Regulation by Endogenous Interleukin-1 of mRNA Expression of Healing-Related Factors in Gastric Ulcers in Rats

SATORU TAKAHASHI, NORIHIRO KOBAYASHI, and SUSUMU OKABE

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto, Japan

Accepted for publication July 16, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

We investigated the role of endogenous interleukin (IL)-1 in the mRNA expression of cyclooxygenase (COX)-1, COX-2, inducible nitric oxide synthase (iNOS), cytokine-induced neutrophil chemoattractant (CINC)-1, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and transforming growth factor (TGF)-β1 in acetic acid-induced gastric ulcers in rats. IL-1β mRNA was not detected in the normal or intact mucosa of ulcerated stomachs, but its expression was induced in the ulcerated tissue. IL-1β immunoreactivity was observed in macrophages/monocytes and fibroblasts in the ulcer base. COX-2, iNOS, and CINC-1 mRNAs were expressed by ulceration. EGF, bFGF, HGF, and TGF-β1 mRNA expression was detected in the normal mucosa, and their levels were significantly elevated by ulceration. In contrast, COX-1 mRNA level did not differ between the normal and ulcerated tissues. In a culture of isolated ulcer bases, block of IL-1 with IL-1 receptor antagonist (IL-1RA) dose-dependently and significantly reduced the mRNA levels of COX-2, iNOS, CINC-1, HGF, and bFGF. In contrast, COX-1, EGF, and TGF-β1 mRNA expression was not affected by IL-1RA. IL-1RA dose-dependently reduced prostaglandin E2 production, total and iNOS activities, neutrophil chemotactic activity, and growth-promoting activity toward gastric epithelial cells in the ulcer base. Finally, the administration of IL-1RA caused a significant impairment of ulcer healing. These results indicate that IL-1, expressed in macrophages/monocytes and fibroblasts in the ulcer base, might up-regulate the mRNA expression of COX-2, iNOS, CINC-1, HGF, and bFGF, thereby contributing to gastric ulcer healing in rats.

Proinflammatory cytokine interleukin (IL)-1 exerts numerous biological effects on multiple cell types and plays a central role in the regulation of inflammatory reactions (Dinarello, 1996). The basis for these IL-1 actions is considered to be the effect on the gene expression of cytokines, growth factors and their receptors, cyclooxygenase (COX)-2, and inducible nitric oxide synthase (iNOS). Kinoshita et al. (1995) and we (Takahashi et al., 1998) reported that IL-1α and IL-1β mRNA expression is induced by gastric ulceration in rats. Other studies also revealed that IL-1 mRNA and protein are highly expressed in the gastric mucosa of Helicobacter pylori-positive patients with gastritis and ulcers (Noach et al., 1994; Yamaoka et al., 1996). In addition, in ulcerated gastric tissue, the production of cytokines other than IL-1, growth factors, and autacoids is also elevated for mucosal regeneration. Consequently, we postulated that IL-1 may regulate mRNA expression of these factors in ulcerated tissue. Therefore, the present study was designed to investigate the effect of locally produced IL-1 on the expression of COX-1, COX-2, iNOS, cytokine-induced neutrophil chemoattractant (CINC-1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and transforming growth factor (TGF)-β1 in gastric ulcers in rats. Because IL-1 has both systemic and local effects (Dinarello, 1996), we examined the effect of IL-1 receptor antagonist (IL-1RA) in a culture of isolated ulcer bases. In addition, the effect of IL-1RA on ulcer healing was examined. Based on the obtained data, we discuss the pharmacological relevancies of IL-1 in gastric ulcer healing.

Materials and Methods

Production of Gastric Ulcers. Male Donryu rats (Nihon SLC, Hamamatsu, Japan), weighing 250 to 300 g, were fasted for 5 h before ulcer induction. Under ether anesthesia, gastric ulcers were induced by submucosal injection of 20% acetic acid (0.04 ml) into the border between the antrum and fundus on the anterior wall of the stomach (Takagi et al., 1969). After closure of the abdomen, the rats were maintained in the usual manner. Because deep and well-defined ulcers were observed 5 days after the acid injection, we defined the fifth day as the day of ulceration (day 0). On the indicated days,
rats were sacrificed and their stomachs were excised. Subsequently, the stomachs were incised along the greater curvature, and the ulcerated area (mm²) was blindly determined under a dissecting microscope (X10; Olympus, Tokyo, Japan).

**Northern Blot Analysis of mRNA Expression.** Rat IL-1β (Hershkowitz et al., 1995), COX-1 (Feng et al., 1993), COX-2 (Feng et al., 1993), iNOS (Lyons et al., 1992), EGF (Fan et al., 1995), bFGF (El-Husseini et al., 1992), HGF (Kinoshita et al., 1995), and TGF-β1 (Siegle et al., 1994) cDNA probes were prepared by means of reverse transcription-polymerase chain reaction. In addition, the primers for rat CINC-1 were 5′-AGCACCAGTGGCTCAAGCACC-3′ and 5′-TGCCGGCTTCTCCGTGTC-3′, and total RNA for the amplification of CINC-1 cDNA was isolated from the spleen of a lipopolysaccharide-infused rat (5 mg/kg, 2 h). The products corresponding to IL-1β [520 base pairs (bp)], COX-1 (887 bp), COX-2 (702 bp), iNOS (651 bp), CINC-1 (591 bp), EGF (566 bp), bFGF (354 bp), HGF (580 bp), and TGF-β1 (396 bp) were purified from polyacrylamide gels and used as probes after their sequences had been confirmed to be completely identical with known ones (with reference to the GenBank and EMBL databases). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe was purchased from Clontech (Palo Alto, CA). The cDNA probes were 32P-labeled by the random primer method (Ready-To-Go; Pharmacia Biotech, Uppsala, Sweden).

Gastric specimens were taken from both intact (posterior side) and ulcerated (anterior side) tissues of stomachs with ulcers and from normal ones without ulcers. Total RNAs were extracted from fresh or cultured tissues by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski and Sacchi, 1987), using TRIZOL (GIBCO BRL, Gaithersburg, MD). Poly(A)⁺ RNAs were purified with Oligotex dT30 (TaKaRa, Kyoto, Japan). Poly(A)⁺ RNAs (0.5 µg) were separated by electrophoresis on 1.2% agarose gels, transferred to nylon membranes (Gene Screen Plus; New England Nuclear, Boston, MA), and then hybridized with 32P-labeled cDNA probes, according to the standard method (Sambrook et al., 1989). The detection and quantification of hybridized mRNAs were carried out with an imaging analyzer (BAS-5000 Mac; Fuji Film, Tokyo, Japan). The levels of mRNAs were expressed as the ratio to GAPDH mRNA (an internal standard).

**Immunohistochemical Analysis of IL-1β Protein.** Gastric specimens were taken from both intact (posterior side) and ulcerated (anterior side) tissues of stomachs with ulcers and from normal ones without ulcers. After they had been fixed with 4% paraformaldehyde (anterior side) tissues of stomachs with ulcers and from normal ones were isolated from the fragments and subjected to Northern blot analysis. Prostaglandin E₂ (PGE₂) production, NOS activity, neutrophil chemotactic activity, and growth-promoting activity were also determined as described below. After a 36-h incubation with IL-1RA, the DNA and RNA contents were nearly the same as those before culture. Histologically, granulation tissues in the ulcer bases remained unchanged, and fibroblasts and immune cells were observed even after the incubation.

**Determination of PGE₂ Production.** After gastric tissues had been cultured, they were washed three times with the medium. They were further incubated in 0.5 ml of the medium at 37°C for 1 h. The amount of PGE₂ in the medium was determined by enzyme immunoassay (PGE₂ EIA kit; Cayman Chemicals, Ann Arbor, MI). When ulcer bases without treatment with IL-1RA were incubated with 10 µM NS-398 (COX-2-selective inhibitor; Taisho Pharmaceutical Co., Tokyo, Japan) or 5 µg/ml IL-1RA, PGE₂ production was also determined. PGE₂ production was expressed as pg PGE₂/mg tissue.

**Determination of NOS Activity.** NOS activity was measured as the conversion of L-[¹⁴C]arginine to [¹⁴C]citrulline, as described by Lamarrque et al. (1998). After gastric tissues had been cultured, they were washed with 10 mM HEPES-NaOH buffer (pH 7.4) containing 1 mM dithiothreitol, 10 µg/ml soybean trypsin inhibitor, 5 µg/ml aprotinin, and 10 µg/ml leupeptin. The tissues were homogenized in 0.5 ml of the same buffer with a Potter-Elvehjem homogenizer (Iuchi, Osaka, Japan), followed by centrifugation at 10,000g for 20 min. The resulting supernatants were incubated in 0.2 ml of 50 mM phosphate buffer (pH 7.4) containing 50 mM L-lysine, 1 mM MgCl₂, 0.2 mM CaCl₂, 1 mM l-citrulline, 0.3 mM NaADP, 3 µM PAD, 3 µM FMN, 3 µM BH₄, and 15 mM L-¹⁴C]arginine (Amersham, Buckinghamshire, UK) at 37°C for 20 min. The incubation was terminated by the addition of 1 ml of Dowex AG 50W-8 (1:1 suspension in water; Sigma Chemical Co.). The resin was allowed to settle for 30 min, and then the radioactivity in the supernatants was measured. In addition, when 1 mM amino guanidine (iNOS-selective inhibitor; Sigma Chemical Co.) or 5 µg/ml IL-1RA was added to the supernatant derived from ulcer bases without treatment with IL-1RA, NOS activity was also determined. Product formation, which was inhibited by 0.2 mM N⁶-nitro-L-arginine methyl ester (Sigma Chemical Co.), was taken as total NOS activity. iNOS activity was taken as that which was inhibited by N⁶-nitro-L-arginine methyl ester but not by 1 mM EGTA. Constitutive NOS activity was calculated as the difference between the total NOS and iNOS activities. NOS activity was expressed as pmol [¹⁴C]citrulline/mg protein/min. Protein concentrations were determined with a protein assay kit (Bio-Rad, Hercules, CA).

**Determination of Neutrophil Chemotactic Activity.** Gastric extracts were prepared according to the method we described previously (Fujita et al., 1998). After gastric tissues had been cultured, they were washed with 10 mM phosphate buffer (pH 7.2) containing 1 mM ethylenediaminetetraacetic acid, 200 mM NaCl, and 0.1 mM phenylmethylsulfonyl fluoride. The tissues were homogenized with a Potter-Elvehjem homogenizer in the same buffer with 1 ml to 100 mg tissue. The homogenates were centrifuged at 20,000g for 1 h, and the supernatants were recovered as gastric extracts.

Peritoneal neutrophils were isolated from normal rats according to the method of Fujita et al. (1998). The purity and viability of neutrophils were both more than 95%, as determined by Diff Quick (International Reagents, Kobe, Japan) staining and the trypan blue dye exclusion method, respectively.

The neutrophil chemotaxis assay was performed according to the modified Boyden method (Falk et al., 1980), using a membrane filter of 5-µm pore size (Neuro Probe, Cabin John, MD). Gastric extracts (20 µl), 100 ng/ml CINC-1 (Immuo Biological Laboratories, Fujioka, Japan), 5 µg/ml IL-1RA, 100 ng/ml human IL-1β (Otsuka Pharmaceutical Co., Tokushima, Japan), or the above buffer alone (as a control for the chemotactic assay) was added to the lower compartment of the chemotactic chamber. Neutrophils (1 x 10⁶ cells) were placed in the upper compartment. The chamber was stood for 1 h at 37°C to allow neutrophil migration. Thereafter, the filter was removed, and the cells were stained with Diff Quick. The numbers of
of gastric tissue with ulcers. High expression of IL-1β mRNA was observed on day 3, with the level decreasing thereafter. The level was well associated with ulcer healing.

Furthermore, we determined the cellular localization of IL-1β protein by immunohistochemical staining (Fig. 2). In the gastric mucosa of the normal stomach, immunoreactivity for IL-1β protein was not detected. Similarly, there were no appreciable signals with anti-IL-1β antibody in the gastric glands around ulcers. In contrast, in the ulcer base, apparent IL-1β immunoreactivity was found in the upper portion. The immunoreactive IL-1β protein was abundant in mononuclear cells, which were positive as to the staining due to macrophage-specific esterase activity. In addition, IL-1β immunoreactivity was observed in fibroblasts (spindle-shaped cells). The number of IL-1β-expressing cells decreased with ulcer healing. When the antibody preincubated with IL-1β was applied to sections, no immunoreactive signals were observed.

mRNA Expression of Healing-Related Factors in Gastric Ulcers. We examined the mRNA expression of COX-1, COX-2, iNOS, CINC, EGF, bFGF, HGF, and TGF-β1 in gastric tissues (Fig. 3). COX-1 mRNA was detected in the normal mucosa and similarly expressed in the ulcerated tissue. In contrast, COX-2, iNOS, and CINC-1 mRNAs were not found in the normal mucosa, but their expression was induced by ulceration. Their levels were higher in the early phase (day 3) than in the late phase of ulcer healing (day 10; i.e., the expression decreased with ulcer healing). Furthermore, mRNA expression of EGF, bFGF, HGF, and TGF-β1 was also promoted by ulceration. The levels of these growth factor mRNAs were significantly higher in the ulcerated tissue than in the normal mucosa. EGF, bFGF, and TGF-β1 mRNA levels remained unchanged during ulcer healing, whereas HGF mRNA expression decreased with the healing.

Role of Endogenous IL-1 in COX-2 mRNA Expression in Gastric Ulcers. To clarify the role of endogenous IL-1 expressed in ulcerated gastric tissue, we examined the effect of IL-1RA on a culture of isolated ulcer bases. Figure 4 shows the effect of IL-1RA on COX mRNA expression and PGE₂ production in the ulcer base. Treatment with IL-1RA caused a dose-dependent and significant decrease in COX-2 mRNA expression of gastric tissue with ulcers. High expression of IL-1β mRNA was observed on day 3, with the level decreasing thereafter. The level was well associated with ulcer healing.

Furthermore, we determined the cellular localization of IL-1β protein by immunohistochemical staining (Fig. 2). In the gastric mucosa of the normal stomach, immunoreactivity for IL-1β protein was not detected. Similarly, there were no appreciable signals with anti-IL-1β antibody in the gastric glands around ulcers. In contrast, in the ulcer base, apparent IL-1β immunoreactivity was found in the upper portion. The immunoreactive IL-1β protein was abundant in mononuclear cells, which were positive as to the staining due to macrophage-specific esterase activity. In addition, IL-1β immunoreactivity was observed in fibroblasts (spindle-shaped cells). The number of IL-1β-expressing cells decreased with ulcer healing. When the antibody preincubated with IL-1β was applied to sections, no immunoreactive signals were observed.

mRNA Expression of Healing-Related Factors in Gastric Ulcers. We examined the mRNA expression of COX-1, COX-2, iNOS, CINC, EGF, bFGF, HGF, and TGF-β1 in gastric tissues (Fig. 3). COX-1 mRNA was detected in the normal mucosa and similarly expressed in the ulcerated tissue. In contrast, COX-2, iNOS, and CINC-1 mRNAs were not found in the normal mucosa, but their expression was induced by ulceration. Their levels were higher in the early phase (day 3) than in the late phase of ulcer healing (day 10; i.e., the expression decreased with ulcer healing). Furthermore, mRNA expression of EGF, bFGF, HGF, and TGF-β1 was also promoted by ulceration. The levels of these growth factor mRNAs were significantly higher in the ulcerated tissue than in the normal mucosa. EGF, bFGF, and TGF-β1 mRNA levels remained unchanged during ulcer healing, whereas HGF mRNA expression decreased with the healing.

Role of Endogenous IL-1 in COX-2 mRNA Expression in Gastric Ulcers. To clarify the role of endogenous IL-1 expressed in ulcerated gastric tissue, we examined the effect of IL-1RA on a culture of isolated ulcer bases. Figure 4 shows the effect of IL-1RA on COX mRNA expression and PGE₂ production in the ulcer base. Treatment with IL-1RA caused a dose-dependent and significant decrease in COX-2 mRNA expression of gastric tissue with ulcers. High expression of IL-1β mRNA was observed on day 3, with the level decreasing thereafter. The level was well associated with ulcer healing.

Furthermore, we determined the cellular localization of IL-1β protein by immunohistochemical staining (Fig. 2). In the gastric mucosa of the normal stomach, immunoreactivity for IL-1β protein was not detected. Similarly, there were no appreciable signals with anti-IL-1β antibody in the gastric glands around ulcers. In contrast, in the ulcer base, apparent IL-1β immunoreactivity was found in the upper portion. The immunoreactive IL-1β protein was abundant in mononuclear cells, which were positive as to the staining due to macrophage-specific esterase activity. In addition, IL-1β immunoreactivity was observed in fibroblasts (spindle-shaped cells). The number of IL-1β-expressing cells decreased with ulcer healing. When the antibody preincubated with IL-1β was applied to sections, no immunoreactive signals were observed.

mRNA Expression of Healing-Related Factors in Gastric Ulcers. We examined the mRNA expression of COX-1, COX-2, iNOS, CINC, EGF, bFGF, HGF, and TGF-β1 in gastric tissues (Fig. 3). COX-1 mRNA was detected in the normal mucosa and similarly expressed in the ulcerated tissue. In contrast, COX-2, iNOS, and CINC-1 mRNAs were not found in the normal mucosa, but their expression was induced by ulceration. Their levels were higher in the early phase (day 3) than in the late phase of ulcer healing (day 10; i.e., the expression decreased with ulcer healing). Furthermore, mRNA expression of EGF, bFGF, HGF, and TGF-β1 was also promoted by ulceration. The levels of these growth factor mRNAs were significantly higher in the ulcerated tissue than in the normal mucosa. EGF, bFGF, and TGF-β1 mRNA levels remained unchanged during ulcer healing, whereas HGF mRNA expression decreased with the healing.

Role of Endogenous IL-1 in COX-2 mRNA Expression in Gastric Ulcers. To clarify the role of endogenous IL-1 expressed in ulcerated gastric tissue, we examined the effect of IL-1RA on a culture of isolated ulcer bases. Figure 4 shows the effect of IL-1RA on COX mRNA expression and PGE₂ production in the ulcer base. Treatment with IL-1RA caused a dose-dependent and significant decrease in COX-2 mRNA expression of gastric tissue with ulcers. High expression of IL-1β mRNA was observed on day 3, with the level decreasing thereafter. The level was well associated with ulcer healing.

Furthermore, we determined the cellular localization of IL-1β protein by immunohistochemical staining (Fig. 2). In the gastric mucosa of the normal stomach, immunoreactivity for IL-1β protein was not detected. Similarly, there were no appreciable signals with anti-IL-1β antibody in the gastric glands around ulcers. In contrast, in the ulcer base, apparent IL-1β immunoreactivity was found in the upper portion. The immunoreactive IL-1β protein was abundant in mononuclear cells, which were positive as to the staining due to macrophage-specific esterase activity. In addition, IL-1β immunoreactivity was observed in fibroblasts (spindle-shaped cells). The number of IL-1β-expressing cells decreased with ulcer healing. When the antibody preincubated with IL-1β was applied to sections, no immunoreactive signals were observed.

mRNA Expression of Healing-Related Factors in Gastric Ulcers. We examined the mRNA expression of COX-1, COX-2, iNOS, CINC, EGF, bFGF, HGF, and TGF-β1 in gastric tissues (Fig. 3). COX-1 mRNA was detected in the normal mucosa and similarly expressed in the ulcerated tissue. In contrast, COX-2, iNOS, and CINC-1 mRNAs were not found in the normal mucosa, but their expression was induced by ulceration. Their levels were higher in the early phase (day 3) than in the late phase of ulcer healing (day 10; i.e., the expression decreased with ulcer healing). Furthermore, mRNA expression of EGF, bFGF, HGF, and TGF-β1 was also promoted by ulceration. The levels of these growth factor mRNAs were significantly higher in the ulcerated tissue than in the normal mucosa. EGF, bFGF, and TGF-β1 mRNA levels remained unchanged during ulcer healing, whereas HGF mRNA expression decreased with the healing.

Role of Endogenous IL-1 in COX-2 mRNA Expression in Gastric Ulcers. To clarify the role of endogenous IL-1 expressed in ulcerated gastric tissue, we examined the effect of IL-1RA on a culture of isolated ulcer bases. Figure 4 shows the effect of IL-1RA on COX mRNA expression and PGE₂ production in the ulcer base. Treatment with IL-1RA caused a dose-dependent and significant decrease in COX-2 mRNA expression of gastric tissue with ulcers. High expression of IL-1β mRNA was observed on day 3, with the level decreasing thereafter. The level was well associated with ulcer healing.

Furthermore, we determined the cellular localization of IL-1β protein by immunohistochemical staining (Fig. 2). In the gastric mucosa of the normal stomach, immunoreactivity for IL-1β protein was not detected. Similarly, there were no appreciable signals with anti-IL-1β antibody in the gastric glands around ulcers. In contrast, in the ulcer base, apparent IL-1β immunoreactivity was found in the upper portion. The immunoreactive IL-1β protein was abundant in mononuclear cells, which were positive as to the staining due to macrophage-specific esterase activity. In addition, IL-1β immunoreactivity was observed in fibroblasts (spindle-shaped cells). The number of IL-1β-expressing cells decreased with ulcer healing. When the antibody preincubated with IL-1β was applied to sections, no immunoreactive signals were observed.
expression. However, IL-1RA failed to inhibit COX-1 mRNA expression. PGE2 production in the normal mucosa was low (105.2 ± 15.9 pg/mg/h), whereas the production was markedly promoted by ulceration. The increased PGE2 production in the ulcer base was associated with the level of COX-2 mRNA. Similar to COX-2 mRNA expression, the increased production was also significantly reduced by IL-1RA in a dose-dependent manner. The inhibition by 5 μg/ml IL-1RA of iNOS mRNA expression and the total and inducible NOS activities were 53.3, 66.8, and 52.6%, respectively, compared with the control. In addition, 1 mM aminoguanidine also reduced the total and inducible NOS activities to nearly the same extents, with the inhibition being about 60 and 70%, respectively. However, 5 μg/ml IL-1RA had no effect on the total and inducible NOS activities when IL-1RA was added to the control tissue in the NOS activity assay.

Role of Endogenous IL-1 in CINC-1 mRNA Expression in Gastric Ulcers. Similarly, we examined the effect of IL-1RA on CINC-1 mRNA expression and neutrophil chemotactic activity in the ulcer base (Fig. 5A). Ulceration-induced CINC mRNA expression was dose-dependently reduced by IL-1RA, with the inhibition by 5 μg/ml IL-1RA being significant (i.e., 32.9%). Neutrophil migration was not promoted by the extract prepared from the normal mucosa but significantly stimulated by the extract prepared from the ulcer base to 290.6% compared with the vehicle (Fig. 5B). Treatment with IL-1RA dose-dependently inhibited the stimulated neutrophil chemotaxis, with the effect of 5 μg/ml IL-1RA being significant (70.2% inhibition). When neutrophils were incubated with 100 ng/ml CINC-1, the migration was significantly stimulated to 356.8%. However, neither 100 ng/ml IL-1RA nor 5 μg/ml IL-1RA affected neutrophil migration.

Role of Endogenous IL-1 in mRNA Expression of Growth Factors in Gastric Ulcers. The effect of IL-1RA on the mRNA expression of EGF, bFGF, HGF, and TGF-β1 is shown in Fig. 7A. The increased level of HGF mRNA was dose-dependently and significantly reduced by treatment with IL-1RA. The inhibition by 10 μg/ml IL-1RA was 59.2% for HGF mRNA expression. IL-1RA also dose-relatedly decreased bFGF mRNA expression, and a significant inhibition by 10 μg/ml IL-1RA was observed (35.6%). In the cases of EGF and TGF-β1 mRNA expression, IL-1RA had no effect.

Furthermore, we examined the effect of IL-1RA on growth-promoting activity in the ulcer base (Fig. 7B). When rat gastric epithelial RGM1 cells were incubated with extracts, prepared from the normal mucosa or the ulcer base, cell growth was assessed as DNA synthesis. The extract prepared from the normal mucosa contained no growth-promoting activity. In contrast, the extract prepared from the ulcer base significantly enhanced the growth of RGM1 cells to 219.9% compared with the vehicle. Treatment of the base with IL-1RA caused a decrease in the growth of RGM1 cells to 171.1%, but 10 ng/ml bFGF, 100 ng/ml IL-1β, and 10 μg/ml IL-1RA had no effect on RGM1 growth.

Role of Endogenous IL-1 in Gastric Ulcer Healing. Finally, we investigated the role of endogenous IL-1 in gas-

Fig. 2. Immunohistochemical detection of IL-1β protein in the gastric tissues of rats. IL-1β-immunoreactivity was found only in the upper portion of the ulcer base. A, normal gastric tissue (×20). B, tissue around ulcers on day 3 (×20). C, upper portion of the ulcer base on day 3 (×100).

Role of Endogenous IL-1 in iNOS mRNA Expression in Gastric Ulcers. We examined the effect of IL-1RA on iNOS mRNA expression and NOS activity in the ulcer base (Fig. 5). IL-1RA dose-dependently and significantly reduced iNOS mRNA expression. In the ulcer base, total NOS activity markedly increased in response to iNOS expression, compared with that in the normal mucosa (11.4 ± 3.2 pmol/mg/min). iNOS activity in the normal mucosa was negligible (2.3 ± 1.8 pmol/mg/min), whereas iNOS activity comprised most of the total NOS activity in the ulcer base. IL-1RA significantly decreased the total and inducible NOS activities in a dose-dependent manner. The inhibition by 5 μg/ml IL-1RA of iNOS mRNA expression and the total and inducible NOS activities were 53.3, 66.8, and 52.6%, respectively, compared with the control. In addition, 1 mM aminoguanidine also reduced the total and inducible NOS activities to nearly the same extents, with the inhibition being about 60 and 70%, respectively. However, 5 μg/ml IL-1RA had no effect on the total and inducible NOS activities when IL-1RA was added to the control tissue in the NOS activity assay.

Role of Endogenous IL-1 in CINC-1 mRNA Expression in Gastric Ulcers. Similarly, we examined the effect of IL-1RA on CINC-1 mRNA expression and neutrophil chemotactic activity in the ulcer base (Fig. 6A). Ulceration-induced CINC mRNA expression was dose-dependently reduced by IL-1RA, with the inhibition by 5 μg/ml IL-1RA being significant (i.e., 32.9%). Neutrophil migration was not promoted by the extract prepared from the normal mucosa but significantly stimulated by the extract prepared from the ulcer base to 290.6% compared with the vehicle (Fig. 6B). Treatment with IL-1RA dose-dependently inhibited the stimulated neutrophil chemotaxis, with the effect of 5 μg/ml IL-1RA being significant (70.2% inhibition). When neutrophils were incubated with 100 ng/ml CINC-1, the migration was significantly stimulated to 356.8%. However, neither 100 ng/ml IL-1RA nor 5 μg/ml IL-1RA affected neutrophil migration.
tric ulcer healing in rats. The effect of exogenously adminis-
tered IL-1RA on ulcer healing was examined. As shown in
Fig. 8, IL-1RA significantly impaired the healing of acetic
acid-induced ulcers in rats, compared with the control. The
ulcerated areas in the IL-1RA-treated and control groups
were 19.4 ± 2.9 and 8.3 ± 1.8 mm², respectively.

Discussion

We reconfirmed that in response to gastric ulceration,
IL-1β mRNA is expressed only in the ulcerated tissue, as
described previously (Takahashi et al., 1998). This is well
consistent with the finding that IL-1β-expressing cells are
localized only in the ulcer base. Considering their existence
in granulation tissue, the cells were morphologically identi-
fied as macrophages/monocytes and fibroblasts. Most IL-1β-
expressing cells were also positive as to staining due to mac-
rophage-specific esterase activity, indicating that IL-1β is
produced mainly by macrophages/monocytes. Macrophages/
monocytes are well known to be dominant IL-1-producing
cells (Dinarello, 1996). In addition, IL-1β mRNA level de-
creased with ulcer healing; it is suggested that the reduction
in infiltration and activation of macrophages/monocytes and
fibroblasts associated with ulcer healing results in the de-
crease in IL-1β expression.

As reported by several researchers, the expression of
COX-2 (Mizuno et al., 1997; Schmassmann et al., 1998;
Shigeta et al., 1998; Takahashi et al., 1998), EGF (Wright et
al., 1990; Konturek et al., 1991), bFGF (Folkman et al., 1991;
Satoh et al., 1997), HGF (Kinoshita et al., 1995; Schmass-
mann et al., 1997), and TGF-β1 (Tominaga et al., 1997;
Takahashi et al., 1998) is induced or promoted by gastric and
duodenal ulceration. In addition, to our knowledge, we de-
scribe for the first time that iNOS and CINC mRNAs are also
expressed only in ulcerated tissue. The mRNA expression of COX-2, iNOS, CINC-1, HGF, and bFGF was inhibited on the block of IL-1 receptor with IL-1RA. These results suggest that during the healing of gastric ulcers, IL-1 may serve as one of the inducers of the mRNA expression of several factors. In addition, it is suggested that IL-1 type 1 receptor is involved in the induction of COX-2, iNOS, CINC-1, HGF, and bFGF mRNA expression, because IL-1RA binds more preferentially to the type 1 receptor than the type 2 receptor (Sims et al., 1993). The significance of the IL-1-induced expression of COX-2, iNOS, CINC-1, HGF, and bFGF is discussed below.

First, our results indicate that IL-1 might induce COX-2 mRNA expression, resulting in increased PGE2 production in gastric ulcers. Many studies revealed that COX-2 mRNA expression is induced by IL-1 in several cell types (Dinarello, 1996; Herschman, 1996). The increased production of PGE2, derived from COX-2, is required for ulcer healing, as described previously (Mizuno et al., 1997; Schmassmann et al., 1998; Shigeta et al., 1998). Accordingly, IL-1 is suggested to contribute to ulcer healing through COX-2 induction and the elevation of PG production.

Second, we found that IL-1 stimulates the induction of iNOS mRNA expression in gastric ulcers. It is well known that IL-1 causes iNOS mRNA expression in several cell types ( Förstermann et al., 1995). In addition, we found that the total NOS activity is markedly elevated in the ulcerated tissue and is due predominantly to iNOS activity. It is generally accepted that at inflammatory sites, NO production is highly increased by iNOS (Förstermann et al., 1995; Dinarello, 1996). These results suggest that iNOS expressed in gastric ulcers provides a considerable amount of NO. Kon-turek et al. (1993) reported that NO plays an important role in gastric ulcer healing. Taken together, it is suggested that IL-1 enhances NO production as well as PG production, thereby contributing to gastric ulcer healing.

Third, we found that CINC-1 mRNA expression is also induced by IL-1 in gastric ulcers. IL-1 is also a potent inducer of CINC expression ( Watanabe and Nakagawa, 1987; Dinarello, 1996). CINC belongs to the IL-8 chemokine family, exhibits potent chemotactic activity in vitro (Shibata et al.,
control. is involved in epithelial regeneration, but it remains unclear the IL-1 action. These results suggest that IL-1-induced HGF decrease in HGF mRNA expression caused by inhibition of promoting activity toward epithelial cells is reduced with the growth in rats. In the present study, we showed that growth- (1997) reported that exogenous HGF fails to accelerate gas-

Fig. 8. Effect of IL-1RA on the healing of acetic acid-induced ulcers in rats. IL-1RA or BSA (control) at 10 μg/animal was administered to the rats with ulcers. On day 10, ulcerated area was determined. The data are presented as mean ± S.E. (n = 6). * significantly different from the control.

whether endogenous HGF by itself plays a major role in ulcer healing.

Fifth, IL-1 might slightly induce bFGF expression in gastric ulcers. bFGF had no effect on epithelial cell growth but is well known to stimulate gastric fibroblast proliferation (Watanabe et al., 1995) and to promote angiogenesis (Folkman et al., 1991; Schmassmann et al., 1995; Satoh et al., 1997). These results suggest that IL-1 participates in the formation of granulation tissue and angiogenesis in the ulcer base via bFGF induction.

Furthermore, it is also suggested that the increased expression of EGF and TGF-β1 mRNAs is not regulated by IL-1 in gastric ulcers. The mechanism by which EGF and TGF-β1 expression is promoted is unknown at present.

Finally, administered IL-1RA significantly impaired the healing of acetic acid-induced ulcers in rats, indicating that endogenous IL-1 plays a crucial role in gastric ulcer healing. Taken together the above results, IL-1 might up-regulate the expression of several healing-related factors, contributing to gastric ulcer healing.

Watanabe et al. (1997) reported that exogenous IL-1β at 1 μg/kg causes recurrence of acetic acid-induced gastric ulcers in rats. In an acetic acid-induced ulcer model, ulcers are spontaneously relapsed after the initial ulcers healed (Takagi et al., 1969). However, they did not determine the IL-1β production or examine the role of endogenous IL-1β in spontaneous recurrence of gastric ulcers. Because IL-1β was injected intraperitoneally in their study, the systemic effect of exogenous IL-1β considerably appeared. Accordingly, it is quite possible that the injurious effect of exogenous IL-1β may be not physiological but artificial. In contrast, the action of locally produced IL-1β was examined in this study. Alternatively, IL-1β may exert opposite effects between ulcer healing and ulcer relapse; IL-1β in active ulcers may induce multiple responses for healing in granulation tissue, whereas IL-1β in healed ulcers may exert a deleterious effect toward the regenerated mucosa.

Overall, we conclude that IL-1, expressed in macrophages/monocytes and fibroblasts in the ulcer base, might up-regulate the mRNA expression of COX-2, iNOS, CINC-1, HGF, and bFGF, thereby contributing to gastric ulcer healing in rats.

Acknowledgments

We thank N. J. Halewood for critical reading of the manuscript and H. Fujita, E. Nakamura, M. Ishikawa, and S. Kitazawa for technical assistance. In addition, we are grateful to Dr. S. Takahashi (Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan) for supporting Northern blot analysis.

References


1995), and acts as a functional chemoattractant for neutrophils in vivo (Watanabe et al., 1991; Utsunomiya et al., 1996). To date, a rat counterpart of human IL-8 has not been identified, and CINC is considered to play a crucial role in the acute neutrophil infiltration in rats (Watanabe et al., 1993). In the present study, neutrophil chemotactic activity in the ulcerated tissue significantly decreased with the reduction in CINC-1 mRNA expression. Exposure of neutrophils to IL-1β failed to induce chemotaxis. We reported that neutrophil infiltration occurs into ulcerated gastric tissue and that both neutrophil chemotactic activity and CINC levels in the ulcerated tissue decrease with ulcer healing (Fujita et al., 1998; Yamada et al., 1999). These results suggest that CINC-1 is involved in neutrophil infiltration into the ulcerated tissue and that IL-1 causes neutrophil infiltration, partly via CINC-1 induction. Our hypothesis is supported by the finding of Utsunomiya et al. (1996) that locally injected IL-1α induces neutrophil chemotaxis indirectly through CINC production in rats. Because the activation and infiltration of neutrophils are essential for the elimination of wounded cells, cell debris, and extracellular matrix proteins before tissue regeneration, the induction of CINC expression by IL-1 is thought to be important for ulcer healing.

Fourth, our results indicate that IL-1 might up-regulate HGF mRNA expression in gastric ulcers. This is supported by the finding that HGF production is promoted by IL-1 in cultured fibroblasts (Tamura et al., 1993). As described previously (Kinoshita et al., 1995), HGF stimulates the growth of rat gastric epithelial cells, thereby probably contributing to mucosal regeneration. In contrast, IL-1β had no effect on epithelial cell growth. In addition, Schmassmann et al. (1997) reported that exogenous HGF fails to accelerate gastric ulcer healing but significantly enhances epithelial cell growth in rats. In the present study, we showed that growth-promoting activity toward epithelial cells is reduced with the decrease in HGF mRNA expression caused by inhibition of the IL-1 action. These results suggest that IL-1-induced HGF is involved in epithelial regeneration, but it remains unclear
IL-1 and Healing-Related Factors in Gastric Ulcers


Send reprint requests to: Satoru Takahashi, Ph.D., Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan. E-mail: takahasi@mb.kyoto-phu.ac.jp