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Review article

P.C. KONTUREK[1], S.J. KONTUREK[2], T. BRZOZOWSKI[2]

HELICOBACTER PYLORI INFECTION IN GASTRIC CANCEROGENESIS

[1]First Department of Medicine, University Erlangen-Nuremberg, Erlangen Germany,
[2]Department of Physiology, Jagiellonian University College of Medicine, Cracow, Poland

Gastric cancer (GS) remains one of the most common cancers worldwide. It is considered as the second most frequent cause of cancer death worldwide, although much geographical variation in incidence exists. Many studies before linked Helicobacter pylori (Hp) which is now considered as an important pathogen, to the risk of developing noncardia GS. This overview attempts to summarize the recent basic and clinical evidence on the link between H. pylori and gastric cancer, after the award of the Nobel Prize for Physiology or Medicine to Drs. J.R. Warren and B.J. Marshall for the first culture and isolation of Hp and the investigation of their relevance to peptic ulcer disease. It become evident that Hp eradication by antibiotic treatment combined with proton pump inhibitor (PPI) serves as the primary chemoprevention strategy to reduce gastric cancer incidence. Moreover, the eradication therapy reduces gastric cancer incidence in patients without any precancerous lesions at the baseline and is most effective before the development of atrophic gastritis. Due to understanding the molecular nature of GC which has been nowadays under intense investigation, our review attempts to highlight recent progress in the field of research on Hp-induced GS. We discuss the geographical diversity in Hp infection and cancer incidence and the mechanistic role of gastrin, cyclooxygenase-2 (COX-2), growth factor, nitric oxide (NO)/NO synthase and E-cadherin/beta-cathenin systems, apoptosis and angiogenesis in Hp-induced gastric carcinogenesis. In addition host-related genetic susceptibility and the role of overexpression of a proinflammatory cytokines and their polymorphism is discussed in the relation to the cascade of events such as gastric atrophy, intestinal metaplasia and dysplasia that finally lead to adenocarcinoma.

Key words: *Helicobacter pylori, gastric cancer, Cag A, Vac A, cyclooxygenase-2, gastrin, nitric oxide, interleukin-1, tumor necrosis factor*

INTRODUCTION

It has been over a century since Walery Jaworski at Cracow University detected a spiral bacteria named *Vibrio rugula*, in the sediment after gastric washing from patients with gastric cancer (GC) and over a quarter of century since Marshall and Warren drew attention to the spiral bacteria, *Helicobacter pylori* (Hp), as a pathogen in various gastric diseases. The Nobel Prize was awarded to Marshall and Warren in 2005 for the discovery of Hp within the gastric mucosa, and its role in gastritis and peptic ulceration pathologies. This prestigious award re-emphasized the clinical importance of their observations. However, Nobel citation was limited only to these pathologies, but the discovery of Hp infection appears to have much wider implication with the greater challenge being understanding its involvement in the development of GC, which is the second most frequent cause of cancer death in men, and GC rates are noted to be twice as high in men as in women. This review focuses on recent clinical and basic research related to the pathophysiology of GC in Hp infected humans and animals. It is widely accepted that Hp infection is followed by the induction of inflammatory changes in gastric mucosa that may persist for decades without causing any gastric disturbances. However, in a small percentage of adult patients it may initiate chronic atrophic inflammatory changes of the *corpus* or *antrum* of the stomach accompanied by the increase in gastrin expression and release, enhancement of cyclooxygenase-2 (COX-2) expression and prostaglandin (PG) generation as well as by numerous morphological and biochemical changes leading to the transformation of mucosal cells into malignant cells. Correa established the cascade of events leading to GC more than 30 years ago and long before the discovery of Hp. Chronic inflammation induced by Hp may progress further through the premalignant stages of gastric atrophy, intestinal metaplasia, dysplasia and finally adenocarcinoma. This association between Hp infection and GC has been confirmed in certain animals species such as gerbils and mice, indicating that Hp infection may lead to GC accompanied by and over-expression of gastrin and other growth factors, enhanced COX-2 - PG system, an increase in generation of pro-inflammatory cytokines with the polymorphism in their genes, apoptosis and angiogenesis with invasion of the malignant cells into the surrounding tissues. This overview provides evidence that Hp infection is the major risk factor in GC and that the eradication of Hp can reverse many biochemical, genetic and epigenetic changes that Hp infection induces in the stomach leading to the development of GC.

GEOGRAPHIC DISTRIBUTION OF HP INFECTION AND GC PREVALENCE

Hp is known to colonize the stomach of about half of the world's population with the prevalence of Hp infection varying within countries and between countries (1-6). In developing countries such as the eastern regions of Asia and in some parts of Latin America, the prevalence of Hp infection is characterized by a rapid rate of acquisition of the infection, usually in childhood so that about 80% of the population is infected by the age of 20 (4, 5). In contrast, in developed countries such as France, USA, UK or Australia, the prevalence of Hp infection in children is low for the ages below 10 years and peaks to about 40% at 30 to 40 years of age. It is important to note that even in the USA the prevalence of Hp infection varies between the subpopulations, being several times higher in some ethnic groups such as African Americans and Asians with lower socioeconomic status during childhood. Within the Polish population the Hp incidence is similar to that in the developing countries, reaching its peak at the age of 40 yrs but then it tends to decline. It is of interest that there is an increase in Hp prevalence with age in both developing and developed countries and this may be attributed either to the new acquisition of infection among the population or to the effect of different birth cohorts, each with a different rate of acquisition in childhood (6-9). In general, the prevalence of Hp infection is related to age, socioeconomic status and country of origin (*Fig. 1*). In the USA, Hp infection is lower among white Americans and those economically advantaged, and remains quite common among the socially disadvantaged groups and in immigrant populations (10). A difference in prevalence among the ethnic groups of similar socio-economic status reflects the components of environment and possible host genetics (9-11). In childhood, the socioeconomic status of the family is the major risk factor for infection.

Although Hp infection is usually chronic and life-long, the spontaneous elimination of the Hp infection was reported both in the developed and underdeveloped countries (5-7). The changes in the Hp infection rate in Russia during the last decade are a dramatic example of how sensitive Hp acquisition is to the improvement of living standards (12). In 1955, the overall prevalence of Hp infection in children living in St. Petersburg was 44%, and 10 years later it declined to 13%, probably due to the significant improvement of household hygienic practices and the use of anti-Hp eradication therapy. A cross-sectional study conducted in children revealed significantly higher Hp infection rate in children residing in rural areas compared to those living in industrial areas (13). Studies in Kazakhstan (14) and Peru (15), showed high Hp infection prevalence in children, confirming that socio-economic conditions, local household hygiene, and the use of Hp contaminated water could be the factors responsible for the high rate of Hp infection in these children.

RELATIONSHIPS BETWEEN HP INFECTION AND ASSOCIATED GASTRIC DISEASES

The colonization of the stomach by Hp results in the development of gastritis in all infected subjects. Hp is a truly an "opportunistic" *bacterium* that uses any available way to gain access to the stomach. The *bacterium* usually enters the stomach by fecal-oral route but it can also be transmitted through Hp contaminated food or water (15, 16). The majority of data supports the notion that the most likely sources of transmission are person-to-person contact in families and/or exposure to a common source of infection such as contaminated water or food (14-16). Support for this notion comes from the studies of children in custodial care, where the prevalence of infection is higher than expected and from studies of crowded families in which there is at least one infected child (7). In Polish adults, a close association was observed between gastric Hp infection, as detected by ^{13}C-urea breath test (UBT), and the presence of the bacteria within the oral cavity accompanied by periodontal disease. This finding suggests that this might facilitate the oro-gastric transmission and colonization of the bacteria in the digestive tract (17).

Hp exerts a trophic influence on the gastric epithelium, but before it can attach to the epithelium surface it has to first cross the thick mucus layer by adhering to the mucosal surface (*Fig. 2*). The presence of unipolar flagella helps to establish the Hp

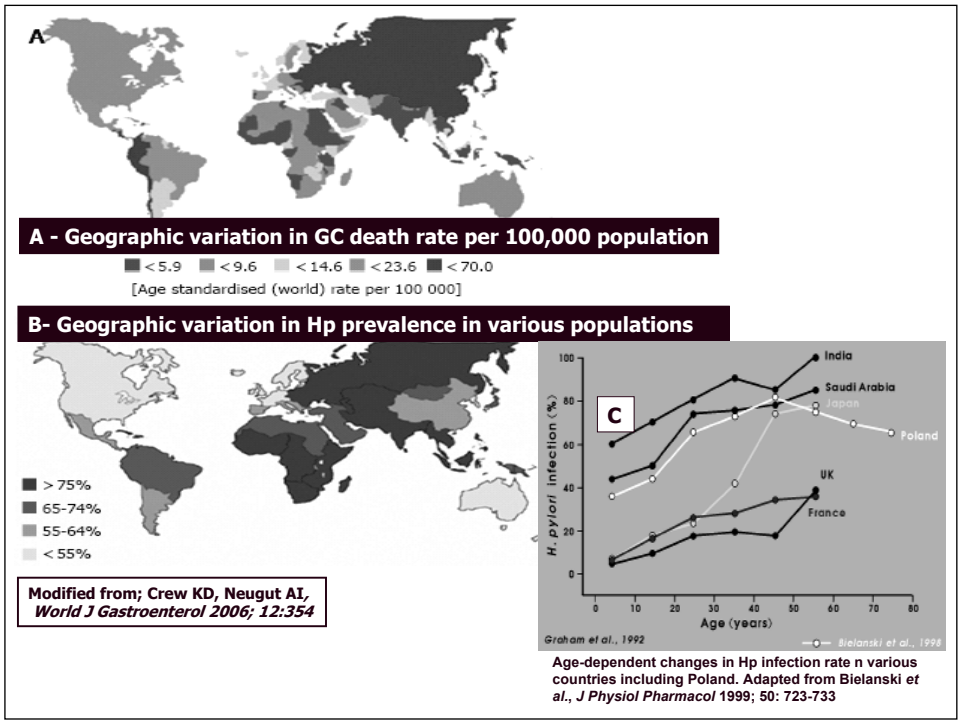

Fig. 1. Geographic world variation in GC cancer death (A) and geographic variation in the incidence of Hp infection (B) (adapted from Crew KD and Neugut AI, *World J Gastroenterol* 2006; 12: 354). Age dependency of Hp infection rate in various countries (adapted from Graham DY, *et al*. *Dig Dis Sci* 1999; 50:723-733) including Poland (adapted from Bielanski W; *J Physiol Pharmacol* 1999; 50: 723-733) (C).

colonization of the stomach and its attachment to the epithelial surface of mucosa in the stomach despite the body attempts to rid itself of the bacterial infection. Due to the flagella, the bacteria moves quickly from the gastric lumen, where low pH is inhospitable to the surface epithelium, where the pH is higher, permitting the optimal Hp growth. It is of interest that mutant Hp strains that are non-motile are unable to colonize the stomach of gnotobiotic piglets (18, 19).

The initial gastric Hp colonization usually occurs in childhood, however a new infection may also occasionally happen in adults. As mentioned before, all infected individuals develop inflammatory and immune responses to Hp, but Hp-associated diseases such as acute gastritis, gastric or duodenal ulcer, non-cardia gastric adenocarcinoma or primary lymphomas occur predominantly in adults. Acute inflammation of the gastric mucosa has been described in a small number of subjects who were deliberately infected with pathogenic Hp strain mainly for research purposes (20-22). Histologically, such acute gastritis is characterized by heavy neutrophil infiltration of the entire gastric mucosa. It has been described previously as an "epidemic hypochlorhydria" observed in volunteers several decades ago who were exposed repeatedly to intragastric pH-electrodes which were contaminated with Hp (22). Such hypochlorhydric gastritis can resolve spontaneously or change into chronic

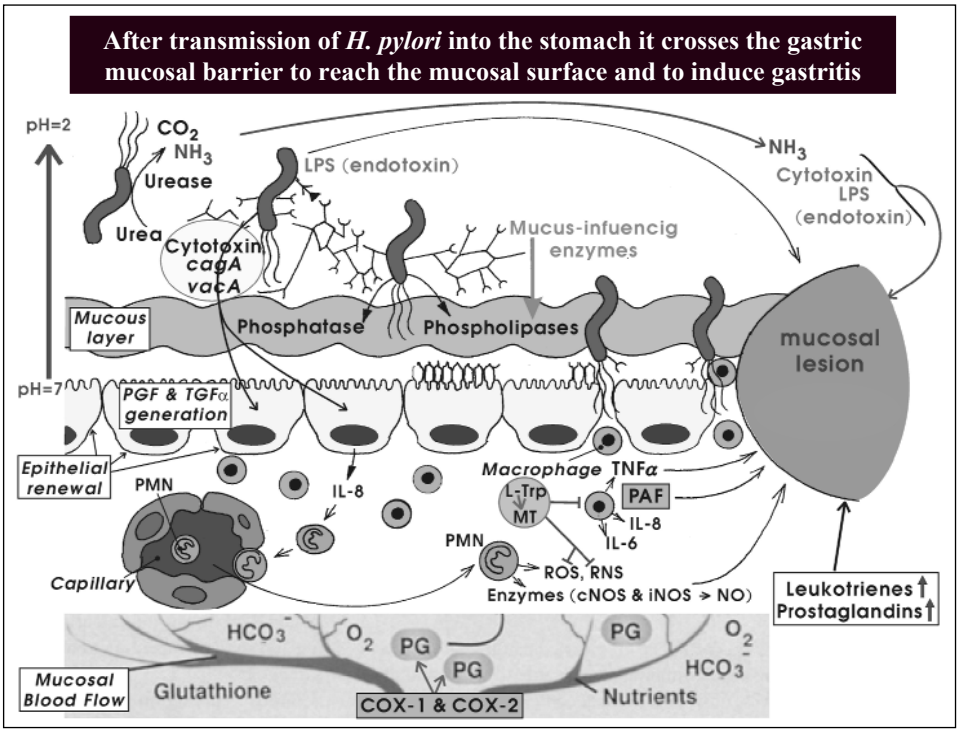

Fig. 2. After transmission of the Hp into the stomach, the bacterium has to pass through the mucosal adherent mucus layer before reaching the surface of mucosal cells and induction of inflammatory changes in the mucosa by releasing the virulence factors and releasing proinflammatory cytokines, free radicals and activation of inflammatory cells (adapted from Konturek PC, *et al. J Physiol Pharmacol* 2006; 57: 5165)

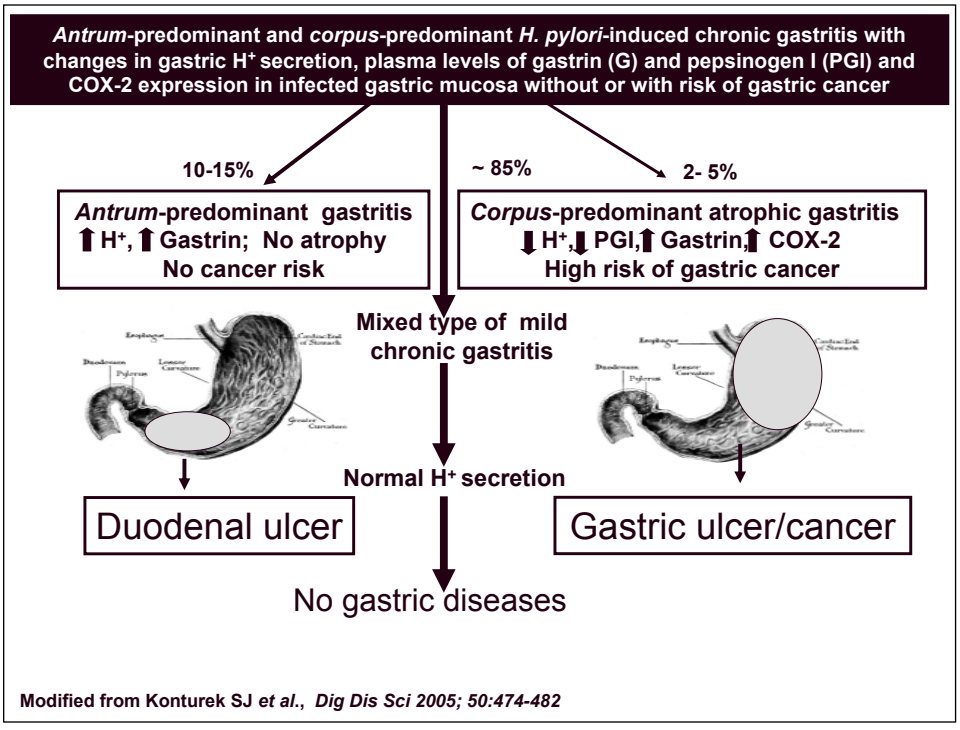

Fig. 3. Hp-induced *antrum*-dependent gastritis with hypercholohydria caused by hypergastrinemia with subsequent duodenal ulcer and *corpus*-dependent atrophic gastritis that may result in gastric ulceration or gastric cancer (adapted from Konturek SJ, *et al., Dig Dis Sci* 2005; 50: 474-482.

gastritis being localized mainly in the antral portion of the stomach (*antrum*-predominant gastritis) or potentially extending to the gastric corpus (*corpus*-predominant gastritis). The severity of inflammation depends on its gastric localization and systemic immune response. Chronic *antrum*-predominant gastritis may be associated with serum hypergastrinemia and gastric hyperchlorhydria, which can be predisposing to duodenal ulceration. Chronic *corpus*-predominant atrophic gastritis is accompanied by hypochlorhydria and hypergastrinemia, which may predispose to gastric adenocarcinoma (*Fig. 3*). This predisposition involves the interaction of three major factors: the virulence of the agent (Hp), the host immune system and the environmental factors. It may occur when gastritis is accompanied by the over-expression of COX-2 protein and subsequent release of prostaglandins (PG), up-regulation of oxidative stress system and over-expression of cytokines such as IL-1β and TNF-α associated with cytokine gene polymorphism. Interestingly, patients with Hp infection that is localized mainly in distal portion of the stomach (*antrum*-predominant gastritis) and those with duodenal ulcer almost never develop GC. This finding may suggest that duodenal ulcer somehow provide a protective mechanism against gastric malignancy (23).

GC incidence rate varies by up to ten-folds throughout the world. Nearly two-thirds of stomach cancers, mainly non-cardia gastric carcinoma occur in developing countries located in Eastern Asia, Eastern Europe and Central and South America. These geographical locations also show a high prevalence of Hp infection. Unexpectedly, low incidence rates of GC are found in South Asia, North and East Africa, North America, Australia and New Zealand where the Hp prevalence is moderate or low (23).

HP VIRULENCE FACTORS IN GASTRITIS AND GASTRIC CANCER

As mentioned previously Hp is specifically adapted to colonize the stomach and survive in its hostile acidic environment, resulting in the induction of gastritis, peptic ulcer or cancer. This particular strain of bacteria is equipped with numerous virulence factors, which helps it to colonize the stomach and subsequently damage its mucosa. Hp possesses enzymes such as urease to maintain a neutral pH in the microenvironment of the gastric lumen. One of the major virulence factors of Hp includes the *cag* pathogenicity island (PAI) encoded virulence factors such as the CagA protein, the vacuolating toxin (VacA), the blood group antigen-binding adhesin (BabA), theouter inflammatory protein (OipA) and IceA (*Fig. 4*). Probably the most important Hp virulence factor with a well established role in the induction of mucosal inflammation is *cag* (cytotoxin-associated gene) in the pathogenicity island (*cag PAI*). Hp strains having the *cag PAI* are more likely to be associated with peptic ulceration or gastric adenocarcinoma than those strains lacking it. The *cag PAI*, a 40 kilobase segment of DNA is a group of about 30 genes, many of which encode components of type IV secretion system (T4SS) that acts as a "molecular syringe" through which cytotoxin-associated antigen (CagA), a protein encoded on the *cag* PAI, is translocated or "injected" into the epithelial cytosol causing powerful changes which is believed to benefit the bacterium. The CagA cytotoxin is a 121-145 kDa immuno-dominant protein that is commonly used as a marker for the entire cag locus in epidemiological studies. Within the Western populations, cagA-positive strains are more commonly associated with peptic ulceration, atrophic gastritis and gastric adenocarcinoma when compared to *cag*-negative strains, but in many high GC populations such as eastern regions of Asia, almost all Hp strains are *cag*-positive. In contrast, only about 60% of Hp strains isolated in Western countries carry the *cag PAI*. Ohnishi *et al*. (24) were first to provide experimental evidence of the potential oncogenicity of CagA *in vivo*. Transgenic expression of CagA in mice led to the development of gastric epithelial hyperplasia and adenocarcinoma of the stomach. Thus, the experimental evidence was obtained that CagA acts as a *bacterium*-derived oncoprotein in the development of the Hp-associated GC.

The question remains how the type IV secretion system translocates the CagA protein into gastric epithelial cells? Recent studies showed that CagF, a chaperone-like protein, which interacts with the C-terminal secretion signal is involved in the

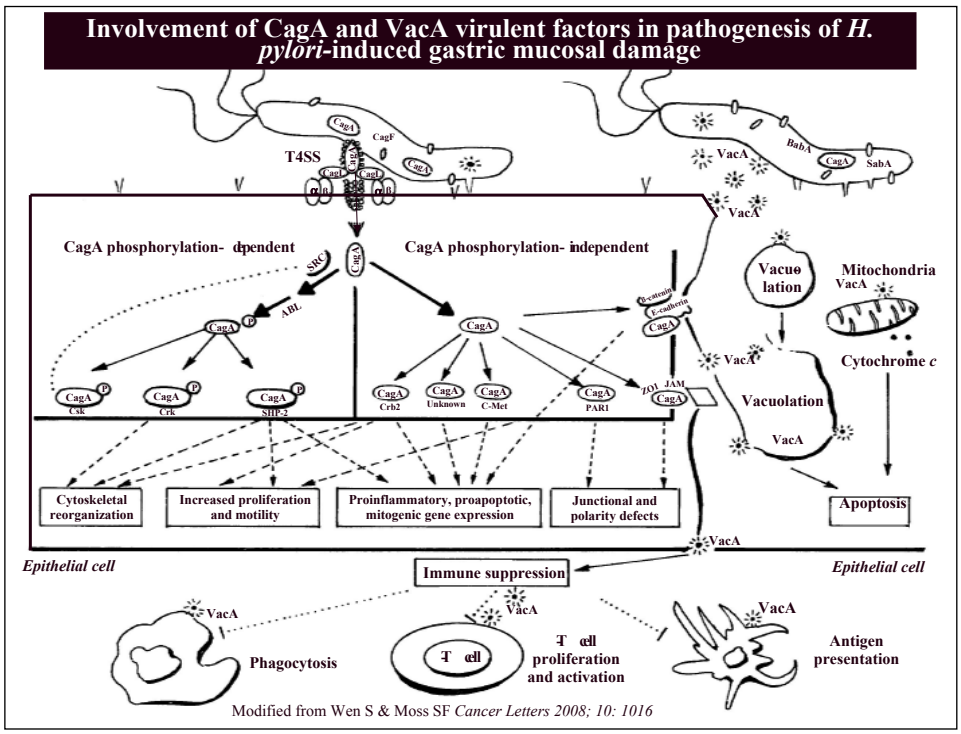

Fig. 4. Involvement of various virulence factors such as CagA and VacA encoded by Hp genes and acting by mucosal cells and immune system resulting in mucosal damage and inflammation (modified from Wen S, Moss SF, *Cancer Letters* 2008; 10: 1016-1019).

early steps of CagA recognition and is crucial for CagA delivery into the mucosal cells (25). CagL, another product of a *cag PAI*, utilizes host integrin alpha5-beta1 as a cell surface receptor (see *Fig. 4*). This CagL-integrin interaction triggers the delivery of CagA into the target cell and subsequently activates focal adhesion kinases (FAKs) and SRC kinases (26). Epithelial cells recognize the translocated CagA as a signaling molecule that is activated by phosphorylation on tyrosine residue by a host, mainly by Src kinases. It then interacts with SRC homology, SH2 domain containing-proteins including the thyrosine phosphatase SHP-2, the C-terminal SRC tyrosine kinase (SCK) and the adaptor protein Crk (see *Fig. 4*). This results in cytoskeletal reorganization and cell elongation - a phenotype that leads to cell scattering and the so-called "hummingbird" morphological changes. It also induces MAP kinase signaling, resulting in abnormal cell proliferation and movement of gastric epithelial cells (27-29). MAP kinase activation also promotes cell cycle progression and this together with the phenotypic changes have been taken as an evidence that CagA-activated SHP-2 may play an important role in the cell transformation and gastric cancer promotion. Phosphorylated CagA binds the adaptor protein Crk proteins, leading to the cytoskeletal reorganization, disruption of the epithelial cell tight junctions and tissue damage. Non-phosphorylated CagA interacts also with certain host cell proteins such as epithelial tight junction-scaffolding protein zonulin (ZO-1) (30), the hepatocyte growth factor receptor C-Met, the cadherin/beta-cathenin, the adaptor protein GRB-5 and kinase PAR1 (31). These CagA-host protein interactions disrupts the tight and adherent junctions, leading to a loss of cell polarity, and inducing pro-inflammatory and mitogenic response-effects that may be important in the formation of gastric carcinogenesis. Tight junctions are important for maintaining paracellular permeability and cell polarity and are also involved in cell motility, cell-cell adhesion and cell proliferation. CagA also directly interacts with E-cadherin, leading to the impairment of E-cadherin/beta-catenin complexes and to cytoplasmic and nuclear accumulation of beta-catenin. Downstream events include transcription of genes involved in intestinal differentiation such as *cdx1*, and *muc2* mucin gene causing transdifferentiation from gastric to intestinal type epithelial cells (32).

Hp strains possessing the cag PAI stimulate gastric epithelial cells to express and release excessive amounts of pro-inflammatory cytokines such as interleukin-8 (IL-8) both *in vivo* and *in vitro* (33-35). The initial step in this process is recognition by intracellular pattern recognition receptor (PRR) nucleotide-binding oligomerisation domain protein 1 (Nod 1) (36). Nod 1 is an innate epithelial detection system for Gram-negative bacteria and has been shown previously to detect intracellular infections. It senses the presence of a unique mucopeptide from Gram-negative bacterial peptidoglican (37, 38). The interaction with Nod1 leads to activation of NFκB and expression of genes encoding for pro-inflammatory cytokines such as IL-8, resulting in chronic inflammation, which is the hallmark of the pathogenesis of Hp-induced GC (*Fig. 5*). El-Omar *et al.* (39, 40) have shown that pro-inflammatory *IL-1* gene cluster polymorphisms (*IL-1B* encoding IL-1B and *IL-IRN* encoding its naturally occurring receptor antagonist) increase the risk of GC and its precursors in the presence of Hp infection. IL-1 is relevant in this disease because it is up regulated in Hp infection, has pro-inflammatory actions and is a powerful gastric acid inhibitor. The pro-inflammatory IL-1 genotypes increased the risk of both intestinal and diffuse type of GC, but the risk is restricted to the non-cardia subsides. This is in keeping with the proposed mechanism for the effect of these polymorphisms in GC, namely the reduction in gastric acid secretion, hypergastrinemia and mucosal damage in the form of atrophic gastritis (*Fig. 6*). Thus, a high IL-1 genotype increases the risk of non-cardia GC, a disease that is characterized by hypochlorhydria, while it has no effect on cancers associated with high acid exposure such as esophageal adenocarcinoma and some cardia cancers. The association between *IL-1* gene cluster polymorphisms and GC was confirmed independently by other groups covering Caucasian, Asian and Spanish populations (41- 43).

Another major Hp virulence determinant that is present in all Hp strains is the *vacuolating cytotoxin gene* (*vacA*), which encodes vacuolating cytotoxin A (VacA) (44-46). VacA exerts multiple effects on epithelial cells resulting in the induction of

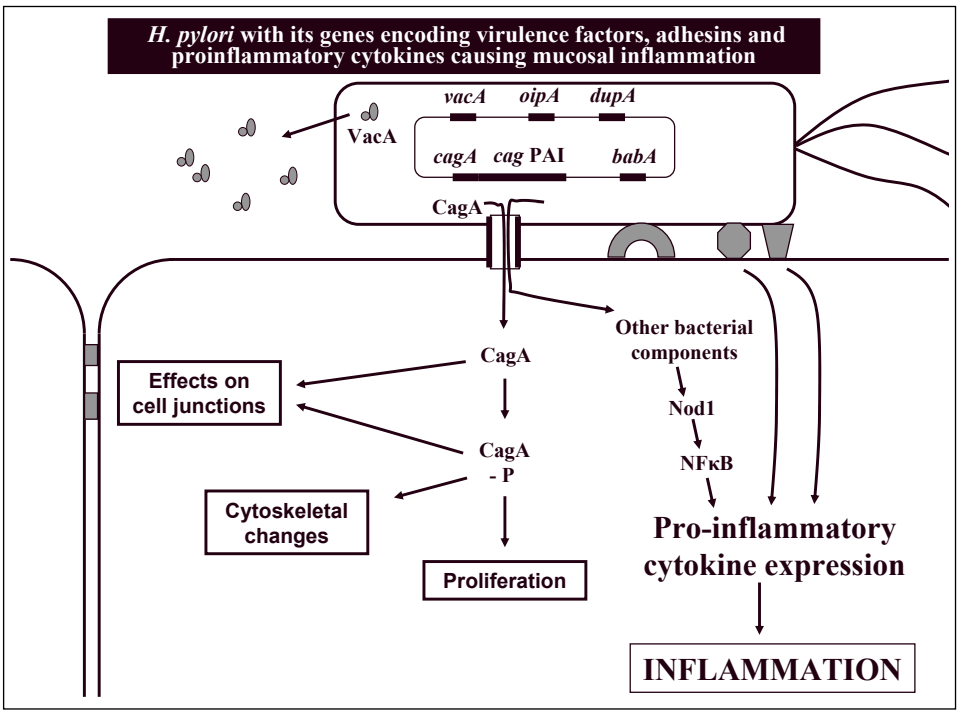

Fig. 5. Schematic presentation of Hp genome with various genes encoding the virulence factor (CagA and VacA) and pro-inflammatory cytokines *e.g.* IL-8 responsible for mucosal inflammation.

vacuole formation in the gastric epithelial cellular membrane, cellular damage, an increase of intracellular permeability and the modulation of apoptosis rather than release of pro-inflammatory cytokines (see *Fig. 4*). Polymorphism also exists among the VacA alleles, resulting in different levels of cytotoxicity (47). The variations in the signal (s) region and in the mid (m) greatly influence the effects of VacA, especially the s region variation which is associated with the vacuolating activity of VacA, whereas the m region of VacA determines the cell specificity of vacuolation by affecting the binding of VacA to the host cells. The VacA polymorphisms are correlated with gastric diseases, particularly with peptic ulceration in Western populations. VacA s1/m1 may be strongly associated with gastro-duodenal diseases and with gastric cancer. East Asian strains are almost universally s1/m1 and are not associated with any specific clinical outcome. Additional reported effects of VacA include the disruption of cytoskeleton architecture through gene modification and damage of cell-cycle related genes (46, 47). Furthermore, VacA exerts multiple effects on the immune system. It can interfere with phagocytosis and antigen presentation and inhibits the proliferation of T cells (48, 49). In general, the VacA polymorphisms are correlated with gastric diseases, particularly with peptic ulcer (48, 49).

Outer membrane inflammatory protein (OipA) is an outer membrane protein of Hp that when expressed together with CagA is associated with an enhanced inflammatory response in the gastric mucosa (50, 51). OipA is universally present in Hp strains in Eastern Asian populations, but in Western strains it is present in less than in 50% of all cases, mainly those inducing asymptomatic chronic gastritis. OipA status of Hp is significantly associated with duodenal ulceration and GC (51). Yamaoka and his colleagues (51) reported that OipA expression is closely associated with gastric inflammation and induction of expression of pro-inflammatory cytokines such as IL-8 and these effects were confirmed in mice and gerbils, but other evidence failed to confirm this association.

Adherence is important for Hp virulence. The intimate attachment observed between Hp and the gastric epithelial cell surface is required for the bacteria to colonize the gastric mucosa and to deliver efficiently the virulence factors such as CagA or VacA into the gastric mucosal cells. Functional receptors for Hp adherence include fucosylated ABO blood group and Lewis b antigens (52-54). The blood group antigens binding adhesin (BabA) is an outer membrane protein, encoded by the *babA2 gene* that binds to Lewis b antigen and ABO antigens and the sialyl-Lewis x/a antigens (55). There are two distinct *babB* alleles (*babA1* and *babA2*) and one highly homogenous gene, *babB*, however; only the *babA2* is functionally active (see *Fig. 5*). The Hp alters expression of its adhesions during infection. The expression of the *babA* gene can be modulated through the recombination between *babA* and *babB*. The adherence of Hp to the gastric mucosal cells mediated by *babA* facilitates Hp colonization, induces mucosal inflammation and promotes expression of sialyl-Lewis x/a. The presence of *babA*, *cagA* and *vacAs1*, referred to as the "triple positive strains" may be associated with duodenal ulcer or gastric adenocarcinoma in Western populations (56).

ASSOCIATION BETWEEN HP INFECTION AND GASTRIC CANCER

As mentioned previously, gastric cancer (GC) remains a major cause of cancer-related mortality in the world despite declining rate of GC incidence in developed countries (57). The epidemiological studies reveal that in the year 2010 it is expected that there will be around 1.1 million new cases of GC and that about 60% of new GC cases will be in developing countries. Despite some advances in detection and treatment of GC, the 5-year survival rate outside Japan, is still only around 20% (57, 58). It is now recognized that proximal or cardia GC incidence is somewhat different from distal or non-cardia cancers. These two subtypes of GC have different epidemiology and pathogenesis, though the picture in still far from clear. It is generally accepted that Hp infection is the most recognized etiological risk factor for GC and its precursors (59, 60).

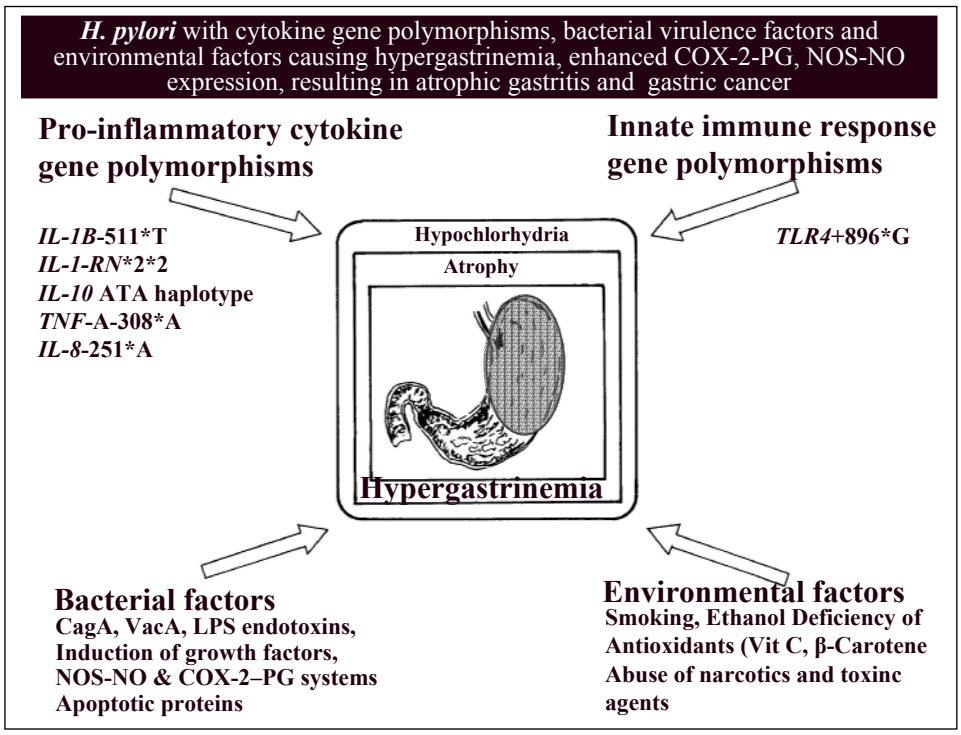

Fig. 6. Proinflammatory cytokine gene polymorphisms. Alteration in immune host system, bacterial virulence factors (CagA and VacA, LPS), and COX-2-prostaglandin and NOS-nitric oxide system as well the environmental factors contributing to atrophic gastritis (hypochlorhydria, hypergastrinemia) and development of gastric cancer.

The International Agency for Research on Cancer (IARC) in its meeting in 1994 in Lyon, France, concluded that there is sufficient evidence to classify Hp a "Group 1 = Definite" human carcinogen (61). Although initially there were some doubts concerning this conclusion, mainly due to a lack of experimental support from animal studies and insufficient epidemiological evidence, but now numerous clinical experimental studies during the last decade have vindicated the IARC conclusion. The strongest evidence came from nested case - control studies based on serological evidence of the Hp infection in samples taken many years before the development of GC. Overall conclusion of six of these meta-analyses (62-67) is that Hp infection is associated with approximately a two fold increased risk of developing GC (range of pooled odds ratios (OR) 1.92-2.56, all with significant 95% confidence intervals (Cl). The strong association between *H. pylori* infection and GC has been obtained in a widely publicized prospective study involving 1526 Japanese patients with duodenal ulcers, gastric ulcers, gastric hyperplasia or nonulcer dyspepsia at entry (62). After a follow-up period of 7.8 years, GC was detected in 2.9% of *H. pylori* positive patients and not in any participant without *H. pylori* infection. Huang *et al*. (63) identified 16 qualified studies with 2284 cases and 2770 controls. Hp and cagA seropositivity significantly increased the risk of GC by 2.28-2.87-folds respectively. Among Hp infected populations, infection with CagA positive strain further increased the risk for GC by 1.64 fold (95% CI , 1. 21-3.32 for non-cardia GC). GC at the gastric cardia was not associated with Hp infection or cagA positive strains of Hp. The same study found that an association between Hp infection and GC was equally strong for intestinal and diffuse cancer [OR 2.49 (95% CI 1.41-4.43)] with a 2.58 [(95% CI (1.47-4.53)]. It should be noted that according to Wong *et al*. (65), the incidence of GC development in the high risk Chinese population, which was subjected to Hp eradication was unexpectedly reduced significantly only in a subgroup of Hp-carriers without pre-cancerous gastric lesions but not in those with pre-cancerous lesions. The study by the Helicobacter and Cancer Collaborative Group headed by Forman (66) analyzed the data from 12 case-control studies nested with a prospective cohort. They reported an overall OR for GC in those who were Hp-positive of 2.36 (95% CI 1.98-2.81). The risk was confirmed for non-cardia cancers (OR 2.97, 95% CI 1.98-2.81) and not for cardia cancers (OR 0.99, 95% Cl 0.72-1.35). Furthermore, they found that the OR for non-cardia GC was stronger when blood samples for Hp serology were collected more than 10 years before cancer diagnosis (OR 5.9, 95% Cl 3.4- 10.3) and they argued that this higher estimate is a more accurate reflection of the risk. Finally, the authors found that Hp infection was associated with an equally increased risk of intestinal and diffuse non-cardia cancer [OR 4.45 (95% CI 2.74 - 7.34) and 3.39 (95% CI 1.70-6.76), respectively (*Table 1*).

The question remains whether all strains of Hp are equally carcinogenic. It has been established that strains possessing CagA are more harmful to the gastric mucosa and thus associated with an increased risk of duodenal ulcer and gastric cancer. As mentioned before, Huang *et al.* (63) based on his meta-analysis on large group of GC cases and controls emphasized that Hp and CagA seropositivity significantly increased the risk of GC. Thus, estimates based on CagA seropositivity gave a stronger relationship between Hp and GC of both histological subtypes, while the risk of non-cardia GC of both histological subtypes is not increased. Infection with CagA-positive strains has been reported by several groups to increase the risk of non-cardia GC, the OR for CagA seropositivity among *H. pylori* positive subjects being 9.7 (67). According to our experience (68), GC linked to Hp infection was accompanied by a 2-3 fold higher CagA seropositivity in GC

Table 1

Epidemiology supports the association between the Hp infection and gastric cancer

- ✓ **The incidence of GC, especially its intestinal-type, is significantly higher in developing countries showing also the highest (over 80%) Hp prevalence (East Asia, Estern Europe and Central and South America)(Graham *at al*., *Dig Dis Sci 1991, 36, 1084;***
- ✓ **Nearly two thirds of GC occur in developing countries; Japan and Korea showing the highest GC rates in the world and also the highest Hp prevalenvce (Yamamoto S., *Jpn J Clin Oncol* 2002; 31: 471);**
- ✓ **Huang *et al.* ,who identified 16 studies with 2284 Hp positive and 2770 controls, found ~ 3 fold higher GC occurrence in Hp positive than in Hp negative controls (*Gastroenterology* 2003; 125: 1636);**
- ✓ **Umehara *at al.* found that Hp eradication completely prevented GC compared to 2.9% of GC in placebo treated infected controls during 7 yr observation (*NEJM* 2001; 345: 784);**
- ✓ **Wong *et al*. found that in high risk China region, in study on 1630 during 7.5 yrs after Hp eradication, the GC incidence was significantly reduced in Hp carriers but without precancerous lesions (*JAMA* 2004; 291: 187);**
- ✓ **Forman's group showed that all GC in high risk countries (*e.g.* Japan) may be attributed to the Hp and subsequent mucosal inflammatory action of Hp (*NEJM* 2008; 395: 448).**

than in age-matched non-GC Hp positive controls, indicating that out of several tested parameters (CagA, plasma gastrin, IL-8, gastric acid secretion), the CagA seropoositivity appears to be one of the best discriminative parameters between GC and controls as determined by multivariable analysis with logical regression and that this parameter might be a useful marker of gastric carcinogenesis (68) (*Fig. 7*).

In summary, the epidemiological evidence strongly supports the notion that Hp infection increases the risk of non-cardia gastric cancer of both histological subtypes while the risk of cardia cancer is not increased. Virulent strains, *e.g. cagA* positive strains further increase the risk of cancer (69).

ANIMAL MODELS IN ASSESSMENT OF THE ROLE OF HP IN GASTRIC CARCINOGENESIS

To clarify the relation between the Hp infection and GC development, several animal models have been developed to induce chronic infection with Hp or *H. felis* (70-74). Rodents were the primary models used to reveal the details of host, bacterial and environmental factors involved in gastric carcinogenesis. Mice are inbred, thus permitting host variables to be carefully controlled. After the Hp colonization, most mouse strains develop only mild inflammation but not GC. However, the reproducible cancer sequence was achieved with *H. felis*,

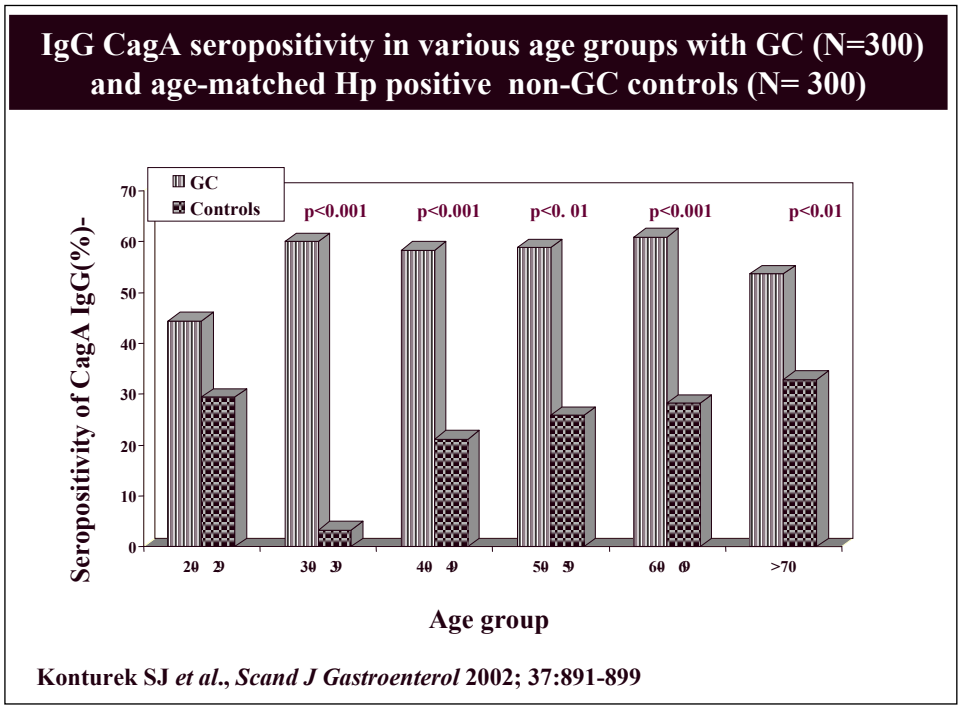

Fig. 7. CagA seropositivity in various age groups with CG and age-matched Hp positive non-GC controls (adapted from Konturek SJ, *et al. Scand J Gastroenterol* 2002; 37: 891-899).

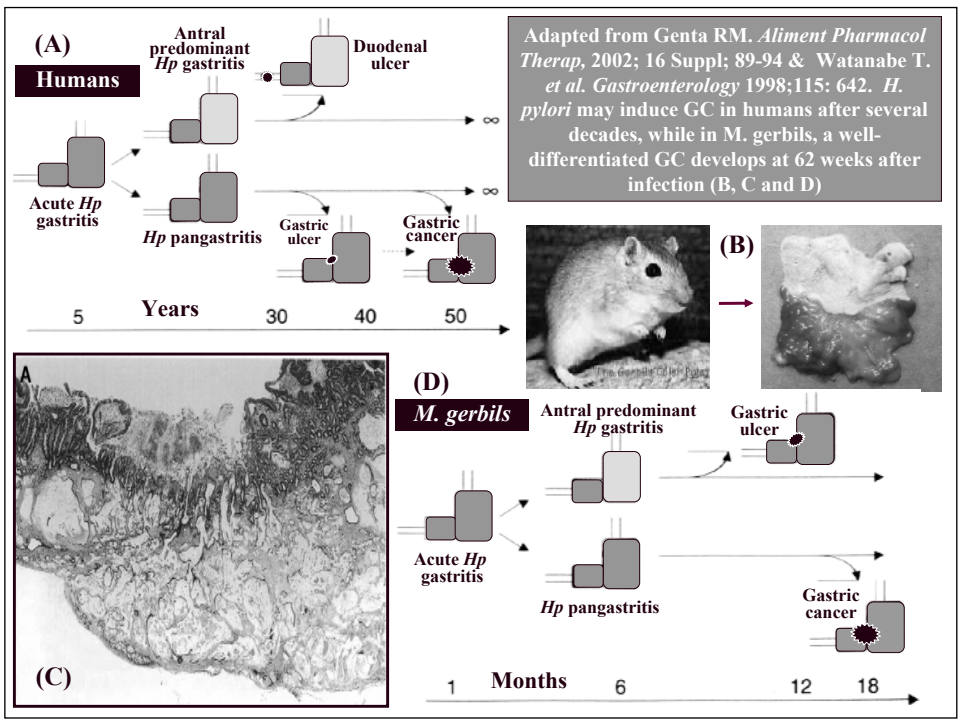

Fig.8. Hp infection in humans may results in atrophic gastritis followed by intestinal metaplasia and dysplasia leading to the development of GC but this requires several decades (A), while in experimental model in Mongolian gerbils the intragastric administration of CagA & VacA positive Hp results in acute/chronic pangastritis and the development of gastric cancer with about 62 wks (adapted from Watanabe, *et al., Gastroenterology* 1998; 115:642.).

which is similar to Hp (73). Alternatively, *Mongolian gerbils*, which are not inbred animals, can develop cancer when colonized with certain strains of Hp. The histopathological stages in gerbil model including gastric atrophy, intestinal metaplasia, dysplasia and adenocarcinoma are similar to those described in humans (70, 74). The fact that following infection, cancer develops without pretreatment with nitro-carcinogens such as N-methyl-N-nitrosourea, indicates that the chronic inflammatory process initiated by this infection is the driving force for neoplasia (70). Among primates, monkeys are the most closely related to humans but high costs, expensive animal facilities and difficult experimental manipulations make them impractical in studies on carcinogenesis induced by Hp. The most practical species for studies on gastric carcinogenesis are gerbils, which allow one to examine the functional alterations of Hp-infected gastric mucosa and the role of various virulence determinants in gastric mucosal damage (74). Brzozowski *et al*. (75, 76) found that *H. pylori* infection in Mongolian gerbils examined at 30 weeks upon *H. pylori* inoculation resulted in functional and pathologic gastric changes such as suppression of acid secretion, impairment of mucosal microcirculation, and alteration of gastrin-somatostatin link. Interestingly, probiotic bacteria (Lacidofil) containing a combination of *L. rhamnosus* and *L. acidophilus* could be a novel approach that might exert a beneficial effect against *H. pylori*-induced carcinogenesis *via* its attenuating the effect on the *H. pylori* colonization, the mucosal inflammation and the impairment of gastrin-somatostatin link. In addition, the probiotic treatment contributed significantly to the normalization of gastric mucosal blood flow and gastric acid secretion, and reversed the over-expression of COX-2 in the *H. pylori*-infected gastric mucosa (76).

Other research has focused on determining the apoptosis (programmed cell death) in *H. pylori*-infected gastric mucosa of Mongolian gerbils, since this issue has not been extensively studied in this experimental model. The expression of Bax protein was markedly enhanced, whereas Bcl-2 protein expression was inhibited in gerbils infected with *H. pylori*. On the other hand, tumor development is associated with the inactivation of Bax and the over-expression of Bcl-2 leading to an overall inhibition of apoptosis. We noticed an evident decrease in the apoptosis, as determined by protein expression of key proteins involved in the apoptosis process before the cancer developed. Interestingly, the evident modulation of apoptosis by *H. pylori* in our study was counteracted by anti-*H. pylori* triple eradication therapy and Lacidofil treatment, suggesting that these therapies might be important in the reversal of the cascade of apoptosis leading to the elimination of unnecessary cells (76). As pointed out by Genta and supported by experimental evidence by Watanabe *et al.* (70), the induction of gastric cancer in experimental animals takes over 1 year after Hp infection and includes first acute gastritis, hypochlorhydria, then pangastritis and finally leading to GC (*Fig. 8*). All these steps are accompanied by the hypergastrinemia, resulting from the damage by VacA of parietal cells, hypochlorhydria and impairment of gastrin-somatostatin link with excessive release of gastrin and its CCK_2-receptors in the inflamed fundic mucosa and by neoplastic cells (23, 70).

GASTRIN, COX-2, GROWTH FACTORS AND ANGIOGENESIS IN HP-INDUCED GASTRIC CANCEROGENSESIS. PROTECTION BY VACCINE?

One host determinant that seems to influence the development of GC with prolong gastric infection with Hp is gastrin, which is known to stimulate the gastric epithelial cell proliferation and cancer cells (23). Hypergastrinemia was found to accompany GC development in gerbils was associated with overexpression of certain growth factors, COX-2-prostaglandin system and anti-apoptotic proteins such as survivin and Bcl2, leading to proliferation of mutated atrophic cells, excessive angiogenesis and formation of gastric tumor (23, 75, 76). Wang *et al*. (77) using transgenic mice that expressed gastrin (NS-GAS mice) observed spontaneous development of GC, which occurred over a long period of time in these animals. Infection with the mouse-adapted Hp strain or the gerbil adapted Hp strain

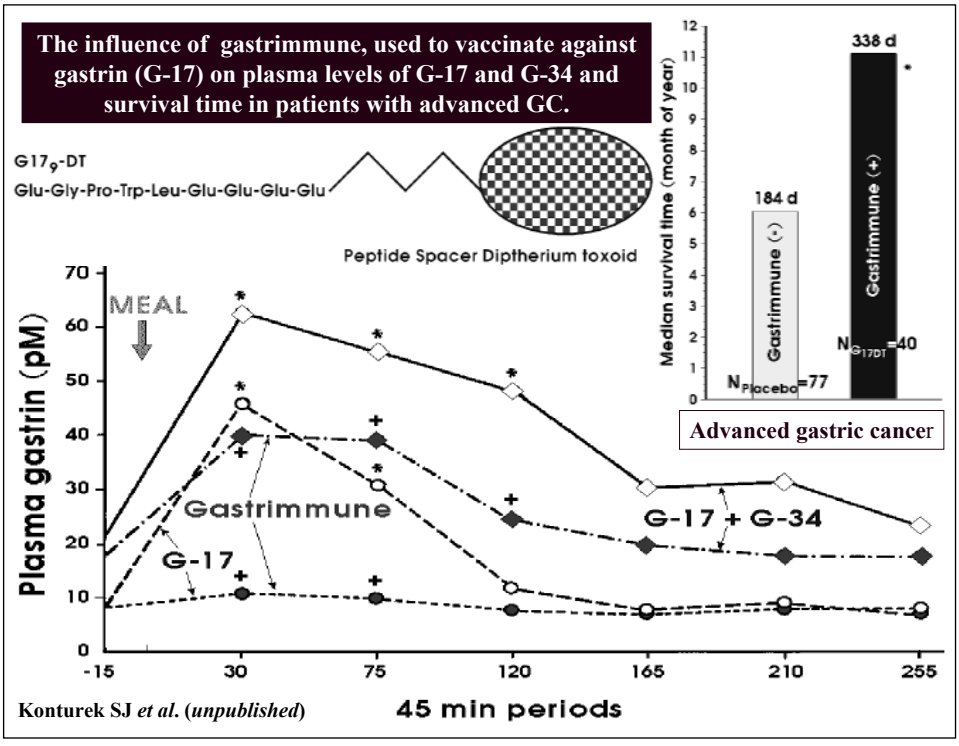

Fig. 9. The influence of gastrimmune, used to vaccinate against gastrn (G-17 and G-34) on basal and postprandial G-17 and G-34 levels and the survival time of patients with advanced GC (unpublished data).

resulted in an acceleration of cancerogensesis, suggesting that chronic hypergastrinemia in these species synergizes with Hp infection and contributes to the eventual parietal cell loss and progression to gastric ulcer (23). Przemeck *et al*. (78) has recently confirmed this using such transgenic INS-GAS mice. Hypergastrinemia was found to render the cells within the gastric fundic epithelium more susceptible to the induction of apoptosis by Hp. This altered susceptibility to apoptosis may be one of major factors predisposing to gastric cancerogenesis in these animals (*Table 2*). As gastrin and its receptors are widely upregulated and involved in the development of gastrointestinal malignancy, attempts have been made to apply active vaccination using antigastrin-17 immunogen (G17DT) to raise the production of endogenous antibodies against gastrin for the purpose of counteracting the carcinogenic action of this hormone in GC (79). Indeed, active vaccination with G17DT was found by our group to reduce the postprandial increase in plasma gastrin in humans (*Fig. 9*). When administered in combination with cisplatin plus 5-fluorouracil in patients with inoperable and metastatic GC G17DT was found to be successful in the prolongation of the overall survival by Ajani *et al*. (80) in the first phase of an international trial (see *Table 2*).

It is of interest to note that gastric culture epithelial cells appear to exhibit the gene expression of gastrin and its receptors and to up-regulate Bcl-2 and survival (81). Ito *et al*. (82) confirmed that GC patients exhibit significantly higher plasma gastrin levels (23) than matched controls and that all GC cell lines express gastrin and gastrin receptors (CCK_2-R), emphasizing the important role of the gastrin-CCK_2-GR system in the development of GC. Furthermore, gastrin stimulated the gene and protein expression of COX-2 and HGF in human culture cancer cells, thereby contributing to tumorigenesis (81). Thus, the Hp infection may contribute to gastric cancerogensesis via induction of expression of gastrin and COX-2 that could account for the stimulation of tumorigenesis, angiogenesis and reduction in apoptosis. The relationship between gastric Hp infection with atrophic gastritis and subsequent hypergastrinemia, increased production of various growth factors, anti-apoptotic proteins as well as COX-2-prostaglandin system involved in gastric cancerogenesis is schematically presented on *Fig. 10*.

Cyclooxygenase (COX) is a key enzyme that converts arachidonic acid to prostanoids that function as important biological mediators. There are three COX isoenzymes (COX-1, COX-2 and COX-3) out of which COX-1 and COX-2 genes have been cloned. The over-expression of COX-1 was found to occur in normal gastric mucosa, whereas COX-2 mRNA and protein are undetectable in normal tissues, but become abundant in activated macrophages and other cells at the site of inflammation as well as in human malignancies and animal models of carcinogenesis (23, 81-85). COX-2 appears to be mutagenic and tumorigenic *in vitro*. In addition, COX-2 over-expression may inhibit apoptosis and increase the invasiveness of malignant cells (81-87). In particular, COX-2 over-expression may inhibit apoptosis and increase invasiveness of malignant cells. In the intestinal-type gastric adenocarcinoma and in precarcinogenic (dysplastic) gastric lesions leading to the development of GC, the lymphatic metastasis, tumor invasion and differentiation of GC can be observed (83-86). CagA positive Hp infection up-regulates the expression of COX-2 in gastric cancer in humans (83, 84). Interestingly, Iwamoto *et al*. (84) showed that exposure of gastric cancer cells (NCTC11637) for 3-6 hrs results in an over-expression of COX-2 and a remarkable increase in the expression PGE_2-receptors as well as the marked release of PGE_2 into the cell medium. This helped to confirm the involvement of the COX-PG system in gastric cancerogenesis (*Fig. 11*). Walduck *et al*. (87) reported that prolong (16 wks) treatment of Hp-infected mice with specific

Table 2

GASTRIN *vs* GASTRIC CANCER

✓Hp infection *per se* and subsequent gastric atrophy, particularly of corpus mucosa, results in the elevation of gastrin concentration in plasma, gastric juice and in the tumor itself (Konturek PC *et al*., *Aliment Pharmacol Therap* 2001; 15, 989; Watson SA *et al*., *Nat Rev Cancer* 2006; 6:936);

✓Hp eradication therapy strongly reduces plasma, gastric juice and GC tumor gastrin concents, suggesting that hypergatrinemia originates both from the antral G-cells and GC cells, that also show the presence of gastrin receptors (CCK_2-R) (Konturek PC *et al*., *DDW* 2001);

✓Gastric cancer cells (Kato III) exposed to gastrin exhibit concentration-dependent expression of HGF, TGFα and anti-apoptotic proteins (BCL_2, and survuvin) suggesting that this hormone predispose to cancerogenesis (Konturek PC *et al*., *J Physiol Pharmacol* 2003; 54:17);

✓Hypergastrinemic transgenic INS-GAS mice show altered susceptibility of gastric mucosal cells to apoptosis after Hp infection, predisposing to carcinogenesis, which could be prevented by pretreatment with CCK_2-R antagonist (Przemeck SMC *et al*., *Regulatory Peptides,* 2008; 146: 147);

✓Vaccination with immunogen containing gastrin (G17DT) prolonged the survival of GC probably due to reduction in plasma gastrin produced by the GC (Ajani JA *et al*., *Cancer* 2006; 106: 1908)

COX-2 blocker (NS-398) reduced mucosal inflammation provoked by Hp infection and reduce the activity of COX-2, but failed to effect the COX-2 expression and bacterial colonization in the stomach. Furthermore, Hp might activate the NFκB, which is an oxidant-sensitive transcription regulator of inducible expression of inflammatory genes such as COX-2 that regulates human GC growth and proliferation (*Table 3*). This NFκB may play a role in the expression of COX-2 by Hp within gastric cancer cells (85-90). As over-expression of COX-2 is accompanied by abundant release of prostaglandins (PG), especially PGE_2, this may contribute to the progression of chronic inflammation and the growth of neoplasia. PGE_2, prominently expressed by T lymphocytes in the gastric mucosa at the boundary between normal mucosa and tumor cells, may play a central role in prostanoid-driven tumorigenesis of this tissue (87). In addition, binding of HGF to its receptors (C-Met) enhances gastric cancer progression and metastasis and upregulates the expression of COX-2 and enhances the release of PGE_2 (90). Hp is known to stimulate COX-2 expression and also to increase PGE_2 synthesis (90) that could be an important factor by which CagA positive Hp strains could increase the risk of GC (*Fig. 11*). The major factor that appears to mediate the COX-2 expression in isolated gastric cancer cells is gastrin, which is also capable of stimulating the expression of growth factors such

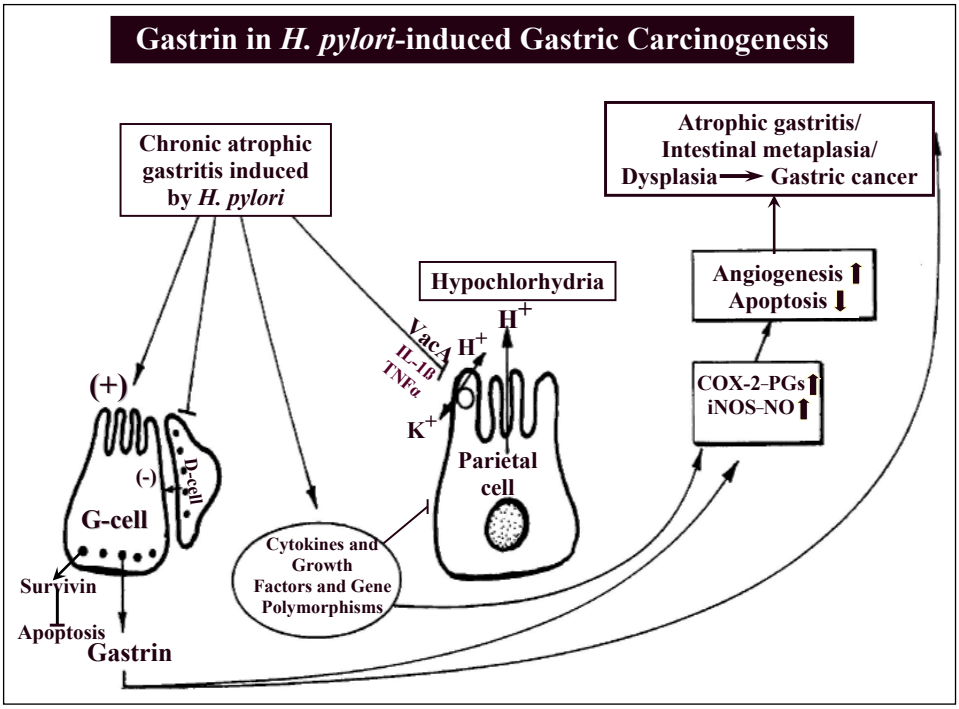

Fig. 10. Role of gastrin in Hp-induced gastric carcinogenesis.

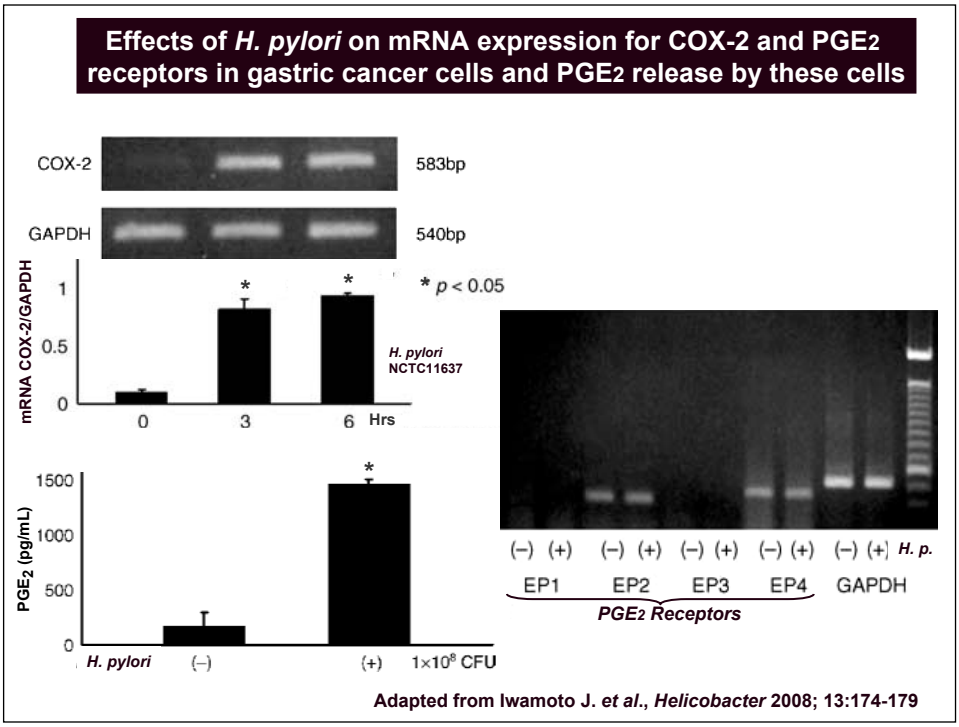

Fig. 11. Effect of Hp infection on COX-2 gene expression 3-6 hrs after exposition of the human gastric cancer cells to Hp and prostaglandin release into the cell medium as well as the expression of PGE_2 receptors (EP_2, EP_4) in these cells (adapted from Iwamoto J, *et al. Helicobacter* 2008; 13: 174-179).

as hepatocyte growth factor (HGF) and antiapoptotic proteins such as BCL_2 (*Fig. 12*). Thus, gastrin released in excessive amounts following Hp infection contributes greatly to the expression of proinflammatory COX-2-prostaglandin system and antiapoptotic proteins contributes to the cancer growth.

The implication of COX-2-PG system in gastric carcinogenesis is supported by numerous studies showing that the inhibition of COX-2 prevents the growth of GC xenografts in nude mice and that aspirin use, which inhibits both COX-1 and COX-2 activity decreases the risk of developing of some types of GC (91). Application of COX-2 selective inhibitors reduces the inflammation, suppresses carcinogenesis in the gastrointestinal tract and might be an effective and important target in the treatment of patients with atrophic gastritis in order to reduce the risk of the occurrence of Hp-related GC (92, 94, 95). Epidemiological studies indicate that the use of aspirin and other non-steroidal anti-inflammatory drugs can block the action of COX-2 (*Fig. 13, Table 3*), thereby reducing the risk of malignancy in the digestive system (96). The clinical use of specific COX-2 inhibitor in the prevention or treatment of GC may be limited because of potential side-effects most notably within the cardiovascular system such as myocardial infarctions and elevation in blood pressure (92, 95).

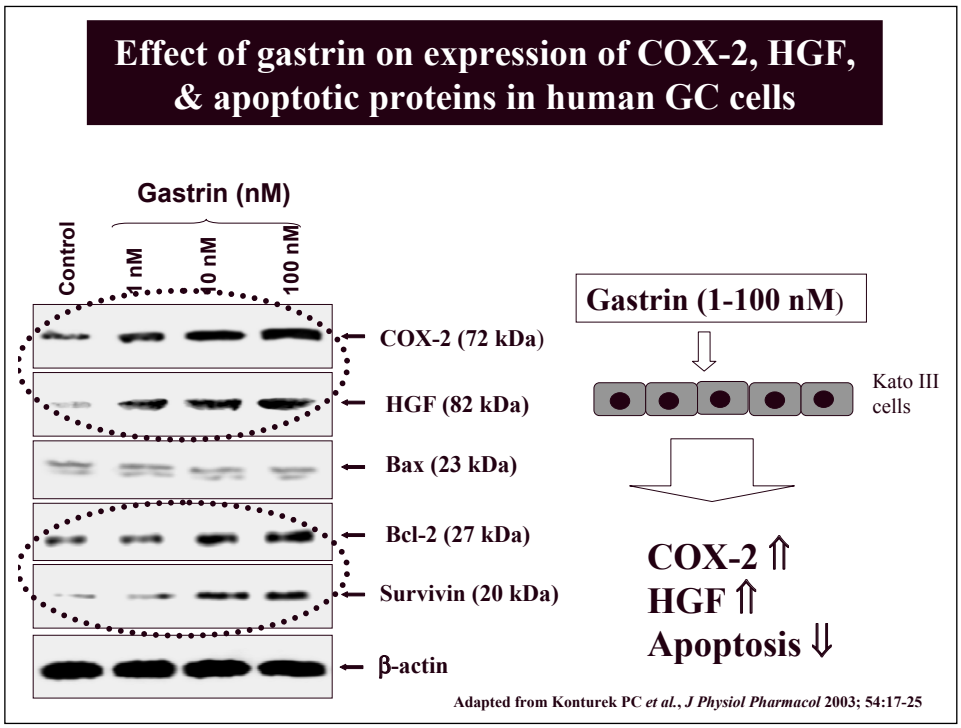

Fig. 12. Exposure of gastric cancer cells to gastrin induces concentration-dependent increase in the expression of COX-2, HGF and anti-apoptotic proteins (adapted from Konturek PC, *et al. J Physiol Pharmacol* 2003; 54: 17-32).

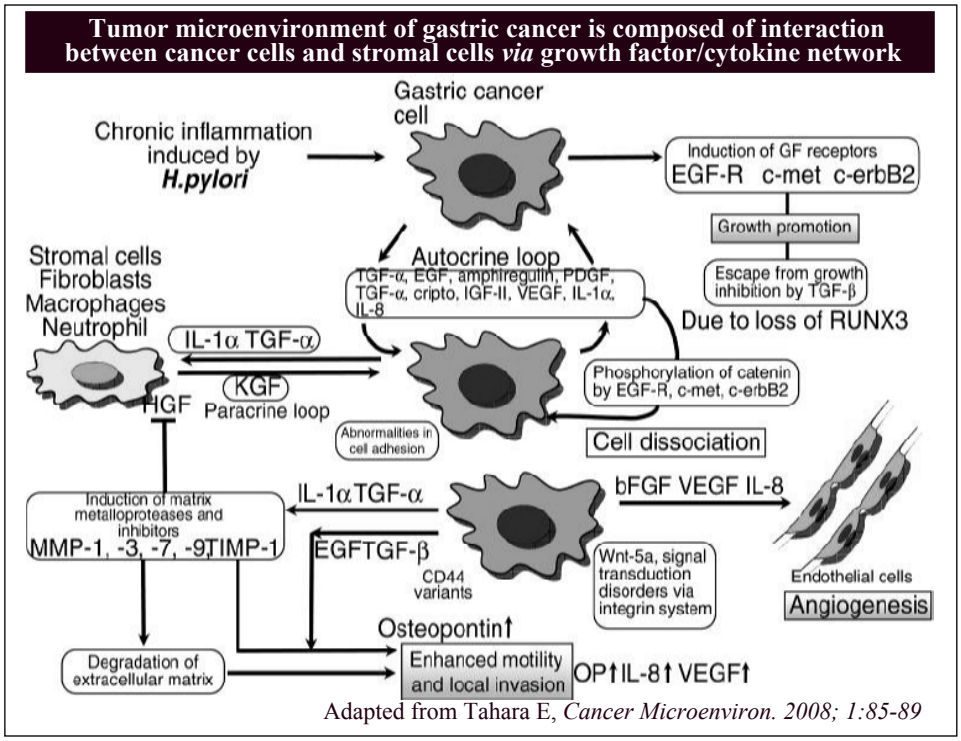

Fig. 13. Tumor microenvironment of gastric cancer is composed of interaction between cancer cells stromal cells *via* growth factor/cytokine network (adapted from Tahara E, *Cancer Microenviron* 2008; 1: 85-91).

In addition to gastrin, several other growth factors have been shown to be co-expressed in the cancerogensesis provoked by Hp infection of the stomach. Increased mucosal gene expression and luminal release of epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) as well as other growth factors were described in gastric cancer patients infected with Hp and these changes were reported to be normalized upon eradication of the bacteria from the stomach (97-100). Similarly, gastric mucosal over-expression and release of HGF, TGF-α and the altered regulation of apoptotic related proteins (Bax/Bcl-2) were found to be associated with gastric carcinogenesis in Hp-infected patients. In addition, the C-Met gene, a proto-oncogenic member of the tyrosine kinase growth factor receptors was found to be over-expressed in over 46% of gastric cancer. Its ligand, HGF/scattered factor was reported to be over-expressed in 67% of gastric cancer (98). Hp activates the C-Met, thus promoting GC (92, 93). Higher expression of C-Met was observed in alpha-fetoprotein-producing gastric cancer and this was associated with decreased apoptosis, liver metastasis and poorer prognosis, possibly due to induction and release of HGF and its receptors that are known to increase mitosis, cell movement and tumor progression (92-94, 100, 101) (*Fig. 13*).

Development of a Hp vaccine would be a new effective strategy for the prevention and treatment of Hp infection. It is of interest to note that a recombinant Hp vaccine comprising of a single subunit antigen can only induce immune response with limited protection and efficacy. In contrast Wu *et al*. (102) immunized Mongolian gerbils with different formulations of three recombinant Hp antigens UreB, HspA and HpaA with two different adjuvants. They demonstrated that the recombinant multi-component vaccine provided effective protection against Hp infection as compared to the single-antigen vaccine. This protective immunity could be closely associated with a predominant Th-2-type response. Thus, UreA, HspA and HpaA were identified as candidate protective antigens that could be used to design the Hp vaccine.

ANGIOGENESIS IN HP-INDUCED GASTRIC CANCER

Amplification and over-expression of various growth factors observed in GC results in the promotion of neovascularization that increases the risk of invasion and metastases. Among the mediators of this tumor angiogenesis is vascular endothelial growth factor (VEGF) that was found to be over-expressed in 54% of GC and which correlates with the depth of invasion into the lymph nodes and liver metastases. The increase in VEGF is accompanied by a marked rise in the induction of interleukin (IL)-6, especially in advanced stage (IV) of tumor (96). IL-6 that modulates VEGF release and action and is accompanied by the increase in the generation of IL-8 that also acts as angiogenic factor in gastric cancer through the interaction with cognate receptors (CXCR1 and CXCR2) on endothelial cells (103-105). IL-8 and VEGF as well as NO derived from over-expressed inducible NO synthase (iNOS) in the tumor (*Fig. 14*), are implicated in Hp-related gastric carcinogenesis, at least in part due to the stimulation of angiogenesis (98).

INDUCIBLE NOS-NO SYSTEM IN GASTRIC CANCEROGENESIS

As indicated above, the angiogenesis is a critical aspect of cancer biology and among the multiple molecular pathways involved in its regulation, the major role is played by endothelial nitric oxide synthase (eNOS or NOS III). This enzyme is expressed in significantly higher levels in both the primary tumors and lymph node metastases than in normal mucosa (106). NO generated in

Table 3

COX-2 -PG vs GASTRIC CANCER

✓Overexpression COX-2 in Hp-infected gastric mucosa and GC tissue is well documeneted (Konturek PC *et al*., *J Physiol Pharmacol* 2006; 57: 51; Ymac D *et al*., *Pathol Res Pract* 2008; 204: 5270);

Co-culture of human MKN-45 gastric cells with Hp increases the expression of COX-2 protein and COX-2 activity within 24 h *via* enhanced phosphorylation of p38MAPK and ATF-2 (Li Q *et al*., *Cancer letters* 2009);

Prolonged (16 wks) treatment of Hp-infected mice with specific COX-2 blocker (NS-398) reduced mucosal inflammation and COX-2 activity and but did not affect COX-2 expression and bacterial colonization in the stomach (Walduck AK *et al.* (*Molecular Cancer* 2009; 8: 22);

Overexpressed COX-2-PG promotes cancerogenesis by the increase of cell proliferation, inhibition of apoptosis, enhanced GC cells invasion and angiogenesis (Huang H *et al*., *J Biomed Sci* 2005; 12: 229);

Use of aspirin and other NSAIDs reduces the risk of non-cardia cancers in large cohort and meta-analysis of 7 yr study (N = 311 115) confirming earlier reports of the benefit of NSAIDs use in anti-cancer treatment (Abnet CC *et al*., *British J Cancer* 2009; 100: 551)

large amounts by this enzyme in GC increases angiogenesis and is vital for the activity of pro-angiogenic cytokines such as VEGF. Sustained production of NO can enhance tumor growth through the increase in blood flow, vascular permeability and tumor vascularization due to the stimulation of endothelial cell growth and migration. Wang *et al*. (106) found that over-expression of eNOS occurs mainly in poorly differentiated GC and correlated with the microvessel density in tumor tissue (see *Fig. 14*). Ohshimura *et al*. (107) indicated that chronic inflammation such as induced by Hp infection in gastric mucosa activates iNOS and numerous other enzymes such myeloperoxidases, NADPH oxidases, esosinophil peroxidases. These enzymes generate numerous potent reactive oxygen (ROS)and nitrogen species (RNS) that can damage DNA and RNA, leading to increased mutations and thus contributing to

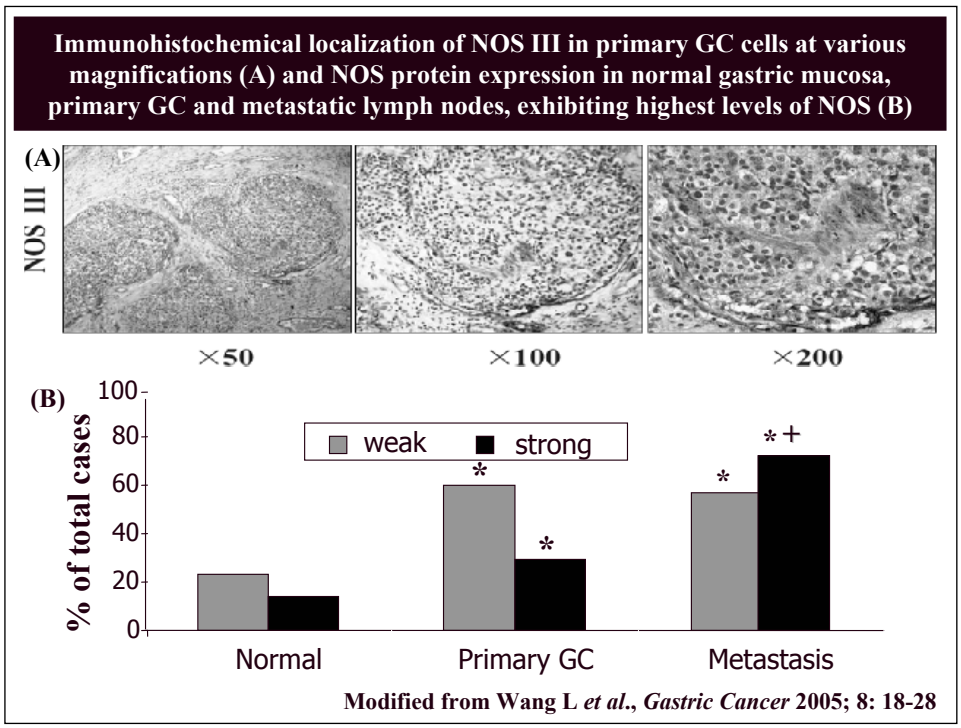

Fig. 14. Immunohistochemical localization of NOS III in primary gastric cancer at various magnification (A) and protein NOS expression in intact mucosa and in primary cancer and in its metastases (adapted from Wang L, *et al. Gastric Cancer* 2005; 8: 18-28).

Table 4

Inducible NOS-NO vs GASTRIC CANCER

*** Overexpression of iNOS in gastric cancer, mainly in poorly differentiated GC, correlates with microvessel density and seems to play a role in tumor angiogenesis and aggressiveness (Wang L *et al*., *Gastric Cancer* 2005;8: 18);**

*** Excessive amounts of NO produced by iNOS together with other reactive species damage DNA and inhibits its repair activities, leading to the loss of cell proliferation and p53 mutant control (Ohishima H *et al*., *Arch Biochem Biophys* 2003; 416: 3);**

*** Expression of iNOS is significantly correated with expression of VEGF, indicating that iNOS enhances tumor progression by stimulating angiogenesis and suppressing immune response of GC (Yamaguchi K, *et al*., Oncology 2005; 68: 471);**

*** According to Heller A. (*Chem Med 2008; 3: 1493),* the overexpression of iNOS with enhanced synthesis of NO, a stable nitrosyl radical (·NO), may be attenuated by TGF-ß1, indicating the role of iNOS-NO in gastric carcinogenesis requires further studies.**

*** In general, iNOS-NO observed in Hp infected mucosa, and particularly in the Hp-related gastric cancer, supports the proposal of Correa that Hp-induced changes in gastric mucosa result from the action of numerous reactive oxygen (ROS) and nitrogen species (RNS) and that cancerogenesis may be considered as a result of exidative stress (Correa P., Houghton J. Gastroenterology 2007; 133: 659) .**

the multistage carcinogenesis process (*Fig. 15*). Yamaguchi *et al.* (108) using advanced gastric carcinoma also found a close correlation between iNOS expression and VEGF expression. Heller (109) confirmed that high nitric oxide level is common to most cancers and that arginase competes with iNOS for arginine, catalyzing its hydrolysis to ornithine and urea. However, certain types of human tumors may express little or no iNOS and this deficiency is caused by the suppression of iNOS expression at the mRNA level by TGF-β1, which has been described also in advanced gastric cancers (*Table 4*). In general, iNOS-NO observed in Hp infected gastric mucosa and particularly in the Hp-originating GC supports the proposal of Correa (110, 111) that Hp induced inflammatory changes in gastric mucosa results from the action of numerous ROS and RNS and, therefore, the carcinogenesis could be considered as a result of oxidative stress (*Table 4*).

CELLULAR ORIGIN OF GASTRIC CANCER INDUCED BY HP

Gastric carcinomas are generally believed to evolve from native gastric mucosa or intestinal metaplastic mucosa that

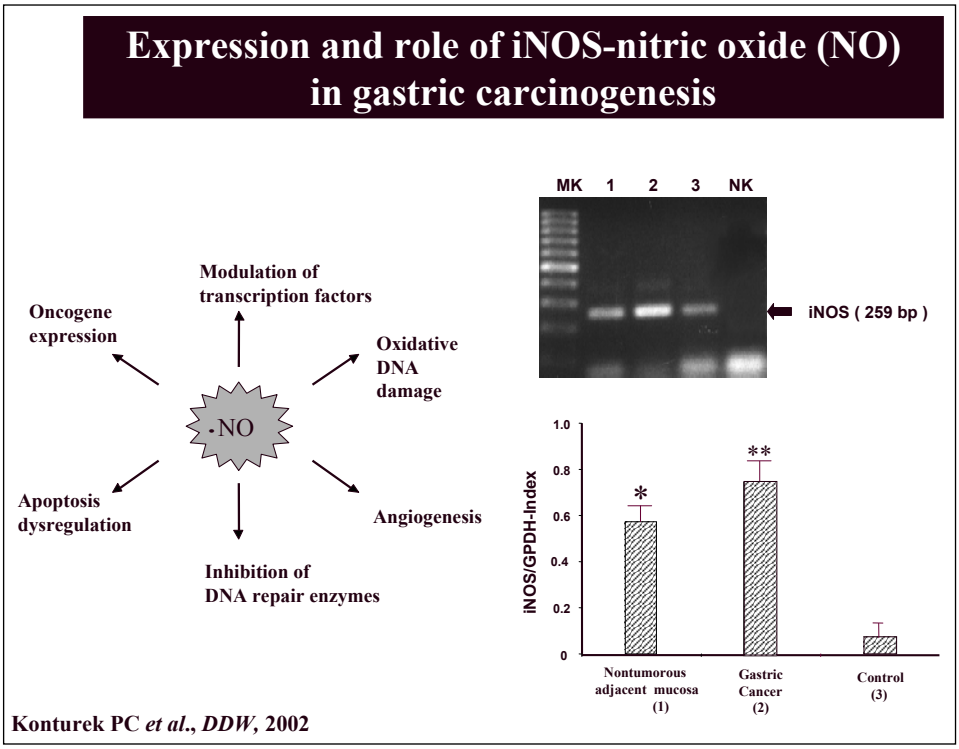

Fig. 15. Expression and role of iNOS-NO system in gastric carcinogenesis (adapted from data presented by Konturek PC, at AGA DDW, 2002).

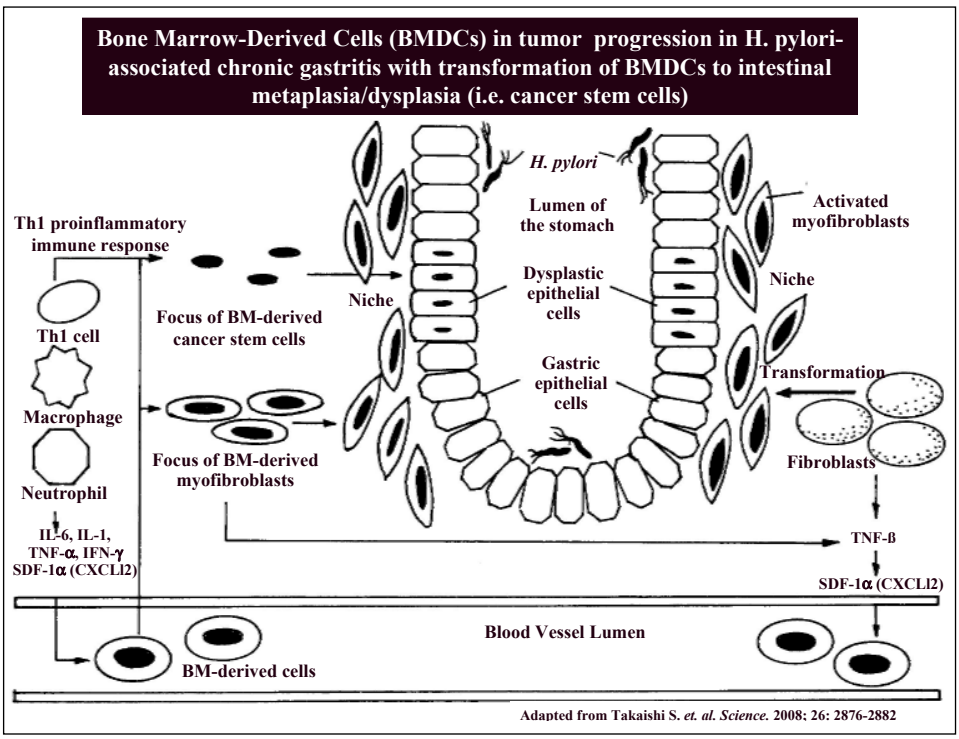

Fig. 16. Bone Marrow-Derived Cell (BMDCs) in tumor progression in Hp-associated chronic gastritis with transformation of BMDCs to intestinal metaplasia/dysplasia (*i.e.* gastric stem cells) (adapted from Takahaishi S, *et al. Science* 2008; 26: 2876-2882).

undergoes various genetic and epigenetic alterations involving either the suppressor pathway defects (defects in tumor suppressor genes) or the mutation pathway (defects in DNA mismatch repair gene) (112). In general, GC can be classified as proximal gastric tumors, commonly known as cardia-type cancer and intestinal-type cancer. According to results of research conducted by Kamangar *et al*. (113), who conducted a prospective nested case-controlled study based on Hp serology, Hp is a strong risk factor for non-cardia or intestinal-type gastric cancer but is inversely associated with the risk of cardia cancer. Thus, the contribution of Hp appears to be implicated in the pathogenesis of non-cardia GC, that histologically can be classified as intestinal- and diffuse-type. Diffuse-type adenocarcinoma affects mostly young men and consists of individually infiltrating neoplastic cells that do not form glandular structures and are not associated with intestinal metaplasia. Most cases of familial GC exhibits a diffuse histopathology and mutations in the *E-cathedrin* (*CDH-1*) gene is the major cause of this disease. Although Hp increases the risk of both types on noncardia gastric cancer, its role in the intestinal-type cancer is well known.

Intestinal type GC typically arises in the setting of chronic gastritis and develops through the intermediate stages of atrophic gastritis, intestinal metaplasia, dysplasia and finally adenocarcinoma. During Hp-induced chronic gastric inflammation, there is an increased tissue turnover that predisposes to an excessive rate of cell proliferation and may result in excessive frequency of mitotic errors and increased rate of mutagenesis. This lengthy process commonly known as the "Correa pathway" is dependent on continued or chronic inflammation (110, 111). Matsumoto *et al.* (114) and Takaishi *et al*. (115) reported recently that Hp infection triggers the expression of activation-induced cytidine deaminase (*AID*) gene, originally linked to immunoglobulin class switching and B lymphocytes hypermutation, but aberrantly expressed in cancer, where it may predispose to point mutations of the *p53*, tumor suppressive gene. Although the mutagenesis of specific genes may be of some importance in the carcinogenesis, recent studies highlighted the important role of immune cells population (*e.g*. macrophages, T cells) and pro-inflammatory cytokines (*e.g*. IL-1β , IL-6, TNF-α) in the pathogenesis of gastric cancer. Given current hypothesis that GC originates from gastric cancer stem cells (CSC) or progenitor cells (116) another possible source of CSC has recently been described, namely bone marrow-derived mesenchymal cells (BMDC) identified in the course of studies employing mouse models of Hp-induced gastric cancer (117). These BMDC seem to possess a wide range of plasticity and may migrate through peripheral organs as a result of their damage or inflammation (*Fig. 16*). The differentiation pattern and growth regulation of these cells may depend upon local environment and cues. Chronic inflammation by Hp attracts the BMCD cells into the mucosa with depleted resident gastric stem cells. After engraftment or settlement in inflamed mucosa the BMDC quickly grow and develop into cancer cells.

SUMMARY AND CONCLUDING REMARKS

Numerous epidemiological studies have shown that about half of world human population is infected with Hp that is associated with progressive gastric inflammation and progression through intestinal metaplasia towards gastric cancer, especially the distal portion of the stomach.

This multi-step progression of gastric carcinoma is sustained predominantly by the action of various Hp-originated virulence factors, particularly CagA, VacA, various growth factors, cytokines and free radicals.

One of the early changes after Hp infection and subsequent atrophic gastritis is the rise of plasma gastrin release caused by the increased release of this hormone from the antral G-cells as well as from the cancer cells, which possess CCK_2-receptors that act in a vicious cycle to enhance the unlimited tumor cells growth, proliferation and metastasis.

As the majority of cases present with advanced disease, conventional therapy has limited efficacy to reduce mortality, emerging modalities provide promise to combat the malignancy. Target-protein based cancer therapy becomes available in clinical practice. As COX-2 is over-expressed in all types of Hp-related gastric cancer, the knockdown of COX-2 by the administration of the inhibitors of COX-2 generation or activity suppresses tumor formation in models of GC. Induction of apoptosis, reduction of angiogenesis and blocking of potassium ion channels may present new mechanisms of COX-2 inhibition.

Excessive expression of gastrin and its release can be counteracted by applying active vaccination using antigastrin-17 immunogen (G17DT) to raise the production of endogenous antibodies against gastrin.

Tumor microenvironment modulation also provides a powerful tool to inhibit cancer development and progress. Osteopontin is a secreted protein involved in the stress response, inflammation and immune response. Inhibition of osteopontin by RNA interfering techniques suppressed tumorigenesis and angiogenesis.

The biochemical changes accompanying gastric carcinogenesis disappear after eradication of HP and this supports the WHO 1994 conclusions that Hp infection is a definite or class 1 carcinogen in the human stomach.

Conflict of interests: None declared.

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Author's address: Professor Peter C. Konturek, M.D. First Department of Medicine, University Erlangen-Nuremberg, Ulmenweg 18, 91054 Erlangen, Germany.