Interleukin 1β Enhances Invasive Ability of Gastric Carcinoma through Nuclear Factor-κB Activation

Naoki Yamanaka,[1] Takashi Morisaki,[1] Hiroshi Nakashima,[1] Akira Tasaki,[1] Makoto Kubo,[1] Hirotaka Kuga,[1] Chihiro Nakahara,[1] Katsuya Nakamura,[1] Hirokazu Noshiro,[3] Takashi Yao,[2] Masazumi Tsuneyoshi,[2] Masao Tanaka,[3] and Mitsuo Katano[1]

Departments of [1]Cancer Therapy and Research, [2]Anatomic Pathology, and [3]Surgery and Oncology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

ABSTRACT

Purpose: **We examined the role of interleukin (IL)-1β in activation of nuclear factor κB (NF-κB) and the biological function of activated NF-κB in gastric carcinoma cells.**

Experimental Design: **Human gastric carcinoma cell line GCTM-1 was used to examine NF-κB activation by immunostaining and electrophoretic mobility shift assay. Matrix metalloproteinase (MMP)-9 expression, which plays an important role in tumor invasion, was assessed by semi-quantitative reverse transcription-PCR, Western blotting, and immunostaining. The invasive ability of GCTM-1 cells was measured by Matrigel invasion assay. *In vivo* expression of IL-1β and MMP-9 and activation of NF-κB in 10 surgically resected gastric carcinoma specimens were examined immunohistochemically.**

Results: **IL-1β enhanced NF-κB activation, MMP-9 expression, and the invasive ability of GCTM-1. A NF-κB inhibitor, pyrrolidine dithiocarbamate, suppressed both MMP-9 expression and invasiveness of IL-1β-treated GCTM-1 cells. IL-1β did not increase the invasive ability of GCTM-1 cells transfected with MMP-9 antisense oligonucleotide. Concomitant expression of IL-1β and nuclear NF-κB was observed in 3 of 10 gastric carcinoma specimens. Cells producing IL-1β were tumor-infiltrating macrophages in two specimens and gastric carcinoma cells in one specimen.**

Conclusions: **One of the molecules that may play a role in NF-κB activation in some gastric carcinomas is IL-1β. The present results suggest that IL-1β increases the invasive ability of carcinoma cells through activation of NF-κB and the resulting MMP-9 expression.**

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Requests for reprints: Mitsuo Katano, Department of Cancer Therapy and Research, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan. Phone: 81-92-642-6941; Fax: 81-92-642-6221; E-mail: mkatano@tumor.med.kyushu-u.ac.jp.

INTRODUCTION

Nuclear factor κB (NF-κB) is a transcription factor that is involved in inflammation (1), cell proliferation (2), angiogenesis (3), and apoptosis (4). In most unstimulated normal cells, NF-κB is sequestered in the cytoplasm and complexed with inhibitor proteins (inhibitor of NF-κB; Ref. 5). Stimulation of cells leads to phosphorylation and degradation of inhibitors of NF-κB and allows translocation of NF-κB, *i.e.*, NF-κB (p65) and/or NF-κB1 (p50), to the nucleus, resulting in expression of target genes (6, 7). In most cases, NF-κB activation is evaluated as the nuclear translocation of NF-κB, especially p65. Recent studies revealed that NF-κB is constitutively activated in several types of carcinoma, including pancreatic (8), breast (9), colorectal (10), and hepatocellular carcinoma (11). We reported previously that NF-κB is constitutively activated in gastric carcinoma and suggested that NF-κB activation is related to the invasive ability of carcinoma cells (12). However, little information is available concerning the *in vivo* roles of NF-κB activation and the mechanisms by which NF-κB is activated in carcinoma tissue.

Generalized or local inflammation may transform the carcinoma cells to more aggressive cells. It has been suggested that chronic inflammation produces a procancer microenvironment that favors survival and growth of carcinoma cells (13–16). Inflammatory infiltrates are thought to produce large amounts of proinflammatory cytokines such as interleukin (IL)-1β at the tumor site. IL-1β induces NF-κB activation in macrophages and neutrophils (17, 18). We hypothesized that IL-1β produced at the tumor site enhances NF-κB activation in carcinoma cells and that NF-κB activation accelerates invasiveness of carcinoma cells by increasing expression of invasion-related molecules. We investigated activation of NF-κB and the resulting expression of matrix metalloproteinase (MMP)-9 in response to IL-1β in a gastric carcinoma cell line.

MATERIALS AND METHODS

Reagents and Antibodies. Recombinant human IL-1β was purchased from Diaclone (Besancon, France). Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB translocation, was purchased from Sigma (Deisenhofen, Germany). The primary antibodies used for immunohistochemistry and Western blot analysis were anti-NF-κB p65 (sc-109; Santa Cruz Biotechnology), anti-MMP-9 (sc-6840; Santa Cruz Biotechnology), and anti-IL-1β (BD Biosciences, Heidelberg, Germany). Secondary antibodies for immunohistochemistry were purchased from Nichirei (Tokyo, Japan), and those labeled with FITC for West-

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ern blot analysis were purchased from Santa Cruz Biotechnology.

Cells. Human gastric adenocarcinoma cell line GCTM-1 was established in our laboratory from ascites of a cancer patient with peritoneal dissemination (19). GCTM-1 cells were maintained in RPMI 1640 (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) at 37°C in an atmosphere of 5% CO_2 in air.

Gastric Carcinoma Specimens. Carcinoma specimens from 10 gastric carcinoma patients who gave informed consent before surgical treatment were fixed with liquid nitrogen immediately after surgery.

Immunohistochemistry. For immunostaining of carcinoma specimens, frozen sections (4 μm) were fixed in acetone for 10 min. For immunostaining of cultured carcinoma cells, GCTM-1 cells (1×10^5 cells/well) were grown on 8-chamber glass slides overnight. Cells were air-dried and fixed in 100% methanol for 5 min at −20°C and then incubated in PBS containing 0.2% Triton X-100 for 10 min. Slides were immersed in 3% H_2O_2 in methanol or 10% normal goat or rabbit serum (Santa Cruz Biotechnology) for 30 min, and each section was then incubated with the optimal concentration of primary antibody for 24–48 h at 4°C. After the sections were incubated with appropriate secondary antibodies, immune complexes were detected with a combination of 3,3′-diaminobenzidine (40 mg/150 ml in PBS; Wako Pure Chemical Industries, Hyogo, Japan) and 0.06% hydrogen peroxide. Specimens were photographed with a digital camera (Binary Planner 4490; Jenoptik, Jena, Germany) and attached to a microscope (BX51; Olympus, Tokyo, Japan). Nuclear staining, which reflects nuclear translocation of p65, was considered a marker of NF-κB activation.

Electrophoretic Mobility Shift Assay. Preparation of nuclear extracts of GCTM-1 cells was performed as described previously (12). Briefly, 1×10^6 GCTM-1 cells cocultured with IL-1β for the indicated times were collected and washed once with PBS. GCTM-1 cells were then homogenized in hypotonic buffer [10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.1% NP40, and 5% protease inhibitor mixture] incubated for 10 min on ice. Nuclei were collected by centrifugation at $800 \times g$ for 5 min, washed once with hypotonic buffer, and resuspended in low-salt buffer [20 mM HEPES (pH 7.9), 0.02 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, and 5% protease inhibitor mixture]. An equal volume of high-salt buffer [20 mM HEPES (pH 7.9), 800 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, and 5% protease inhibitor mixture] was added by vortex mixing. Nuclei were incubated for 30 min on ice and centrifuged at $13{,}000 \times g$ for 30 min. The supernatants were collected.

Nuclear protein extracts of GCTM-1 cells were analyzed by electrophoretic mobility shift analysis for NF-κB nuclear translocation as described previously (12). Nuclear protein extracts of 1×10^6 cells were incubated for 30 min at 37°C with binding buffer [60 mM HEPES (pH 7.5), 180 mM KCl, 15 mM $MgCl_2$, 0.6 mM EDTA, and 24% glycerol], poly(deoxyinosinic-deoxycytidylic acid) (Amersham Pharmacia Biotech ABs, Uppsala, Sweden), and ^{32}P-labeled double-stranded oligonucleotide containing the NF-κB binding motif (5′-AGT-TGA-GGG-GAC-TTT-CCC-AGG-C-3′; Promega). Mixtures were loaded onto 4% polyacrylamide gels and separated by electrophoresis in 0.25× Tris-borate EDTA running buffer. Oligomer-protein complexes were visualized by autoradiography. Intensity of the NF-κB band was determined with NIH Image version 1.60 software (NIH Division of Computer Research and Technology).

Semiquantitative Reverse Transcription-PCR. Total RNA was extracted from GCTM-1 cells with the guanidinium thiocyanate-phenol-chloroform single-step method (20) and quantified by spectrophotometry (Ultrospec 2100 Pro; Amersham Pharmacia Biotech, Cambridge, United Kingdom). IL-1β sense (5′-CAG-TGA-AAT-GAT-GGC-TTA-TTA-C-3′) and antisense (5′-CTT-TCA-ACA-CGC-AGG-ACA-GGT-3′) primers yield a 548-bp product (21). MMP-9 sense (5′-TGG-GCT-ACG-TGA-CCT-ATG-ACA-T-3′) and antisense (5′-GCC-CAG-CCC-ACC-TCC-ACT-CCT-C-3′) primers yield a 172-bp product. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense (5′-CCA-CCC-ATG-GCA-AAT-TCC-ATG-GCA-3′) and antisense (5′-TCT-AGA-CGG-CAG-GTC-AGG-TCC-ACC-3′) primers gave rise to a 593-bp product (22). IL-1β amplification conditions consisted of an initial denaturation for 2 min at 95°C followed by 30 cycles of 45 s at 94°C, 45 s at 55°C, and 1 min at 72°C (21). MMP-9 and GAPDH amplification conditions comprised an initial denaturation for 2 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C (22). Amplification of each gene was in the linear range. The PCR products were separated on ethidium bromide-stained 2% agarose gels. Semiquantitative analysis was done with Molecular Imager FX Pro (Bio-Rad) to obtain MMP-9/GAPDH ratios.

Western Blot Analysis. GCTM-1 cells (5×10^6) cultured as described above (23) were collected and lysed with extraction buffer [10 mM HEPES (pH 7.9), 1.5 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1% SDS, 0.5 mM phenylmethylsulfonyl fluoride, and double-distilled water]. After 30 min at 4°C, cell debris was removed by centrifugation at $10{,}000 \times g$ for 10 min, and the supernatant was collected. Protein concentrations were measured with an Ultrospec 2100 Pro. Protein (100 μg) was mixed with SDS sample buffer, separated by SDS-PAGE (10% acrylamide), transferred to polyvinylidene difluoride membrane (Bio-Rad), and blocked with 0.1% Tween 20/5% skim milk for 1 h at room temperature. Membranes were incubated with anti-MMP-9 antibody for 1 h. Membranes were washed three times with 0.1% Tween 20 in PBS and stained with the appropriate secondary antibody. For quantification, the bands were scanned with Molecular Imager FX Pro, and densitometry was done with NIH Image version 1.60 software.

MMP-9 Antisense Oligonucleotide Treatment. MMP-9 antisense oligonucleotide (5′-CAG-GGG-CTG-CCA-GAG-GCT-CAT-3′; Sigma Genosys Japan, Hokkaido, Japan) or scramble oligonucleotide (5′-GCG-AGC-TAG-GAC-TGT-GCA-GCC-3′) was transfected into GCTM-1 cells with LipofectAMINE Plus Reagent (Invitrogen) following a procedure protocol (24). Oligonucleotide-LipofectAMINE complexes were added to GCTM-1 cells (1×10^6) suspended in serum-free medium and incubated for 5 h. An equal volume of 15% fetal bovine serum-RPMI 1640 was then added, and cells were incubated for 24 h.

Matrigel Invasion Assay. Invasiveness of GCTM-1 cells was assessed as the invasion of cells through Matrigel-

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coated transwell inserts (22). Briefly, the upper surface of the filter (pore size, 8.0 μm; BD Biosciences) was coated with basement membrane Matrigel (BD Biosciences) at a concentration of 250 μg/cm^2 and air-dried overnight at room temperature. GCTM-1 cells were suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 with 10% fetal bovine serum and treated with IL-1β (5 ng/ml) for 12 h. Cells were washed twice with PBS to remove IL-1β, 1×10^5 cells were added to the upper chamber, and 2% Matrigel-containing RPMI 1640 (400 μl) was added to the lower chamber. Cells were incubated in a water-saturated 5% CO_2 atmosphere at 37°C for 48 h. Cells that transverse the Matrigel adhere to the opposite surface of the filter. After incubation, the filter was fixed with 100% methanol and stained with Giemsa, after which cells on the upper surface were removed completely with a cotton swab. GCTM-1 cells that had migrated from the upper to the lower side of the filter were counted under light microscopy at a magnification of ×200. Tumor cell invasiveness was defined as the mean cell number of cells in five microscopic fields.

Statistical Analysis. Student's *t* test was used for statistical analyses. Calculations were carried out with StatView software (Abacus Concepts). All results with a $P < 0.05$ were considered statistically significant.

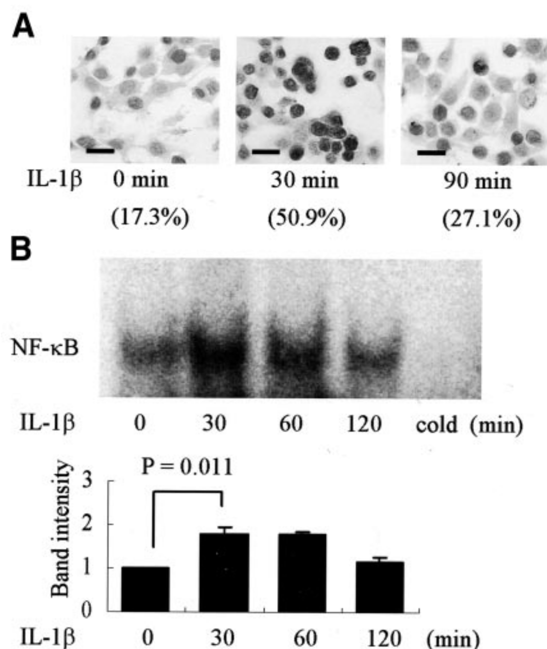

Fig. 1 Activation of nuclear factor-κB (NF-κB) p65 in interleukin (IL)-1β-stimulated gastric carcinoma cell line GCTM-1. *A,* photographic images of immunohistochemical staining of p65 at various points after stimulation of GCTM-1 cells with 5 ng/ml IL-1β. Cells showing nuclear translocation of NF-κB were counted and presented as the percentage of positive cells. *Bar,* 20 μm. *B, top panel,* NF-κB binding activity of GCTM-1 cells treated with IL-1β (5 ng/ml) for the indicated times was examined by electrophoretic mobility shift analysis. *B, bottom panel,* band intensities of NF-κB were quantified with NIH Image, and the value represents the mean ± SD from three different experiments. The control band intensity was indicated as 1.0.

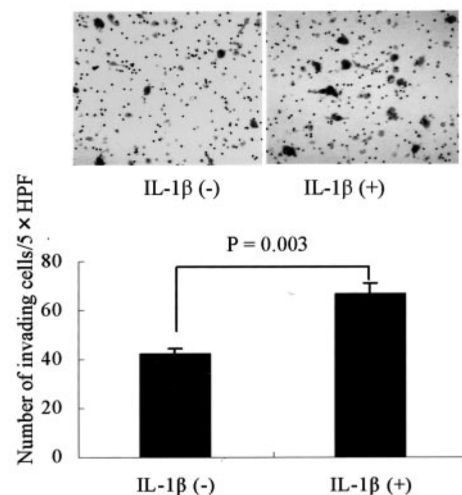

Fig. 2 Effect of interleukin (IL)-1β on the *in vitro* invasiveness of GCTM-1 cells. GCTM-1 cells were incubated with or without IL-1β (5 ng/ml) for 12 h. After incubation, cells were washed with PBS to remove IL-1β. IL-1β-treated GCTM-1 cells (2×10^5 cells/well) were seeded in a Matrigel-precoated invasion chamber and incubated at 37°C for 48 h. The number of cells that invaded the Matrigel were examined and counted under light microscopy (×200). Data are expressed as the mean ± SD of the number of cells counted in five randomly selected fields. Representative data from one of three independent experiments are shown.

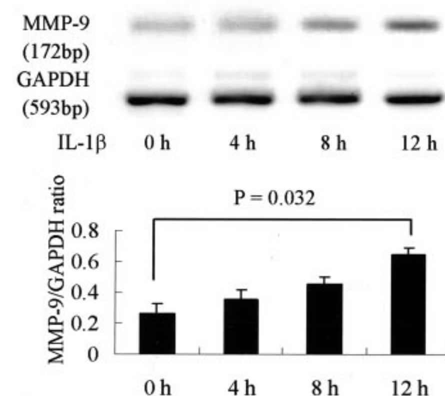

Fig. 3 Effect of interleukin-1β on expression of matrix metalloproteinase (MMP)-9 mRNA levels in GCTM-1 cells. *Top panel,* total RNA of MMP-9 isolated from GCTM-1 cells treated with interleukin-1β (5 ng/ml) for the indicated times was assessed by semiquantitative reverse transcription-PCR. *Bottom panel,* band intensities were quantified with NIH Image, and the data are presented as the ratio of MMP-9 to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The value represents the mean ± SD from three different experiments.

RESULTS

IL-1β Induced NF-κB Activation in GCTM-1 Cells. On the basis of our previous study, 5 ng/ml IL-1β was used throughout this study (12). Nuclear staining of NF-κB p65 in GCTM-1 cells was enhanced within 30 min of GCTM-1 exposure to 5 ng/ml IL-1β (Fig. 1*A*). We also investigated the effect

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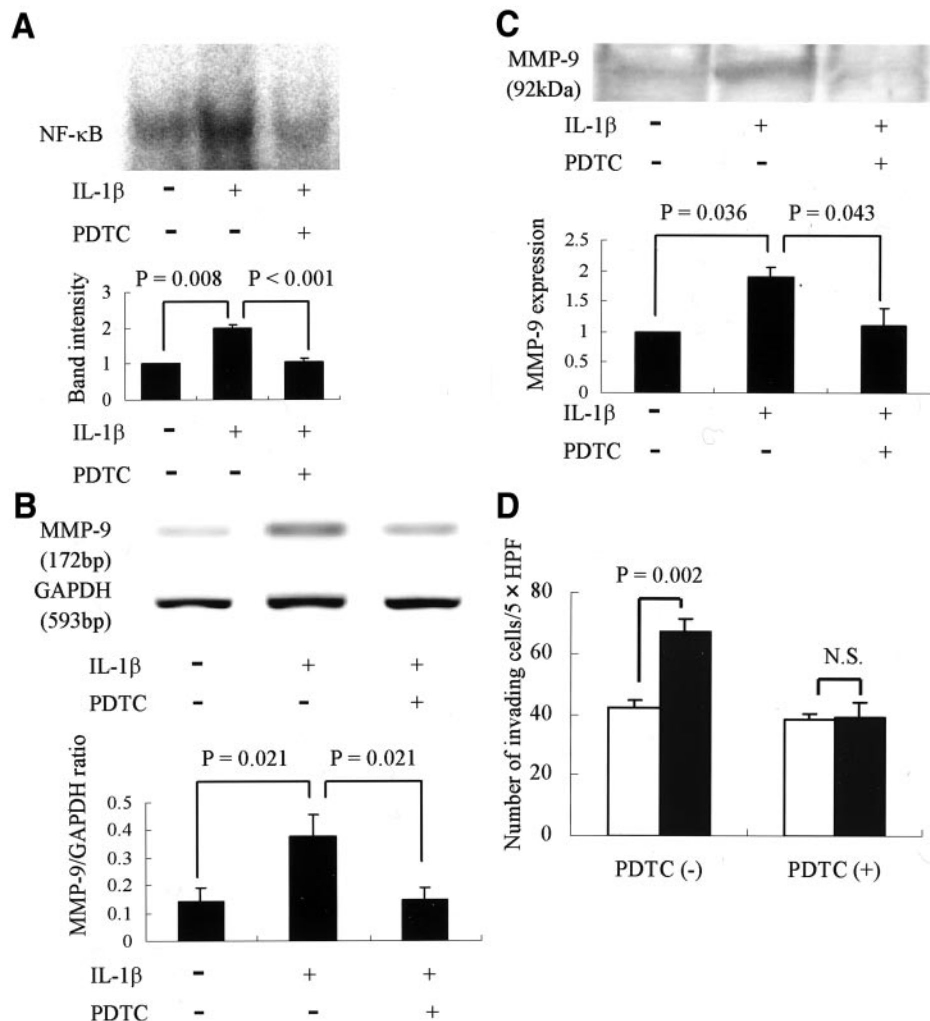

Fig. 4 Inhibition of interleukin (IL)-1β-induced matrix metalloproteinase (MMP)-9 expression and invasion of GCTM-1 cells by PDTC. GCTM-1 cells were preincubated with PDTC (10 μM) for 1 h before the addition of IL-1β (5 ng/ml). *A*, *top panel,* nuclear factor-κB binding activity 30 min after the addition of IL-1β was examined by electrophoretic mobility shift analysis. *A, bottom panel,* band intensities of nuclear factor-κB were quantified with NIH Image, and the values represent the mean ± SD from three different experiments. The control band intensity was indicated as 1.0. *B, top panel,* total RNA of MMP-9 isolated from GCTM-1 cells 12 h after the addition of IL-1β was assessed by semiquantitative reverse transcription-PCR. *B, bottom panel,* band intensities were quantified with NIH Image, and the data are presented as the ratio of MMP-9 to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Data represent the mean ± SD from three different experiments. *C, top panel,* MMP-9 expression 18 h after the addition of IL-1β was examined by Western blotting. *C, bottom panel,* band intensities were quantified with NIH Image, and the data represent the mean ± SD from three different experiments. The control band intensity was indicated as 1.0. *D,* invasiveness of cells was assessed by a Matrigel invasion assay. Cells that had invaded the Matrigel were counted under light microscopy (×200). Data are expressed as the mean ± SD of the number of cells counted in five randomly selected fields (□, control cells; ■, IL-1β-treated cells). Representative data from one of three independent experiments are shown.

of IL-1β on nuclear translocation of NF-κB p65 by electrophoretic mobility shift analysis. When GCTM-1 cells were treated with IL-1β for 30 min, NF-κB binding to DNA clearly increased (Fig. 1*B*).

IL-1β Enhanced the Invasive Ability of GCTM-1 Cells through MMP-9 Expression. We examined whether IL-1β enhances invasive ability of GCTM-1 cells using a Matrigel invasion assay. Pretreatment of GCTM-1 cells with 5 ng/ml IL-1β significantly increased the invasive ability of these cells ($P = 0.003$, Fig. 2). We then examined whether the enhanced invasive ability of GCTM-1 cells induced by IL-1β is associated with MMP-9. IL-1β increased levels of MMP-9 mRNA in GCTM-1 cells within 8 h (Fig. 3).

NF-κB Inhibitor, PDTC, Suppressed MMP-9 Expression and Invasive Ability of GCTM-1 Cells Induced by IL-1β. To confirm the relation between NF-κB activation, MMP-9 expression, and enhanced invasive ability, we examined the effect of a specific NF-κB inhibitor, PDTC, on IL-1β-induced changes (25). PDTC (10 μM) had no effect on cell viability (data not shown). PDTC (10 μM) was added to GCTM-1 cells 1 h before treatment with IL-1β, and expression of MMP-9 was analyzed. PDTC inhibited nuclear translocation

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of NF-κB p65 induced by IL-1β (Fig. 4*A*). In the Matrigel invasion assay, PDTC was added to GCTM-1 cultures 1 h before treatment with IL-1β for 12 h. GCTM-1 cells were washed immediately before the Matrigel invasion assay to remove both PDTC and IL-1β. PDTC suppressed MMP-9 expression at both the mRNA and protein levels (Fig. 4, *B* and *C*). Enhanced invasive ability of GCTM-1 cells induced by IL-1β was also suppressed by PDTC (Fig. 4*D*).

IL-1β Did Not Increase the Invasive Ability of GCTM-1 Cells Treated with MMP-9 Antisense Oligonucleotide. To verify the effect of MMP-9 on the enhanced invasive ability of GCTM-1 cells induced by IL-1β, we performed Matrigel invasion assays with MMP-9 antisense oligonucleotide-transfected GCTM-1 cells. The invasive ability of MMP-9-antisense oligonucleotide-transfected GCTM-1 cells was not affected by IL-1β (Fig. 5).

Macrophages Infiltrating Gastric Carcinoma Tissue and/or Gastric Carcinoma Cells Produced IL-1β. Finally, we examined whether IL-1β is produced at the tumor site. IL-1β immunoreactivity was clearly observed in three of the 10 carcinoma specimens. Cells producing IL-1β were tumor-infiltrating macrophages in two specimens and gastric carcinoma cells in one specimen. In these three specimens, both NF-κB activation and MMP-9 expression were found (Fig. 6).

DISCUSSION

Here we report that in gastric carcinoma tissue IL-1β produced at the tumor site may play a role in NF-κB activation and that this activation increases expression of MMP-9, resulting in increased invasiveness of carcinoma cells.

We reported previously that NF-κB is constitutively activated in gastric carcinoma and suggested that NF-κB activation may be linked to the invasive abilities of gastric carcinoma cells (12). In fact, several researchers have described a close relation between NF-κB activation and increased expression of invasion-related molecules, such as MMPs, by carcinoma cells *in vitro* (22, 26, 27). In the present study, we first examined constitutive activation of NF-κB in gastric carcinoma cells. Because IL-1β induces NF-κB activation in gastric carcinoma HTB-135 cells (12), we hypothesized that IL-1β might activate NF-κB in gastric carcinoma tissue. IL-1β in fact induced NF-κB activation in gastric carcinoma GCTM-1 cells. It is generally accepted that microbial and nonmicrobial products induce IL-1β secretion by macrophages (28, 29) and that macrophages frequently infiltrate carcinoma tissue (30–33). It has also been suggested that tumor-infiltrating macrophages are activated by transforming growth factor β1 secreted by tumor cells to produce IL-1β (34). IL-1β is secreted by a variety of tumors, including melanoma (35), sarcoma (36), hepatoblastoma (37), ovarian carcinoma (38), colorectal carcinoma (30, 39), pancreatic carcinoma (40), and gastric carcinoma (41, 42). Although these findings suggest that IL-1β may be secreted locally at the site of the tumor, direct evidence that IL-1β activates NF-κB in gastric carcinoma tissue has not been reported. GCTM-1 cells do not express IL-1β at both the mRNA and protein levels (data not shown). In 10 surgically resected gastric carcinoma specimens, IL-1β secretion from tumor-infiltrating macrophages and gastric carcinoma cells was observed in two specimens and one specimen, respectively (Fig. 6). These findings provide evidence of an association between autocrine secretion of IL-1β from carcinoma cells and activation of NF-κB in gastric carcinoma tissue.

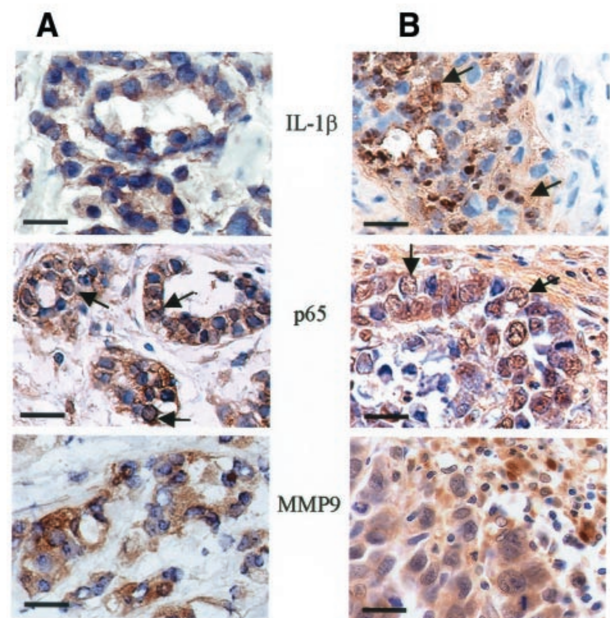

Fig. 6 Expression of nuclear factor-κB (NF-κB) p65, interleukin (IL)-1β, and matrix metalloproteinase (MMP)-9 at the tumor site of gastric carcinoma specimens (*A* and *B*). Expression of these three molecules was examined by immunostaining. *A,* photographic images of autocrine IL-1β secretion by gastric carcinoma cells, NF-κB p65 nuclear translocation in gastric carcinoma cells (*arrow*), and MMP-9 expression by gastric carcinoma cells. *B,* photographic images of paracrine IL-1β secretion by tumor-infiltrating macrophages (*arrow*), NF-κB p65 nuclear translocation in gastric carcinoma cells (*arrow*), and MMP-9 expression by gastric carcinoma cells and stromal cells. *Bar*, 20 μm.

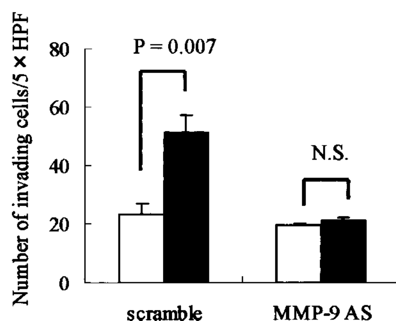

Fig. 5 Effect of interleukin (IL)-1β on the invasive ability of matrix metalloproteinase 9 antisense oligonucleotide-transfected GCTM-1 cells. GCTM-1 cells treated with scramble or matrix metalloproteinase 9 antisense oligonucleotide were incubated with IL-1β (5 ng/ml) for 12 h. After incubation, cells were washed with PBS to remove the IL-1β. IL-1β-treated tumor cells (2×10^5 cells/well) were then seeded into a Matrigel-precoated invasion chamber and incubated at 37°C for 48 h. Cells that had invaded the Matrigel were examined and counted under light microscopy (×200). Data are expressed as the mean ± SD of the number of cells counted in five randomly selected fields (□, control cells; ■, IL-1β-treated cells). Representative data from one of three independent experiments are shown.

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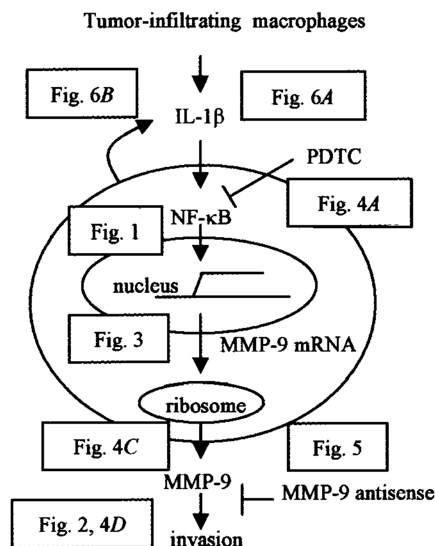

Fig. 7 Proposed mechanism for the enhancement of invasive ability of carcinoma cells by interleukin 1β. In some carcinoma cells, interleukin 1β secreted in a paracrine and/or autocrine fashion activates nuclear factor-κB and promotes the invasive ability through production of matrix metalloproteinase 9.

We then sought to clarify the biological function of IL-1β-induced activation of NF-κB induced in GCTM-1 cells. Arlt *et al.* (40) reported that NF-κB activation induced by IL-1β protects pancreatic carcinoma cell lines from apoptosis. Several studies have shown a close relation between NF-κB activation and expression of invasion-related molecules (26), suggesting that invasive ability is modified by NF-κB activation induced by IL-1β. Many factors, including MMP-2, MMP-9 (43, 44), urokinase-type plasminogen activator (45), and plasminogen activator inhibitor-1 (46), contribute to tumor invasion. It has been reported that an inhibitor of MMP-9 reduces invasion by some carcinoma cells (47–49), suggesting an essential role of MMP-9 in invasion. However, it was not known whether IL-1β increases MMP-9 expression or the invasive ability of carcinoma cells. We show for the first time that IL-1β increases both MMP-9 expression and the invasive ability of GCTM-1 cells and that these phenomena are both suppressed by NF-κB inhibitor PDTC (Fig. 4, *A–C*). IL-1β had no effect on the invasive ability of GCTM-1 cells treated with MMP-9 antisense oligonucleotide (Fig. 5). Our findings are summarized in Fig. 7. Briefly, tumor-infiltrating macrophages and/or carcinoma cells express IL-1β at the site of the tumors. IL-1β induces NF-κB activation, which then induces transcription of MMP-9 mRNA and production of MMP-9 protein. Up-regulation of MMP-9 enhances the ability of GCTM-1 cells to invade a collagen matrix.

In conclusion, NF-κB activation in gastric carcinoma cells is enhanced by IL-1β secreted by tumor-infiltrating macrophages (paracrine) and/or carcinoma cells (autocrine). NF-κB activation induced by IL-1β contributes to increased invasive ability of gastric carcinoma cells through overexpression of MMP-9. These findings suggest that inflammation at the site of a tumor may play some roles in the metastasis of tumor cells. In cases in which IL-1β may play a significant role in NF-κB activation of tumor cells, NF-κB inhibitor, neutralizing antibody to IL-1β, or anti-inflammatory agents may offer new therapeutic strategies against invasive gastric carcinoma.

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