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Human recombinant interleukin-1α increases biosynthesis of collagenase and hyaluronic acid in cultured human chorionic cells

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The influence of human recombinant interleukin-1α (hrIL-1) on biosynthesis of collagenase and glycosaminoglycans was investigated with fibroblast-like cells of human chorionic membrane. hrIL-1 stimulated cells to produce procollagenase in a dose-dependent manner. Furthermore, it similarly accelerated both biosynthesis and secretion of hyaluronic acid in chorionic cells, but did not modulate the biosynthesis of sulfated glycosaminoglycans. Therefore, the relative concentration of hyaluronic acid vs total glycosaminoglycans increased significantly. These results are connected with the decrease in tensile strength observed in ruptured fetal membranes. Thus, it is proposed that IL-1 from effused leukocytes in fetal membranes plays an important role in connective tissue metabolism, especially in premature rupture of membranes with chorioamnionitis.

Interleukin-1α, recombinant; Collagenase; Collagen; Hyaluronic acid; (Human chorionic cell)

1. INTRODUCTION

Interleukin-1 is known to exert a number of biological effects in vivo and in vitro [1]. It has been shown to be one of the inflammatory mediators [1] and to participate in connective tissue matrix catabolism [2]. The degradation of extracellular matrix components induced by IL-1 is believed to be due to the accelerated production of proteolytic enzymes by connective tissue cells, especially synovial fibroblasts and chondrocytes [3–5]. Different effects of IL-1, however, on the biosynthesis of glycosaminoglycans are observed in those cells [6,7]. Furthermore, the precise influence of IL-1 on the connective tissue metabolism in the other cells has not been fully understood.

Chorioamniotic membranes are known to consist mainly of collagen [8] and glycosaminoglycans [9], and these connective tissue components are considered to maintain the elasticity of the membranes [10]. Therefore, it is supposed that profound biochemical changes in the connective tissue of membranes are closely connected with a reduction in elasticity of prematurely ruptured fetal membranes and also of normally ruptured ones at term pregnancy. However, at present, the precise mechanism for the reduction of tensile strength of those membranes has not been clarified. Maternal leukocytes might be a major cause of this reduction, since effusion of maternal ones is frequently observed on prematurely ruptured fetal membranes [12,13] and normally ruptured ones at term pregnancy [14]. In order to clarify the correlation between leukocytes and the reduction of tensile strength of the above membranes, we have examined the effect of a leukocyte factor, IL-1, on the connective tissue metabolism in human chorionic cells.

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Abbreviations: IL-1, interleukin-1; hrIL-1, human recombinant interleukin-1α; MEM, Eagle's minimum essential medium; FBS, fetal bovine serum; PBS(−), Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline; HBSS(−), Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution

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Here, we report that hrIL-1 enhances the production of collagenase and hyaluronic acid in human chorionic cells, and that IL-1 plays an important role in connective tissue metabolism of fetal membranes.

2. MATERIALS AND METHODS

The following reagents were obtained commercially: MEM from Grand Island Biological (Grand Island, NY); FBS from Whittaker M.A. Bioproducts (Walkersville, MD); D-[6-^{3}H]glucosamine hydrochloride (22.6 Ci/mmol) from The Radiochemical Center (Amersham, Bucks, England); chondroitin AC lyase (EC 4.2.2.5) and chondroitin ABC lyase (EC 4.2.2.4) from Seikagaku Kogyo (Tokyo). hrIL-1 (2×10^7 U/mg) was kindly donated by Dainippon Pharmaceutical Co. (Suita, Osaka). Other reagents used were the same as described [15].

2.1. *Culture of human chorionic cells*

Human fetal membranes were collected at delivery from term pregnancies. The chorionic membrane was separated manually from the amniotic membranes and washed sufficiently with PBS(−)/penicillin G (200 U/ml) and streptomycin (200 μg/ml). Chorionic cells were prepared and cultured as described for uterine cervical cells of rabbits [15,16]. The membranes were digested with bacterial collagenase in HBSS(−)/antibiotics for 20 min at 37°C. The digest was passed through a nylon mesh and cells were then collected by low-speed centrifugation and washed twice with HBSS(−) followed by MEM/10% (v/v) FBS/antibiotics. The cells were maintained to confluency in a culture of MEM/10% (v/v) FBS under 5% CO_2-95% air at 37°C. In this series of experiments, human chorionic cells were used at passage level 1–6.

2.2. *Collagenase production*

Subcultured human chorionic cells were suspended in MEM/10% (v/v) FBS, placed in 35-mm Corning plastic dishes (1.5 ml/dish, 5×10^4 cells) and maintained to confluency. For production of collagenase, the culture medium was changed to MEM/0.2% (w/v) lactalbumin hydrolysate. The culture media harvested were centrifuged at $1300 \times g$ and then stored at −20°C until use.

2.3. *Biosynthesis of glycosaminoglycans by human chorionic cells*

Confluent chorionic cells in 35-mm plastic dishes were incubated with 10 μCi D-[6-^{3}H]glucosamine hydrochloride for 1–24 h in a culture medium of MEM/antibiotics. The incubation medium was removed from each dish and cells on the dish washed twice with PBS(−). The medium and washes were combined and designated as the medium fraction. The residue (cell fraction) was treated with 0.5 M NaOH for 24 h at room temperature and then collected. Extraction of glycosaminoglycans from each fraction, digestion of glycosaminoglycans with chondroitin AB lyase and ABC lyase, and estimation of unsaturated disaccharides were performed as in [17].

2.4. *Assay for collagenase activity*

Collagenase was assayed by the fibril assay using [^{14}C]-acetylated collagen and total collagenase activity determined by the activation of procollagenase with trypsin as in [15]. One unit of collagenase hydrolyzes 1 μg substrate/min at 37°C.

3. RESULTS

When confluent human chorionic cells were treated with various amounts of hrIL-1, cells produced much collagenase activity as a latent form and the apparent collagenase activity in culture media increased in a dose-dependent manner as shown in fig.1. Untreated cells, however, did not produce any collagenolytic activity. The maximal effect of hrIL-1 was observed at around 0.25 ng/ml. On the other hand, measuring the synthesis of [^{3}H]proline-labeled macromolecules indicated that hrIL-1 did not modulate the syntheses of collagen and non-collagenous protein in chorionic cells (not shown).

When confluent chorionic cells were incubated with D-[6-^{3}H]glucosamine, cells secreted about 75% of the synthesized glycosaminoglycans into the medium, and the total radioactivity of glycosaminoglycans synthesized increased approximately linearly throughout the incubation period (up to 24 h, not shown).

hrIL-1 enhanced significantly the accumulation

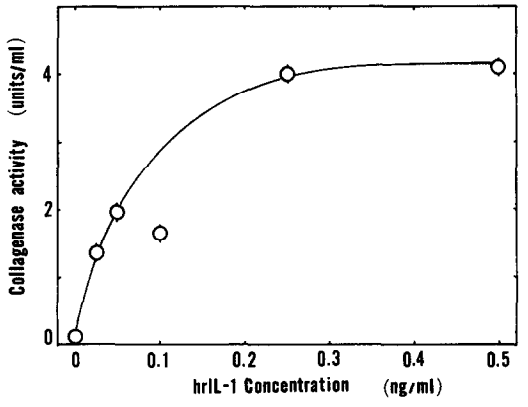

Fig.1. Effect of hrIL-1 on the production of collagenolytic activity by human chorionic cells. Chorionic cells at the fourth passage were maintained to confluence as described in the text and cells were then treated with hrIL-1 in 0.2% (w/v) lactalbumin hydrolysate/MEM for 48 h. Aliquots (100 μl) of the medium were assayed for total collagenolytic activity as described in the text. Data are represented as means ± SD of 4 dishes.

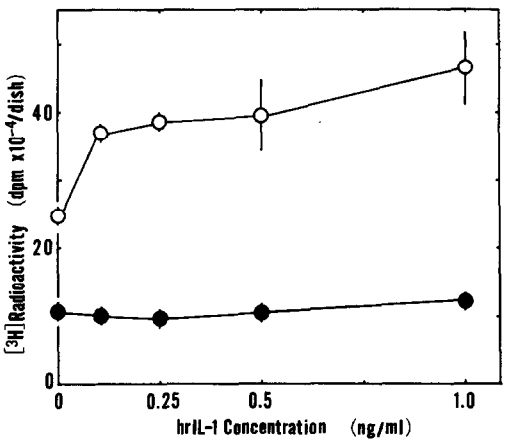

Fig.2. Dose-dependent effect of hrIL-1 on the production of glycosaminoglycans by human chorionic cells. Chorionic cells at fifth passage were grown to confluence in 1.5 ml of 10% (v/v) FBS/MEM, and cells were then treated with various concentrations of hrIL-1 for 24 h. The medium was then changed to FBS-free/MEM containing hrIL-1 and cells were labeled with 10 μCi D-[6-^{3}H]glucosamine for 24 h. Glycosaminoglycans in cell (●) and medium (○) fractions were extracted and purified as described in the text. Data are shown as the means ± SD of 4 dishes.

of extracellular glycosaminoglycans in a dose-dependent manner, indicating that hrIL-1 accelerates both biosynthesis and secretion of glycosaminoglycans in chorionic cells as shown in fig.2. Furthermore, the effect of hrIL-1 on the composition of glycosaminoglycans synthesized in human chorionic cells was examined. As shown in table 1, hrIL-1 accelerated specifically the biosynthesis of hyaluronic acid and thus increased significantly its ratio to total glycosaminoglycans. In contrast, the ratios of chondroitin sulfate and dermatan sulfate were decreased markedly compared to each control.

4. DISCUSSION

It has been reported that collagen content in prematurely ruptured amniotic membranes is characteristically low in comparison with those without premature rupturing [18,19]. In addition, Skinner and Liggins [9] have reported the marked decreases in concentration of sulfated glycosaminoglycans and collagen in term and post-term amnions and in some cases of premature rupture of membranes. In contrast, the hyaluronate concentration in those amnions was obviously increased. Therefore, they concluded that these changes can be explained well by connective tissue remodeling which causes the decrease in tensile strength of fetal membranes. They also proposed that in some clinical disorders such as amnionitis, some factors of the inflammatory process may initiate the remodeling of connective tissue [9]. In this respect, it is interesting to note that effusion of maternal polymorphonuclear leukocytes into the chorionic membrane has been observed with the progression of amniotic infection [20]. In addition, it is known that effusion of maternal macrophages is observed

Table 1

Effect of hrIL-1 on the composition of glycosaminoglycans produced by human chorionic cells

	Glycosaminoglycans (dpm × 10^{-4}/dish)				
	Chs	DS	HA	HS	Total
Total (cell and medium fractions)					
Control	5.34 ± 0.22 (18.8 ± 0.3)	4.21 ± 0.32 (14.7 ± 0.3)	18.67 ± 1.35 (65.2 ± 1.0)	0.38 ± 0.24 (1.4 ± 0.9)	28.62 ± 1.66
hrIL-1	5.49 ± 0.25 (9.0 ± 0.4)[b]	5.64 ± 0.34 (9.3 ± 0.6)[a]	48.75 ± 0.09[b] (80.1 ± 0.1)[b]	0.99 ± 0.22 (1.6 ± 0.3)	60.86 ± 0.19[b]

Control and hrIL-1 (1 ng/ml)-treated samples in fig.2 were employed for this assay. Results are calculated from descending paper chromatography of chondroitin AC lyase- or ABC lyase-digested glycosaminoglycans. Data are shown as means ± SD of 4 dishes. Relative composition is represented in parentheses. Chs, chondroitin sulfate; DS, dermatan sulfate; HA, hyaluronic acid; HS, heparan sulfate. [a] and [b]: significantly different from each control ($p < 0.05$ and $p < 0.01$)

in normal chorionic membrane at term pregnancy [14] and that placenta at post-parturition is a source of IL-1 [21]. These observations suggest the significance of IL-1 during the rupture of fetal membranes, since both monocytes/macrophages [1] and polymorphonuclear leukocytes [22] are recognized as being typical IL-1-producing cells.

When fibroblast-like cells from human chorion were treated with hrIL-1, collagenase production was enhanced in a dose-dependent manner as reported in synovial fibroblasts and chondrocytes [3–5], indicating that IL-1 promotes collagenolysis in human chorionic membrane. In addition, IL-1 preferentially stimulates the biosynthesis and secretion of hyaluronic acid in these cells without affecting those of sulfated glycosaminoglycans. Therefore, the relative concentration of hyaluronate vs total glycosaminoglycans inevitably increased. This promoting effect of IL-1 results in further acceleration of reducing the tensile strength of the fetal membranes, because of the highly hydrophilic nature of hyaluronic acid and the significant decrease in dermatan sulfate which interacts strongly with collagens. These effects of IL-1 on connective tissue are identical with those observed in prematurely ruptured fetal membranes and normally ruptured ones at term pregnancy [9].

In conclusion, IL-1 may play an important role in connective tissue metabolism in fetal membranes with chorioamnionitis, and also participate in connective tissue catabolism in normal rupturing of fetal membranes at term pregnancy.

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