Gastric malt-lymphoma, gastrin and cyclooxygenases

P.C. Konturek[1], S.J. Konturek, P. Pierzchalski, T. Starzyńska[2], K. Marlicz[1], A. Hartwich[3], M. Zuchowicz[3], Z. Darasz[3], D. Papież, E.G. Hahn[1]

Department of Physiology, University College of Medicine, Cracow, Poland. (1) Department of Medicine, University Erlangen-Nuremberg, Erlangen, Germany ; (2) Department of Gastroenterology, Pomeranian Academy of Medicine, Szczecin ; (3) Department of Surgery of District Hospital and Institute of Oncology, Cracow, Poland.

Abstract

Malt-lymphoma, gastrin and COX-2 interaction. **Low grade, mucosal associated lymphoid tissue (MALT)-lymphoma is an unique among gastric malignancies where causal involvement of *Helicobacter pylori (H. pylori)* infection has been proposed based on complete regression of the tumor following the eradication therapy. In this report ten primary, low-grade MALT-lymphomas have been examined before and 6 months after one week of successful eradication therapy (clarithromycin + amoxicillin + omeprazole). Gastric biopsy samples from tumor and intact *antrum* and *corpus* mucosa were obtained during endoscopy before and after eradication for assessment of expression of gastrin and gastrin receptor (CCK_B-R) as well as cyclooxygenase (COX)-1 and COX-2 using RT-PCR. The gastric lumen and serum gastrin and mucosal and tumor tissue PGE_2 biosynthesis were determined by RIA before and after *H. pylori* eradication. Eradication of *H. pylori* resulted in complete endoscopic and histological remission of MALT-lymphoma in 9 out of 10 patients as assessed 6 months after this eradication. Before eradication, the mRNA expression for gastrin and CCK_B-R as well as mRNA expression for COX-1 and COX-2 were observed in tumor tissue and infected mucosa, while *corpus* mucosa expressed only CCK_B-R and antrum mucosa only gastrin. Six months upon the eradication when MALT-lymphoma completely regressed both endoscopically and histologically in 9 of 10 tested subjects, the expression of gastrin and COX-2 disappeared from the former area of MALT-lymphoma tumor. Gastrin mRNA remained detectable only in *antrum* mucosa, CCK_B-R mRNA in *corpus* mucosa and COX-1 mRNA both in *antrum* and *corpus* mucosa. Gastric luminal and serum gastrin levels and gastric mucosa and tumor PGE_2, which were greatly elevated before eradication, became normalized after this procedure. This study demonstrates that low-grade MALT-lymphoma is linked to *H. pylori* infection which may promote the expression and excessive release of gastrin and COX-2 expression that could be involved in the pathogenesis of MALT-lymphoma.** (Acta gastroenterol. belg., **2002**, 65, **17-23**).

Key words : *helicobacter pylori,* gastrin, cyclooxygenase, histamine, prostaglandins, MALT-lymphoma.

Introduction

Among the major ten achievements in gastroenterology during last century proposed by 50 most active clinicians and leading researches, the discovery of *Helicobacter pylori (H. pylori*) infection has been considered as the most important achievement before such as fiberoptic endoscopy, imaging radiography and computed tomography, vaccination against hepatitis A and B, molecular biology of colon cancer and others (1).

H. pylori infection is recognized as chronic and transmissible disease in which various clinical disorders develop slowly during decades starting with inoculation of the bacteria in the mucus layer of the distal portion of the stomach. Subsequent *antritis* occurring in most infected patients may lead to hypergastrinemia, increased acid secretion and eventually gastroduodenal ulcerations. The infection of the *corpus* mucosa also results in hypergastrinemia but mainly due to the atrophic gastritis of gastric *corpus* and decrease in gastric acid secretion leading to gastric cancerogenesis (2-5). Hypergastrinemia initially results in hyperproliferation of gastric and colonic epithelial cells but then may cause mucosal atrophy (5-8). Carcinogenesis promoted by *H. pylori* infection starts with genomic instability and loss of tumor-suppressor genes such as *p53*, resulting in mutations in intracellular signaling protein such as K-ras and creating oncogenes that generate unremitting stimulus for cellular proliferation and tumor growth as originally proposed by Correa (9).

Perhaps the most important clinical and biochemical findings in *H. pylori*-related gastric tumorigenesis include ; 1. high (> 90%) prevalence of *H. pylori* usually accompanied by CagA seropositivity initiating *antritis* and activation of the antral G-cells to produce and release excessive amounts of gastrin that is the most potent growth factor for gastric mucosal as well as tumor cells, and 2. an unexpected overexpression of cyclooxygenase-2 in these tumors supported by clinical observations that aspirin or some other nonsteroidal anti-inflammatory agents (NSAID) even at low doses sharply reduce the risk of dying of gastric and colorectal cancers (10-12).

H. pylori infection is directly responsible for the causation of special pathogenic changes in gastric mucosa such as the occurrence of lymphoid tissue, normally non present in this mucosa (13-17). The eradication of *H. pylori* by combined antibiotic therapy was reported by several investigators to result in almost, complete endoscopic regression of the majority of MALT lymphoma tumors and in the disappearance in the gastric mucosa of lymphoma follicles and lymphoepithelial distractions (14,18,19). Thus, the link between *H. pylori* infection and the onset of MALT-lymphoma has been established. Our recent study (20) confirmed that ; gastric MALT-lymphoma is closely linked to *H. pylori*

Address correspondence to : Prof Dr. S.J. Konturek, Department of Physiology, University College of Medicine, 16 Grzegorzecka str, 31-531 Krakow, Poland.
Presented at session 8th UEGW in Brussels, November 28, 2000.

CagA seropositive infection and that gastrin and its receptors (CCK_B-R) detected in tumor tissue might be implicated in the pathogenesis of gastric MALT-lymphoma.

As mentioned previously, NSAID are effective in reducing the mortality of gastric and colorectal cancer patients (10-12) and these dramatic effects of COX-inhibitors may be of some therapeutic potential in these patients. These impressive results have been attributed to the suppression by NSAID of the COX-2-activity in MALT-lymphoma patients. Unlike COX-1, which is constitutively expressed in the intact gastric mucosa to maintain its homeostasis, COX-2 is not ordinary expressed in the gastric mucosa but can be induced by various growth factors and cytokines in the inflamed or cancer tissue to release large amounts of PGE_2 (21, 22).

This study was undertaken to verify 1) whether infection of *H. pylori*, especially that expressing CagA, is prevalent in MALT-lymphoma patients ; 2) whether this infection induces an expression of gastrin and its receptors in tumor tissue and gastric mucosa, and 3) whether an overexpression of COX-2 occurs in the tumor tissue and results in enhancement of prostaglandin (PG) generation. For this purpose we investigated 10 primary, low grade gastric MALT-lymphoma before and six months after successful *H. pylori* eradication using triple therapy. We confirmed that MALT-lymphoma tissue overexpresses gastrin and its receptors but also expresses COX-2 resulting in greatly increased generation of PG that could contribute to the pathogenesis of the MALT-lymphoma.

Material and methods

The studies were carried out on 10 histologically verified low grade MALT-lymphomas (5 men and 5 women) with the median age of 56 years (range 38 to 67 years) and 40 control volunteers (20 men, 20 women) with the median age of 54 years (range from 38 to 65 years) with moderate dyspeptic symptoms similar to those reported by MALT-lymphoma patients. Patient symptoms, age, gender, tumor site and its histology were recorded at the Department of Gastroenterology Clinic of Pomeranian Medical Academy, Szczecin, District Hospital and Cancer Institute, Cracow and Department of Physiology, Jagiellonian University School, Cracow, Poland. Stage and histological type of gastric tumor as well as those in gastric *antrum* and fundic *corpus* were assessed by routine examination of paraffin sections obtained from surgical specimens. All lymphoma tumors were located in distal portion of the fundus of the stomach. All the tumors were identified histologically as low-grade primary gastric MALT-lymphomas (13). All subjects gave informed consent and the project was reviewed by the Ethics Committee of Pomeranian Academy of Medicine, Szczecin, Poland, University College of Medicine, Cracow, Poland and Department of Medicine, University of Erlangen-Nurenberg, Erlangen, Germany.

Assessment of H. pylori and CagA status cytokines

The *H. pylori* infection status was assessed by our modification of capsulated minidose ^{13}C-Urea Breath test (UBT) as described earlier (23,24) and by determination of IgG antibodies to *H. pylori* by enzyme linked immunosorbent assay (ELISA) using commercially available kit (EIAGEN *H. pylori* IgG, Clone Systems, Italy). Titers higher than 15 AU/ml were considered positive (following the manufacturer recommendations). IgG antibodies against CagA were detected by ELISA using recombinant CagA kindly provided Ora Vax, Cambridge, USA as described previously (24). Serum IL-8 and TNFα were also measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Biosource Europe S.A., Belgium) in accordance to manufacturer's instructions.

RNA extraction and reverse-transcriptase polymerase chain reaction (RT-PCR) to detect mRNA (mRNA) for gastrin and gastrin receptor (CCK_B) and for COX-1 and COX-2

Seven large biopsy samples of the tumor area and of macroscopically intact mucosa of *corpus* and *antrum*. at the site opposite to the tumor were taken during endoscopy. One sample of each of these three examined area was placed in buffered paraffin and then used to define histologically the types of the lymphoma, the histology of surrounding intact mucosa and the presence of *H. pylori*. Four mucosal samples (~ 50 mg) (separately from *corpus* and *antrum*) and four tumor samples (~ 50 mg) taken using large biopsy forceps were frozen immediately in the liquid nitrogen for the detection of the signals for mRNA of gastrin and gastrin receptors (CCK_B-R) using RT-PCR as described before (7) as well as for mRNA of COX-1 and mRNA COX-2 expression using, RT-PCR analysis as described before (25).

Total RNA was extracted from biopsy samples using TRIzol Reagent in a single-step method. Aliquoted RNA samples were stored at -80°C until analysis. Single stranded cDNA was generated from 5 µg of total cellular RNA using StrataScript reverse transcriptase (Stratagene, Heidelberg, Germany) and oligo-(dT)-primers (Stratagene, Heidelberg, Germany). Briefly, 5 µg of total RNA was uncoiled by heating (65°C for 5 min) and then reverse by transcribed into complementary DNA (cDNA) in a 50 µl reaction mixture that contained 50 U of Moloney murine leukemia virus reverse transcriptase (NMLV-RT), 0.3 µg oligo-(dt)primer, 1 µl RNase Block Ribonuclease Inhibitor (40 U/µl), 2 µl of a 100 mmol/l mixture of deoxyadenosine triphosphate (dATP), deoxyribothymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 5 µl 10 × RT buffer (10 mM/l Tris-HCl, pH = 8.3, 50 mM KCl, 5 mM $MgCl_2$). The resultant cDNA (2 µl) was amplified in a 50 µl reaction volume containing 0.3 µl (2.5 U) Taq

polymerase, 200 mM (each) dNTP (Pharmacia, Germany), 1.5 mM/l $MgCl_2$, 5 µl 10 × polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH = 8.3) and primers used at final concentration of 0.5 µM. The mixture was overlaid with 25 µl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT) at the following specifications ; 45-s denaturation at 94°C, 45-s annealing at 60°C and 2-min primer extension at 72°C. The nucleotide sequence of the primers for gastrin and CCK_B receptors and for COX-1 and COX-2 and β-actin were based on the basis of the published cDNA. The primers were synthesized by GIBCO BRL/Life Technologies, Eggenstein, Germany. The sequences of oligonucleotide primers for the RT-PCR used in this study are identical to those published previously (20, 24).

Polymerase chain-reaction (PCR) products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Location of a predicted product was confirmed by using GIBCO 100-bp ladder as a standard size marker. Electrophoresed products were scanned by densitometry and the relative value of mRNA for gastrin and CCK_B receptors and COX-1 and COX-2 over β-actin was calculated in each sample.

Determination of tissue generation of orostaglandins

Two samples of the fundic and antral mucosa and tumor tissue were taken and used immediately for the determination of PGE_2 generation and gastrin content using the specific radioimmuno assay for PGE_2 and gastrin in homogenized tissue samples (tissue samples were homogenized in phosphate buffer at pH 7.7 and 0°C for 10 s with Ultra-Turrax T-25, Ika Labortechnik, Staufen, Germany) as described before (5).

Testing of gastric acid secretion and plasma and luminal gastrin level

Blood samples were also collected at the initial examination and from peripheral vein under basal conditions and following histamine (25 µg/kg-h) stimulation and after the separation of the serum was stored at -80°C for gastrin RIA as described before (24,26). Also the samples of gastric juice aspirated carefully during initial and postreatment gastroscopy were collected under basal conditions, immediately neutralized to pH 7.5 using 200 mM NaOH and stored at -80°C until gastrin radioimmunoassay as described before (24,26). The serum samples, the neutralized gastric juice and homogenized tissue samples were processed as described for gastrin radioimmunoassay using gastrin antiserum No 4562 recognizing amidated C-terminal sequence of all biologically active gastrin (G-17 and G-34) (kindly donated by Professor J.E. Rehfeld of Copenhagen, Denmark) and used in the final dilution of 1:140 000. The antibody used recognized G-17 and G-34 equally. The sensitivity of the present assay was 2.5 pmol/mL serum equivalent to human G-17.

Gastric acid outputs were measured in 10 MALT-lymphoma patients and 40 controls at separate test day during 30 min-infusion of a standard dose of histamine (25 µg/kg-h i.v.). Gastric juice samples were aspirated continuously and gastric aliquots were collected at 10 min intervals. Blood samples were withdrawn just before and at the end of 30 min of histamine infusion. The serum was separated from blood cells, frozen at -80°C and kept for gastrin RIA. Gastric acid secretion is expressed as mmoles (per h) of acid secreted after histamine stimulation. Serum I1-8 and TNFα were measured by an enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Biosource Europe S.A., Belgium) in accordance with manufacturer's instructions described previously (7).

Data Analysis

The major groups were the *H. pylori*-, CagA-seropositive and serum and gastric luminal gastrin concentrations in patients with MALT lymphoma, and controls. In general, rank sum test, Sperman's rank test order correlation was used for relation between independent variables. P value of less than 0.05 was accepted as significant.

Results

The *H. pylori* seropositivity was detected in all 10 MALT lymphoma patients (100%) and in 23 out of 40 age- and gender-matched control subjects (57.5%). The difference in prevalence of *H. pylori* seropositivity between gastric MALT lymphomas and controls was statistically significant. The CagA seropositivity in lymphoma patients was found in 6 of 10 patients (60%) and it was more than twice as high as in controls (30%) (Table 1). The UBT results fully agreed with serology results and showed an overall *H. pylori* positivity in 90% of lymphoma patients and in 50% in controls. Eradication of *H. pylori* by triple therapy (Amoxycillin, Clarythromycin and Omeprazole) resulted in the negative UBT in 9 of 10 MALT-lymphoma patients.

Serum levels of both IL-8 and TNFα were significantly higher in MALT lymphoma patients as compared to the controls. Following *H. pylori* eradication, plasma IL-8 and TNFα fell to the levels that were significantly lower than those recorded in *H. pylori* positive MALT lymphoma patients and only slightly higher than in controls (Table 1).

Serum level of gastrin in MALT lymphoma was about 147 ± 18 pM and this was about eight folds higher than that in controls (18 ± 4 pM). In MALT lymphoma patients the gastric luminal gastrin concentration was many folds higher than in control subjects (Table 1). This difference was highly statistically significant. Gastrin content in MALT lymphoma tissue averaged

Table 1. — **Fasting serum and gastric luminal gastrin concentrations and tumor gastrin concentrations and basal and histamine-induced maximal acid output in 10 MALT lymphoma patients before and after the eradication of *H. pylori* and in 40 control subjects. Means ± SEM. Asterisk indicates significant change as compared to values obtained before eradication. Cross indicates significant change as compared to that obtained in tests without histamine infusion. NT - not tested.**

Type of examination	Before eradication	After eradication	Controls
	MALT-lymphoma		
Hp seropositive, (%)	100%	90%	57.5
CagA seropositive	60%	60%	30%
IL-8 (pg/ml)	17.2 ± 2.2	3.9* ± 1.5*	2.1
TNFα (pg. ml)	64.1 ± 10.5	7.2* ± 2. 1	2.5
Serum gastrin	147 ± 18 pM	27 ± 5 pM*	18 ± 4 pM
Luminal gastrin	2450 ± 560 pM	58 ± 17 pM*	32 ± 7 pM
Serum gastrin during	210 ± 42 +	58 ± 10*+	36 ± 7+
Tumor tissue gastrin	48 ± 7 ng/g	5 ± 1 ng/g*	NT
Antral mucosal gastrin	5.7 ± 1.2 ng/g	3.8 ± 0.6 ng/g*	4.1 ± 0.5
Basal acid output	1.2 ± 0.6 mmol/h	0.9 ± 0.4 mmol/h	5.3 ± 1.2
Maximal acid output	14.5 ± 2.9 mmol/h	10.9 ± 1.8 mmol/h*	30.5 ± 6.0

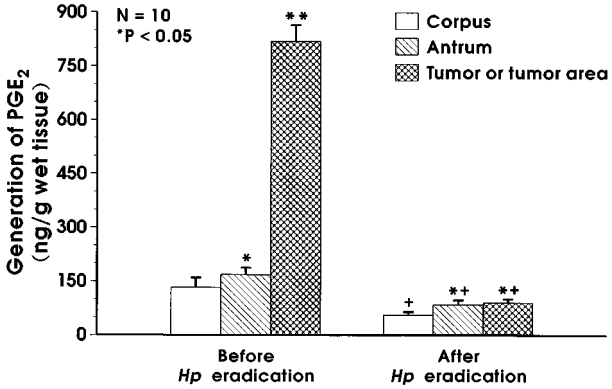

Fig. 1. — PGE_2 generation in gastric MALT lymphoma tumor, antral and fundic mucosa before and after eradication of *H. pylori*. Asterisk indicates significant difference when compared to the value recorded in gastric *corpus* and former tumor area. Double cross indicates significant decrease below the values recorded in the stomach before Hp eradication.

48 ± 7 ng/g of tissue weight and it was about 8 times higher than that in antral mucosa of the same subjects (5.7 ± 1.2). Tissue gastrin content in fundic mucosa was below the level of detection by our RIA used and this was omitted for the sake of clarity (Table I).

Maximal gastric acid output (MAO) induced by histamine in MALT-lymphoma patients was about 14.5 ± 2.9 mmol/h and it was about 50% of that produced by histamine in controls (30.5 ± 6.0). Following *H. pylori* eradication, basal gastric acid secretion remained unchanged while that induced by histamine showed significant reduction (by about 25%) as compared to the value recorded before the eradication. Histamine infusion resulted in a significant rise in serum gastrin level both in MALT lymphoma patients and in controls but in the formers both basal and histamine-stimulated serum gastrin were significantly higher than those in control subjects (Table 1).

PGE_2 generation in MALT lymphoma tumor tissue was about 6 times as high as that in *H. pylori* infected antral mucosa, which showed only slightly but significantly higher generation than that in fundic mucosa of these patients (Fig. 1). Following eradication therapy the biopsy samples taken from the same area where six months earlier tumor was localized by endoscopy showed similar generation of PGE_2 as that in surrounding gastric *antrum* mucosa.

Mucosal generation of PGE_2 in *H. pylori*-eradicated gastric *antrum* and *corpus* mucosa of MALT-lymphoma patients showed only small but significant decrease as compared to that before the *H. pylori* eradication.

The tumor tissue and *antrum* mucosa of all MALT lymphoma patients but not their corpus mucosa showed an upregulated gastrin mRNA, while signal for gastrin receptor (CCK_B-R) mRNA was detected both in the MALT lymphoma tissue and corpus mucosa. The ratio of gastrin mRNA to β-actin mRNA was significantly higher in the tumor tissue than in antrum mucosa but it was negligible in the corpus mucosa. The ratio of CCK_B-R mRNA to β-actin RNA was nearly zero in antral mucosa while the values of this ratio in tumor tissue and corpus mucosa was highly elevated and not significantly different from each other (Fig. 2). Following eradication therapy, the gastrin and its receptor mRNA expression disappeared from the previous site of gastric tumor. The mRNA expression for gastrin in *antrum* and for CCK_B-R expression in *corpus* of the stomach after the eradication was slightly but significantly reduced as compared to that observed before the therapy.

Expression of COX-1 remained at similar level in MALT-lymphoma and in the mucosa of *antrum* or *corpus* before and after eradication of *H. pylori*. COX-2 mRNA expression in *H. pylori* infected patients was markedly increased in the tumor tissue of MALT-lymphoma and also exhibited a detectable signals in the *antrum* and *corpus* of *H. pylori* infected patients. Following the eradication therapy, the signal of COX-2 mRNA in *antrum* and *corpus* almost completely disappeared (Fig. 3).

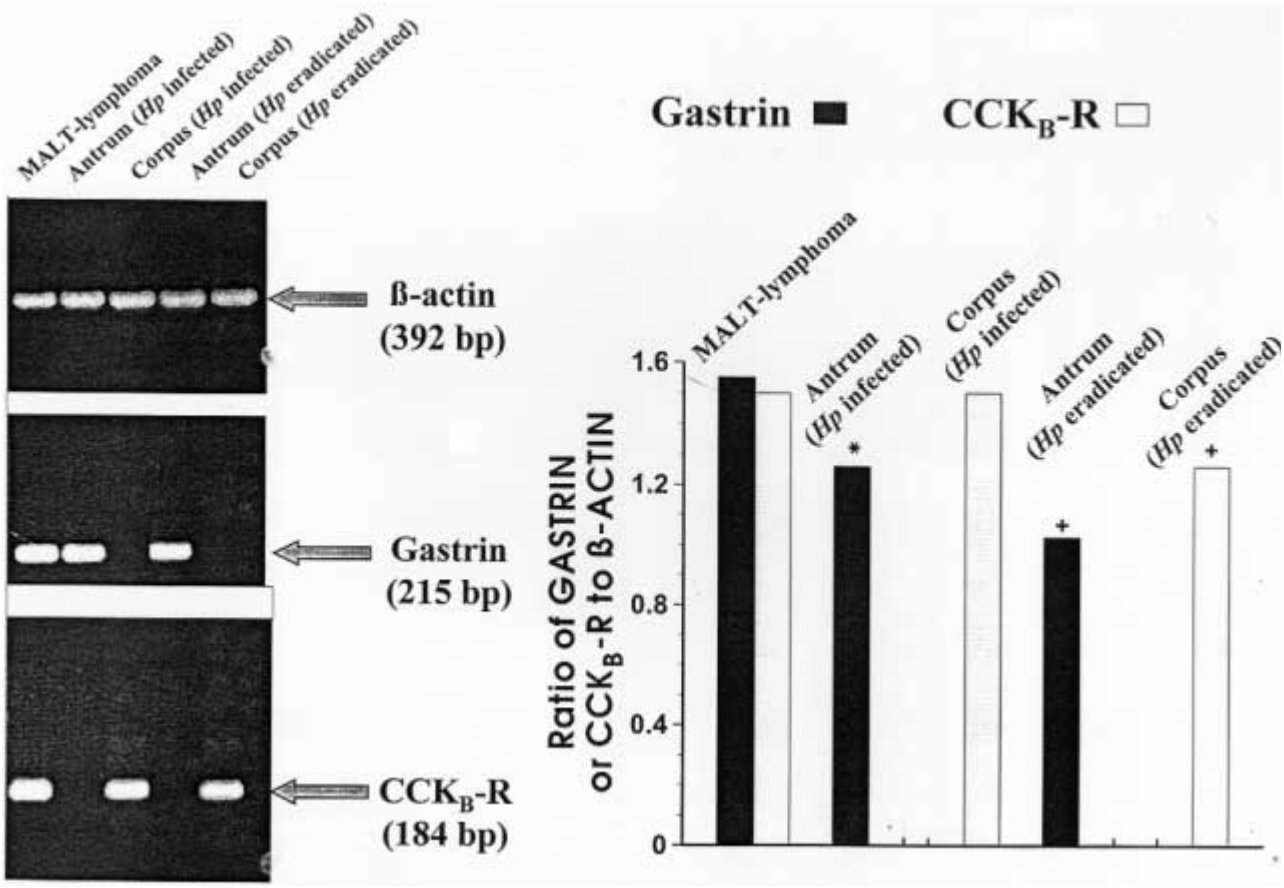

Fig. 2. — Messenger RNA expression for β-actin (upper panel), for gastrin (middle panel) and for gastrin receptors (CCK_B-R) (left panel) as well as the ratio (in columns) of gastrin and CCK_B-R mRNA to β-actin mRNA in MALT lymphoma tissue and in mucosa of gastric (corpus) and antrum before and after Hp eradication. Asterisk indicates significant difference when compared to the value recorded in infected mucosa. Arrow – expected PCR product. MALT-lymphoma tissue. Cross indicates significant decrease below the value recorded in Hp infected mucosa.

Discussion

This study confirms previous findings that low grade MALT lymphoma is strongly associated with CagA-positive *H. pylori* infection and that this tumor exhibits an overexpression of mRNA for gastrin and its receptors (CCK_B-R) accompanied by greatly increased levels of gastrin in the serum and gastrin juice as well as in the MALT-lymphoma tissue. Furthermore, MALT-lymphoma was found to overexpress COX-2 and to exhibit high capability to generate PGE_2. Eradication of *H. pylori* resulted in complete regression of tumor and normalization of mRNA expression for gastrin, CCK_B-R and COX-2 and PGE_2 generation.

Primary, low grade gastrointestinal lymphoma is known to exhibit no evidence of extradigestive involvement except the regional lymph nodes and it may occur more frequently in certain populations including patients with *H. pylori* infection. patients with congenital immunodeficiency states, HIV individuals and those undergoing immuno-suppressive therapy (11,13). Since all our Malt-lymphoma patients were infected with *H. pylori*, it is reasonably to assume that the major etiologic factor of MALT lymphoma was the spiral bacterium. The observations of this study and previous reports (15,16) that the majority of MALT lymphoma patients are CagA positive emphasize the crucial role of so called "pathogenicity islet" virulent factors of *H. pylori* in the pathogenesis of these gastric tumors.

Although the question of how this germ and its virulent factors are capable to transform and activate the B-cells of the gastric mucosa associated lymphoid tissue (MALT) into malignant lymphoma cells remains open, this study provides an evidence that such low grade MALT lymphoma is accompanied by an overexpression of both gastrin and CCK_B-R mRNA and that the tumor tissue produces large amounts of immunoreactive gastrin. Furthermore, this tumor appears to be capable of releasing large quantities of gastrin into the gastric lumen, as confirmed by direct measurement of immunoreactive gastrin in gastric content collected from the stomach. Thus, low grade MALT-lymphoma expresses and releases large quantities of gastrin and this hormone due to its action on gastrin receptors could be responsible, at least in part, for the rapid proliferation of lymphoma cells and tumor growth (7,20). It should be

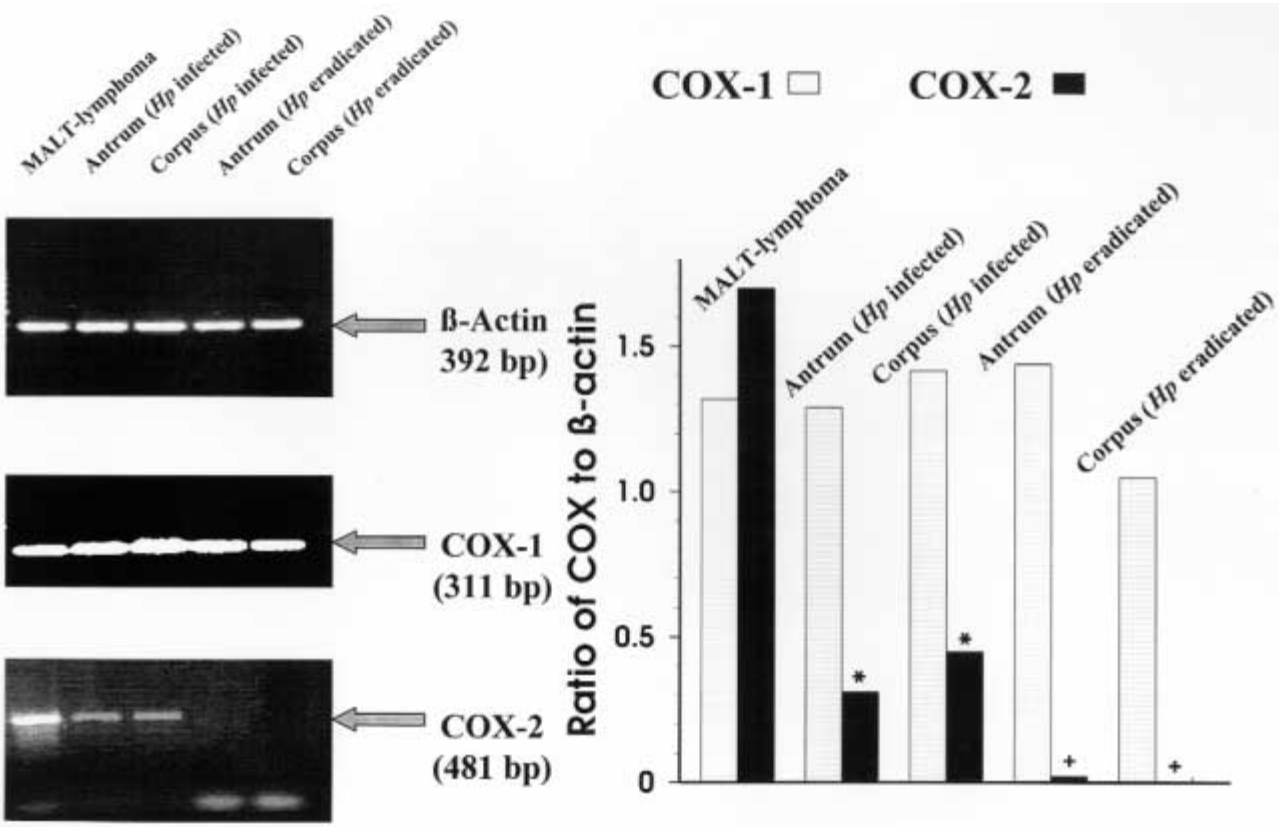

Fig. 3. — Messenger RNA expression for β-actin, for COX-1 and for COX-2 (left panel) as well as the ratio (in columns) of COX-1 and COX-2 mRNA to β-actin mRNA in MALT lymphoma tissue and gastric corpus and antrum mucosa before and after eradication of *H. pylori*. Arrow - expected PCR product size). Asterisk indicates significant decrease below the value recorded in infected mucosa. PCR product (bp) size. Asterisk indicates significant decrease below the value recorded in MALT lymphoma. Cross indicates significant decrease below the value recorded in Hp infected *antrum* and *corpus*.

mentioned that enhanced histochemical expression of gastrin along with other biologically active substances such as serotonin, glucagon, and synaptophysin was already reported (27) but the possible role of gastrin in the growth of MALT lymphoma has not been only recently considered.

The major evidence linking the *H. pylori* infection with MALT lymphoma originates from numerous studies showing independently that gastric lymphoma can be effectively cured with complete regression of the tumors by eradication of this germ (16-18). Since it is well established that plasma levels of gastrin drop almost immediately after the eradication of the bacteria (5,6), it is likely that *H. pylori* provides a direct drive for the enhanced expression and release of gastrin by MALT lymphoma cells. This is supported by our recent *in vitro* studies using cultured lymphoid-originated cell line showing enhanced gastrin or its receptor expression following exposure to alive *H. pylori* but not its compounds such as CagA or lipopolysaccharides (unpublished data).

As *H. pylori* alone is capable to stimulate the G-cells to produce gastrin possibly through the action of Nα-methyl histamine (28,29), we decided to assess whether simple histamine dihydrochloride, used for testing gastric acid secretory capacity, is also effective in the release of gastrin. Indeed, the serum and luminal gastrin significantly increased following histamine stimulation in all *H. pylori*-infected MALT-lymphoma patients, supporting the notion that *H. pylori* may stimulate the production of gastrin by MALT lymphoma cells through the release of histamine metabolite.

The consequence of the acceptance of the crucial role of the *H. pylori* in the pathogenesis of MALT lymphoma is the necessity of prompt eradication of this bacterium to remove the stimulus of lymphoma cell proliferation and growth. An alternative approach could be to treat these patients by neutralizing locally produced gastrin with gastrin antibodies obtained by appropriate vaccination with antigen containing gastrin (using gastrimmune a gastrin-possessing antigen) to produce specific anti-gastrin antiserum. Such approach was found to be effective in prolongation of the survival of mouse with peritoneal tumor induced by injection into the peritoneal cavity of cancer cells capable of producing gastrin in endocrineparacrine pathway (30) but it is unknown whether such treatment could be helpful in the treatment of MALT lymphoma.

The major finding of this report is the observation that MALT lymphoma is capable of mRNA expressing

for both COX-1 and COX-2 that are responsible for the excessive generation of PGE_2 detected in the tumor tissue. Our finding agrees with previous observations that gastric adenocarcinoma can express COX-2 (21,22), but our study demonstrates that it is also true with respect to MALT lymphoma which does not originate from the epithelial cells but from the activated lymphocytes B. It is likely that increased release of gastrin and possible other growth promoting factors (28) and cytokines such as IL-8 or TNFα contribute to the activation and proliferation of lymphocytes B in gastric MALT lymphoma. It is also possible that using novel, highly effective blockers of COX-2 such as rofecoxib, it will be possible to suppress the tumor growth and its invasiveness but this requires experimental and clinical studies.

References

1. JANOWITZ H.D., ABITTAN C.S., FIEDLER L.M. A gastrointestinal list for the millenium. *J. Clin. Gastroenterol*., 1999, **29** (44) : 336-338.
2. WEBB P.M., FORMAN D. Helicobacter pylori as a risk factor for cancer. *Balliere's Clin. Gastroenterol*., 1995, **9** : 563-582.
3. BLASER M.J., PEREZ-PEREZ G.I., KLEANTHOUS H., COVER T.L., PEEK R.M., CHYOU P.H., STEMMERMAN G.N., NOMURA A. Infection with Helicobacter pylori strains possessing cagA associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*., 1995, **55** : 2111-2115.
4. DANESH J. Helicobacter pylori infection and gastric cancer : systematic review of the epidemiological studies. *Aliment Pharmacol. Ther.*, 1999, **13** : 851-856.
5. KONTUREK P.C., BIELAŃSKI W., BOBRZYŃSKI A., HAHN E.G., KONTUREK S.J. Gastric mucosal expression and luminal release of growth factors in gastric carcinoma and duodenal ulcer patients before and after eradication of Helicobacter pylori. *J. Physiol. Pharmacol*., 1997, **48** : 375-382.
6. KONTUREK P.C., BIELAŃSKI W., KONTUREK S.J. Helicobacter pylori - associated gastric pathology. *J. Physiol. Pharmacol*., 1999, **50** : 695-710.
7. KONTUREK P.C., KONTUREK S.J., BIELAŃSKI W., KARCZEWSKA E., PIERZCHALSKI P., DUDA A., STARZYŃSKA T., MARLICZ K., POPIELA T., HARTWICH A., HAHN E.C. Role of gastrin in gastric cancerogenesis in H. pylori infected humans. *J. Physiol. Pharmacol*., 1999, **50** : 857-873.
8. SINGH P., DAI B., WU H., OWILIA A. Role of autocrine and endocrine gastrin-like peptides in colonic carcinogenesis. *Current Opinion in Gastroenterology*, 2000, **16** : 68-77.
9. CORREA P. Human gastric cancerogenesis : a multistep process. *Cancer Res*., 1992, **52** : 6735-6740.
10. THUN M.J. Aspirin and gastrointestinal cancer. *Adv. Exp. Med. Biol*., 1997, **400A** : 395-402.
11. CRYER B., FELDMAN M. Effects of very low dose daily, long-term aspirin therapy on gastric, duodenal and rectal prostaglandin levels and on mucosal injury in healthy humans. *Gastroenterology*, 1999, **117** : 17-25.
12. SHENG H., SHAO J., KIRKLAND S.C., ISAKSON P., COFFEY R.J., MORROW J., BEAUCHAMP R.D., DEBOIS R.N. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase. *J. Clin. Invest*., 1997, **99** : 2254-2259.
13. COTRAN R.S. Neoplasia. *In* : Pathologic Basic of Disease. VI USA - W.B. Saunders Co, 1999, 260-327.
14. GENTA R.M., HAMNER H.W., GRAHAM D.Y. Gastric lymphoid follicles in Helicobacter pylori infection : frequency, distribution, and response to triple therapy. *Hum. Pathol*., 1993, **24** : 577-583.
15. WOTHERSPOON A.C., ORTIZ-HIDALGO C., FALZON M.R., ISAACSON P.G. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet*, 1991, **338** : 1175-1176.
16. BOUZOURENE H., HAEFLINGER T., DELACRETAZ F., SARAGA E. The role of Helicobacter pylori in primary gastric MALT lymphoma. *Histopathology*, 1999, **34** : 118-123.
17. ECK M., SCHMAUSSER B., HAAS R., GREINER A., CZUB S., MULLER-HERMELINK H.K. MALT type lymphoma of the stomach is associated with Helicobacter pylori strains expressing the CagA protein. *Gastroenterology*, 1997, **112** : 1482-1486.
18. MONTALBAN C., MANZANAL A., BOIXEDA D., REDONDO C., ALVAREZ I., CALLEJA J.L., BELLAS C. Helicobacter pylori eradication for the treatment of low-grade gastric MALT lymphoma : follow-up together with sequential molecular studies. *Ann. Once*, 1997, **8** : 37-39.
19. PACKMAN M., MONGER A., RUDOLPH B. Progression of gastric MALT lymphoma after eradication of Helicobacter pylori is predicted by endosonographic staging. MALT Lymphoma Study Group. *Gastroenterology*, 1977, **113** : 1087-1090
20. KONTUREK P.C., KONTUREK S.J., STARZYŃSKA T., MARLICZ K., BIELAŃSKI W., PIERZCHALSKI P., KARCZEWSKA E., HARTWICH A., REMBIASZ K., ŁAWNICZAK M., ZIEMNIAK W., HAHN E.C. Helicobacter pylori – gastrin link to MALT-lymphoma. *Alim. Pharmacol. Therap*., 2000, **14** : 1311-1318.
21. LIPKY P.E. Role of cyclooxygenase-1 and -2 in health and disease. *Am. J. Orthip*., 1999, **28** : 8-12.
22. WILLIAMS C.S., MANN M., DUBOIS R.N. The role of cyclooxygenases in inflammation, cancer and development. *Oncognese*, 1999, **18** (55) : 1708-1716.
23. BIELAŃSKI W., KONTUREK S.J. New approach to ^{13}C urea breath test : capsule-based modification with low-dosed of ^{13}C-urea breath test in the diagnostic of Helicobacter pylori infection. *J. Physiol. Pharmacol*., 1996, **47** : 545-553.
24. KONTUREK J.W., BIELAŃSKI W., KONTUREK S.J., DOMSCHKE W. Eradication of Helicobacter pylori and gastrin-somatostatin link in duodenal ulcer patients. *J. Physiol. Pharmacol*., 1996, **47** : 161-176.
25. TATSUGUCHI A., SAKAMOTO C., WADA K., AKAMATSU T., TSUKUI T., MIYAKE K., FUTAGAMI S., KISHIDA T., FUKUDA Y., YAMANAKA N., KOBYASHI M. Localization of cyclooxygenase 1 and cyclooxygenase 2 in Helicobacter pylori-related gastritis and gastric ulcer tissues in humans. *Gut*, 2000, **46** : 782-789.
26. KONTUREK S.J., BIELAŃSKI W., SOLOMON T.E. Effects of an antral mucosectomy, L-364,718 and atropine on cephalic phase of gastric and pancreatic secretion in dogs. *Gastroenterology*, 1990, **98** : 47-55.
27. MC GREGOR D.H., CHERIAN R., WESTON A.P., LAWSON L., MC ANAW M.P. Adenocarcinoid of ileum and appendix, incidentally discovered during exploratory laparotomy for gastric MALT lymphoma, with subsequent diffuse prostatic metastases : report of a case with light, immunohistochemical, and electron microscopic studies. *Dig. Dis. Sci*., 1999, **44** : 87-95.
28. BLISS P.W., HEALEY Z.V., AREBI N., CALAM J. Nα-methyl histamine and histamine stimulate gastrin release from rabbit G-cells via histamine H_2-receptors. *Aliment Pharmacol. Ther.*, 1999, **13** : 1669-1674.
29. KONTUREK P.C., KONTUREK S.J., SITO E., KRORICIEN N., OBTUTOWIA W., BIELAŃSKI W., HAHN E.G. Luminal Nα-methyl histamine stimulates gastric acid secretion in duodenal ulces patients via releasing gastrin. *Eur. J. Pharmacol.*
30 WATSON S.A., MORRIS T.M., VARRO A., MICHAELI D., SMITH A.M. A comparison of the therapeutic effectiveness of gastrin neutralization in two human gastric cancer models : relation to endocrine/autocrine gastrin mediated growth. *Gut*, 1999, **45** : 812-817.
31. KONTUREK P.C., KONTUREK S.J., STACHURA J., DUDA A., MEIDNER H., STARZYŃSKA T., SULEKOVA Z., KARCZEWSKA E., MARLICZ K., HAHN E.G. Expression of hepatocyte growth factor, tranforming growth factor, apoptosis related proteins Bax and Bcl2 in human gastric cancer. *Aliment Pharmacol. Ther.*, 2001, **15** : 989-999.