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Inhibitory Effect of Macrophage-Derived Factors on the Recovery of Wounds Induced in Rat Gastric Epithelial Monolayers

Eiji Nakamura, Satoru Takahashi, Makoto Ishikawa and Susumu Okabe†*

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan

ABSTRACT. The effect of macrophage supernatant on the recovery of wounds induced in rat gastric epithelial RGM1 monolayers was investigated. The repair of wounds induced in the monolayers of RGM1 cells was accelerated time-dependently by 10 ng/mL of transforming growth factor-α (TGF-α). TGF-α also significantly stimulated DNA synthesis in RGM1 cells for 24 hr. Upon treatment of the cells with the macrophage supernatant, spontaneous and TGF-α-stimulated restoration was inhibited in a time- and concentration-dependent manner. After 24 hr, TGF-α-enhanced restoration was eliminated completely by the supernatant at 10^6 cells/mL. Similarly, the macrophage supernatant suppressed the spontaneous and TGF-α-stimulated DNA syntheses in a concentration-dependent manner. The macrophage supernatant at 10^6 cells/mL contained 0.4 ng/mL of interleukin-1β (IL-1β). Interleukin-1 receptor antagonist (IL-1RA) reversed the inhibition induced by the macrophage supernatant in a concentration-dependent manner. Nonetheless, pretreatment with IL-1RA had no effects on the spontaneous and TGF-α-stimulated DNA syntheses. Reverse transcription–polymerase chain reaction analysis revealed that RGM1 cells express mRNA for IL-1 receptor type 1, but not for type 2. These results indicate that macrophages can inhibit the spontaneous and TGF-α-stimulated recovery of wounds induced in gastric epithelial monolayers. The inhibitory effects of the supernatant are suggested to be partially mediated through a IL-1β/IL-1 receptor type 1 pathway. BIOCHEM PHARMACOL **58**;7:1221–1227, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. macrophage; wound repair; gastric epithelial cells; interleukin-1β; interleukin-1 receptors; growth factor

Gastric epithelial cell layers serve as a physical barrier against aggressive factors such as acid, pepsin, and *Helicobacter pylori* (*H. pylori*) [1]. During the healing process of gastric mucosal lesions, the migration and proliferation of the epithelial cells are stimulated to promptly reseal the wounded area [2]. It has been reported that such responses are promoted by growth factors, such as epidermal growth factor and TGF-α‡ [2–4]. These growth factors are produced locally around the wounds [2, 5, 6], and have been found to increase the proliferation and migration of gastric epithelial cells in *in vitro* experiments [7, 8]. Consequently, growth factors are considered to participate in the repair of the gastric mucosa.

Infiltrated macrophages/monocytes have been observed extensively in the gastric mucosa of patients with chronic gastritis and/or gastric ulcers [9–11]. IL-1β, which is produced mainly by macrophages/monocytes, plays an important role in regulating the inflammatory response [12]. We have reported previously that IL-1β inhibits the recovery of wounds enhanced by growth factors induced in gastric epithelial monolayers [13]. It follows that macrophages/monocytes may produce inhibitory factors that attenuate epithelial cell functions, and consequently delay the healing of the damaged mucosa.

In the present study, we examined whether macrophage supernatant inhibits the recovery of wounds induced in rat gastric epithelial monolayers. As a result, we found that macrophage supernatant potently impairs repair of the wound. In addition, potential participation of IL-1β in the inhibitory effects of the supernatant was also studied. IL-1RA is a naturally occurring IL-1 inhibitor that blocks the binding of IL-1 to its type I receptor (IL-1R1) and has been utilized for the elucidation of the role of endogenous IL-1 [12]. It follows that the effect of IL-1RA on the macrophage supernatant-induced inhibition of wound repair was also examined.

* Present address: Dr. Eiji Nakamura, Department of Surgery RW-875, Beth Israel Deaconess Medical Center-East, 330 Brookline Ave., Boston, MA 02215. Tel. (617) 667-3564; FAX (617) 975-5328; E-mail: enakamur@caregroup.harvard.edu

† Corresponding author.

‡ *Abbreviations:* TGF, transforming growth factor; IL, interleukin; IL-1RA, IL-1 receptor antagonist; LDH, lactate dehydrogenase; IL-1R1, IL-1 receptor type 1; IL-1R2, IL-1 receptor type 2; TNF, tumor necrosis factor; RT–PCR, reverse transcription–polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; and DMEM/F12, Dulbecco's modified Eagle's medium: Coon's modified Ham's F12 (1:1) medium.

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MATERIALS AND METHODS

Materials

Recombinant human TGF-α, TGF-β, and IL-1RA were purchased from Pepro Tech. Recombinant human IL-1β was provided by the Otsuka Pharmaceutical Co. The above agents were dissolved in Ca^{2+}, Mg^{2+}-free PBS containing 2.5 mg/mL of BSA. All other chemicals used in the study were of reagent grade.

RGM1 Cell Cultures

The rat gastric epithelial cell line RGM1, established by Dr. H. Matsui (Institute of Physical and Chemical Science (RIKEN) Cell Bank, Institute of Clinical Medicine, University of Tsukuba) [14, 15], was maintained in DMEM/F12 supplemented with heat-inactivated 20% fetal bovine serum (FBS; GIBCO BRL), 100 U/mL of penicillin, 100 U/mL of streptomycin, and 0.25 μg/mL of amphotericin B. Cells (8×10^4 cells in 0.5 mL of medium) were inoculated in 24-well plates (Corning Costar), cultured for 24 hr, and then starved for 24 hr in a culture medium (DMEM/F12 containing 0.25% FBS and 25 mM HEPES; pH 7.4) at 37° under 5% CO_2 in air.

Isolation of Rat Macrophages

Macrophages were isolated from the peritoneal cavity of male Wistar rats (Nihon SLC). Four days after an i.p. injection of 4% thioglycollate medium (Difco Laboratories), the exudate was collected by flushing the peritoneal cavity with PBS. The cells were centrifuged and washed with PBS, and subsequently suspended in a culture medium containing 10% FBS. After incubating the cells in 60-mm culture dishes for 2 hr at 37° under 5% CO_2 in air, the cells were washed three times with PBS to remove non-adherent cells. The adherent cells were incubated in a culture medium for 2 hr at 37° under 5% CO_2 in air. The procedure yielded 95% of macrophages, and almost all of the cells were viable by the trypan blue dye exclusion test. Finally, the medium was collected and centrifuged (400 *g* for 5 min at 4°), and the resulting supernatants were filtered through a 0.45-μm pore size membrane (Corning Costar). The samples were stored at −80° until used.

Restoration Assay

Epithelial restoration was assessed by the method of Watanabe *et al.* [16] with a slight modification. After the formation of a confluent RGM1 cell monolayer, a round artificial wound of constant size (a cell-free area, 2.5 mm^2) was induced in the center of the cell monolayer using a pencil-type mixer with a rotating silicon tip (Pencil mixer; luchi). Then the cells were washed with PBS, and the indicated agents were added into the wells. The restoration process was monitored at specified intervals under an inverted phase contrast microscope equipped with an auto-focusing photograph system (PM-10AD; Olympus). Change in the cell-free area was determined quantitatively with a planimeter (X-plan 360i; Ushikata).

Determination of DNA Synthesis

We have confirmed previously that there is a positive correlation between DNA synthesis and the number of cells [13]. Consequently, DNA synthesis was determined by means of the [^{3}H]thymidine incorporation assay. Briefly, RGM1 cells were incubated for 24 hr with the indicated agents and/or the vehicles in the presence of [^{3}H]thymidine (7.4 kBq, 2.22 to 3.2 TBq/mmol; Amersham). After washing the cells twice with 0.5 mL of 10% trichloroacetic acid, the cells were solubilized with 0.1 mL of 0.3 N NaOH at 37° for 20 min. The radioactivity (25 μL) in the lysate was measured with a liquid scintillation counter (Beckman Instruments Inc.). In the case of blocking the effects of IL-1β, cells were pretreated with IL-1RA 2 hr before the addition of the other agents.

Determination of Cell Viability

Cell viability was determined by the release of LDH. After treatment with the indicated agents and/or the vehicles for appropriate intervals at 37°, the culture medium was collected for the quantification of LDH activity released into the medium. The remaining cells were solubilized with culture medium containing 1% Triton X-100 for determination of intracellular LDH. LDH activity was determined spectrophotometrically by measuring the rate of the oxidation of NADH in the presence of pyruvate as described previously [17].

Messenger RNA Analysis by RT–PCR

Total cellular RNA was isolated from RGM1 cells and the placenta of female Wistar rats [18] by TRIZOL Reagent (GIBCO BRL). First strand complementary DNA was prepared from 5 μg of the total RNA using Moloney murine leukemia virus reverse transcriptase (RT; GIBCO BRL) according to the GIBCO BRL procedure. Rat L-1R1 primers were designed according to the EMBL database. The RNA sequence for the rat IL-1R2 has not been published. Thus, the IL-1R2 primers were designed based on the high sequence homology between mouse and human IL-1R2 sequences. PCR was performed using the following specific primers for rat IL-1R1 (sense: 5′-CAAGACCCCCATATCAGCGGA-3′, antisense: 5′-GGGTCAGCTTCCATCGCTTCAT-3′, PCR product: 534 bp), mouse IL-1R2 (sense: 5′-CCAGGAATACAACATCACTAGG-3′, antisense: 5′-GCCAGCGCAATGCTCCAGGA-3′, PCR product: 463 bp), and rat GAPDH (sense: 5′-CCAGTATGATTCTACCCACGGCAA-3′, antisense: 5′-ATACTTGGCAGGTTTCTCCAGGCG-3′, PCR product: 625 bp) [19] with a PCR thermal cycler (TaKaRa Biomedical). After sample denaturation at 94° for 1 min, PCR was

performed for 30 cycles consisting of denaturation at 94° for 1 min, annealing at 60° for 1 min, and extension at 72° for 1 min. The amplification was terminated by a 15-min final extension step at 72°. After aliquots of reactants were subjected to 2% agarose gel electrophoresis, the PCR products were visualized by ethidium bromide staining.

Determination of IL-1β and TGF-β Content in the Macrophage Supernatant

The amounts of IL-1β and TGF-β produced by macrophages after incubation for 2 hr were quantified by a sandwich ELISA (Biosource International for IL-1β and R & D Systems for TGF-β), as described by the manufacturers.

Statistical Analysis

Data are presented as means ± SEM for the cultures. Statistical differences were evaluated using Dunnett's multiple comparison test and Student's *t*-test, with a value of $P < 0.05$ regarded as significant.

RESULTS

Effect of Macrophage Supernatant on Restoration of RGM1 Cells

The sizes of the artificial wounds were nearly consistent among the wells. Immediately after induction of the wounds, the cells at the edges of the wounds in the vehicle-treated group began to form lamellipodia, which migrated toward the centers of the wounds. The sizes of the cell-free areas decreased with time after induction of the wound. The lamellipodia disappeared after complete recovery of the wounds. In the vehicle-treated group, the wounds were repaired gradually, and the areas of the wounds decreased to 62.8% of the initial values after 24 hr (Fig. 1A).

To examine the effects of macrophage-derived factors on the repair of wounds, the supernatant of macrophages incubated for 2 hr was prepared. Treatment with macrophage supernatant induced concentration-dependent suppression of the spontaneous recovery of the wounds after 12 hr. Significant inhibition by the supernatant at concentrations greater than 10^5 cells/mL was observed after 12 hr.

TGF-α at 10 ng/mL significantly promoted wound restoration, even after 6 hr (Fig. 1B). The cell-free area was reduced to 16.2% 24 hr after induction of the wounds. Treatment with the macrophage supernatant caused an inhibition of TGF-α-stimulated restoration in a concentration-dependent manner. Even after 6 hr, the supernatant from 5×10^4 cells/mL, and more, significantly inhibited the restoration enhanced by TGF-α. After 24 hr, the macrophage supernatant at concentrations greater than 10^5 cells/mL completely eliminated TGF-α-stimulated restoration. The cell-free area was larger than that observed in the vehicle group.

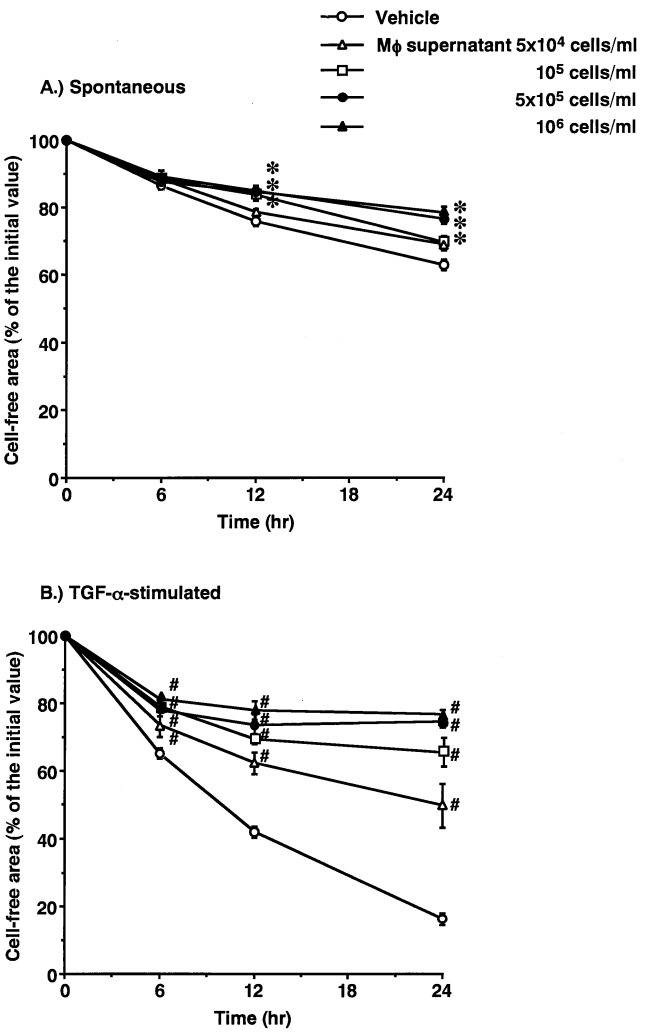

FIG. 1. Concentration-dependent effect of the macrophage supernatant on spontaneous (A) and TGF-α-stimulated (B) restoration of wounds induced in the monolayers of RGM1 cells. After the induction of the wounds, cells were incubated with the indicated concentrations of the macrophage supernatant in the presence and absence of TGF-α (10 ng/mL). It should be noted that the macrophage supernatant suppressed both the spontaneous and the stimulated restoration of wounds in a concentration-dependent manner. Data are presented as means ± SEM (N = 4). The initial cell-free area was 2.5 ± 0.2 mm². Mϕ indicates macrophage. Key: (*, #) statistically significant differences from the vehicle in spontaneous and in TGF-α-stimulated, respectively, at $P < 0.05$.

Effect of Macrophage Supernatant on DNA Synthesis of RGM1 Cells

The macrophage supernatant both with and without 10 ng/mL of TGF-α had no effect on RGM1 cell viability compared with the vehicle or TGF-α alone, as judged by LDH activity. However, the macrophage supernatant suppressed the spontaneous increase in DNA synthesis in a concentration-dependent manner (Fig. 2). Significant inhibition by the supernatant was observed at more than 5×10^4 cells/mL. Upon treatment of the cells with TGF-α at 10 ng/mL for 24 hr, the DNA synthesis was enhanced signif-

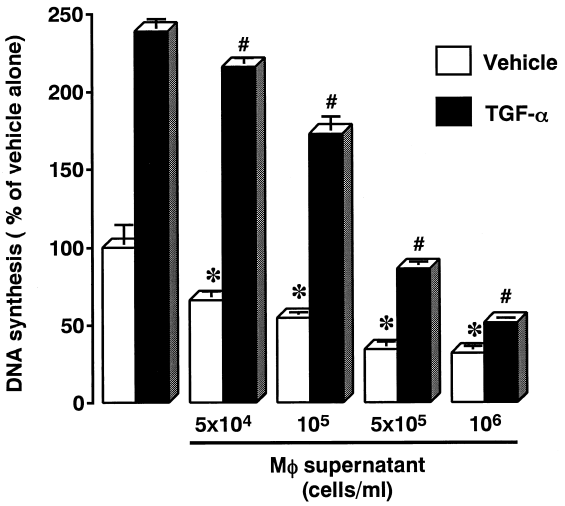

FIG. 2. Effect of the macrophage supernatant on spontaneous and TGF-α-stimulated DNA syntheses by RGM1 cells. Cells were incubated with the indicated concentrations of the macrophage supernatant in the presence and absence of TGF-α (10 ng/mL) for 24 hr. DNA synthesis was determined. It should be noted that the macrophage supernatant inhibited both the spontaneous and the stimulated DNA synthesis in a concentration-dependent manner. Data are presented as means ± SEM (N = 4). The absolute value was 6839 ± 807 dpm in the vehicle group. Key: (*, #) statistically significant differences from the vehicle and TGF-α alone, respectively, at $P < 0.05$.

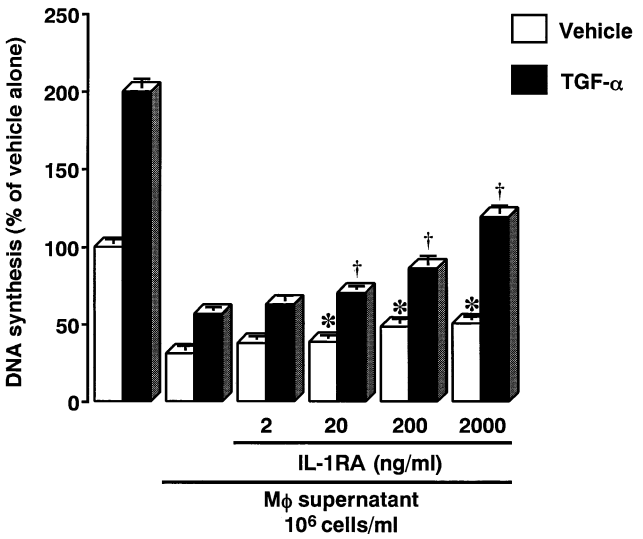

FIG. 3. Effect of IL-1RA on the inhibitory action of the macrophage supernatant on spontaneous and the TGF-α-stimulated DNA syntheses by RGM1 cells. Cells were preincubated with the indicated concentrations of IL-1RA, and then incubated with 10^6 cells/mL of the macrophage supernatant in the presence and absence of TGF-α (10 ng/mL) for 24 hr. DNA synthesis was determined. Data are presented as means ± SEM (N = 4). The absolute value was 4655 ± 99 dpm in the vehicle group. Key: (*, †) statistically significant differences from the macrophage supernatant alone and the macrophage supernatant with TGF-α, respectively, at $P < 0.05$.

icantly (approximately 2.5-fold). The stimulated DNA synthesis also was inhibited by the supernatant in a concentration-dependent manner. A significant inhibitory effect by the supernatant was observed even at 5×10^4 cells/mL; the effect was augmented further at higher concentrations. At 10^6 cells/mL, both the spontaneous and the TGF-α-stimulated DNA syntheses were suppressed to 31.8 and 21.3% of the corresponding control values, respectively.

Effects of IL-1RA on Inhibition Induced by IL-1β and Macrophage Supernatant

The macrophage supernatant at 10^6 cells/mL contained 412.2 ± 0.2 ng/mL of IL-1β, as determined by ELISA. We confirmed that exogenous IL-1β at 0.4 ng/mL inhibits the TGF-α-stimulated restoration after 24 hr. The suppression was 50.7%. In addition, treatment with 0.4 ng/mL of IL-1β caused significant inhibition (62.1%) of the TGF-α-stimulated DNA synthesis. Thus, using IL-1RA, we examined whether the IL-1β secreted from macrophages participates in the inhibitory effects of the supernatant on the restoration and DNA synthesis. IL-1RA blocked the inhibitory effects of IL-1β at 0.4 ng/mL in a concentration-dependent manner. At 2000 ng/mL, IL-1RA completely eliminated the inhibitory effect of 0.4 ng/mL of IL-1β on DNA synthesis.

The macrophage supernatant-induced inhibition of the spontaneous and the TGF-α-stimulated DNA syntheses was ameliorated by IL-1RA in a concentration-dependent manner (Fig. 3). A significant recovery of DNA synthesis was obtained in both the spontaneous and the TGF-α-stimulated condition by treatment with IL-1RA at more than 20 ng/mL. IL-1RA at 2000 ng/mL restored DNA synthesis by 19.9 and 62.5% in the spontaneous and the TGF-α-stimulated conditions, respectively.

IL-1RA at 2000 ng/mL also blocked the macrophage-induced inhibition of the wound repair (Fig. 4). In the spontaneous condition, complete recovery was observed throughout 24 hr. In the TGF-α-stimulated condition, however, the recovery was complete within 6 hr, yet after 12 hr IL-1RA failed to completely eliminate the inhibitory effect of the macrophage supernatant. The recovery was approximately 70% after 24 hr. In both the spontaneous and the TGF-α-stimulated conditions, pretreatment with IL-1RA at 2000 ng/mL, in the absence of the supernatant, did not affect the restoration or DNA synthesis.

Effect of TGF-β on DNA synthesis of RGM1 Cells

The macrophage supernatant at 10^6 cells/mL contained 8.1 ± 2.2 pg/mL of TGF-β, as determined by ELISA. Exogenous TGF-β at 0.01 ng/mL did not suppress the spontaneous and the TGF-α-stimulated DNA syntheses by RGM1 cells. In addition, TGF-β at 0.01 ng/mL did not facilitate IL-1β (0.4 ng/mL)-induced inhibition of the spontaneous or TGF-α-stimulated DNA synthesis.

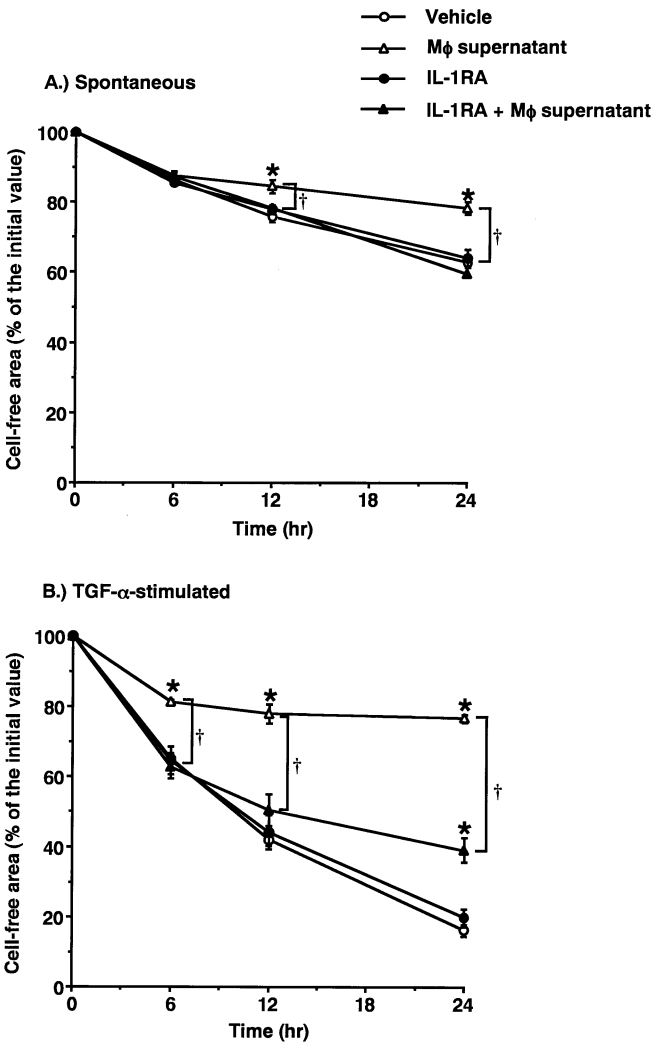

FIG. 4. Effect of IL-1RA on the inhibitory action of the macrophage supernatant on the the spontaneous (A) and TGF-α-stimulated (B) restoration of wounds induced in the monolayers of RGM1 cells. Cells were preincubated with 2000 ng/mL of IL-1RA, and then incubated with 10^6 cells/mL of the macrophage supernatant in the presence and absence of TGF-α (10 ng/mL) for 24 hr. Data are presented as means ± SEM (N = 4). The initial cell-free area was 2.5 ± 0.2 mm^2. Key: (*, †) statistically significant differences from the vehicle and the macrophage supernatant, respectively, at $P < 0.05$.

Expression of IL-1R1 Messenger RNA in RGM1 Cells

Figure 5 illustrates the RT–PCR analysis for the expression of mRNA for the two types of IL-1 receptors. Rat placenta was used as a positive control for the expression of IL-1R1 and IL-1R2 mRNA. The products for IL-1R1 and IL-1R2 were 534 and 463 bp, respectively, the sizes of which are the same as those predicted. RGM1 cells constitutively expressed mRNA for IL-1R1. In contrast, IL-1R2 mRNA was not detected in RGM1 cells.

DISCUSSION

In the present study, we clearly demonstrated that a macrophage supernatant inhibits both restoration and DNA synthesis by RGM1 cells. It should be noted that the inhibition was observed even under the TGF-α-stimulated condition. The concentration of TGF-α used for the study is considered to be within the physiological range reported recently [20]. Both Watanabe *et al.* [16] and this laboratory [13] previously reported that the recovery of round wounds induced in gastric epithelial cell monolayers consists of cell migration during the early phase, followed by cell proliferation. In addition, the stimulatory effects of TGF-α have been observed after 6 hr in the case of migration and after 12 hr in the case of proliferation [13]. In this study, the inhibition of TGF-α-stimulated restoration by macrophage supernatant was even observed after 6 hr. The macrophage supernatant thus delayed the recovery of wounds by inhibiting not only proliferation but also migration by RGM1 cells. It is well known that growth factors participate in the repair of gastric mucosal injury and gastric homeostasis by promoting epithelial proliferation and migration [2–8]. In addition, in the gastric mucosa of patients with chronic gastric diseases, the infiltration of macrophages has been observed frequently [9–11]. Consequently, it could reasonably be suggested that macrophages play a role in the destruction of the gastric epithelial repair system during chronic inflammation.

It has been reported previously that macrophages secrete cytotoxic factors, such as reactive oxygen species [21]. Accordingly, it remains possible that the cytotoxic factors are contained in the prepared supernatant. Nonetheless, in the present study, 24-hr treatment with the macrophage supernatant did not cause release of LDH into the medium in either the presence or absence of TGF-α. Consequently, inhibition of the restoration and proliferation does not result from cytotoxicity induced by the supernatant. In the preliminary experiment, after RGM1 cells had been pretreated with macrophage supernatant for 6 hr and then washed, they were incubated with TGF-α for 24 hr. There were no significant differences of DNA syntheses between the above described cells and the cells treated with both the supernatant and TGF-α. Therefore, it is also ruled out that the inhibition of TGF-α-stimulations induced by macrophage supernatant might be due to the degradation of TGF-α by proteases released from the macrophages.

We have already reported that IL-1β inhibits TGF-α-stimulated restoration and proliferation by RGM1 cells [13]. In addition, it has been shown that IL-1β is produced primarily by macrophages/monocytes in inflammation [12]. In this study, measurement by ELISA revealed the presence of 0.4 ng/mL of IL-1β in the supernatant of macrophages. We reconfirmed that the concentration of IL-1β in the supernatant is sufficient to inhibit the restoration and proliferation by RGM1 cells [13]. Consequently, it was speculated that IL-1β secreted from cultured macrophages might participate in the inhibitory effects of the supernatant. IL-1RA exhibited a concentration-dependent recovery from inhibition of the restoration and DNA synthesis induced by the macrophage supernatant. However, IL-1RA did not affect the spontaneous and TGF-α-stimulated

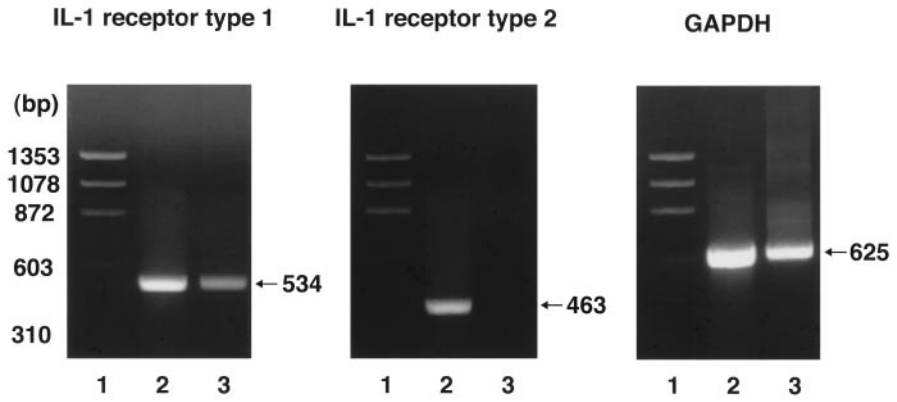

FIG. 5. RT–PCR analysis of IL-1R1 and IL-1R2 mRNA expression in RGM1 cells and rat placenta. The numbers below the figures indicate (1) marker, (2) placenta, and (3) RGM1 cells. Arrows indicate the expected sizes of the PCR products for IL-1R1 (534 bp), for IL-1R2 (463 bp), and for GAPDH (625 bp). The values on the left indicate the sizes of the standard marker.

restoration and proliferation in the absence of the macrophage supernatant. These results indicate that IL-1 is involved in the inhibitory effects of the macrophage supernatant.

The blocking effects of IL-1RA on the macrophage supernatant-induced inhibition of the restoration and DNA synthesis were inadequate even at 2000 ng/mL. In contrast, IL-1RA at the same concentration completely eliminated the effects of IL-1β (0.4 ng/mL). These results suggest that there might exist macrophage-derived inhibitory factors other than IL-1β. It is known that macrophages produce IL-1α as well as IL-1β and that IL-1RA also blocks the effects of IL-1α [22, 23]. It is reasonable to suggest that IL-1α may also be involved in the inhibitory effects of the macrophage supernatant. Yet, there are several differences between the effects of IL-1α and IL-1β [12]. It is necessary to examine whether IL-1α exerts inhibitory effects similar to those of IL-1β on RGM1 cells. Alternatively, inhibitory factors other than IL-1β may be present in the macrophage supernatant. For example, TGF-β is reported to suppress the proliferation of rabbit gastric epithelial cells [7, 8]. In this study, it was shown that the macrophage supernatant contained 0.01 ng/mL of TGF-β. However, exogenous TGF-β at 0.01 ng/mL did not suppress the spontaneous or the TGF-α-stimulated DNA synthesis by RGM1 cells. In addition, TGF-β did not facilitate IL-1β-induced inhibition of the spontaneous or TGF-α-stimulated DNA synthesis. Therefore, it is excluded that TGF-β may be involved in the inhibitory effects of macrophage supernatant. It is well known that the biological effects of TNF-α, which is also produced by macrophages, are similar to those of IL-1β [12, 21]. However, TNF-α exhibited no inhibitory effects on the restoration or proliferation by RGM1 cells (unpublished data). Therefore, it is unlikely that TNF-α participates in the inhibitory effects of the macrophage supernatant. It is also conceivable that the factors that strengthen IL-1β-induced inhibition may be released from macrophages. Further studies are needed to elucidate factors other than IL-1β involved in the inhibitory effects of macrophage supernatant.

In this study, we found that gastric epithelial cells express mRNA for IL-1R1, but not for IL-1R2. These results strongly suggest that the inhibitory effects of IL-1, which is released from macrophages, are mediated via IL-1R1 on RGM1 cells. This is consistent with the result that the inhibitory effects of the macrophage supernatant were attenuated by treatment with IL-1RA, since IL-1RA prefers IL-1R1 to IL-1R2 [24].

It has been reported that *H. pylori*-derived factors potently stimulate IL-1β production by monocytes/macrophages prepared from the lamina propria of the gastric mucosa and blood [25, 26]. In addition, it has also been reported that mucosal levels of IL-1β are significantly higher in *H. pylori*-infected patients than in *H. pylori*-negative patients [27, 28]. Therefore, it is reasonable to suggest that the IL-1β locally produced by macrophages may play an important role in the development of *H. pylori*-induced gastric inflammation. Further studies on gastrointestinal macrophages and *H. pylori*-derived factors are needed.

RGM1 cells are derived from the gastric mucosa of normal rats and exhibit characteristics of gastric epithelial cells [14, 15]. In addition, we confirmed that IL-1β inhibits the growth factor-stimulated proliferation of gastric epithelial cells isolated from rabbit stomachs (manuscript in preparation). Thus, it would appear that the inhibitory effects of IL-1β may be generally applied to gastric epithelial cells.

In summary, this study demonstrated that macrophages can inhibit the spontaneous and TGF-α-stimulated recovery of wounds induced in gastric epithelial monolayers. The effects of the macrophage supernatant are due to the inhibition of both cell migration and proliferation, and appear to be partly mediated through an IL-1β/IL-1R1 pathway.

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