Effects of IL-1β on the proliferation and apoptosis of gastric epithelial cells and acid secretion from isolated rabbit parietal cells

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Abstract. The aim of the present study was to explore the effects of IL-1β on the proliferation and apoptosis of gastric epithelial cells and acid secretion from isolated rabbit parietal cells. The mechanisms by which these effects are mediated were also investigated. Parietal cells were isolated from rabbit gastric mucosa by elutriation. The AGS human gastric cancer cell line, the GES-1 human gastric epithelial cell line and parietal cells were treated with interleukin (IL)-1β in the presence or absence of *Helicobacter pylori* (*H. pylori*) for the times indicated. MTT assay and flow cytometry (FCM) were used to determine the levels of proliferation and apoptosis. The expression levels of cyclooxygenase-2 (COX-2) mRNA and protein were examined by RT-PCR and FCM. Acid secretion by parietal cells was examined using ^{14}C-aminopyrine (^{14}C-AP) accumulation. H^+/K^+ATPase α subunit mRNA expression was assessed by RT-PCR. The results demonstrated that IL-1β (10 ng/ml) stimulated cellular proliferation and inhibited *H. pylori*-induced apoptosis in GES-1 and AGS cell lines. IL-1β (10 ng/ml) upregulated the mRNA and protein expression of COX-2 in GES-1 and AGS cells. Acid secretion stimulated by histamine was identified as significantly inhibited and mRNA expression of H^+/K^+ATPase α subunit was downregulated by treatment with IL-1β (10 ng/ml) for 30 min and 16 h compared with the control in isolated rabbit parietal cells. The present study demonstrates that IL-1β plays a significant role in *H. pylori*-induced gastric carcinogenesis through 2 main mechanisms: i) IL-1β may interfere in gastric epithelial cell growth by upregulating COX-2 expression; ii) IL-1β may inhibit the acid secretion from parietal cells by downregulating H^+/K^+ATPase expression.

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Introduction

Helicobacter pylori (*H. pylori*) infection is associated with divergent clinical outcomes that range from simple asymptomatic gastritis to more serious conditions, including peptic ulcer disease and gastric neoplasia (1). A number of previous studies have focused on the role of bacterial virulence factors in the pathogenesis of these diseases. Although these factors undoubtedly contribute to the degree of tissue damage, the 2 key outcomes, gastric cancer and duodenal ulcer disease, have yet to be distinguished (2). Therefore, understanding of host genetic factors that may be relevant to this process must be developed further.

The key pathophysiological event in *H. pylori* infection is initiation of a gastric mucosal inflammatory response, which is mediated and regulated by a large number of pro-inflammatory cytokines, particularly interleukin (IL)-1β, IL-6 and IL-8 (3,4). Previous studies have demonstrated that the host genetic polymorphisms of IL-1β are relevant to *H. pylori*-associated gastric cancer. Polymorphisms in the IL-1β gene that correlate with increased levels of the cytokine have been identified to increase the risk of hypochlorhydria and gastric atrophy in response to *H. pylori* infection, therefore increasing the risk of gastric cancer itself (5-7). To this end, IL-1β, as a potent pro-inflammatory cytokine, may be involved in the host response to *H. pylori* infection. At present, the molecular mechanisms associated with the correlation between the risk of gastric cancer and the polymorphisms of IL-1β remain unclear.

Disturbance of the balance between proliferation and apoptosis of gastric epithelial cells is considered to interfere with the integrity of gastric mucosa and promote the development of gastric carcinogenesis (8,9). Gastric acid hyposecretion also correlates with increased risk of gastric cancer (5). Therefore, in the present study, we investigated the effects of exogenous IL-1β on proliferation and apoptosis of gastric epithelial cells and acid secretion of isolated rabbit parietal cells in order to explore the role of IL-1β in *H. pylori*-associated diseases and

to identify the mechanisms involved in *H. pylori*-induced gastric carcinogenesis.

Materials and methods

Gastric epithelial cell culture. The human gastric cancer cell line, AGS, and the human gastric epithelial cell line, GES-1, were maintained in RPMI-1640 medium containing 10% FBS and in MCDB-153 medium supplemented with 10% FBS, respectively, at 37˚C with 5% CO_2 and 95% air in a humidified incubator. The 2 cell lines were preserved in our laboratory. Cells were serum-starved for 12 h prior to treatment and then treated with either vehicle or test reagents for the indicated time.

Parietal cell preparation. Rabbit parietal cells were isolated and enriched from male New Zealand rabbits (2±0.2 kg) using a modification of previously described methods (10). Briefly, gastric fundic mucosa was digested with sequential exposure to type I crude collagenase (30-40 mg/100 ml; Sigma-Aldrich, St. Louis, MO, USA) and EDTA (1-2 mmol/l). Parietal cells were enriched from the crude suspension by the standard centrifugal elutriation technique using a Beckman JE6B elutriation system. For selected experiments, further purification of parietal cells was performed using continuous density gradient centrifugation with 50% Percoll (Pharmacia, Piscataway, NJ, USA). Parietal cells were enriched to >70% homogeneity as determined by hematoxylin and eosin staining and >95% viability as determined by trypan blue exclusion.

Harvested cells from the parietal cell-enriched fractions were collected by brief centrifugation and resuspended in complete culture medium (Ham's F12/DMEM, 1:1). Cells were cultured at 37˚C in 5% CO_2, 95% air for 12 h prior to treatment and then treated with either vehicle or IL-1β (PeproTech, Rocky Hill, NJ, USA) for the indicated time.

H. pylori preparation. Cytotoxin-associated gene A (CagA)-positive and cytotoxin-producing *H. pylori* (NCTC 11637) strain was used in the present study. Bacteria were grown under microaerophilic conditions on Columbia agar plates (supplemented with 8% sheep blood) for 72 h, harvested and resuspended in RPMI-1640 medium. Bacterial concentrations were standardized by optical density measurement at 600 nm and validated by serial dilution. OD_{600} of ~1.0 corresponded to a bacterial concentration of $1.5x10^8$ cfu/ml.

MTT assay. Cell proliferation was analyzed using MTT assay. Cells were seeded on a 96-well plate at $1.0x10^4$ and $0.5x10^4$ cells/well for GES-1 and AGS cells, respectively and incubated with increasing concentrations of IL-1β (0.1, 1.0 and 10 ng/ml) for 24 h in serum-free culture medium. Each sample had 6 replicates. MTT (0.5 mg/ml) was added and the reaction mixture was incubated for 4 h at 37˚C. Following MTT incubation, cells were lysed in 150 µl of 10% DMSO and the absorbance at 490 nm was measured using an automatic plate reader (Bio-Rad, Hercules, CA, USA). Viable cell number was expressed as a percentage of control: MTT assay (% control) = $OD_{test}/OD_{control}$.

Assessment of cell apoptosis. AGS and GES-1 cells were treated with either vehicle or test reagents for 24 h prior to assessment of apoptosis. Cells floating in the culture medium were collected by centrifugation and adherent cells were harvested by incubation with 1% trypsin for 1-2 min at 70-80% confluence. Following washing with ice-cold PBS, cells were suspended in 70% ethanol and kept at 4˚C for 30 min. Fixation was terminated by washing twice with PBS and the cells were stained with propidium iodide (100 µg/ml) at 4˚C for 30 min. The cell suspension was filtered through 50-µm nylon mesh and DNA fluorescence was analyzed by flow cytometry (FCM; Beckman Coulter, Miami, FL, USA). A minimum of 10,000 events were measured per sample. Apoptosis was detected by the appearance of a sub-G1 fraction of fragmented nuclei in the analysis. Apoptosis was expressed as a percentage of the control and caluculated as: apoptotic cell $(\%)_{test}$/apoptotic cell $(\%)_{control}$.

Detection of mRNA by RT-PCR. Using a RNA extraction kit according to the manufacturer's instructions, total cellular RNA was isolated from AGS/GES-1 and parietal cells, which were treated with vehicle or IL-1β (10 ng/ml) for indicated times prior to isolation. RNA (2 µg) from each sample was reverse transcribed using Supercript II RT system (Invitrogen Life Technologies, Carlsbad, CA, USA) in a total reaction volume of 20 µl and the resulting cDNA was amplified by PCR. The PCR primer sequences (F, forward; R, reverse) and PCR product size were as follows: cyclooxygenase-2 (COX-2)-F, 5'-TTCAAATGAGATTGTGGGAAAATTGCT-3' and COX-2-R, 5'-AGATCATCTCTGCCTGAGTATCTT-3', 305 bp; β-actin-F, 5'-CCAGAGCAAGAGAGGTATCC-3', β-actin-R, 5'-CTGTGGTGGTGAAGCTGTAG-3', 463 bp; H^+/K^+ATPase α subunit-F, 5'-ACTCTGGGCTCCACGTCG-3', H^+/K^+ATPase α subunit-R, 5'-AGGATGGAGCTGCAGCGC-3', 470 bp; UBCP-F, 5'-AGAAGAAGTCTTACACCACTC-3', UBCP-R, 5'-GTAAGTCAGACAACATTTGCC-3', 203 bp. For COX-2/β-actin, the PCR mixture was heated to 95˚C for 5 min and amplification was performed for 35 cycles: denaturation at 95˚C for 50 sec, annealing at 58˚C for 60 sec and extension at 72˚C for 60 sec. Following the final cycle, the reactions were incubated at 72˚C for an additional 10 min. For H^+/K^+ATPase α subunit/UBCP, amplification was performed for 35 cycles: denaturation at 95˚C for 60 sec, annealing at 62˚C for 60 sec and extension at 72˚C for 60 sec. PCR products were electrophoretically separated on 1.5% agarose gels containing 0.5 µg/ml ethidium bromide and visualized under ultraviolet transillumination. Quantification of COX-2 and H^+/K^+ATPase ATPase α subunit PCR products were standardized in comparison with the housekeeping gene, β-actin, and UBCP products, respectively, by densitometry. The mRNA expression levels of the control group was expressed as 100%. Levels of mRNA expression were presented as a percentage of the control.

Analysis of protein expression by FCM. Goat anti-COX-2 IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as primary antibody and FITC-labeled rabbit anti-goat IgG antibody was used as secondary antibody for indirect immunofluorescence according to the manufacturer's instructions. Briefly, AGS and GES-1 cells, treated with the vehicle or IL-1β (10 ng/ml) for the indicated times, were washed with PBS and fixed in 4% paraformaldehyde

for 40 min. Following fixation, cells were washed twice and treated with 0.2% Triton X-100 containing 5% FBS on ice for 10 min. Cells were washed again with PBS and then incubated with a 1:50 dilution of anti-COX-2 antibody at 4˚C. Following incubation for 40 min, cells were washed twice, further incubated with a 1:100 dilution of the FITC-labeled secondary antibody at 4˚C for 40 min, washed twice and filtrated through a 50-μm nylon mesh. Specific fluorescence was measured by FCM. For data acquisition, a gate was set on intact cells by forward/side scatter analysis and a minimum of 10,000 events were analyzed. Protein expression levels are presented as the mean fluorescence intensity (MFI) and expressed as the relative MFI following correction for non-specific fluorescence using the isotype control ($MFI_{COX-2}/MFI_{isotype\ control}$).

Measurement of acid secretion. Intracellular accumulation of ^{14}C-aminopyrine (^{14}C-AP) was used as an indirect measurement of functional acid secretory activity by parietal cells (11,12). Cultured parietal cells, treated with vehicle or IL-1β (10 ng/ml) for the indicated times, were washed with EBSS containing 0.2% BSA, 2 mmol/l glutamine, 20 mmol/l HEPES (pH 7.4) to remove dead and non-adherent cells and resuspended in the medium described above at $1.0x10^6$ cells/ml. ^{14}C-AP (0.1 μCi; GE Healthcare Biosciences, Pittsburgh, PA, USA) was added to 1.0 ml of the cell suspension and the mixture was equilibrated at 37˚C for 15 min. Following thorough mixing of the cells, secretagogue histamine (10^{-4} mmol/l; Sigma-Aldrich) was added to stimulate the parietal cells to uptake ^{14}C-AP and the cells were incubated at 37˚C for 30 min in an atmosphere of 5% CO_2 and 95% air. Incubation was terminated by rapidly removing the medium by centrifugation and washing twice with EBSS solution. The cell pellet was lysed with 0.5 ml 1% Triton X-100. Aliquots of cell lysates were counted in a Beckman LS-6800 liquid scintillation counter with dinitrophenol (DNP) correction. DNP (0.1 mmol/l) was added separately to assess non-specific incorporation and values were subtracted from the test and control values. ^{14}C-AP uptake was expressed as a percentage of control and calculated as: (^{14}C-AP uptake of IL-1β group - ^{14}C-AP uptake of DNP group)/(^{14}C-AP uptake of control group - ^{14}C-AP uptake of DNP group).

Statistical analysis. Values are expressed as the means ± SD of at least 6 independent experiments. Six replicates were performed in each experiment. The Student's t-test, one-way ANOVA and Mann-Whitney U test were performed for statistical evaluation of the data using the SPSS 17.0 statistical software package. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of increasing concentrations of IL-1β on cell proliferation in AGS and GES-1 cells. Cell proliferation was increased in AGS and GES-1 cells by increasing the concentration of IL-1β (0.1, 1.0 and 10 ng/ml). A significant increase in the proliferation rate in response to IL-1β (10 ng/ml) was identified compared with the control group in AGS and GES-1 cells (P<0.05; Fig. 1).

Effect of IL-1β on H. pylori-induced apoptosis in AGS and GES-1 cells. The apoptosis of AGS and GES-1 cells was

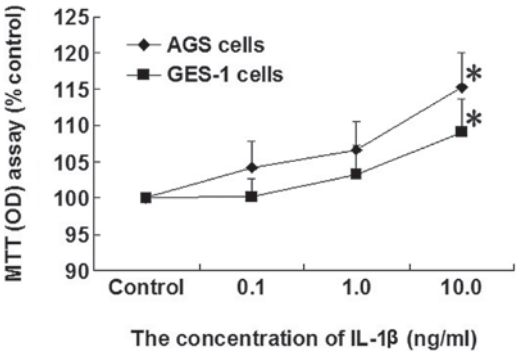

Figure 1. Effect of IL-1β on proliferation of AGS and GES-1. AGS and GES-1 cells were treated with various concentrations of IL-1β (0.1, 1.0 and 10 ng/ml) for 24 h. Level of proliferation was examined by MTT assay. Optical density of control group was expressed as 100%. Results were expressed as a percentage of the basal control. All data are expressed as the means ± SD (n=6). *P<0.05, compared with the control group.

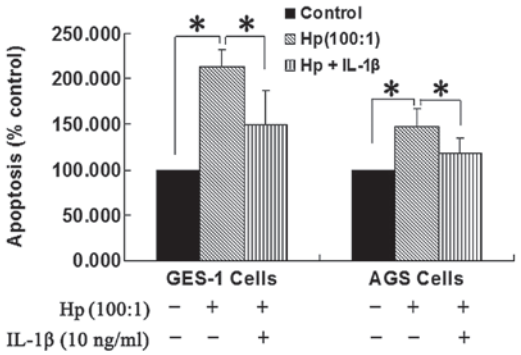

Figure 2. Inhibitory effects of IL-1β on *H. pylori*-induced apoptosis in AGS and GES-1 cells. AGS and GES-1 cells were exposed to *H. pylori* (CFU/cell=100/1) with or without IL-1β (10 ng/ml) for 24 h. The level of apoptosis was examined by flow cytometry. The apoptotic rate of the control group was expressed as 100%. Results are expressed as a percentage of the basal control. All data were expressed as the means ± SD (n=6). *P<0.05.

significantly increased in the *H. pylori* group compared with the control (P<0.05; Fig. 2). IL-1β (10 ng/ml) exposure for 24 h significantly attenuated the *H. pylori*-induced apoptosis by 61.1 and 56% in the AGS cells and GES-1 cells, respectively, compared with the *H. pylori* group (P<0.05; Fig. 2).

Effect of IL-1β on expression of COX-2 mRNA in AGS and GES-1 cells. Compared with the control group, mRNA expression of COX-2 in the AGS cell line was significantly upregulated following treatment with IL-1β (10 ng/ml) for 8 h (P<0.05; Fig. 3). A similar result was obtained in the GES-1 cell line (P<0.05).

Effect of IL-1β on expression of COX-2 protein in AGS and GES-1 cells. As demonstrated in Fig. 4, the protein expression of COX-2 was significantly increased following treatment with IL-1β (10 ng/ml) for 8 h in the AGS and GES-1 cell lines compared with the control group (all P<0.05). These results indicated that the effect of IL-1β on COX-2 protein expression was consistent with the effect on COX-2 mRNA expression.

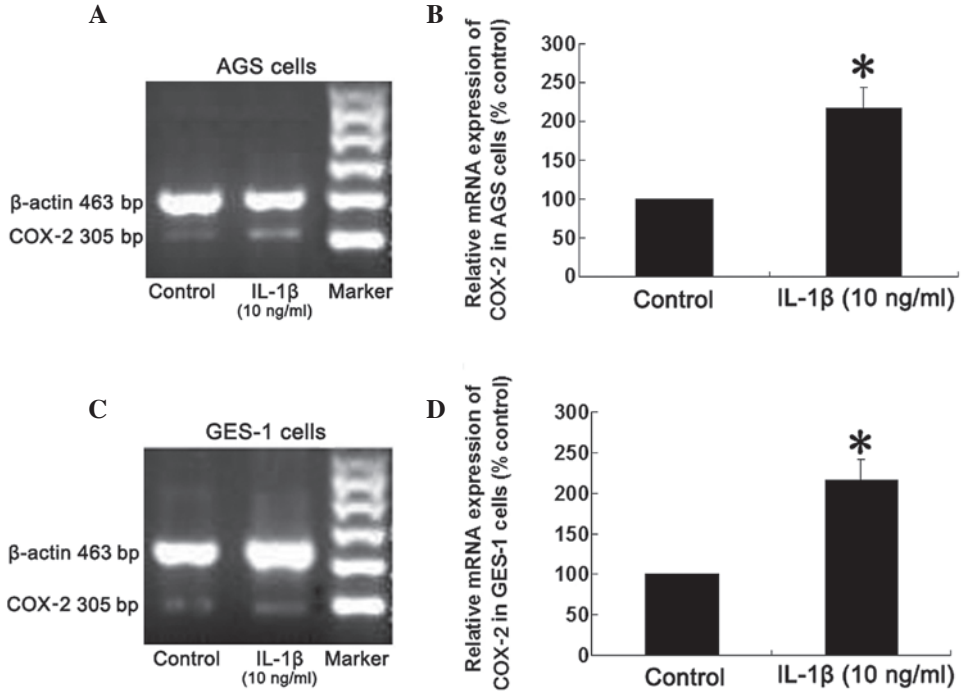

Figure 3. Effects of IL-1β on the expression of COX-2 mRNA in (A and B) AGS and (C and D) GES-1 cells. AGS and GES-1 cells were treated with IL-1β (10 ng/ml) for 8 h. Level of mRNA expression was determined by RT-PCR and the mRNA expression of the control group was expressed as 100%. Results are expressed as a percentage of the control. Data are expressed as the means ± SD (n=6). *P<0.05, compared with the control group.

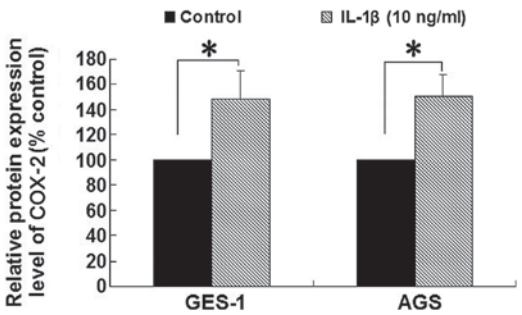

Figure 4. Effect of IL-1β on protein expression of COX-2 in AGS and GES-1 cells. AGS and GES-1 cells were treated with IL-1β (10 ng/ml) for 8 h. Level of protein expression was determined by flow cytometry. Protein expression of the control group is expressed as 100%. Results are expressed as a percentage of the control. Data are presented as the means ± SD (n=6), *P<0.05.

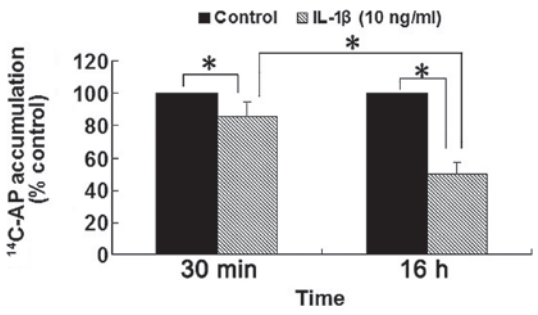

Figure 5. Inhibitory effect of IL-1β on acid secretion induced by histamine in isolated rabbit parietal cells. Isolated parietal cells were pre-incubated with IL-1β (10 ng/ml) for 30 min or 16 h prior to stimulation by histamine (10^{-4} mol/l) for 30 min. Level of acid secretion was reflected by ^{14}C-aminopyrine (^{14}C-AP) accumulation and the acid secretion of the control group is expressed as 100%. Results are expressed as a percentage of the basal control. Data are expressed as the means ± SD (n=6). *P<0.05.

Effect of IL-1β on acid secretion in isolated rabbit parietal cells. The acid secretion stimulated by histamine significantly inhibited by 14 and 50% following treatment with IL-1β (10 ng/ml) for 30 min and 16 h, respectively, compared with the control group in isolated rabbit parietal cells (all P<0.05; Fig. 5). Compared with the group treated with IL-1β (10 ng/ml) for 30 min, acid secretion stimulated by histamine was significantly decreased in the group treated with IL-1β (10 ng/ml) for 16 h. These results demonstrated that IL-1β (10 ng/ml) inhibited acid secretion stimulated by histamine in a time-dependent manner in isolated rabbit parietal cells.

Effect of IL-1β on H^+/K^+ATPase α subunit mRNA expression in isolated rabbit parietal cells. As demonstrated in Fig. 6, the mRNA expression of H^+/K^+ATPase α subunit in isolated rabbit parietal cells was downregulated by 11 and 29% following treatment with IL-1β (10 ng/ml) for 30 min and 16 h, respectively, compared with the control group (all P<0.05). Compared with the group treated with IL-1β (10 ng/ml) for 30 min, the mRNA expression of the H^+/K^+ATPase α subunit was significantly decreased in the group treated with IL-1β (10 ng/ml) for 16 h. These results indicated that IL-1β (10 ng/ml) downregulated the mRNA expression of H^+/K^+ATPase α subunit in a time-dependent manner in isolated rabbit parietal cells.

Discussion

Previous studies have demonstrated that human gastric mucosa expresses COX-2 protein at low levels; however, the expression of COX-2 is induced by *H. pylori*-associated premalignant and malignant gastric lesions and correlates with the depth of

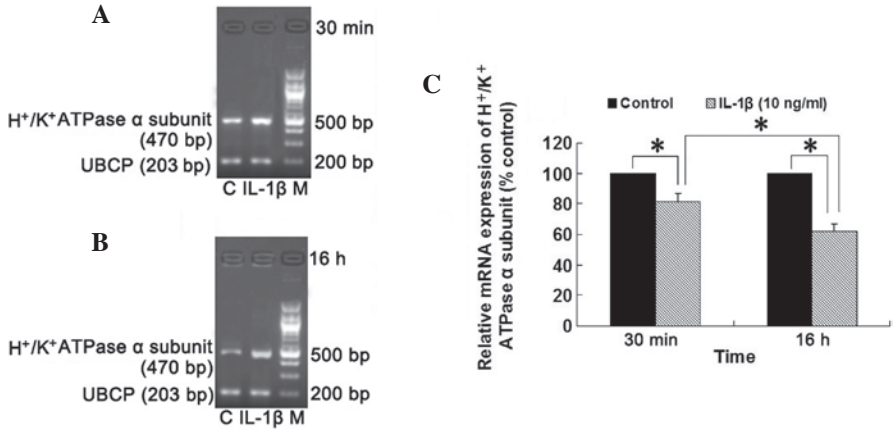

Figure 6. IL-1β downregulated H^+/K^+ATPase α subunit mRNA expression in isolated rabbit parietal cells. Isolated parietal cells were incubated with IL-1β (10 ng/ml) for (A) 30 min or (B) 16 h. C, control; IL-1β, IL-1β (10 ng/ml) group; M, marker. (C) The level of mRNA expression was determined by RT-PCR. The mRNA expression of the control group is expressed as 100%. Results are expressed as percentage of the control. Data are expressed as the means ± SD (n=6). *P<0.05.

mucosal invasion, lymphatic invasion and metastasis in human gastric carcinoma (11-13). Specific and non-specific inhibitors of COX-2 suppressed proliferation of cell lines that expressed high levels of COX-2. However, these inhibitors exerted minimal effects on proliferation of the cell lines expressing lower levels of COX-2 (14). In addition, COX-2 inhibitors suppressed growth of gastric cancer xenografts by induction of apoptosis and suppression of neoplastic cell replication (15). These results indicate that COX-2 is important for the development of gastric cancer. The present study identified the basal COX-2 expression in the transformed human gastric cancer cell line (AGS) and human gastric epithelial cell line (GES-1) using RT-PCR.

Although the mechanism of COX-2 regulation of cancer development remains unclear, existing data indicate that COX-2 expression is associated with stimulation of cellular proliferation and resistance to apoptosis. Previously, COX-2 inhibitor treatment was observed to induce apoptosis, suppress cellular proliferation, downregulate Bcl-2 expression and suppress the growth of H-ras-transformed cells (16-18). In addition, overexpression of COX-2 may induce expression of epidermal growth factor receptor and metalloproteinase and decrease expression of E-cadherin and transforming growth factor-β receptor (15,19). These alterations are correlated with enhanced tumorigenic potential and increased tumor invasiveness.

COX-2 expression is associated with intensive infiltration of inflammatory cells in *H. pylori*-infected gastric mucosa where substantial amounts of cytokines are induced, including IL-1β (20,21). A previous study by Zhang *et al* demonstrated that *H. pylori* isogenic mutants specifically lacking picA or picB, molecules responsible for cytokine production in gastric cells, are less effective in upregulation of COX-2 mRNA expression (22). These results indicate that picA and picB may contribute to increased COX-2 expression and stimulation of cytokine production. In the present study, the effect of exogenous IL-1β on COX-2 expression of gastric epithelial cells was analyzed. The results demonstrated that IL-1β induced the expression of COX-2 mRNA and protein in GES-1 and AGS cell lines. COX-2 expression induced by IL-1β may be mediated by activation of multiple intracellular signaling pathways, including p44/42 and p38 MAPK, JNK and NF-κB (23,24). The results of the present study revealed that IL-1β enhanced cellular proliferation and attenuated *H. pylori*-induced apoptosis in GES-1 and AGS cell lines, consistent with the observation that IL-1β induced expression of COX-2. Therefore, we conclude that *H. pylori*-associated IL-1β may stimulate cellular proliferation, inhibit *H. pylori*-induced apoptosis and mediate gastric carcinogenesis through upregulation of COX-2 expression. Additional mechanisms by which IL-1β affects carcinogenesis may include IL-1β induction of angiogenin mRNA and protein expression. Angiogenin is a proangiogenic molecule associated with neovascularization of cancer tissue. IL-1β may also induce carcinogenesis by stimulation of metalloproteinases, thought to be important mediators of metastasis (25,26). Maihöfner *et al* revealed that expression of COX-2 was consistent with expression of IL-1β in human colorectal cancer tissue, however, the association between COX-2 and IL-1β in human gastric cancer tissue requires additional investigation (27).

In humans, *H. pylori* infection may cause acute epidemic gastritis associated with hypochlorhydria. In certain individuals, chronic *H. pylori* infection causes body-predominant gastritis and profound suppression of gastric acid secretion that is partially reversible with eradication therapy (28-30). The degree of acid suppression depends on the distribution of *H. pylori* infection, scores for activity and inflammation of gastritis in the body, the number of *H. pylori* and the grade of colonization (31). In the present study, ^{14}C-AP accumulation was performed to determine the effects of exogenous IL-1β on acid secretion in isolated parietal cells from rabbits. The results demonstrated that IL-1β exposure for 30 min or 16 h inhibited histamine-stimulated acid secretion, accompanied by downregulation of H^+/K^+ATPase mRNA expression. The inhibitory potency of IL-1β was time-dependent, as preincubation of parietal cells with IL-1β for longer time intervals resulted in increased inhibition of ^{14}C-AP accumulation and downregulation of H^+/K^+ATPase mRNA expression. Since specific H^+/K^+ATPase in parietal cell is the key mediator of the final stages of gastric acid secretion, a decrease in the level of H^+/K^+ATPase is hypothesized to lead to a reduc-

tion of acid secretion (32). The present results indicate that IL-1β may inhibit gastric acid secretion by downregulating expression of H^+/K^+ATPase. However, the possibility that IL-1β performs antisecretory functions in parietal cells by blocking H^+/K^+ATPase activity cannot be ruled out at present. Accumulating evidence indicates that the inhibitory action of IL-1β is mediated by multiple intracellular signaling pathways, including pertussis toxin and tyrosine kinase-dependent and independent pathways (33,34).

Additional *in vivo* studies are consistent with the present *in vitro* results. In Mongolian gerbils inoculated orally with *H. pylori* for 6 and 12 weeks, serum gastrin levels were increased and gastric acid output was decreased. These alterations correlated with elevation of IL-1β mRNA levels in gastric mucosa; however, gastric acid output and serum gastrin level returned to control levels following recombinant human IL-1 receptor antagonist (rhIL-1ra) injection. In *H. pylori*-associated enlarged fold gastritis, increased IL-1β release from gastric body mucosa was correlated with decreased basal and tetragastrin-stimulated acid output, whereas IL-1β release was significantly decreased with concomitant increase in gastric acid secretion following eradication of *H. pylori*. In patients infected with *H. pylori*, a significant correlation was observed between IL-1β mRNA expression in gastric fundic gland mucosa and gastric juice pH. Significant decreases in the amount of IL-1β mRNA, gastric juice pH and serum gastrin levels were observed in patients with eradication of *H. pylori*, whereas no significant changes were observed in patients without eradication (35). These results indicate that *H. pylori* infection induces IL-1β expression and suppresses acid secretion.

In addition to the direct effects on parietal cells, the mechanisms by which the inhibitory effects of IL-1β on acid secretion are mediated *in vivo* are: i) IL-1β induces apoptosis in enterochromaffin-like cells (ECL cells) or inhibits gastric histamine secretion and synthesis from ECL cells, leading to the reduction in acid secretion stimulated by histamine (36,37). ii) IL-1β mediates increased prostaglandin E2 (PGE_2) production via the overexpression of COX-2. PGE_2 functions as a potent inhibitor of gastric acid secretion by directly retarding the secretory function of parietal cells or reducing histamine release from ECL cells. Moreover, PGE_2 stimulates bicarbonate secretion from gastric epithelial cells, which may contribute to a decrease in gastric acidity (38-40). A previous study in *H. pylori*-infected mice demonstrated that increased PGE_2 produced by overexpression of COX-2 stimulated cytokines (IL-1β) induced by *H. pylori* infection, demonstrating the importance of PGE_2 in gastric acid hyposecretion by *H. pylori* infection (41).

One of the mechanisms involved in the development of gastric cancer by *H. pylori* infection is associated with long-term acid hyposecretion. Patients with hyposecretion are exposed to hypergastrinemia, bacterial toxins, N-nitroso compounds and products of inflammation, including reactive oxygen radicals and nitrogen oxygen species, all well-known mutagens or carcinogens (5,7). In hosts with low basal secretory capacity and high IL-1β phenotypes, *H. pylori* is prone to colonization of a wider niche involving the acid secretory corpus region, resulting in higher levels of IL-1β production, resulting in additional inhibition of acid secretion, a more aggressive body gastritis and acceleration of gastric cancer development. A previous study in Mongolian gerbils with low basal acid output and genetic predisposition demonstrated that gerbils developed corpus atrophy, intestinal metaplasia and were particularly prone to developing gastric cancer when chronically colonized by *H. pylori* infection (42). These observations are consistent with a phenotype identified in human individuals associated with increased risk of gastric cancer with high IL-1β phenotypes.

In conclusion, the results from the present study suggest that IL-1β may be a key mediator in *H. pylori*-induced gastric carcinogenesis and a prime candidate as a host genetic factor that may alter the risk of gastric cancer. The present study demonstrates that IL-1β induced by *H. pylori* infection is associated with the 2 mechanisms involved in gastric carcinogenesis: i) IL-1β may promote cellular proliferation, inhibit *H. pylori*-induced apoptosis by upregulating COX-2 expression and lead to the disturbance of gastric epithelial cell growth and ii) IL-1β may inhibit acid secretion from parietal cells by downregulating H^+/K^+ATPase expression.

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