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The relation of *Helicobacter pylori* to gastric adenocarcinoma and lymphoma: pathophysiology, epidemiology, screening, clinical presentation, treatment, and prevention

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Helicobacter pylori infection may be the most common chronic bacterial infection worldwide. Presently, it colonizes about 50% of the world's population, most commonly in individuals in developing countries [1]. According to serologic studies, the infection rate among 45-year-old men in California during the 1960s was more than 60% [2]. By extrapolation, *H pylori* infection was probably nearly universally present in humans at the beginning of the twentieth century [3].

Currently *H pylori* infects most people in developing countries, and 20% to 40% of individuals in Western countries [1]. Subgroups of the population in Australia and the United States have a high prevalence, such as immigrants from third world countries and indigenous populations [4,5]. A high prevalence of *H pylori* nearly always correlates with low socioeconomic status [6].

The association between *H pylori* and gastric cancer is an extrapolation from the known association between gastritis and gastric cancer. From postmortem series and analyses of gastrectomy specimens, various forms of

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gastritis were strongly associated with gastric cancer [7]. As it became evident that *H pylori* was the most important cause of gastritis, a causative role of *H pylori* on gastric cancer was postulated in 1983 [8].

Presently, gastric cancer is one of the most frequent cancers in developing countries and about the seventh most common in developed countries [9]. In developing countries, gastric cancer is even more common than cancers of the lung and liver. The gastric cancer incidence in most countries where *H pylori* is common is at least 40 per 100,000 per annum, but the gastric cancer incidence ranges from 50 to 100 persons per 100,000 per annum in Japan, Korea, and Colombia [9].

Gastric cancer was once the most common cancer in the United States, but the incidence decreased from more than 50 per 100,000 per annum in 1930 to approximately 7 per 100,000 per annum in 1980 [10]. The gradual decline of gastric cancer was attributed initially to dietary changes (ie, the introduction of refrigeration and widespread access to fresh fruit and vegetables after 1930). The prevalence of *H pylori* in the United States remained high until about 1970, so the major reduction in gastric cancer incidence between 1930 and 1970 cannot be attributed to *H pylori* infection, except if it relates to a lower incidence of *H pylori* in very young children (discussed below) [2,11].

The first global analysis of the association between cancer and *H pylori* was described by the Eurogast Study Group in 1993 [12]. They analyzed the mortality from gastric cancer in many countries, and showed mortality was significantly correlated with the prevalence of *H pylori*. The study may have erred in estimating the relative risk attributed to *H pylori*, because histologic types of cancer were not well defined and proximal versus distal gastric cancers were not separated. Huang et al [13] found that cardial cancer was much less likely to be related to *H pylori*, with a relative risk of only 1.2 versus 3.1 for antral cancer.

The mortality from gastric cancer is approximately 800,000 per annum worldwide. The global mortality is shown as a function of *H pylori* infection rates in Fig. 1. About 50% to 75% of the mortality from gastric cancer is attributable to *H pylori*. Although most gastric cancers occur after age 50, many individuals develop gastric cancer at an early age, even in their 20s, in countries with a high incidence of gastric cancer, such as Japan. The age-related incidence of gastric cancer in Japan is shown in Fig. 2.

Japan is a developed country with a high gastric cancer rate. David Graham states ''The Japanese have a first world economy and a third world stomach.'' This phenomenon probably reflects the emergence of the Japanese economy from a postwar depression to a modern era at about 1970, after which acquisition of *H pylori* in Japan was greatly reduced. For example, persons below the age of 30 in Japan now have a much lower prevalence of infection with *H pylori* than their parents (25% vs 60%). Because of the magnitude of the gastric cancer problem, annual screening endoscopy was instituted in Japan after age 40 to detect and resect early

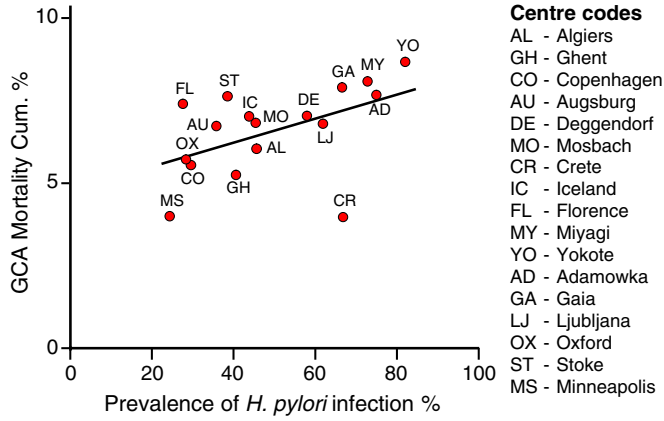

Fig. 1. Global correlation between *H pylori* and gastric cancer mortality. The graph plots prevalence of *H pylori* in the adult population against deaths from gastric adenocarcinoma. Such data do not separate various histologic types of cancer, and ignore cancers that are detected early and cured. (*From* EUROGAST Study Group. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet 1993;341:1359–62; with permission.)

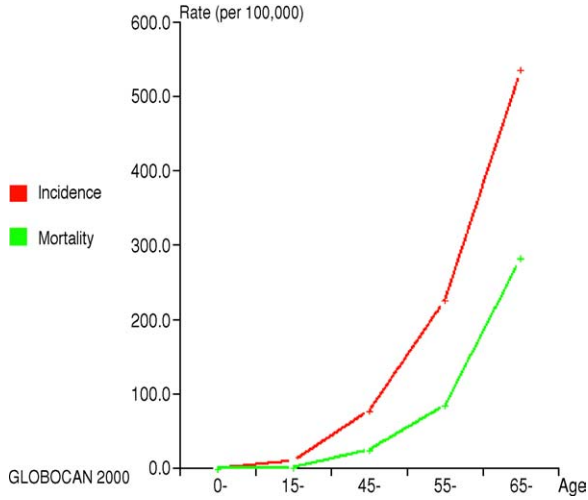

Fig. 2. Age-related incidence and mortality from gastric cancer for Japanese males. More than 2800 new cases of stomach cancer occur in persons under the age of 44 years. (*Data from* Ferlay J, Bray F, Pisani P, et al. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide. Version 1.0. Lyon: IARCPress; 2001.)

gastric cancer. Such a screening program decreases the gastric cancer mortality by two thirds in compliant persons, but costs more than US$1 billion per annum in Japan. The detection rate of gastric cancer is 0.13%, creating value of $300,000 for each 55-year old saved, at a cost of only $50,000 [14]. Despite these screening programs, approximately 12,000 Japanese die from gastric cancer per year.

History of the discovery of *Helicobacter pylori*

Helicobacter pylori was discovered by Marshall and Warren [15]. In studies from 1979 to 1983, they observed a very strong association between *H pylori* and gastritis and between *H pylori* and duodenal ulcer. They developed the hypothesis that *H pylori* caused gastritis, which could be a precursor to both peptic ulcer and gastric cancer. This hypothesis explained why ulcers recurred when acid-suppressing drugs were discontinued. It followed that eradicating *H pylori* might cure peptic ulcers.

They recognized that most of the literature on gastritis was actually describing *H pylori* gastritis. There was some confusion between the autoimmune gastritis of pernicious anemia, which also caused atrophic gastritis and which had an end-stage lesion histologically similar to that caused by *H pylori*. It is now known that the late stages of *H pylori* infection may cause gastric atrophy with intestinal metaplasia, and that this lesion probably predisposes an individual to gastric cancer.

Helicobacter pylori epidemiology

In 1900 most of the world's population probably had gastric infection with *H pylori* [3]. Since then the prevalence of the infection has decreased to approximately 25% of the population in Western countries and an even lower percentage for individuals born after 1970. Several studies have documented a rapid decline in *H pylori* prevalence in the United States between 1970 and 1990, with similar changes in Australia [11,16]. In Japan, where the transition to a developed country with a modern standard of hygiene was delayed for 20 years by the Second World War, individuals born after 1980 have a very low prevalence of *H pylori* [17].

Seroepidemiologic and urea breath test studies performed between 1985 and 1995 confirmed the decline in *H pylori* incidence in many countries, except for developing countries with poor economies. For example, the incidence of new *H pylori* infection in young children remained at 25% per annum in Africa, so most children became infected by 5 years of age [18]. Conversely, in developed countries such as Japan and Australia, new infections in young children were uncommon, so that the prevalence of infection in children and young adults became low [19]. This resulted in distinct prevalence curves, as illustrated in Fig. 3A, for developing versus developed countries. In Western countries, persons born before 1950 tend to have a high prevalence, reflecting a high infection rate in that country when

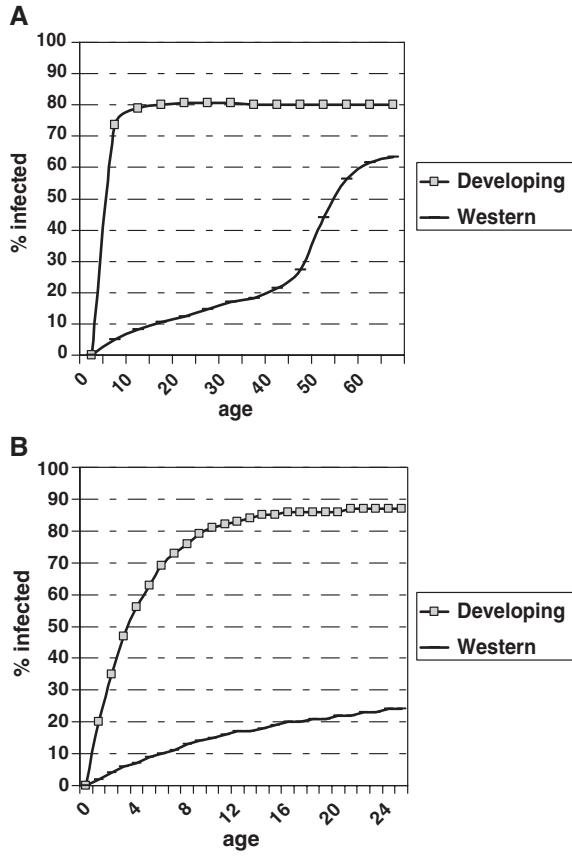

Fig. 3. Epidemiology of *H pylori*: Western versus developing countries. (*A*) Whole of life. (*B*) Childhood. The upper graph shows that even in a Western country, older persons have a high prevalence of *H pylori* because they acquired the infection in childhood. The lower graph illustrates a developing country where the infection rate is 20% per annum and the loss of the infection rate is 3% per annum.

those individuals were children. For example, the United Kingdom and the United States had a prevalence of more than 50% in 50 year olds, versus about 25% in 20 year olds when these data was first examined in the 1980s [1,2,11]. Fig. 3B shows the contrasting epidemiologic curves from a developing country, represented in West African studies, where most children are infected by age 3 [20].

Initially the Western (developed) pattern (Fig. 3) was thought to be caused by a 1% incidence of new *H pylori* infections throughout life, but further studies showed that the prevalence of infection in any particular birth cohort was fixed. The lower incidence (ie, lower acquisition rate of new infections) caused children in these population studies to have a lower prevalence. The prevalence of *H pylori* in children serves as a surrogate

marker for the incidence (ie, if an environmental source for *H pylori* exists and the incidence is high, children become infected by age 5). This incidence also reflects the reinfection rate with *H pylori*, which is high only where environmental sources of *H pylori*, such as drinking water (in Peru), are present [21]. The lowest prevalence in children now exists in New Zealand, Japan, and Australian-born white Australians [22].

The mode of transmission of *H pylori* is not clearly understood. In Peru, for example, the high incidence of *H pylori* has been linked to contaminated water. Using ultrasensitive techniques, which combine magnetic bead separation and polymerase chain reaction, some studies have detected *H pylori* genes in drinking water [23]. In Peru individuals have a high reinfection rate after eradication treatment with new strains of *H pylori* [24]. In addition, young children are infected by the age of 5. These factors render it likely that water-borne transmission of *H pylori* is common in Peru. In other areas, particularly in Africa, the high prevalence of *H pylori* may be related to fecal-oral transmission secondary to poor general hygiene. Where young children are generally infected, they act as an amplifier, transmitting infection to their siblings and possibly to adults. Even in the United Kingdom, the prevalence of *H pylori* in adults correlates with the standard of living of those persons in childhood [25]. Important factors include the number of children present in the family, the sharing of beds among siblings as children, the absence of hot running water in the house, and the number of persons per room in the household [26].

Initially, the high prevalence of *H pylori* in developing countries suggested a genetic predisposition for infection; however, the global epidemiology shows that the standard of living is the most important risk factor. For example, the white populations in Estonia and Russia had a very high prevalence of *H pylori* before 1990, whereas genetically similar populations in Finland and Sweden had a Western prevalence [27]. In the United States and Australia, the prevalence of *H pylori* also correlated with family income [6,28], at least for the white population in the United States, but in African-Americans, the *H pylori* prevalence did not correlate with income. A high childhood incidence of *H pylori* is related to the socioeconomic conditions of childhood, so that affluent African-American adults probably lived in relatively poor conditions in early childhood, a time when they would have acquired *H pylori* and carried it for the rest of their lives [29]. The prevalence of *H pylori* also relates to the percentage of immigrants in a country. For example, immigrants to the United States from Latin America carry their birth-country's prevalence, usually 60% or more [1,22,30].

Taxonomy and morphology

H pylori is the type species of a new genus called "*Helicobacter*" because of its helical or spiral forms. The organism is typically 3.5 μm long, with 1.5 wavelengths present with a diameter of 0.6 μm. It has about seven flagella,

usually coming from one end of the bacterium. These are sheathed, which distinguishes them from the *Campylobacters*. *Helicobacters* are microaerophilic, colonize the mucus layer of the gut, and usually produce urease. These features allow the organism to survive for a time in the gastric lumen by generating a local environment high in ammonia, which is able to neutralize some of the hydrogen ions present in the gastric juice. *H pylori* can survive for a time after ingestion allowing it to attach and penetrate the mucus layer and to adhere to various carbohydrate, lipid, and protein moieties on the epithelial cells. The oxygen saturation in this part of the mucosa is microaerophilic, between aerobic and anaerobic.

Recently the *H pylori* taxonomy has been analyzed by the relatedness of the 16S RNA gene sequence. Using this method, *Helicobacters* clearly form their own genus with at least 30 known species. Most of the species are urease, catalase, and oxidase positive. *Helicobacters* are found particularly in the stomach of carnivores and are seen in cats, dogs, ferrets, and even animals as diverse as dolphins and whales [31]. *Helicobacters* are closely related to *Campylobacter*, but also to *Wolinella succinogenes*, an organism found in the cow rumen.

Detailed sequencing studies of *H pylori* housekeeping genes (essential genes found in all *H pylori* organisms) have revealed that the evolution of *H pylori* parallels the evolution and migration of humans during the past 50,000 years [32]. *H pylori* seems to have accompanied mankind since he left Africa in the ice age. An initial puzzle was that *H pylori* isolated in South America were usually of the Spanish subtype. When studied further, it was found that indigenous tribes in the deep Amazon occasionally carried *H pylori* strains of Asian type [33]. It seems that *H pylori* followed Siberian tribes into Alaska and down to South America 30,000 years ago. These ancient strains of *H pylori* were then outnumbered during the Spanish colonization of South America, so most South Americans now have European strains.

Helicobacter pylori is noninvasive, rarely penetrating beyond the epithelial cell basement membrane. It can, however, damage epithelial cells in several ways, the most important of which is by toxins. The CagA toxin is part of the *cag* pathogenicity island (*cag* PAI), a group of approximately 30 genes used by the organism to produce a type IV secretion system, analogous to a hypodermic syringe, which injects the CagA toxin into epithelial cells [34,35]. The prototype type IV secretion system is found in the plant bacterium *Agrobacterium*, which uses a pilus-like structure to inject proteins and sometimes genetic material into host cells. This causes the host cell to modify its metabolism and nourish the parasite. The *cag* PAI has a G + C abundance of 35% compared with 38% to 45% for the rest of the *H pylori* chromosome suggesting that the island was acquired in entirety from another organism [34].

Once inside epithelial cells, CagA hijacks the proliferative machinery of the cell, causing it to become rather plastic and lose its square epithelial cell

structure [36]. This presumably supplies increased nutrition to the attached *H pylori* organism. *H pylori* also produces VacA toxin, a nonspecific membrane pore, which causes dilatation of subcellular organelles in the epithelial cell leading to the light and electron microscopic appearance of vacuoles within the cell [37,38].

In Western countries, only 60% of *H pylori* strains carry the *cag* PAI and, for some unknown reason, harmful forms of the VacA toxin are associated with the presence of the *cag* PAI [39,40]. In developing countries, however, more than 90% of infected individuals carry a toxin-producing *cag* PAI [41], with associated toxic versions of VacA. Because of their increased virulence and perhaps greater ability to extract nutrition from gastric epithelium, these "toxigenic type 1" strains cause increased levels of inflammation and heavier colonization with *H pylori* [40]. Whereas all *H pylori* cause gastritis in the stomach, the activity of the gastritis is mildly up-regulated in toxigenic strains. Possibly for this reason, these strains are more often associated with peptic ulcer and gastric cancer [42].

CagA toxin binds to the SH2-containing protein tyrosine phosphate SHP-2 in a tyrosine phosphorylation-dependent manner and stimulates phosphatase activity. Affected cells lose their square epithelial structure and become more motile and amorphous, a structure less compatible with the integrity of gastric mucosa. Hatakeyama [43] suggests that, given the positive regulatory roles of SHP-2 in both cell proliferation and cell movement, the CagA-SHP-2 interaction may play a role in the oncogenic transformation associated with CagA-positive *H pylori* infection.

VacA, the vacuolating toxin, is present in all *H pylori*, but the toxin has various subtypes that affect pathogenicity. VacA has an end "signal sequence" and a "middle sequence" in which most of its variability occurs. The variable parts have a mosaic structure such that toxin subtypes can be characterized into s1, s2, m1, and m2 [44]. The most virulent *H pylori* strains carry the s1 and m1 subtype, and this VacA toxin type is also most likely to be associated with the presence of the *cag* PAI.

After passage through the bacterial cell wall VacA forms a hexamer in the presence of low pH and inserts itself into the cell membrane of the epithelial cells to form a pore [45]. Ultimately subcellular structures are attacked leading to vacuolization. In addition to the s1 subtype, toxicity of VacA also depends on the tightness of the attachment of *H pylori* to epithelial cells [46]. VacA induces apoptosis in the epithelial cells, an effect dependent on the presence of acid and ammonium chloride [47]. The apoptotic effect of *H pylori* is mediated in the usual manner by host proteins ARF or p53 [48].

BabA, another surface exposed protein, is actually an adhesin that attaches *H pylori* to carbohydrate structures on the epithelial cells. BabA, by mediating adherence to epithelial cells, affects the virulence of the other toxins, CagA and VacA, which are somewhat contact dependent [42,46]. The sequence of the BabA gene can be used to define subtypes of *H pylori* in the same way that VacA and housekeeping gene sequences are used.

Because BabA binds to Lewis B antigens, it is relevant that *H pylori* also produces carbohydrate groups related to human blood group structures present in the mucosa. *H pylori* is classified into Lewis X and Lewis Y subtypes. These antigens on its surface may allow it to partly mimic the gastric epithelium and possibly evade the immune response. The subject has been extensively reviewed by Blaser and Atherton [49].

Natural history of *Helicobacter pylori* infection

Helicobacter pylori is usually acquired in childhood. Infection is contracted by drinking contaminated water or by contact with fecal material or vomitus. The individual may develop vague epigastric discomfort associated with some nausea and vomiting. During the acute infection, *H pylori* colonizes the gastric mucosa and causes severe acute inflammation. Although the data on acute infection are limited, transient hyperacidity, acute ulceration, increased mucus production, and hypochlorhydria have been described during acute infection [50–53].

After approximately 3 days, the individual may become achlorhydric because the parietal cells in the proximal stomach are injured and unable to secrete acid. This effect is probably caused by a direct toxic effect of *H pylori*, or possibly because of the acute inflammation because interlukin-1 powerfully inhibits acid secretion. Soon after infection, the immune system is triggered and 4 weeks after the initial colonization, antibodies appear in the blood. Initially the antibodies are IgM, but after 6 to 12 weeks most colonized individuals produce IgG (95%–100%) and IgA (80%). Antibody levels are relatively stable in adulthood, but are particularly high in individuals who develop gastric cancer.

After the initial acute inflammation, the gastric mucosa becomes infiltrated with chronic inflammatory cells (ie, lymphocytes, plasma cells, and macrophages), which become more prominent as the polymorphonuclear infiltration declines. Ultimately most of the inflammatory cells in the mucosa are mononuclear chronic inflammatory cells, including both B and T lymphocytes. Specific antibodies are produced by B lymphocytes in the gastric mucosa. In addition, a small number of neutrophils remain, and these typically aggregate around the gland necks as neutrophils migrate between the epithelial cells toward *H pylori* organisms in the glands and in the mucus layer. Neutrophil migration is particularly prominent in the duodenum, causing small ''pimples'' with aggregates of neutrophils migrating through the mucosa. This may be the precursor lesion to duodenal ulcer.

As the chronic inflammation becomes predominant and antibody production increases, acid secretion may return, but some individuals remain achlorhydric. This probably relates to ongoing inflammation caused by *H pylori* deep in the glands of the acid-secreting gastric corpus. The *H pylori* infection in the corpus, however, is not as robust as that in the

antrum. Components of the normal diet and antibiotics can mildly inhibit *H pylori* colonization, enough to allow some acid secretion to return and flush the *H pylori* bacteria out of the corpus glands and out of the deep parts of the acid-secreting gastric mucosa [52]. This results in a typical ''Western'' pattern of *H pylori* infection with a mild, superficial gastritis present in the proximal stomach, where acid is secreted, and a more active gastritis in the antrum. Normal acid secretion with inflamed antral mucosa is typical for duodenal ulcer. *H pylori* colonization of the duodenal bulb is also present in many ulcer patients because at least two thirds of individuals have some gastric mucosa in that region, either antral-type mucosa secreting only mucus (gastric metaplasia), or islands of gastric corpus mucosa, which contain parietal cells and secrete acid (gastric heterotopia). Duodenal ulcers occur because *H pylori* colonizes these areas in the duodenal bulb. A different mucosal environment there and the propensity for this mucosa to produce a florid neutrophilic inflammatory response are also important factors in ulcer development.

After many years, chronic inflammation of the antrum, and to a lesser extent the corpus, damages the epithelium so that parts of the normal gastric tissue are replaced by intestinal-type mucosa (intestinal metaplasia). This is characterized histologically by brush border cells and goblet cells if the metaplasia is complete, or by less well-differentiated epithelial cells if the metaplasia is incomplete. These antral changes gradually migrate into the corpus. The process is often multifocal with intestinal metaplasia arising simultaneously at many locations in the corpus. As the normal mucosa is replaced with chronic inflammatory cells and simple antral type glands, the stomach develops atrophic gastritis, the final precursor lesion of gastric cancer. Typically, individuals who develop gastric cancer have hypochlorhydria or achlorhydria because of replacement of most of the parietal cells. Often gastric contents become putrid because of the lack of acid.

Treatment of *Helicobacter pylori* infection

H pylori infection is reliably eradicated only with antibiotic therapy. In most treatment regimens gastric acidity is removed with a proton pump inhibitor. The degree of acid suppression varies somewhat in individuals depending on genetic polymorphisms, which affect the metabolism and the half-life of proton pump inhibitors, particularly omeprazole. Nevertheless, marked acid suppression enables antibiotics, such as amoxicillin, to work. Two drug regimens of a high-dose proton pump inhibitor with 3 g of amoxicillin daily for 7 to 14 days eradicate 50% to 75% of infections [54–56].

Addition of a second antibiotic, usually clarithromycin, increases the cure rate to 90% [57]. Side effects, most notably a bitter taste in the mouth from clarithromycin, are tolerable. When individuals are allergic to penicillin, amoxicillin should be replaced with metronidazole, and this so-called

"Bazzoli therapy" has been shown to cure nearly 90% of *H pylori* infections in Italian studies, making it the first choice of therapy in some regions [58].

Twenty-five percent to 50% of *H pylori* strains are resistant to metronidazole, so alternative regimens are required in penicillin-allergic patients. Recently, a successful regimen has rabeprazole combined with levofloxacin and rifabutin. Wong et al [59] eradicated more than 85% of *H pylori* infections in persons who had failed other therapies.

One of the earliest treatments for *H pylori* used bismuth subcitrate or subsalycitate (DeNol or Pepto-Bismol) combined with tetracycline and metronidazole [60,61]. By adding a histamine (H2)-blocker or proton pump inhibitor to this regimen, high cure rates are still achieved and this combination often overcomes metronidazole resistance for unknown reasons. A typical treatment regimen is DeNol, 1 tablet four times a day, a proton pump inhibitor, such as rabeprazole, 20 mg twice a day, tetracycline, 500 mg four times a day, and metronidazole, 400 mg three times a day for 7 to 14 days [59]. This treatment is inexpensive, useful for penicillin-allergic patients, and for retreatment after other therapy has failed.

Association of *Helicobacter pylori* infection with gastric carcinoma

Epidemiology and history of gastric cancer

About 800,000 people die from gastric cancer annually worldwide, including about 500,000 males and 300,000 females [9]. As shown in Fig. 4, the incidence of gastric cancer in some countries greatly exceeds the mortality because of effective screening programs.

At least 10,000 deaths from gastric cancer occur in Japan each year. In addition, gastric adenocarcinoma is not rare in individuals less than 40 years old in Japan. Because it is usually associated with hypochlorhydria, gastric cancer has a long asymptomatic stage and produces symptoms only when the tumor is large or when metastasis has occurred. For this reason, effective protection from gastric cancer mortality must either prevent the disease or remove early nonmetastatic tumors. This latter program has been underway for 40 years in Japan where persons more than 40 years old may choose to have an annual endoscopic examination and resection of any suspicious lesion. Many cancers are thereby discovered as carcinoma in situ and are curable. Nevertheless, removal of one gastric cancer signals a patient at increased risk of future cancers, with metachronous gastric cancers appearing in about 2% of individuals per annum [62].

Most countries with high gastric cancer rates have a high prevalence of *H pylori*. The exception to this rule, the "African enigma," is that African countries, although highly infected with *H pylori*, do not have a very high incidence of gastric cancer. In Fig. 4, this is demonstrated for Kenya, a country with a relatively low incidence of gastric cancer despite a high prevalence of *H pylori*.

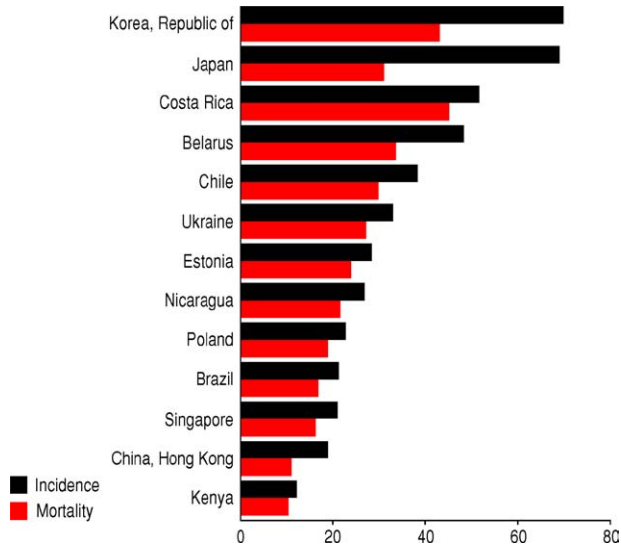

Fig. 4. Mortality versus incidence of gastric cancer by country. The listed countries are sorted both by cancer mortality and by cancer incidence. Note that in Japan, incidence is far greater than mortality. This is because effective screening programs detect gastric cancer at an early stage and cure is possible. Although high gastric cancer usually correlates with *H pylori* prevalence, there are some notable exceptions. Kenya is the representative African country with a rather low gastric cancer rate but a high *H pylori* prevalence. This paradox, referred to as the "African enigma" by some, demonstrates that cancer causation from *H pylori* must be modulated by many factors. These could be dietary, ethnic, and bacterial strain related. (*Data from* Ferlay J, Bray F, Pisani P, et al. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide. Version 1.0. Lyon: IARCPress; 2001.)

Evidence of an association

Experimental evidence of a causative role for *H pylori* in gastric adenocarcinoma comes from small animal studies. Yokota et al [63] established *H pylori* infection in Mongolian gerbils. These infected animals develop severe gastritis and intestinal metaplasia [63,64]. Watanabe [65] in a prospective study found that 37% of infected Mongolian gerbils developed adenocarcinoma and several developed carcinoids during 62 weeks. In this study, the whole gastric mucosa became inflamed and hyperplastic with most animals developing gastric ulcers by the 26th week of infection. Infected animals then developed intestinal metaplasia, especially in the pyloric region and most commonly in association with gastric ulcers. Of 27 animals still alive at the end of the study, 10 had developed adenocarcinoma and all of these cancers were in the prepyloric area. All tumors were well-differentiated intestinal type epithelial adenocarcinomas. Three carcinoids developed in fundic mucosa and one of these was invasive.

Compared with other studies, Watanabe's gerbils [66,67] had a very high incidence of adenocarcinoma, and most other experiments have required additional carcinogens, such as *N*-methyl-*N*-nitrosourea or *N*-methyl-*N*-nitro-*N*-nitrosguanidine, to demonstrate potentiation of a carcinogenic effect by *H pylori*. With this methodology, *H pylori* and other *Helicobacters* have been shown to be carcinogenic in mouse models of the infection [68]. Sugiyama et al [69] showed that *H pylori* potentiates the carcinogenic effect of low levels of known carcinogens, such as *N*-methyl-*N*-nitrosourea. When low-dose *N*-methyl-*N*-nitrosourea was given in drinking water for 6 weeks followed by *H pylori* infection, 33% of animals developed adenocarcinoma. Control groups with solely *H pylori* or *N*-methyl-*N*-nitrosourea did not develop cancer.

Other evidence of carcinogenicity comes from transgenic models. Hypergastrinemic (INS-GAS) mice usually develop gastric adenocarcinoma in old age. When *H pylori* is given, cancer is preceded by gastritis and intestinal metaplasia, but surprisingly, cancers tend to be accelerated by *H pylori* only in male mice. This may parallel the human condition where males are about twice as likely to develop gastric carcinoma [68]. In this study some of the carcinogenic effect was attributed to the *cag* PAI, more specifically the *cagE* component, which is an ATPase driving the type IV secretion system. C*agE* defective isolates of *H pylori* still generated cancers, but these cancers were greatly delayed compared with wild-type *cag*-positive *H pylori* strains [68].

The animal models of *H pylori* carcinogenesis are consistent with the concept of *H pylori*–associated gastritis leading to epithelial cell changes (intestinal metaplasia), a type of mucosa, which accumulates genetic defects. Whether this mucosa is precancerous or merely a marker of carcinogenesis is unknown. Nevertheless, individuals with gastric metaplasia are more likely to develop adenocarcinoma.

As in other cancers, *H pylori*–induced cancer might be more likely if programmed cell death (apoptosis) is defective in some way, so that proliferative signals proceed unchecked. The p53 gene instigates apoptosis and mutations disabling p53 are implicated in many cancers [70]. The p19Arf tumor-suppressor is an activator of p53. There is some evidence that p53 or ARF are induced when *H pylori* infects epithelial cells [48].

In summary, numerous studies hint that *H pylori* can trigger carcinogenesis. These hints cannot, however, be amalgamated into a coherent hypothesis. It seems *H pylori* triggers a proliferative response in the epithelium, perhaps related to the delivery of CagA into the epithelial cells, whereas components of *H pylori* such as CagA and VacA cause increased apoptosis to balance the proliferative effect. Although these events are not necessarily carcinogenic, the associated inflammation, reactive oxygen species, nitric oxide, HCl, and ammonia may generate known carcinogens, such as nitrosodimethylamine [71,72]. Ultimately, mutations occur (eg, in the p53 gene), which give *H pylori*–infected epithelial cells a proliferative

advantage. The final steps toward malignancy might not require the continued presence of *H pylori*.

Epidemiologic evidence

Controlled bioptic studies analyzing the relationship between gastritis, gastric cancer, and *H pylori* have been reported from a few sources. According to Sipponen and Marshall [73], a gradual decline in gastritis and, presumably, *H pylori* between 1930 and 1980 was accompanied by a logarithmic decline in gastric cancer in each birth cohort. In any birth cohort, cancer risk in later years correlates with the childhood incidence of gastritis rather than the ultimate prevalence. This has also been shown in Iran where areas of high cancer incidence have high childhood prevalence [74]. Although interventional data are scarce, one study from Japan suggests that progression of gastritis to cancer is halved by infection eradication. Uemura et al [62], in a prospective unblinded study, documented a decreased risk of recurrent adenocarcinoma of the stomach in persons in whom *H pylori* had been eradicated after mucosal resection of a carcinoma during a screening program. There was a threefold difference between the treated and untreated groups, but the numbers were too small to achieve statistical significance.

To improve the statistical power of such studies, surrogate markers for cancer risk, (eg, intestinal metaplasia) are assayed. The effect of *H pylori* eradication on the subsequent development or exacerbation of intestinal metaplasia was reported by Leung et al [75] in Chinese patients from Hong Kong. In this prospective, randomized, blinded study, eradication of *H pylori* in persons with already established intestinal metaplasia resulted in a 50% decline in exacerbation of the lesion during more than 5 years of follow-up as compared with untreated controls. Carcinomas developed in both *H pylori* treated and untreated individuals, but only 10 cancers developed in the study, so it was insufficiently powered to demonstrate statistical significance. This study showed that the precursor lesion for gastric cancer was diminished but not eliminated after eradication of *H pylori*. Possibly, once the metaplasia has become widespread in gastric mucosa, eradication of *H pylori* makes little difference to this lesion or its malignant potential. If intestinal metaplasia is proved to be a true surrogate marker for gastric cancer, future studies might not need to be as large as those designed to detect actual gastric cancer development.

The duodenal ulcer paradox

A paradox in the supposed etiologic role of *H pylori* in gastric cancer is the lack of association between gastric cancer and duodenal ulcer despite the strong association between duodenal ulcer and *H pylori* infection. In the United States, Sweden, and Japan the incidence of gastric carcinoma is not increased in individuals with duodenal ulcer [2,62]. In fact, Parsonnet et al

[2] and Hansson et al [76] reported that duodenal ulcer affords protection from gastric cancer. A high or normal acid level (evidenced by the presence of duodenal ulcer) seems to protect *H pylori*–positive individuals from the carcinogenic effect of *H pylori*, even though changes such as intestinal metaplasia still occur, albeit less commonly, in duodenal ulcer patients [77].

Serologic and pathologic evidence

Several studies have shown that *H pylori* is strongly associated with the development of initial cancer lesions. Serologic studies demonstrate an association between *H pylori* and cancer. Crabtree et al [78] reported that 70% of individuals with gastric cancer had IgG antibody to *H pylori*; however, if the gastric mucosa was cultured in vitro, another 20% of individuals gave evidence of past *H pylori* infection manifested by the generation of specific antibodies from the cultured gastric mucosa. The diminished prevalence in clinical cases of gastric cancer may have been explained by Asaka et al [79], who showed that the prevalence of *H pylori* in late gastric cancer was 85%, but the prevalence of *H pylori* in early gastric cancer was significantly higher at 93%. This suggests *H pylori* is associated with early gastric cancer, but once clinically significant cancers develop, the gastric milieu is so altered that *H pylori* is eradicated either by the immune system or by competition from other more vigorous organisms that colonize the stomach with hypochlorhydria.

Intestinal metaplasia is usually present in the border of single gastric ulcers, and nearly universally present in the stomach when adenocarcinoma is present. The classification of gastric carcinoma is based on the work of Lauren [80], who described two main types. The most common is intestinal adenocarcinoma in which glandular structures predominate [79]. In the second diffuse type, the malignant cells tend to be signet ring type and a more diffuse infiltrated lesion is likely.

Both histologic types are increased in incidence in persons with *H pylori*, but the risk of the intestinal type is higher in *H pylori*–positive individuals. Similarly, the intestinal type is responsible for the increased rate of gastric cancer in males [81]. The location of the gastric cancer is also affected by *H pylori* in that the bacterium predisposes to cancer of the distal stomach, rather than the gastric cardia. But this difference has not shown up in Japan, perhaps because the prevalence of *H pylori* was high in all types [82].

Pathologic precursors

Chronic atrophic gastritis associated with *H pylori* is initially more severe in the antrum, but worsens from superficial to more profound gastritis as the antral inflammation migrates up to the proximal stomach over many years [83]. Depending on the duration of the infection and the severity of the inflammation, intestinal metaplasia develops at a rate of 1% to 2% per annum to yield a lifetime risk of 50% to 75% [73,84].

The rate of cancer developing in chronic gastritis and intestinal metaplasia seems to vary depending on the distribution of the *H pylori* bacteria and the metaplasia. Extensive intestinal metaplasia, especially metaplasia extending along the lesser curve from pylorus to the cardia, entails the highest risk of cancer, in the range of 5- to 12-fold higher [85]. It used to be said that cancer risk also depended on whether the intestinal metaplasia was complete (similar to normal intestinal mucosa with goblet cells and brush border present), or incomplete (similar to colonic mucosa with no brush border and sulphomucins present), with the latter being more common in cancer patients [86]. Recent studies have not found this association, however, suggesting that the more immature metaplasia type merely reflects more extensive disease and sampling variation in a patchy mucosal process [87,88].

The most immediate precursor of gastric cancer is carcinoma in situ which is a type of early gastric cancer. Detecting these lesions is the mainstay of gastric cancer screening in Japan because about half of these lesions can be detected and resected endoscopically by injecting saline beneath the mucosa and performing resection with a cautery loop (endoscopic mucosectomy). In a prospective but unblinded study of 132 patients with early gastric cancer, Uemura and Okamoto [89] eradicated *H pylori* in half their patients at the time the early gastric cancer was resected. In the next 2 years, a low recurrence of adenocarcinoma (0 of 65) was seen compared with the controls in whom *H pylori* was not eradicated (7 of 67).

Molecular genetics and carcinogenesis

For gastric malignancy to develop, mutations are required that increase cell turnover, promote cell immortality, and inhibit the demise of abnormal cells. Although many differences have been documented between normal and *H pylori*–infected gastric mucosa, most of these relate to up- or down-regulation of normally present genes. These changes do not necessarily translate into a malignant potential because they are driven by the continued presence of *H pylori*. More likely, *H pylori* increases the chance of a mutation occurring by stimulating cell proliferation and inflammation in gastric mucosa.

The carcinogenic effect of *H pylori* must be small because the lifetime cancer risk of *H pylori* infection is about 5%, or about 0.1% per annum. Attempts to duplicate an etiologic mechanism in vitro might fail merely because the events being measured are rare. Similarly, because cancer risk varies so much in humans, animal models of gastric cancer might bear little relevance to the human condition. A set of events could occur that allow *H pylori* to cause gastric cancer. Because these events transpire over 40 to 60 years, a causative role for gastric cancer is very plausible.

Cell turnover might be increased by increased stimulation or decreased inhibition of cell division. Because *H pylori* causes apoptosis, the loss of cells

from the mucosa would naturally result in decreased contact inhibition causing neighboring epithelial cells to commence cell division. This is an attractive hypothesis because it does not assume that that gastric cancer is fathered by the "risk lesion" of intestinal metaplasia. Attempts to find increasing grades of mutation from normal, through gastritis and metaplasia, to gastric cancer, have yielded unconvincing results. The adenocarcinoma sequence typical in colon cancer does not seem to be a major pathway for gastric adenocarcinoma.

Lee et al [90] found that gastric adenomas have a far higher prevalence of *APC* gene mutations (76%) than dysplastic lesions or adenocarcinomas (3%–4%). This provides strong evidence that adenomas are not precursors of gastric adenocarcinoma. Similarly, microsatellite instability was increased in dysplastic lesions associated with adenocarcinoma, but was only present in a minority of intestinal type adenocarcinomas and was very uncommon in diffuse adenocarcinomas. In support of a minor role for microsatellite instability, Ottini et al [91] found by microdissection that microsatellite instability in the tumor correlated with microsatellite instability in associated dysplasia and metaplasia. These data were supported by Ling et al [92] who reported increasing microsatellite instability across a range of precursor lesions but only to a maximum of 38% in gastric cancer. Microsatellite instability only seemed relevant for antral intestinal cancer. Dysplasia might occasionally lead to intestinal cancer, but rarely to diffuse cancer. More likely, an associated but hitherto unknown precursor cell makes the transition to either diffuse or intestinal type cancer.

Supporting data comes from Boussioutas et al [93] who compared gene expression in gastric cancers to adjacent noncancerous gastric mucosa. They found that chronic gastritis altered the pattern of mitochondrial DNA expression, consistent with colonization by *H pylori* and the known ability of VacA toxin to damage mitochondria [94,95]. Intestinal metaplasia merely showed the pattern of DNA expression seen in intestinal epithelium, however, and these "intestinal" genes were rarely overexpressed in cancer tissue. The findings did not support a transition from intestinal metaplasia to cancer. *H pylori* cannot attach to intestinal epithelium [96], and a process driven by *H pylori* should not continue once intestinal metaplasia has developed.

Diffuse gastric cancer is thought to be less differentiated than intestinal-type gastric cancer [80]. Mutation in the Beta-catenin gene is more common in intestinal-type gastric cancer [97], whereas mutation of its upstream activator CDH1 (E-cadherin) is more common in diffuse gastric cancer [98–100]. CDH1 is especially interesting because germ-line (hereditary) mutations in CDH1 result in familial diffuse gastric cancer [99]. In experimental models, loss of E-cadherin function induces loss of adherens junctions and impairment of cell adhesiveness and cell proliferation signaling pathways. This leads to loss of cellular polarity and contact inhibition, unregulated growth, and invasion of adjacent tissue [101,102].

H pylori probably increases cell migration by increasing levels of Rho-GTP and may interfere with adherence by causing translocation of E-cadherin to intracellular vesicles [103]. According to Conlin [103], ''destabilizing epithelial cell adherence is one of the factors increasing the risk of *H pylori*-infected individuals developing gastric cancer''. How this leads to cancer is not obvious. Although *H pylori* has been shown to down-regulate CDH1 [104], which might cause epithelial cells to ''look malignant,'' this could help *H pylori* cause malignancy only by triggering increased proliferation in these and adjacent cells, in the presence of mutagens. Again, mucosal inflammation and nitrosamine could be more important.

H pylori elicits, yet evades, a Th1 (T-helper cell 1 mediated) immune response possibly by interfering with Stat1 signaling, the mechanism that activates epithelial and Th1 cells after they have been externally triggered by interferon-γ. The effect depends on contact between *H pylori* and the epithelial cell. In animal models lacking this pathway, mucosal inflammation is reduced and bacterial colonization is increased [105,106].

The mutagenic effects of superoxide radicals might not translate into high cancer rates because DNA repair mechanisms are highly efficient [107]. If *H pylori* interfered with DNA repair, mutagenic effects might occur. It may be relevant that *H pylori* decreases expression of mismatch repair genes in gastric epithelial cells [108].

Labeling studies show that *H pylori* increases cell turnover to about twice that of normal gastric mucosa [109]. A similar increase, however, is also present in *H pylori*–negative gastritis and intestinal metaplasia [110]. This increased cell turnover probably follows increased apoptosis of epithelial cells; otherwise, all *H pylori* infections would be associated with tumors. In support of this, Petersson et al [111] observed an increase of the p53 tumor suppressor gene in *H pylori* gastritis. p53 causes apoptosis (programmed cell death), usually triggered by events that have or might cause DNA damage. Mutation or loss of p53 is an important contributor to many cancers.

A special link seems to exist between p53 and oxyradical damage. Oxyradicals cause mutation at CpG sites in DNA and the pattern of DNA damage caused by H_2O_2 to the p53 gene mimics that seen for p53 mutations in various inflammatory conditions, such as copper overload in the liver [112] or even *Helicobacter* gastritis [113].

Oda et al [114] provides evidence for p53 gene mutation as an associative or causative factor in the etiology of *H pylori*–associated gastric cancer. He found that mutations accumulated in the p53 genes of gastric mucosa during 1.5 to 7.5 years in *H pylori*–infected Japanese monkeys. These mutations were associated with increased mucosal atrophy.

Murakami et al [115], however, found that 53% of *H pylori*–infected human mucosa and 100% of infected monkey mucosa contained mutations of the p53 gene, but their method was unable to detect mutations of the p53 gene in the Mongolian gerbil. The evidence that p53 mutations play a role in *H pylori*–generated gastric cancer is presently weak because the most

important animal model, the gerbil, so far has not been shown to have p53 mutations.

Proliferative effects of CagA toxin

Immediately after injection into the epithelial cell, the CagA protein is tyrosine phosphorylated by the host cell Src kinase. The phosphorylated CagA (CagA = P) interacts with at least three signal transduction pathways in the gastric cell.

First, CagA = P binds to SHP-2 (for SH2 homology-domain-containing tyrosine phosphatase), which acts as a growth factor and causes pseudopodia formation, called the "hummingbird" phenotype [116]. Activated SHP-2 drives the MAPK signaling pathway [116] to cause changes in the cell cytoskeleton and cell proliferation [117]. Higashi et al [117] has demonstrated that East Asian CagA (Japanese-Chinese) contains the optimal reaction site motif for CagA binding to SHP-2 and may be more pathogenic [118]. Similarly, Argent et al [118] found that the number of phosphorylation motifs present on the *CagA* gene determined the level of phosphorylation and the propensity to induce the hummingbird phenotype.

Second, even without phosphorylation, CagA binds to Grb2 (growth factor receptor-bound protein 2), which also has an SH2 domain to activate the RAS-MEK-ERK pathway, responsible for a type of proliferation and motility termed "scattering" [119].

Third, CagA = P binds to Csk (C-terminal Src kinase), also an SH2 domain. This inactivates the Src family of protein-tyrosine kinases leading to less tyrosine phosphorylation. By preventing formation of CagA = P, the whole chain of events is muted [120]. This effect may have a self-regulatory function because overdrive of the other pathways might otherwise cause apoptosis.

Blaser and Atherton [49] note that by activating growth but inhibiting apoptosis, CagA allows attached *H pylori* to persist longer on "aged" epithelial cells. CagA is very important in this process. According to Guillemin et al [121], Cag PAI–negative *H pylori* hardly interact with AGS cancer cells. Cag PAI–negative strains are also highly infectious and pathogenic, however, at least in the acute human experiment described by Graham et al [51].

Dietary associations with *H pylori* are most obvious in areas where nitrate or salty foods are dietary components [122]. The ability of *H pylori* to produce ammonia and hypochlorhydria has generated the hypothesis that ammonia in the presence of reactive oxygen species can cause the formation of carcinogens. For example, myeloperoxidase in neutrophils catalyses the oxidation of chloride by H_2O_2 to yield hypochlorous acid (HClO). The interaction between HClO and *H pylori*–derived NH_3 produces monochloramine (NH_2Cl) which is diffusible, lipophilic, and highly reactive. In gastric cell line MKN45, Suzuki et al [123] found that NH_2Cl induced apoptosis. Superoxide injury also causes lipid peroxidation and the generation of

malondialdehyde [124], which can cause frame-shift mutations in mammalian cells. This synergizes with other defects. Malondialdehyde increases the sensitivity to frameshift mutation in mismatch-repair deficient cells [125].

Even without dietary nitrate, generation of nitrite in gastric mucosa is possible in the presence of *H pylori*. The bacterium up regulates the iNOS in mucosal macrophages, and nitric oxide may be a precursor to nitrosamine production [126]. *H pylori* protects itself from iNOS-related monocyte killing by removing arginine, the substrate for iNOS, so that NO production is greatly impaired [127].

H pylori does not fix nitrate to nitrite so it is not directly implicated as a nitrosamine source from dietary nitrate. Other bacteria, however, that colonize the achlorhydric stomach produce nitrite. *H pylori* may facilitate nitrite and nitroso production by causing atrophic gastritis and hypochlorhydria, which allows other bacteria to grow. Production of nitrosamines is best when gastric juice has a pH of about 3.0 [128], so a fluctuating pH with intermittent bacterial colonization may be the ideal setting for production of nitrosamines. In individuals with atrophic gastritis and borderline acid secretion, the gastric pH fluctuates depending on diet and mealtimes.

The possible link between nitric oxide and nitrosation provides a mechanism for production of nitrosamine without dietary nitrate through lifelong chronic inflammation [71]. Vitamin C inhibits formation of nitrosamine. This inhibition could explain the possible benefits of fresh fruit and vegetables, and the decrease in gastric cancer between 1930 and 1970, before the decrease in *H pylori* infection prevalence. *H pylori* also seems to lower vitamin C levels in gastric juice [129], but no prospective data exist showing that vitamin C protects against gastric cancer.

Risk factors for gastric carcinoma

Before the discovery of *H pylori*, it was known that the incidence of gastric cancer varied widely and was falling dramatically in Western countries. Many dietary factors were suspected but these were usually related to socioeconomic status and ethnicity. The only consistent risk factors were high nitrate levels in drinking water or food, and high salt diets [130]. Smoking doubles the gastric cancer risk in all persons, and in persons infected with *H pylori* [131]. Alcohol has been anecdotally associated with gastritis, but this probably reflects the generally lower socioeconomic class of alcoholics prone to *H pylori* infection. There is no association between aspirin and gastric carcinoma, or aspirin and *H pylori* infection. Aspirin and nonsteroidal anti-inflammatory drugs seem to cause a noninflammatory type of gastric mucosal injury, which is not believed to be carcinogenic.

Helicobacter pylori *eradication and gastric cancer prevention*

Occasional studies have documented that eradication of *H pylori* is associated with diminution of intestinal metaplasia [132]. Although investigators

may use atrophic gastritis and intestinal metaplasia interchangeably, the histologic diagnosis of atrophic gastritis based on biopsy samples should be treated with scepticism because of sampling errors in characterizing a widespread gastric process [133]. Investigators have recognized the difficulty in assessing the degree of intestinal metaplasia, often a patchy process, on the basis of a few tiny mucosal biopsies. Even when intestinal metaplasia stays the same, the bioptic studies may identify about 30% of patients in whom it seems to improve and about 30% in whom it seems to have worsened. Numerous biopsy specimens are necessary on several occasions over many years to analyze this issue. Alternatively, large numbers of patients can be used. Leung et al [75] reported that most persons with intestinal metaplasia underwent some deterioration over time regardless of the persistence or eradication of *H pylori*, but the deterioration was less severe in individuals with eradication of *H pylori* infection.

Screening for Helicobacter pylori *infection*

Parsonnet et al [134] have promulgated a rational screening strategy for gastric carcinoma based on the cost effectiveness of screening in different age groups and populations. *H pylori* treatment seems to be cost effective in populations with moderate to high gastric cancer risk and moderate to high *H pylori* infection prevalence provided the presence of infection is screened for by a noninvasive test, such as serology or breath testing. This process is begun at age 50 and subjects are treated with one course of effective antibiotic therapy (cure rate greater than 80%) without follow-up. This strategy is predicated on the assumption that the carcinogenic process triggered by *H pylori* is not self-perpetuating; if gastric cancer has not developed, eradication of *H pylori* interrupts the malignant pathway and reduces the cancer risk by at least 30%.

Parsonnet et al [134] calculated that, depending on the individual cancer risk, most gastric cancer could be prevented for a cost per quality-adjusted life year ranging from $5000 (high risk [eg, Japanese]) to $25,000 (low risk [eg, white USA]). This cost per quality-adjusted life year is much less than that for surveillance of Barrett's esophagus, screening colonoscopy for colon cancer, or Pap smear for cervical cancer [135]. Because of cost discounting, it is less cost effective to interrupt *H pylori* infection in asymptomatic children for whom the malignant risk may be 40 years or more in the future, by which time more effective, simpler, and cheaper means of diagnosis and treatment will likely be available.

Surveillance for gastric cancer in infected patients

It is believed that *H pylori* eradication protects against gastric cancer. Surveillance and estimation of gastric cancer risk is separately analyzed in individuals with persistent *H pylori* that cannot be eradicated and individuals with eradicated *H pylori* who might still carry a residual cancer

risk. The former group of patients has allergies to antibiotics, has a multiply resistant strain, or is intolerant of antibiotic therapy.

Individuals in whom *H pylori* cannot be eradicated need to be educated about their cancer risk. Although cancer risk cannot be precisely quantified on the basis of the presence of intestinal metaplasia, histologic biopsy of the antrum and corpus in eight areas (antrum and corpus, along the lesser curve, greater curve, anterior wall, and posterior wall) can determine whether atrophic gastritis and intestinal metaplasia are present or extensive. The presence of these lesions increases the cancer risk. Aspiration of gastric juice and measurement of gastric pH may help assess the cancer risk. If the gastric pH is less than 2, indicating robust acid secretion, the cancer risk is probably low even if some intestinal metaplasia is present. Additionally, it is reassuring if the patient has mild gastritis in the corpus mucosa. If the patient has a current or recent duodenal ulcer, the cancer risk is low. Gastric polyps or a chronic gastric ulcer, unassociated with nonsteroidal anti-inflammatory drugs, increase the cancer risk. A diet rich in fresh fruit, vegetables, and possibly daily vitamin C supplementation should decrease the cancer risk associated with ongoing *H pylori* infection. If intestinal metaplasia is present, surveillance should be considered, particularly in individuals with a strong family history of gastric cancer. One might attempt further trials of *H pylori* eradication using an older treatment regimen, such as bismuth, tetracycline, and furazolidone.

Individuals without *H pylori* infection with normal gastric histology have a near zero gastric cancer risk. If intestinal metaplasia or atrophy is present, gastric pH can indicate whether the risk is high (acid absent), or low (gastric pH less than 2.0). Finally, patients can take vitamin C supplements. If widespread intestinal metaplasia is present or dysplasia, endoscopic surveillance is warranted. Annual endoscopy with biopsy surveillance should be considered in such individuals with a strong family history of gastric cancer.

Novel experimental and controversial concepts

Opinion as to the risk of *H pylori* as causative agent for gastric cancer is diverse. On one hand, relative risks associated with *H pylori* in epidemiologic studies range from two to six. East Asian strains of *H pylori* are more carcinogenic and have a relative risk of from 5- to 20-fold. Similarly, the risk of gastric cancer for young Japanese is about 20-fold higher for those with *H pylori* versus those without *H pylori*. Host interleukin genetic polymorphisms can intensify inflammation, impair acid secretion, and raise the relative risk another fourfold. The difference in risk between an uninfected individual and an individual infected with a relatively pathogenic strain of *H pylori* in a susceptible individual may be 100-fold.

Brenner and Rothenbacher [136,137] note that in the long-term follow-up study of Japanese with *H pylori* by Uemura et al [62], no gastric cancers occurred in a cohort of 250 individuals with *H pylori* infection who had

suffered from duodenal ulcer. All cancers occurred in individuals without duodenal ulcers. This remarkable dichotomy implies that the risk of cancer from *H pylori* is near zero when acid secretion is maintained. Other factors associated with gastric atrophy and hypochlorhydria are more likely to be the proximate cause of gastric cancer. It should be possible to protect against gastric cancer by preventing gastric achlorhydria. The other theoretical consideration is whether *H pylori* eradication arrests the carcinogenic process or merely slows down the inevitable once the process has been initiated.

Hypochlorhydria (gastric atrophy and marked intestinal metaplasia) signals a nonreversible process. Early gastric cancers are highly associated with *H pylori*, but late gastric cancers are less strongly associated, that is, only 70% or less of individuals have active *H pylori* infection. The infection may have disappeared after the development of the gastric cancer, eradicated perhaps by mucus production, or disappeared before the development of the gastric cancer, in which case the cancer risk factors were already well-established in that individual.

Association of *Helicobacter pylori* with gastric lymphoma

Wotherspoon et al [138] demonstrated an association between *H pylori* and mucosa-associated lymphoid tissue (MALT) lymphoma. They found that 90% or more of individuals with this lymphoma were infected with *H pylori*. After one individual underwent regression of this lymphoma following eradication of *H pylori*, the investigators treated these lymphomas with *H pylori* eradication and observed that most of the lymphomas went into clinical and endoscopic remission following eradication of *H pylori*. They and others have reported ongoing success in more than 100 patients [139].

Pathology

MALT lymphoma is the most common gastrointestinal and gastric lymphoma. It is typically an indolent, small B-cell lymphocytic lymphoma, in which the mucosa is infiltrated with numerous apparent large lymphoid follicles and is diffusely infiltrated with lymphocytes, extending into the epithelium with so-called "lymphoepithelial lesions." The lymphocytes migrate between the epithelial cells, obliterate the mucus-secreting glands, and distort the normal mucosal architecture.

The clinical presentation of gastric MALT lymphoma is similar to that of gastric ulcer. Symptoms range from dyspepsia to vomiting and gastrointestinal bleeding. Weight loss and an abdominal mass are more likely with high-grade lymphoma [140]. Endoscopy reveals lumpiness and irregularity of the mucosa. Sometimes multiple ulcerations occur. The lesion can be focal or widespread throughout the stomach. The diagnosis depends on the

dense infiltration of lymphocytes with the lymphoepithelial lesions. *H pylori* may be diagnosed by serology, by biopsy of infiltrated tissue, or preferably by detection of the organism on nearby normal appearing mucosa.

This gastric lymphoma is usually slowly progressive and for several years may be manifested merely by nonhealing gastric ulcers. Ultimately, it becomes less well-differentiated and may metastasize to lymphoid tissue outside the stomach. At this stage the lymphoma may no longer respond favorably to eradication of *H pylori* because the malignant lymphocytes have become more autonomous. In a few cases, however, MALT lymphoma tissue outside the stomach, in the tonsil or salivary gland [141], has regressed after the underlying gastric MALT lymphoma has been treated by eradication of *H pylori*.

Pathophysiology

Recent volunteer studies have documented that lymphoid follicles can appear in gastric mucosa within weeks of acute infection [51]. Lymphoid follicles are present in at least 30% of persons with long-standing *H pylori* infection. These small lymphoid follicles are distinct from lymphocytic infiltration in the lamina propria and the epithelium. These benign lymphoid follicles are presumed to be the progenitors of MALT lymphoma.

MALT lymphoma is far less common than gastric adenocarcinoma. Its prevalence in various countries parallels the prevalence of gastric *H pylori* infection. Most large series of MALT lymphoma studies have come from countries with a high prevalence of *H pylori* infection, most notably Spain and Germany [142,143].

Crabtree and Spencer [144] studied the ability of *H pylori* to produce mitogenic effects on T cells isolated from gastric mucosa in in vitro studies of cultured biopsies. They demonstrated that T cells proliferated in MALT lymphoma tissue when *H pylori* antigens were present.

Sometimes, the diagnosis of MALT lymphoma is somewhat ambiguous because gastric tissue is infiltrated with lymphocytes and lymphoid follicles are present, but extensive lymphoepithelial lesions are lacking. In this situation, the subtype of immunoglobulin-secreting cells can be estimated by flow cytometry. An extreme predominance of one immunologic subtype suggests that the lesion is malignant. Prognosis is indicated by the B-cell type (eg, predominantly CD19- and CD20-positive cells confer a worse prognosis) [145]. In addition, polymerase chain reaction studies can search for monoclonality of the immunoglobulin heavy chain gene, detected as a distinct DNA band (monoclonal expansion) rather than the normal smear pattern of B cells (many subpopulations with different sized DNA products). The monoclonality depends on the predominance of the malignant lymphocytes over normal lymphocytes generated by the immune reaction (gastritis) to chronic *H pylori* infection. Monoclonality is not always initially present with MALT lymphoma, and may persist after the

lymphoma clinically and histologically remits after *H pylori* eradication. Thiede et al [146] detected immunoglobulin heavy chain monoclonal bands in 50 of 65 (77%) analyzed patients at diagnosis. After achieving complete histologic remission, monoclonality remained in 49% as detected by polymerase chain reaction. Its continued presence emphasizes that persons with MALT lymphoma remain at risk if *H pylori* is not eradicated, if the same infection recurs, or if a new infection occurs.

Epidemiology

The incidence of gastric MALT lymphoma is approximately 1 per 100,000 per annum [147]. The incidence depends on the prevalence of *H pylori*.

Clinical evaluation

If at endoscopy the gastric mucosa appears to be abnormal, all abnormal areas should be biopsied. Typically, the diagnosis of MALT lymphoma is suspected on the first histologic sample and the patient is recalled for further endoscopic and bioptic evaluation, including biopsies of the affected and normal areas, and specimens for immunohistochemistry, flow cytometry, and polymerase chain reaction evaluation. Once the diagnosis is confirmed, staging studies are required, particularly ear-nose-throat examination of Waldeyer's ring lymphoid tissue, CT, or MRI scan to exclude lesions outside the stomach [147]. Regardless of the histologic type, eradication of *H pylori* should be performed with other proved therapy.

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