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Human recombinant interleukin-1α-mediated stimulation of procollagenase production and suppression of biosynthesis of tissue inhibitor of metalloproteinases in rabbit uterine cervical fibroblasts

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Influence of human recombinant interleukin-1 (hrIL-1) on collagen metabolism was investigated with rabbit uterine cervical fibroblasts. Enzyme-linked immunosorbent assays for collagenase and tissue inhibitor of metalloproteinases (TIMP) indicated that hrIL-1 participates in both stimulation of procollagenase production and suppression of TIMP synthesis by uterine cervical cells. IL-1 did not modulate collagen synthesis. In addition, the sensitivity to IL-1 of uterine cervix from ovariectomized rabbits was augmented by estradiol-17β treatment. Thus it is proposed that IL-1 accelerates collagenolysis in the cervical tissue and its effect on uterine cervix is hormonally regulated.

Recombinant interleukin-1α; Procollagenase; Tissue inhibitor of metalloproteinase; Estradiol-17β; (Rabbit uterine cervix)

1. INTRODUCTION

Interleukin-1 (IL-1) is known to exert numerous biological effects on various target cells [1]. In addition to lymphocyte proliferation activity, IL-1 has been reported to promote the production of collagenase [2–4], other matrix metalloproteinases [5] and plasminogen activator [6,7] from synovial fibroblasts or chondrocytes. Therefore IL-1 is considered to play an important role in the destruction process of articular cartilage in arthritis. Recently we have reported that uterine cervical fibroblasts from pregnant rabbits also retain sensitivity to IL-1-like factors in macrophage-conditioned medium and produce a large amount of collagenase in response to the conditioned medium [8,9]. However, at present, the precise influence of IL-1 on the collagenase production has not been clarified. This is due to the fact that collagenase and its endogenous inhibitor (TIMP) are coordinately secreted from cells [2,10–12], and in most reports, the effect of IL-1 on collagenase production has been studied simply by measuring apparent collagenase activity without consideration of the amount of TIMP produced by the cells. In order to investigate the influence of IL-1 on collagenase and TIMP production by rabbit uterine cervix more precisely, we have measured the actual amount of collagenase and TIMP proteins produced using specific ELISA for each component.

Here, we report that hrIL-1 accelerates the production of collagenase and simultaneously depresses the biosynthesis of TIMP in rabbit uterine cervical fibroblasts, and that the sensitivity

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Abbreviations: hrIL-1, human recombinant interleukin-1α; FCS, fetal calf serum; MEM, Eagle's minimum essential medium; DMEM, Dulbecco's modified Eagle's medium; TIMP, tissue inhibitor of metalloproteinases; LAH, lactalbumin hydrolysate; ELISA, enzyme-linked immunosorbent assay

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of the rabbit uterine cervix to IL-1 is modulated by estrogen.

2. MATERIALS AND METHODS

MEM and DMEM (4.5 g glucose/l) were purchased from Grand Island Biological, Co., Grand Island, NY, USA. FCS was obtained from Boehringer-Mannheim, GmbH, Mannheim, FRG. hrIL-1 (1×10^7 units/mg) was kindly donated by Dr M. Yamada, Research Laboratories, Dainippon Pharmaceutical Co., Suita, Osaka, Japan. Other reagents used were of analytical reagent grade.

2.1. *Culture of uterine cervical fibroblasts and uterine cervical explants*

Nippon white rabbits were used for all experiments in this series. Uterine cervical fibroblasts were established from rabbits at 23-days gestation and maintained in a culture of MEM/10% (v/v) FCS as described previously [8,13]. For the collagenase and TIMP production, the culture medium was changed to MEM/0.2% (w/v) LAH after confluence. Uterine cervical explants from non-pregnant and ovariectomized rabbits were maintained in DMEM as described previously [9,14]. The culture media harvested from cell and explant cultures were centrifuged at $1300 \times g$ and then stored at $-20°C$ until use.

2.2. *Assay for collagenase activity*

Collagenase was assayed by the fibril assay using [^{14}C]acetylated collagen and the total collagenase activity was determined by the activation of procollagenase with trypsin as described previously [13]. One unit of collagenase hydrolyzes 1 μg substrate/min at 37°C.

2.3. *ELISA for procollagenase and TIMP*

Sheep anti-(rabbit synovial procollagenase) antibody was prepared as described previously [15]. Procollagenase complexed with the antibody was determined by using alkaline phosphatase conjugated donkey anti-(sheep IgG)IgG in accordance with standard procedures of ELISA. TIMP was also determined by a sandwich enzyme immunoassay set up with a pair of the monoclonal antibodies prepared against bovine dental pulp collagenase inhibitor [16] which was recently proved to be a TIMP [17]. Rabbit TIMP content was calculated using a standard curve for the bovine form, assuming that they both had the same affinity for antibodies.

2.4. *Incorporation of [^{3}H]proline into collagen and [^{3}H]thymidine uptake by DNA*

Radiolabeling and determination of the radiolabeled collagen were carried out as described previously [18]. Incorporation of [^{3}H]thymidine into DNA was also determined as described in the previous paper [18].

3. RESULTS AND DISCUSSION

When the uterine cervical fibroblasts from pregnant rabbits were treated with various amounts of hrIL-1, an apparent collagenase activity in culture media increased in a dose-dependent manner (fig.1A). The maximal effect was observed at the concentration around 0.5 ng/ml. ELISA for procollagenase demonstrated that hrIL-1 stimulated the procollagenase production in a similar dose-dependent manner, although the increase of procollagenase protein was less than that in the collagenase activity (fig.1B). Higher apparent collagenolytic activity detected in the culture media may be due to synergistic effects of collagenase and other gelatinolytic metalloproteinases coordinatedly secreted from the cells as reported by others [19,20] as well as due to the decreased syn-

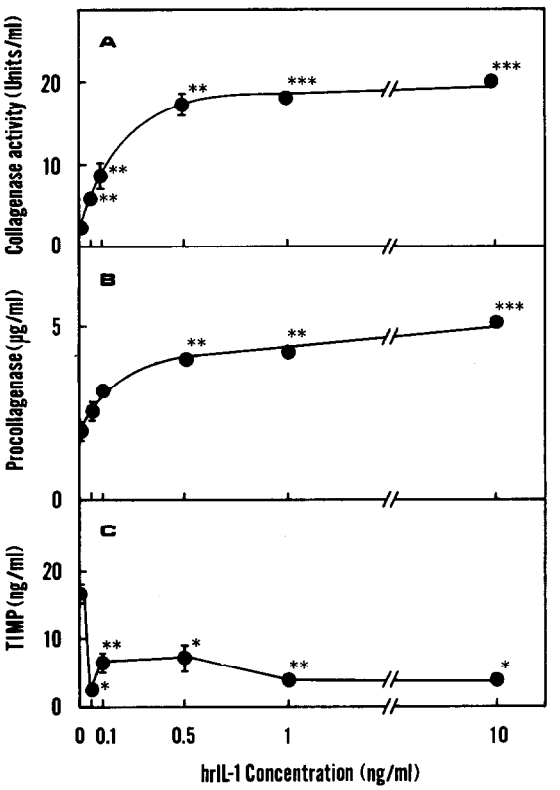

Fig.1. Effect of hrIL-1 on the production of procollagenase and TIMP by rabbit uterine cervical fibroblasts. Cervical fibroblasts from pregnant rabbit were maintained to the confluence as described in the text, and then cells were treated with hrIL-1 in 0.2% (w/v) LAH/MEM. Aliquots (100 μl) of the medium accumulated from 3 to 5 days culture were assayed for collagenolytic activity, collagenase protein and TIMP as described in the text. Data are represented as the mean ± SD of 3 dishes. *, ** and ***, significantly different from control ($p <$ 0.05, 0.01 and 0.001, respectively). (A) Collagenolytic activity; (B) collagenase protein and (C) TIMP.

thesis of TIMP (see below). Nevertheless, the results clearly indicated that hrIL-1 directly modulates the biosynthesis of procollagenase.

On the other hand, the treatment of cervical fibroblasts with hrIL-1 suppressed the production of TIMP to about 20% of the control as shown in fig.1C. With human fetal lung fibroblasts, however, IL-1 has been shown to increase the biosynthesis of TIMP in parallel to procollagenase production [2]. This discrepancy may be due to the difference in species of cell types, i.e., rabbit uterine cervical vs. human fetal lung fibroblasts.

It has been reported that IL-1 regulates the proliferation of fibroblasts [21], but its precise effect has not been clarified yet since the IL-1 preparation used was a partially purified material. We examined, therefore, the influence of hrIL-1 on the proliferation of cervical fibroblasts by measuring the incorporation of [^{3}H]thymidine into DNA. As shown in table 1, hrIL-1 did not exert any effect on the proliferation of cervical fibroblasts both at growth and stationary phases. A similar observation was reported with rat costal chondrocytes [22].

During the uterine cervical ripening and dilation, most obvious changes are the increase in tissue collagenase and decrease in collagen concentration [23–25]. Therefore, we examined whether such physiological changes are attributed to the increased collagenase production and/or to the modulation of collagen biosynthesis. The effect of hrIL-1 on collagen synthesis and secretion was measured by incorporation of [^{3}H]proline to bacterial collagenase-sensitive intracellular and extracellular proteins. Table 2 shows that hrIL-1 did not alter either synthesis or secretion of collagen in cervical fibroblasts. Neither was noncollagenous protein synthesis affected by hrIL-1 treatment.

Regulation of sensitivity of rabbit uterine cervix against IL-1 was also examined by using cervical explant cultures of ovariectomized, ovariectomized-estrogen primed and non-pregnant rabbits. As shown in fig.2, hrIL-1 stimulated all three groups of explants to produce increased amounts of collagenase activity. However, the increased levels of collagenase activity in ovariectomized rabbit primed with estradiol-17β were 3-times greater than those of ovariectomized- or non-pregnant rabbits. Another set of experiments confirmed the effect of estradiol-17β on IL-1 sensitivity. The cervices from ovariectomized-estrogen-primed rabbits were effectively stimulated by

Table 1

Effect of hrIL-1 on the proliferation of rabbit uterine cervical fibroblasts

Group	[^{3}H]Thymidine incorporation into DNA (dpm × 10^{-3}/well)
Expt I (growth phase)	
Control	7.7 ± 0.6
hrIL-1	8.6 ± 1.5
Expt II (stationary phase)	
Control	35.8 ± 3.9
hrIL-1	37.5 ± 2.7

Uterine cervical fibroblasts from rabbit with 23 days gestation were suspended at densities of 8×10^4 cells/1.5 ml of 10% (v/v) FCS/MEM and plated in 35 mm dish. Expt I: 2 days later, the medium was changed to 1% (v/v) FCS/MEM and cells were treated with hrIL-1 (50 ng/ml) for 2 days and then labeled with 1 μCi/ml of [^{3}H]thymidine for final 3 h. Expt II: Cells were grown in 10% (v/v) FCS/MEM to confluence, then cells were treated with hrIL-1 and labeled with [^{3}H]thymidine as in expt I. Data are shown as the mean ± SD of 4 dishes

Table 2

Effect of hrIL-1 on the synthesis of collagen by rabbit uterine cervical fibroblasts

	hrIL-1 (ng/ml)	Collagen (dpm × 10^{-4}/dish)	Noncollagenous protein (dpm × 10^{-4}/dish)	Ratio of collagen (%)
Cell:				
Control	–	3.5 ± 0.3	39.4 ± 1.8	1.62 ± 0.08
hrIL-1	50	3.5 ± 0.1	40.6 ± 2.0	1.57 ± 0.08
	100	3.3 ± 0.1	37.5 ± 1.3	1.58 ± 0.04
Medium:				
Control	–	5.0 ± 0.2	7.4 ± 0.1	11.23 ± 0.57
hrIL-1	50	5.6 ± 0.1	7.1 ± 0.1	13.27 ± 0.59
	100	5.0 ± 0.1	6.1 ± 0.2	13.04 ± 0.53

Cervical fibroblasts were suspended and plated as described in table 1, and grown to the confluence in 10% (v/v) FCS/MEM, and then cells were treated with hrIL-1 for 2 days. The medium was then changed to serum-free/MEM/0.1 mM ascorbate/0.5 μM β-aminopropionitrile containing hrIL-1 and cells were labeled with 7 μCi/ml of [^{3}H]proline for 4 h. Collagen and noncollagenous protein were determined by the digestion with purified bacterial collagenase. Ratio of collagen was calculated with the assumption that the collagen has an imino acid content 5.4-fold higher than that of other proteins. Data shown are as the mean ± SD of 4 dishes

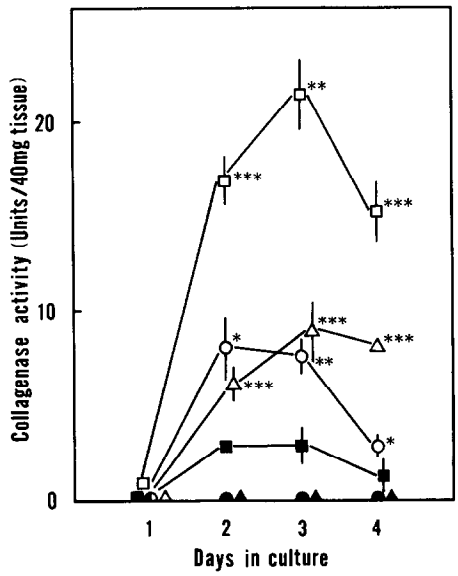

Fig.2. Effect of estradiol-17β priming in vivo on hrIL-1-dependent increase in collagenase production by rabbit uterine cervix. Rabbits were bilaterally ovariectomized and after 4.5 months of the ovariectomy they were primed with estradiol-17β (100 μg/rabbit, sc) once daily for 7 days. On the eighth day cervical tissue was removed and cultured in DMEM as described in the text. Tissue explants were cultured with or without hrIL-1 (50 ng/ml) in media changed daily for 4 days. Aliquots (100 μl) of the medium were assayed for collagenase activity. Data are shown as the mean ± SD of 3 dishes. *, ** and ***, significantly different from each control ($p < 0.05$, 0.01 and 0.001, respectively). (○) or (●), ovariectomized rabbit with or without hrIL-1; (□) or (■), ovariectomized rabbit primed with estradiol-17β with or without hrIL-1; and (△) or (▲), non-pregnant rabbit with or without hrIL-1.

hrIL-1 and produced a large amount of collagenase, but in this experiment ovariectomized rabbits without estrogen-priming did not retain the sensitivity to IL-1 (not shown). These results suggest that the sensitivity against IL-1 in the uterine cervix is hormonally controlled by estradiol-17β. Biological effects of IL-1 are considered to be expressed via the binding of IL-1 to receptors, and IL-1 receptors have been found in various IL-1 target cells [26,27]. Although the exact mechanism is yet to be investigated, the expression of cell surface IL-1 receptors in uterine cervix may be regulated by estrogen.

In conclusion, IL-1 participates in collagenolysis in rabbit uterine cervix by stimulating collagenase production and suppressing TIMP synthesis. The sensitivity to IL-1 of this tissue was augmented by estradiol-17β, suggesting that degradation of collagen during cervical ripening and dilation is hormonally regulated.

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