Gastrin induces CXC chemokine expression in gastric epithelial cells through activation of NF-κB

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Hiraoka, Shintaro, Yoshiji Miyazaki, Shinji Kitamura, Miyuki Toyota, Tatsuya Kiyohara, Yasuhsisa Shinomura, Naofumi Mukaida, and Yuji Matsuzawa. Gastrin induces CXC chemokine expression in gastric epithelial cells through activation of NF-κB. Am J Physiol Gastrointest Liver Physiol 281: G735–G742, 2001.—Although hypergastrinemia is frequently observed in individuals with a chronic Helicobacter pylori infection, its pathophysiological significance in gastric mucosal inflammation is unclear. The present study was designed to determine if gastrin induces the expression of CXC chemokines in gastric epithelial cells. Human and rat gastric epithelial cells, transfected with gastrin receptor, were stimulated with gastrin. The expression of mRNAs for human interleukin-8 (IL-8) and rat cytokine-induced neutrophil chemoattractant-1 and release of human IL-8 protein were then determined by Northern blot analysis and ELISA, respectively. Gastrin not only induced the expression of mRNAs for these chemokines but also stimulated IL-8 protein release. A luciferase assay using IL-8 promoter genes showed that nuclear factor (NF)-κB is absolutely required and activator protein-1 (AP-1) is partly required for the maximum induction of IL-8 by gastrin. An electrophoretic mobility shift assay revealed that gastrin is capable of activating both NF-κB and AP-1. In addition, the inhibition of NF-κB abrogated gastrin-induced chemokine expression. These results suggest that gastrin is capable of upregulating CXC chemokines in gastric epithelial cells and therefore may contribute to the progression of the inflammatory process in the stomach.

mucosa; inflammation; chemokines; transcription factors

GASTRIC EPITHELIAL CELLS PRODUCE chemokines such as interleukin (IL)-8, cytokine-induced neutrophil chemoattractant-1 (CINC-1), and monocyte chemoattractant protein 1 (MCP-1) (1, 19, 30, 32, 40), which are potent chemotactic and activating factors for leukocytes (3). IL-8 and CINC-1 belong to the CXC chemokine family, whereas MCP-1 is a prototype of CC chemokines. IL-8 plays a significant role in several types of human gastric injury through the attraction and activation of neutrophils. Although a rat counterpart of IL-8 has not yet been identified, CINC-1 is thought to play a pivotal role in rat gastric injury.

Presently available evidence suggests that IL-8 may play a significant role in the pathogenesis of gastritis, which is closely associated with Helicobacter pylori infection (1, 19, 30, 32, 40). Several studies have shown that H. pylori is capable of upregulating epithelial cell IL-8 production in gastric epithelial cell lines (42). Furthermore, gastric mucosal levels of IL-8 are increased in parallel with the histological severity of gastritis (2, 32). Prolonged production of IL-8 by gastric epithelial cells could result in the recruitment of leukocytes to gastric tissues (35). Infiltreated leukocytes would produce a number of proinflammatory cytokines, reactive oxygen, and chemical mediators, which would further contribute to the progression of inflammatory processes.

Gastrin is a peptide hormone that stimulates gastric acid secretion and the growth of fundic mucosa in the stomach (38). Hypergastrinemia is found not only in patients with gastrin-producing endocrine cell tumors or with type A atrophic gastritis (6) but also in individuals with H. pylori-associated gastritis (6). It has been reported that proinflammatory cytokines including IL-1β, tumor necrosis factor (TNF)-α, and IL-8 (4, 5) are able to stimulate gastrin release from G cells. In addition, IL-1β, which can also act as a potent inhibitor of acid production, may cause hypochlorhydria, which results in hypergastrinemia (46).

Although the findings of some investigators suggest that hypergastrinemia may contribute to the progression of chronic atrophic gastritis (8, 39), the pathophysiological significance of gastrin in the inflammatory and immune process has not been well studied. The purpose of the present study was to examine the effect of gastrin on expression of CXC chemokines in human and rat gastric epithelial cells. Since epithelial cell lines originating from gastric epithelial cells and expressing gastrin receptor have not been identified, we decided to produce such cell lines by DNA transfection.

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We previously developed a rat gastric epithelial cell line that stably expresses human gastrin receptor. In the present study, we report on the transfection of the gastrin receptor cDNA into MKN28 cells, a human gastric cancer cell line. Using these cell lines, we have shown that gastrin is capable of upregulating production of CXC chemokines through the activation of the transcription factors nuclear factor (NF)-κB and activator protein-1 (AP-1). This represents the first report that demonstrates that gastrin induces chemokine expression in gastric epithelial cells.

MATERIALS AND METHODS

Reagents. RPMI-1640 medium, DMEM, FCS, and Genecitin (G418) were obtained from Gibco BRL (Grand Island, NY). Gastrin 17, cholecystokinin-8 (CCK-8), and somatostatin were purchased from the Peptide Institute (Osaka, Japan). IL-1β was a generous gift from Otsuka Pharmaceutical (Tokushima, Japan). TNF-α, pyrrolidine dithiocarbamate (PDTC), 12-O-tetradecanoylphorbol 13-acetate (TPA), staurosporine, and PD 98059, a selective mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor, were obtained from Sigma (St. Louis, MO). Selective gastrin/CCK-B-receptor antagonist L-740093 (28) and epidermal growth factor (EGF) receptor-specific tyrphostin AG1478 (17) were donated by Merck Sharp & Dohme Research Laboratories (Rahway, NJ) and Dr. A. Levitzki (Hebrew University, Jerusalem, Israel), respectively. Radiochemicals ([α-32P]dCTP and [γ-32P] ATP) were purchased from Dupont NEN (Boston, MA).

Cell lines and transfection. MKN28 cells, a human gastric adenocarcinoma cell line (21), were obtained from the Japanese Cancer Research Cell Bank (no. JCRB0253). These cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 U/ml of penicillin G, and 100 μg/ml streptomycin. MKN28 cells were transfected according to the liposomemediated transfection method (LipofectAMINE PLUS; Life Technologies, Rockville, MD) with pSV2-neo alone or pSV2-neo plus pSVhGAS-R, a plasmid containing the full-length human CCK-B/gastrin receptor cDNA, which was kindly provided by Dr. T. Horiuchi (Exploratory Research Laboratories, Daichi Pharmaceutical, Tokyo, Japan). Cells were selected for their ability to grow in the presence of 1.0 mg/ml G418.

RGaR9 cells were established in our laboratory (20) by transfection of the full-length human CCK-B/gastrin receptor cDNA to RGM1 cells, a rat gastric epithelial cell line, obtained from the Riken Cell Bank (Tsukuba, Japan) (13, 16). The cells were grown in DMEM supplemented with 10% FCS, 100 U/ml of penicillin G, and 100 μg/ml streptomycin.

Bacterial preparations. We have used the urease-positive T ox + CagA + wild-type H. pylori 60190 strain (ATCC 49503). An extract of water-soluble proteins were prepared as described (4). In the experiments with bacterial suspensions, cells were incubated with bacterial preparations at a concentration of 1 × 10⁸ CFU/ml in DMEM supplemented with 5% FCS.

Mitogenic assays. The cells were seeded onto 96-well plates, grown to confluence, and incubated in serum-free medium for 48 h. The cells were subsequently incubated for 18 h with human gastrin 17 or without the CCK-B/gastrin receptor antagonist L-740093 and were labeled with 1 μCi/ml [3H]thymidine (Amersham, Arlington Heights, IL) 18–22 h later. The cells were washed three times with PBS and harvested by trypsinization, and the incorporated [3H]thymidine was measured by a Beta system (Pharmacia, Uppsala, Sweden).

Northern blot analysis. The cells, which were grown in 90-mm dishes until they reached confluence, were serum deprived for 24 h and then stimulated with 10⁻⁸ M human gastrin 17, 10⁻⁸ M human CCK-8, 10⁻⁸ M human somatostatin, 10 ng/ml IL-1β, or 10 ng/ml TNF-α in a serum-free medium. Each reagent was dissolved in 10 μl distilled water. The control cells were treated with a medium containing 10 μl distilled water alone. After 0, 0.5, 1, 3, 5, 7, 24, or 48 h, total RNA was extracted by the guanidinium thiocyanate method (7). Approximately 15 μg of each RNA was electrophoresed onto 1.0% agarose/2.2 M formaldehyde denaturing gels, transferred to Hybond-N⁺ membranes (Amersham, Arlington, IL), and ultraviolet cross-linked (1,200 J/M²). Hybridization was performed using cDNA probes, which were labeled by random priming (Multiprime DNA labeling system; Amersham) with [α-32P]dCTP in Rapid-hyb buffer (Amersham). After hybridization, the membranes were exposed to Kodak Xomat AR film (Eastman Kodak, Rochester, NY). In some experiments, the cells were preincubated with the gastrin/CCK-B receptor antagonist L-740093 (10 nM), the inhibitor of NF-κB, PDTC (10⁻¹⁵–10⁻⁵ μM), the selective MEK inhibitor PD-98059 (50 nM), or the EGF receptor-specific tyrphostin AG1478 (250 nM). To determine the role of protein kinase C (PKC) in gastrin-induced chemokine expression, the cells were preincubated with staurosporine (100 nM) for 1 h or with TPA (500 nM) for 24 h before gastrin 17 treatment.

The probes included a 0.45-kb EcoRI-EcoRI fragment of human IL-8 cDNA, a 0.9-kb EcoRI-BamHI fragment of rat CINC-1 cDNA (a gift from Dr. F. Konishi, Toyama Medical and Pharmaceutical University), and a 1.1-kb XbaI-HindIII fragment of rat glyceraldehyde-3-phosphate dehydrogenase cDNA (a gift from Dr. T. Nakamura, Osaka University Medical School).

Measurement of human IL-8 production by ELISA. Cells were seeded and grown to confluence in 24-well plates. The cells were then stimulated with 10⁻⁸ M gastrin 17, 10 ng/ml IL-1β, 10 ng/ml TNF-α, or H. pylori extract (1 × 10⁷ CFU/ml) in a medium containing 5% FCS. In several experiments, the cells were preincubated with L-740093 (10 nM) or PDTC (10⁻¹⁵–10⁻⁵ μM) 1 h before stimulation. After the cells were incubated for 24 h, the concentration of human IL-8 in the conditioned media was evaluated by using a commercially available ELISA kit (BioSource International, Camarillo, CA) according to the manufacturer’s guidelines.

 Luciferase assay. The 5' flanking region of the IL-8 gene, spanning from bp -133 to +44 was subcloned into a luciferase expression vector as previously described, and site-directed mutagenesis of the NF-κB, AP-1, and NF-IL-6 binding sites was then carried out (14). For the luciferase assay, cells were transfected with 5 μg of each luciferase vector along with 1 μg of pRL-SV40 expression vector as an internal control by using LipofectAMINE PLUS. After a 24-h incubation period, the cells were treated with or without 10⁻⁸ M gastrin 17 for 24 h. The cells were then lysed with 1× luciferase lysis buffer (Toyo Ink, Tokyo, Japan). Luciferase activity was measured using the PicaGene reagent kit (Toyo Ink) in a Lumat LB9501 luminometer (Berthold, Wildbad, Germany). The enzyme activity was normalized for efficiency of transfection on the basis of SeaPansy luciferase activity, and relative values were determined. Transfection experiments were carried out three times independently, and the average of these values was calculated.
Expression of gastrin/CCK-B receptor mRNA by transfected MKN28 cells. The plasmid pSVhGAS-R containing a full-length human CCK-B/gastrin receptor cDNA was transfected into MKN28 cells. Forty-three G418-resistant clones were picked and screened for the expression of human CCK-B/gastrin receptor mRNA. We found that one of these clones, designated MKGR26, strongly expressed the 2.7-kb gastrin receptor mRNA (Fig. 1A). In contrast, the parental MKN28 cells did not express CCK-B/gastrin receptor. The following experiments were performed with MKGR26 cells. When we assayed the effects of gastrin 17 on cell growth, we found that gastrin dose-dependently increased the incorporation of \( ^{3}H \)-thymidine into MKGR26 cells (Fig. 1B). However, the effects of gastrin on MKGR26 cells were inhibited by treatment of these cells with the CCK-B/gastrin receptor antagonist L-740093 (Fig. 1B).

Effect of gastrin on the expression of chemokine transcripts. We next examined the issue of whether gastrin 17 alters the expression of mRNAs for chemokines in MKGR26 cells and RGaR9 cells. IL-8 mRNA was induced in MKGR26 cells within 1 h, reached a peak level at 5 h, and then gradually decreased after the cells were incubated with \( 10^{-8} \) M gastrin 17 (Fig. 2A). In contrast, neither parental MKN28 cells nor cells

Electrophoretic mobility shift assay. Nuclear proteins were extracted from the cells which had been incubated in the presence or absence of \( 10^{-8} \) M gastrin for 0.5 h as described by Dignam et al. (9). Protein concentrations were determined using the method involving bichinonic acid (BCA protein assay reagent, Pierce, Rockford, IL) (34). Electrophoretic mobility shift assay (EMSA) was performed using gel shift assay systems (Promega, Madison, WI) according to the manufacturer’s guidelines. Double-stranded oligonucleotide probes for NF-κB (5′-AGTTGAGGGACTTCCCAGGC-3′) and AP-1 (5′-CGCTTGATGAGCAGCGGA A-3′) were labeled with \( [γ^{32}P]ATP \). Each 10 μg of nuclear proteins were incubated with a labeled probe (5 × 10^4 cpm) and 0.5 μg/ml poly(dI-dC) in 10 μl of binding buffer (4% glycerol with (in mM) 1 MgCl₂, 0.5 EDTA, 0.5 dithiothreitol, 50 NaCl, and 10 Tris-HCl, pH 7.5) for 20 min at room temperature. Samples were loaded onto 4% polyacrylamide gel (acyrloamid/N,N′-methylen bisacryomide, 30:1) with 0.5× Tris borate buffer. After electrophoresis, the gels were dried and exposed to Kodak XOMAT AR film.

Statistical analysis. Data are expressed as means ± SE. Statistical comparisons between groups were performed with the Mann-Whitney U-test. A \( P \) value of \(<0.05\) was considered to be statistically significant.
transfected with pSV2-neo alone showed any response to gastrin 17 (data not shown). CINC-1 mRNA was induced in RGaR9 cells within 30 min, reached a peak level at 1 h, and then decreased after the cells were incubated with 10⁻⁸ M gastrin 17 (Fig. 2B). Gastrin-induced expression of IL-8 mRNA was dose dependent and inhibited by preincubation with the gastrin receptor antagonist L-740093 (Fig. 2C). Gastrin and CCK-8 are equally effective on IL-8 mRNA expression (Fig. 2D). However, somatostatin did not affect IL-8 mRNA expression (Fig. 2D).

We then determined the effects of gastrin on the expression of chemokines induced by IL-1β or TNF-α. As expected, both IL-1β and TNF-α dramatically upregulated the expression of IL-8 and CINC-1 transcripts (Fig. 3). Gastrin enhanced the effect of IL-1β or TNF-α on the expression of chemokine mRNA (Fig. 3).

Effect of gastrin on the expression of IL-8 protein. We also determined the effect of gastrin on IL-8 levels in the culture media of MKGR26 cells. Treatment with gastrin dose dependently increased the concentration of IL-8 in the culture media (Fig. 4A). This effect of gastrin on IL-8 secretion was abolished by pretreatment with the selective CCK-B/gastrin receptor antagonist L-740093 (Fig. 4A). IL-1β, TNF-α, and H. pylori extract also significantly increased IL-8 levels in the culture media (Fig. 4B). Synergistic interaction was observed between gastrin and IL-1β, TNF-α, or H. pylori relative to the production of IL-8 (Fig. 4B).

Transcriptional regulation of the IL-8 gene by gastrin. It is known that the expression of IL-8 is regulated at the transcriptional level (22, 23). As a result, the effects of gastrin on IL-8 gene transcription were examined. Gastrin enhanced luciferase activities in MKGR26 cells that had been transfected with the luciferase expression vector containing a minimally essential promoter region of the IL-8 gene (bp -133 to +44) by threefold (Fig. 5). This promoter region contains three important cis-acting elements for IL-8 gene transcription, namely the NF-κB, AP-1, and NF-IL-6 binding sites. The contribution of each element to the gastrin-induced activation of IL-8 gene transcription was next examined. The mutation of the NF-κB-binding site completely abolished gastrin-induced enhancement in luciferase activities (Fig. 5). The mutation of the AP-1 binding site partially inhibited gastrin-induced enhancement, as evidenced by luciferase activities, whereas the mutation of the NF-IL-6 binding site had no effect on luciferase activities (Fig. 5). These results indicate that the NF-κB binding site is absolutely required and the AP-1 binding site is partially required for gastrin-induced IL-8 gene transcription.

Gastrin increased NF-κB- and AP-1-specific binding activities in MKGR26 cells. Because the NF-κB and AP-1 binding sites appeared to be required for the gastrin-induced transcription of the IL-8 gene, EMSA was performed to determine whether gastrin is capable of activating NF-κB and AP-1. Treatment with gastrin activates NF-κB within 30 min, and this effect was abrogated by pretreatment with L-740093 or PDTC (Fig. 6A). Although AP-1 appeared to be activated to a considerable extent in this cell line without gastrin stimulation, the presence of gastrin further increased AP-1 binding activity significantly. This activation was diminished when the cells were pretreated with L-740093. Pretreatment with PDTC, however, had no

Fig. 3. A: effect of gastrin 17 on IL-1β- or tumor necrosis factor (TNF)-α-induced IL-8 mRNA expression. MKGR26 cells were incubated for 5 h under the following conditions: lane 1, untreated; lane 2, gastrin 17 (10⁻⁸ M); lane 3, IL-1β (10 ng/ml); lane 4, gastrin 17 and IL-1β; lane 5, TNF-α (10 ng/ml); lane 6, gastrin 17 and TNF-α. B: effect of gastrin 17 on IL-1β- or TNF-α-induced cytokine-induced neutrophil chemoattractant (CINC)-1 mRNA expression. RGaR9 cells were incubated for 1 h under the following conditions: lane 1, untreated; lane 2, gastrin 17 (10⁻⁸ M); lane 3, IL-1β (10 ng/ml); lane 4, gastrin 17 and IL-1β; lane 5, TNF-α (10 ng/ml); lane 6, gastrin 17 and TNF-α.
significant effect on the gastrin-induced increase in AP-1 binding activity (Fig. 6B).

Effects of inhibition of NF-κB on gastrin-induced chemokine expression. To further clarify the involvement of NF-κB on the extent of chemokine expression, we examined the effect of PDTC on the gastrin-induced expression of chemokines. Pretreatment with PDTC inhibited the expression of IL-8 transcripts in MKGR26 cells (Fig. 7A) as well as that of CINC-1 mRNA in RGaR9 cells (Fig. 7B) in a dose-dependent manner. Pretreatment with PDTC also abolished the release of IL-8 after gastrin stimulation in MKGR26 cells (Fig. 8).

Effects of inhibition of protein kinases on gastrin-induced chemokine expression. It is known that CCK-B/gastrin receptor-mediated signals involve the activation of PKC and MAPK (33, 43). In an earlier report, we indicated that gastrin induces the expression of EGF-like growth factors, which, in turn, activate EGF receptor (20). As a result, we examined the effects of the MEK inhibitor PD-98059, the EGF receptor tyrosine kinase inhibitor AG1478, and PKC inhibitor staurosporine on the gastrin-induced expression of chemokines. As expected, the gastrin receptor antagonist L-740093 completely inhibited the effect of gastrin, whereas PD-98059, AG1478, and staurosporine partially inhibited gastrin-induced expression of IL-8 or CINC-1 transcripts in MKGR26 cells and RGaR9 cells (Fig. 9). Downregulation of PKC by pretreatment with TPA also partially inhibited gastrin induction of CINC-1 in RGaR9 cells (Fig. 9B).

DISCUSSION

The present study shows that gastrin induces the expression of chemokines, including IL-8 and CINC-1, in gastric epithelial cells that have been transfected...
with the gastrin receptor. Chemokines represent a recently described family of inflammatory cytokines that have leukocyte chemotactic and activating properties (3). The chemokine superfamily has been divided into two major subgroups: the CXC chemokines and CC chemokines. In general, the CXC chemokines, which include IL-8 and CINC-1, appear to primarily affect neutrophils, whereas CC chemokines, which include MCP-1, functionally act on monocytes and lymphocytes (3). Of these chemokines, IL-8 is thought to be important in the initiating and perpetuating of the inflammatory process of \( H. pylori \)-associated gastritis (1, 19, 30, 32, 40). Previous studies have shown that different strains of \( H. pylori \) are able to induce the expression of IL-8 in parallel with their pathogenicity in gastric epithelial cell lines (31). In addition, mucosal IL-8 correlates with the infiltration of polymorphonuclear cells in \( H. pylori \)-associated gastritis (35). It is generally recognized that hypergastrinemia often occurs early in the course of \( H. pylori \) infection (24, 25, 36). The present study also shows that gastrin dramatically enhances the \( H. pylori \)-induced release of IL-8. Thus gastrin may modulate the expression of IL-8 and contribute to the progression of inflammatory processes during \( H. pylori \) infection.

Prolonged IL-8 expression by gastric epithelial cells could result in mucosal infiltrates, which produce large amounts of proinflammatory cytokines such as IL-1\( \beta \) and TNF-\( \alpha \) (26, 37, 45). These cytokines may, in turn, stimulate the expression of epithelial chemokines and further promote the inflammatory and immune process. The data herein show that gastrin dramatically potentiates IL-8 expression in gastric epithelial cells induced by \( H. pylori \), IL-1\( \beta \), or TNF-\( \alpha \). Hypergastrinemia associated with \( H. pylori \) infection often is resolved after eradication of the infection (25, 36) and therefore is recognized to be secondary to gastric inflammation. In fact, IL-1\( \beta \) and TNF-\( \alpha \) are capable of stimulating the release of gastrin from G cells. In addition, IL-1\( \beta \), which can also act as a potent inhibitor of acid production, may cause hypochlorhydria, which results in hypergastrinemia (46). Our results suggest that hypergastrinemia is not only a result of inflammation but also may promote gastric inflammation synergistically with the inflammatory cytokines.

The expression of the IL-8 gene is primarily controlled at the transcriptional level (22, 23). A nucleotide sequence analysis of the 5’-regulatory region of the IL-8 gene shows the presence of potential binding sites for several transcription factors, including NF-kB, AP-1, and NF-IL-6 (22, 23, 44). It is known that the transcription of the IL-8 gene requires either a combination of NF-kB and AP-1 or of NF-kB and NF-IL-6, depending on the type of cells (1, 19, 44). In our experiments, the NF-kB binding site was found to be absolutely required, whereas the AP-1 binding site was partially required for the gastrin-induced transcription of the IL-8 gene. EMSA showed that gastrin enhances the binding activities of both NF-kB and AP-1. PDTC, a well-known inhibitor of I-\( \kappa \)B degradation (18), abro-

Fig. 8. Effects of the NF-\( \kappa \)B inhibitor PDTC on the production of gastrin-induced chemokines as assessed by ELISA. The cells were pretreated with PDTC (30 \( \mu \)M) for 1 h and stimulated with 10\(^{-8}\) M gastrin. After 24 h, the culture supernatant was examined for IL-8 production by ELISA. Values are means \( \pm \) SE (n = 6 for each data point). *P < 0.05 vs. control.

Fig. 9. Involvement of mitogen-activated protein kinase kinase (MEK), epidermal growth factor (EGF) receptor, and protein kinase C (PKC) in the gastrin-induced mRNA expression of chemokines. MKGR26 cells (A) and RGaR9 cells (B) were incubated under the following conditions for 5 h and 1 h, respectively. For both panels, the following doses were used: lane 1, untreated; lane 2, 10\(^{-8}\) M gastrin; lane 3, gastrin with 10 nM L-740093; lane 4, gastrin with 50 nM PD-98059; lane 5, gastrin with 250 nM AG1478; lane 6, gastrin with 100 nM staurosporine; lane 7, gastrin with 100 nM staurosporine (Stauro).
gated not only the gastrin-induced activation of NF-κB but also the expression of IL-8 mRNA and protein as well. These results suggest that gastrin induces IL-8 expression via the activation of NF-κB and AP-1 and that the activation of NF-κB is indispensable for gastrin-induced IL-8 expression. Similarly, NF-κB appears to be required for the gastrin-induced expression of CINC-1.

The gastrin receptor is identical to the CCK-B receptor, and both CCK-A and gastrin/CCK-B receptors belong to the family of G-protein-coupled receptors (33, 43). As expected, the present study shows that CCK-8 and gastrin are equally effective in inducing the expression of IL-8 mRNA. The binding of an agonist to CCK-A or CCK-B receptor activates phospholipase C, which catalyses the hydrolysis of phosphatidylinositol biphosphate, thus generating inositol 1,4,5-triphosphate and diacylglycerol, which mobilize intracellular Ca2+ and activate PKC, respectively (33, 43). It is interesting to note that recent studies indicate that CCK is capable of activating NF-κB and inducing chemokines in pancreatic acinar cells via the CCK-A receptor (11, 12). In vivo experiments also showed that supramaximal stimulation of CCK-A receptors causes acute pancreatitis, suggesting that NF-κB activation is an important early event (10, 27). Since the activation of PKC appears to be required for CCK-induced chemokine expression in pancreatic acinar cells, the effect of the PKC inhibitor staurosporine on gastrin-induced chemokine expression was tested. Staurosporine was found to significantly inhibit the gastrin-induced expression of IL-8 and CINC-1, suggesting that this effect is, in part, mediated by a PKC-dependent pathway.

Gastrin receptor-mediated signals also involve the activation of MAPK and the upregulation of c-jun and c-fos (33, 43). We previously showed (20) that gastrin induces EGFr-like growth factors as autocrine/paracrine growth factors through a MAPK pathway. It has also been reported that EGF can activate NF-κB and induce IL-8 secretion in certain epithelial cell lines (15, 29). Our present study shows that the selective EGF receptor kinase inhibitor AG1478 as well as MEK inhibitor PD-98059 significantly inhibits the induction of chemokines by gastrin. Thus EGFr-like growth factors may partly mediate the effect of gastrin on chemokine expression.

In conclusion, the present study demonstrates for the first time that gastrin induces IL-8 production in gastric epithelial cells through the activation of NF-κB and AP-1. Our findings suggest a possible role of gastrin in the development of gastric inflammation. Further studies are needed to clarify the involvement of gastrin in the inflammatory and immune process of H. pylori-associated chronic gastritis.

REFERENCES


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