housekeeping genes classified them in two groups, hpEurope and hspEAsia, and provided evidence supporting three ancient human migrations: 1) from India, introducing HpEurope into Southeast Asia, 2) from China, carrying hspEAsia, and 3) from Southern China into Thailand carrying hpAsia2. Their findings also support 2 recent migrations within the last 200 years: 1) from Chinese to Thailand and Malaysia spreading hspEAsia strains and 2) from Indians to Malaysia carrying hpAsia2 and hpEurope.[47] A study of *H. pylori* isolates from Malaysia classified them as hpEastAsia, hpAsia2 or hpEurope, and revealed a new subpopulation, hspIndia, within hpAsia2.[48]

In Oceania, there is evidence of two ancient migrations: one reached New Guinea and Australia, and a second, more recent, extended through Melanesia and from there to the Polynesian islands. These migrations were accompanied by two distinct *H. pylori* populations, hpSahul and hspMaori, respectively.[41] After the European settling, the hpEurope predominates.[49]

THE AMERICAS PRE- AND POST- COLUMBUS

It is thought that when humans traveled across the Bering Strait from Asia to North America, they were carrying *H. pylori*, and therefore, their descendants today should be carrying *H. pylori* strains with Asian genotypes. Consistent with this hypothesis, multiple studies including Native North American and South American *H. pylori* strains have found evidence of genetic similarity with Asian strains.[43,50-53] The genetic diversity of the original settlers of the Americas has significantly increased mainly through the genetic flow of Europeans and Africans over the last 500 years. This is a fact extensively reflected in the *H. pylori* populations.

1. The colonization of the Americas

The Spanish Crown sponsored three trips of Columbus, starting in 1492, to what they thought was a new route to the "Indias." But instead of India they found the new American continent, at that time unknown to the Europeans or the Asians. The Spanish settlers brought not only political and social changes; they also transformed most Amerindian into mestizo populations (mixture of Amerindian and European ancestry). At present, the Amerindian component varies greatly, in proportion to the density of the original native population. In Mexico and Guatemala, where the density of the original population is greater, the Amerindian component is greater than in areas such as Southern Brazil and Central Colombia, with lower density of original native populations.[54,55] In general, mestizos in Latin America are mostly colonized by hpEurope strains and the original hpAmerind strains seem to be restricted to a minority of isolated Amerindian populations. As mentioned above, the *H. pylori* genome variants reflect the human demographic evolution.

2. The slave trade

H. pylori infection is ubiquitous in Africa, but gastric ulcers and gastric cancer are rare events.[56] In the 16th century Portuguese merchants started exporting slaves from Africa to the Americas. African slaves had been brought to Europe for centuries, mostly from the Muslim dominated north coast. They were well educated and had a tendency to rebellion, which limited the trade. Slavery was also a traditional practice within some indigenous African societies.[57] Various African kings operated several forms of slavery. When the foreign traders came with a demand for labor, West Africans swiftly developed an organized trade.[57] The exportation of African slaves to the Americas lasted about four centuries, reaching the largest numbers when the British, Spanish, French and Dutch traders joined the business. It is estimated that about 11 million slaves were forcefully recruited for the Atlantic trade. At least one million died during the voyage or soon after, mainly from malnutrition and diseases encountered during the forced marches and in slave camps. Most slaves came from West Africa and included multitude of ethnic groups representing north, central and southern West Africa.[58] The identification of ancestry specific markers has allowed investigators to build reasonable hypotheses about the predominant *H. pylori* genotypes in the Americas before and after the Spanish expeditions to the New World.[49,53] The original predominant *H. pylori* strains were apparently hspAmerind, a subpopulation derived from hpEastAsia. After Columbus and the following Atlantic slave trade, such strains are rare and there is a predominance of hpEurope in mestizos and hspWAfrica in African Americans and mulattoes (mixture of African and European ancestry).

In South America, *H. pylori* strains from Amerindians in the Venezuelan Amazon were compared to strains from a mestizo population from Caracas by examining three independent highly polymorphic *H. pylori* genetic loci. Amerindian strains were found to have genotypes related to East Asia, while the mestizo population harbored strains with Western patterns.[52]

Recent studies including *H. pylori* isolates from Mexican indigenous groups show unique polymorphisms of the *cagA* and *vacA* genes revealing Asian, European and African sequences.[50] Concatenated analysis of housekeeping genes showed that although many isolates clustered within the European groups, some strains clustered in a position intermediate between East Asian and European groups. They represent evidence supporting that Amerindian strains are being displaced by European strains. This novel Amerindian group had been previously found in isolates from indigenous populations in Colombia (Huitotos), Venezuela (Piaroas), and Peru (Shimaa).[50,51,59-61] However, mesti-

zos from the Americas (of mixed Asian and European ancestry) harbor HpEurope strains.[51] The low diversity of Amerindian strains may be linked to their apparent tendency to disappear when colonizing non-Amerindian hosts.

In Colombia, inhabitants of the high-altitude Andes Mountains have very high incidence rates of gastric cancer compared to inhabitants of the Pacific coast. Both populations have similarly high prevalence of *H. pylori* infection. People living in the mountains are mostly mestizos (of Amerindian descent with European admixture), whereas people living on the Pacific coast are predominantly mulattos (mixed African and European ancestry). The prevalence of *cagA+ vacA* s1m1 *H. pylori* strains in the high risk region is only slightly lower than in the low-risk region.[62] Using MLST of seven housekeeping genes, we analyzed *cagA+ vacA* s1m1 strains from both regions of Colombia and compared them to 380 reference strains.[63] All strains of the high risk region (Andes Mountains) were classified as hpEurope (n=35), while among strains from the Pacific coast, 34.5% (n=10) were classified as hpEurope, and the remaining 65.5% (n=19) were classified as hpAfrica1 (3 hspSAfrica, 16 hspWAfrica) (Fig. 2). In this study, strains of European ancestry were associated with more advanced precancerous gastric lesions and greater oxidative damage in gastric mucosa than strains of African ancestry.[63] Known differences in gastric cancer rates may, at least partially, be explained by *H. pylori* genotypes: high rates in mestizos colonized with more virulent hpEurope strains, as documented in the Colombian Andes Mountains, and low rates in African descendents colonized with the less virulent hspAfrica1 strains, as documented in dwellers of the Colombian Pacific coast.

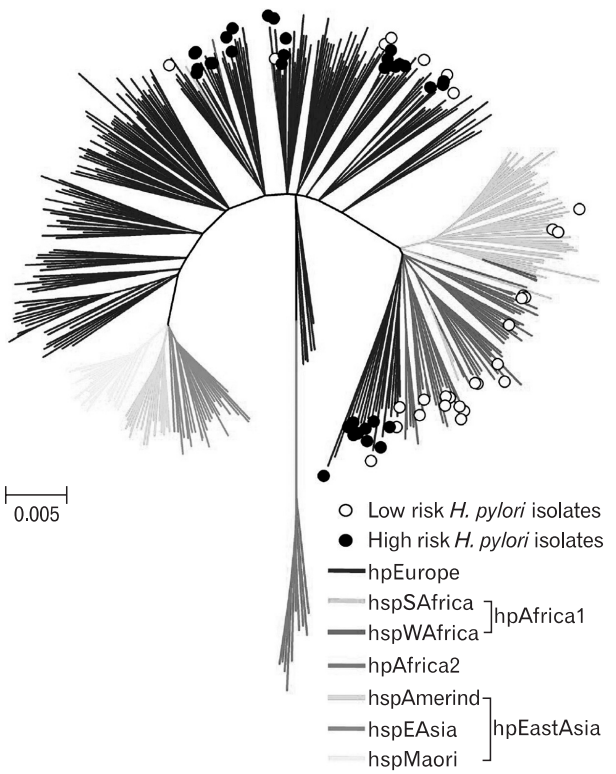

Fig. 2. Phylogeography of *H. pylori*. Neighbor-joining tree of 64 *cagA+ vacA* s1m1 Colombian *H. pylori* isolates from the high-risk (black circles) and low-risk (open circles) regions, along with 380 reference strains that were previously classified into distinct ancestral haplogroups. Branches are drawn to scale to represent evolutionary distance. The colors of the branches represent the classification of strains into distinct populations (From de Sablet T, et al. Gut 2011;60:1189-1195, with permission).[63]

In the United States, African Americans have gastric cancer rates approximately twice those of Caucasians.[64] This phenomenon is probably not related to *H. pylori* genotypes but more related to the prevalence of *H. pylori* infection in these two ethnic groups, approximately double in African Americans than in Caucasians.[65] Gastric cancer rates in U.S. African Americans are much lower than those of mestizos in Latin America.

SECULAR (CENTURIES OLD) TRENDS OF GASTRIC CANCER: TWO EPIDEMICS?

In epidemiologic terms, an epidemic is defined as an unexpected increase in the frequency of a disease. Chronologically, epidemics display a bell-shaped curve: an ascending portion, a plateau and descending portion. Sonnenberg[66,67] has analyzed time trends of mortality from peptic ulcer and gastric cancer in several countries in terms of their birth cohort pattern. The rates rose in generations born during the 18th century until the mid-19th century and then have declined in all subsequent generations. He links the decline of mortality rates to the decreasing prevalence of *H. pylori* infection in the general population. But, in his words, the sudden increase in mortality for these *H. pylori* related a disease within a relatively short time during the 19th century remains an enigma. It might be that the enigma is related to the secular evolution of the *H. pylori* genome, with the acquisition of the *cag* PAI, which occurred both in Asia and the West.[44] Sonnenberg's graphs also show that the steady decline in cancer rates stops for cohorts born around the third decade of the twentieth century, for which increasing rates are observed. This "second" epidemic is driven by younger persons and has previously been detected in Spain and the United States.[68,69] The causes of this apparently incipient epidemic are unknown. There is no evidence suggesting that a recent evolution of the *H. pylori* genome has taken place. The generalized abuse of antacid medications is a phenomenon that preceded this new epidemic, but a causal link to the new epidemic has not been established.

EPILOGUE

The co-evolution of *Homo sapiens* and *H. pylori* throughout millennia may be coming to an end, especially in affluent societies in which marked improvements in home and food sanitation have taken place and antibiotics may be abused to treat minor or to "prevent" infections. The prevalence of the infection

has been declining steadily, especially in younger individuals. This results in cohorts that have never been infected with *H. pylori*. In most subjects, *H. pylori* is commensal, not related to disease. It is hypothesized that the loss of the natural balance between the two species may result in disease and the total absence of *H. pylori* for life has been linked to new epidemics being observed at the present time. One such epidemic is the adenocarcinoma of the lower esophagus, linked to reflux esophagitis and Barrett's esophagus. These may reflect relatively excessive gastric acid secretion, which could be linked to the absence of *H. pylori* infection. Other diseases apparently increasing in frequency lately are allergic disease such as asthma and autoimmune diseases. It has been proposed that these new epidemics may be related to the so-called hygiene hypothesis.[70] Exposure to *H. pylori* and other infections during childhood may drive a Th1 (pro-inflammatory) immune response that may set a pattern of response to later antigenic stimuli. In the absence of infections, antigenic stimuli after the childhood years may lead to Th2 (allergic) immune responses, linked to asthma. This hypothesis has been recently supported in an animal model.[71]

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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