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HUMAN RECOMBINANT INTERLEUKIN 1-MEDIATED SUPPRESSION OF GLYCOSAMINOGLYCAN SYNTHESIS IN CULTURED RAT COSTAL CHONDROCYTES

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SUMMARY : Effects of human recombinant interleukin 1 (IL-1) on the synthesis of glycosaminoglycan were examined with cultured rat costal chondrocytes. Incorporation of [^{35}S]sulfate into glycosaminoglycan was strikingly diminished by the addition of IL-1 in a dose- and time- dependent manner. When the cells were cultured with 340 μg/ml of IL-1 for 72 hr, the synthesis of glycosaminoglycan was inhibited to 10 % of the control. On the other hand, IL-1 had no effect on the morphology and proliferation of the chondrocytes. The suppression of glycosaminoglycan synthesis remained unchanged after the addition of indomethacin, indicating that the effect of IL-1 is independent of the enhanced synthesis of prostaglandins.

Interleukin-1 (IL-1), a polypeptide hormone released from macrophages and blood mononuclear cells, exerts a number of biological activities (1, 2). In addition to the thymocyte proliferating activity, IL-1 has been reported to stimulate the release of collagenase, proteoglycanase and prostaglandin E_2 (PGE_2) from articular chondrocytes or synovial cells (3-5). Furthermore, IL-1 enhances bone resorption (6) and cartilage degradation (4, 7). Therefore, IL-1 is considered to play an important role in pathological destruction of articular cartilage, particularly in the case of rheumatoid arthritis. However, crude or partially purified IL-1 fraction has been used in the experiments of the above reports. Therefore, whether the same molecule as that exhibiting the thymocyte proliferating activity influences on chondrocytes or synovial cells has been obscure.

Recent developments in the molecular biology have led to the success in the cloning and expression of the gene for murine and human IL-1 (8, 9). Human recombinant IL-1 (hrIL-1) has two species, IL-1α and IL-1β. Recombinant murine IL-1 has been reported to stimulate bone resorption (10),

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and hrIL-1α stimulates human synovial cells to produce collagenase and PGE_2 (11). However, few studies have been reported as to the effect of recombinant IL-1 on chondrocytes. In the present study, the effect of hrIL-1 on the incorporation of [^{35}S]sulfate into sulfated glycosaminoglycan (GAG), which is a specific GAG released from differentiated chondrocytes, was examined.

MATERIALS AND METHODS

Chondrocytes were obtained from growth cartilage of the ribs of young (4 weeks old) male Wistar rats, according to the method of Shimomura et al.(12). The isolated cells were plated on a microwell with a diameter of 16 mm (Falcon) at a density of 7 x 10^4 cells per one well, and have grown in Ham F-12 medium (Nissui Seiyaku Co., Ltd.) supplemented with 10 % fetal calf serum (FCS), penicillin (100 u/ml) and streptomycin (50 u/ml) at 37°C under 5 % CO_2 in air. The synthesis of GAG was examined by measuring the incorporation of [^{35}S]sulfate into the precipitates with cetylpyridinium chloride after treatment with pronase E (13). Briefly, after the chondrocytes had been grown to confluence, the cells were cultured for 72 h in a fresh medium containing 3 % FCS and hrIL-1α (kindly supplied from Dainippon Seiyaku, Co., Japan). The hrIL-1 had the specific activity of 2.5 x 10^7 u/mg for thymocyte proliferating activity. The methods for cloning of cDNA for human IL-1 and its expression in E. coli were described previously by Furutani et al. (14). The cells were labeled with 2.5 μCi/ml of [^{35}S]sulfate for the final 3 or 20 h of the culture time. At the end of labelling period, the cells were harvested in 1 ml of 0.15 N NaOH with a rubber policeman. Following the neutralization with 6N HCl, the harvested materials were digested with pronase E. After digestion, the precipitates with cetylpyridinium chloride were collected on a Millipore filter (pore size, 0.45 μm), and the radioactivity on the filter was counted in a liquid scintillation spectrometer (Packard Tri-Carb 3302).

RESULTS

When chondrocytes were cultured for 72 h in the presence of hrIL-1 (340 ng/ml), the incorporation of [^{35}S]sulfate into GAG was markedly inhibited. Fig. 1 shows the dose-dependence of hrIL-1-induced inhibition of GAG synthesis. Half-maximal and near maximal effects were observed with 10 and 100 ng/ml, respectively. At maximal dose of hrIL-1, the GAG synthesis was decreased to about 10 % of the control. The dose-dependence of IL-1 on the suppression of GAG synthesis was much the same with that of thymocyte-activating activity (data not shown).

Fig. 2 shows the effect of the length of exposure of chondrocytes to hrIL-1 on suppression of GAG synthesis. The synthesis of GAG was progressively suppressed as the culture period was prolonged, although no

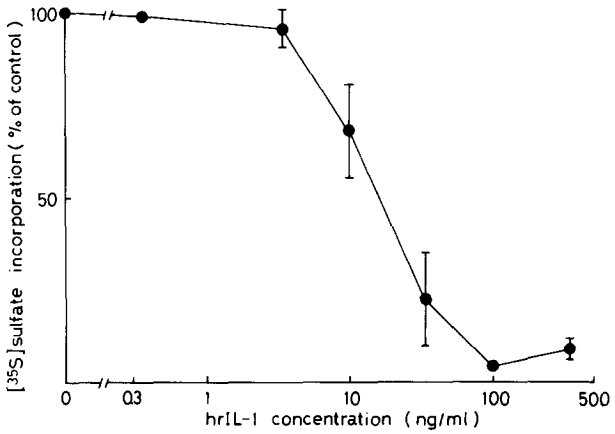


Fig. 1. Suppression of [^{35}S]sulfate incorporation into GAG by hrIL-1.
After the cells grew to become confluent, hrIL-1 was added to the medium at the concentrations indicated in the abscissa. The cells were cultured for 72 h and labeled with 2.5 μCi/ml of [^{35}S]sulfate for final 20 h of culture period. Results are expressed as % of the control value in the absence of IL-1. The points and vertical bars represent the mean ± S.E. for 3-7 experiments.

apparent suppression was observed for initial 24 h. The maximal suppression was obtained by exposing the chondrocytes to hrIL-1 for 72 h or more.

IL-1 has been reported to stimulate the proliferation of fibroblasts (15). We examined whether this is also found to be the case with chondro-

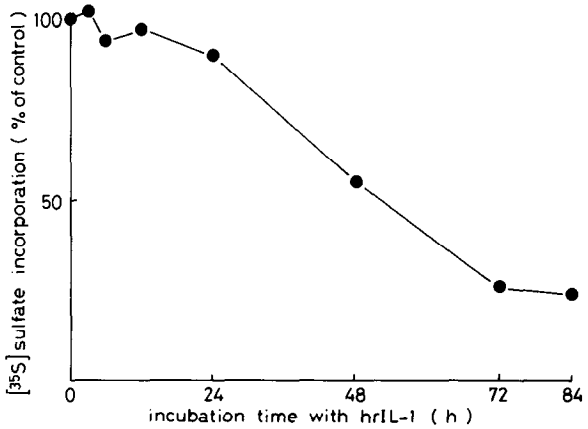
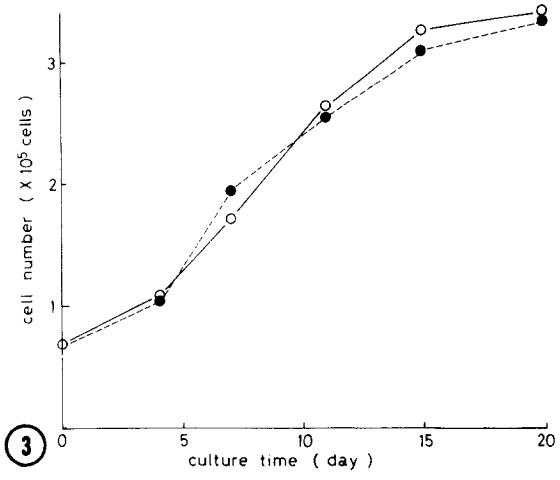


Fig. 2. Effect of the length of exposure to hrIL-1 on suppression of GAG synthesis.
After the cells became confluent, the cells were cultured for 84 h in a fresh medium. The hrIL-1 (340 ng/ml) was included in the culture medium for 3, 6, 12, 24, 48, 72 or 84 h before the end of culture. The cells were labeled with 2.5 μCi/ml of [^{35}S]sulfate for the final 3 h. Results are expressed as % of the control value ([^{35}S]sulfate incorporation in the absence of IL-1 for 84 h). Each point represents the mean of two experiments.



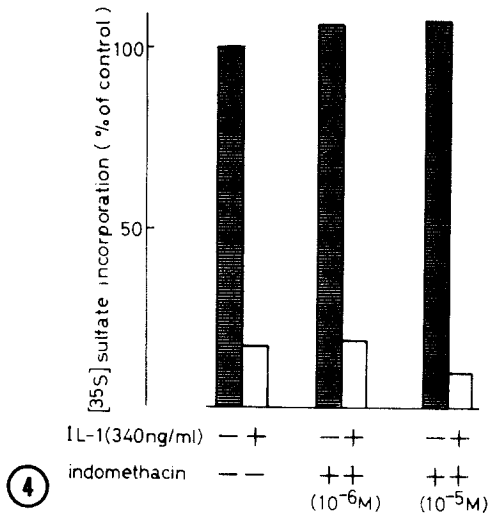


Fig. 3. Effect of hrIL-1 on the proliferation of chondrocytes.
The chondrocytes were seeded at 7 x 10^4 cells/16 mm well and hrIL-1 (136 ng/ml) was added when medium was renewed (day 0, 3, 6 and 10). The cell number was counted under a phase contrast microscope in a Bürker-Türk's counter. (○—○): Control, (●---●): IL-1.

Fig. 4. Effect of hrIL-1 and indomethacin on the incorporation of [^{35}S] sulfate into GAG.
Results are expressed as % of the control values. Each column represents the mean of two experiments.

cytes. As shown in Fig. 3, hrIL-1 (136 ng/ml) had no effect on the chondrocyte proliferation. Vitamin A or phorbol ester, which are known to induce de-differentiation, i.e., transformation of polygonal-shaped cells to spindle-shaped fibroblastic cells, decrease the GAG synthesis (16, 17). Therefore, the possible morphological change of chondrocytes to hrIL-1 was also examined. The hrIL-1 induced no morphological change of polygonal chondrocytes (data not shown).

It was reported that articular chondrocytes produced increasing amounts of PGE_2 in the presence of IL-1 (3), and consequently the PGE_2 inhibited the synthesis of GAG (18, 19). Therefore, we carried out the experiments with indomethacin, a cyclo-oxygenase inhibitor to examine whether the inhibition of PG production could modify the IL-1 effect on GAG synthesis. As shown in Fig. 4, the inhibitory effect of hrIL-1 on the GAG synthesis was not affected by the addition of indomethacin. The result indicates that the IL-1 effect may not be mediated through the enhanced production of PGE_2, though we have not measured PGE_2 produced by IL-1.

DISCUSSION

The results show that hrIL-1 suppresses the incorporation of [^{35}S] sulfate into GAG in cultured rat costal chondrocytes. This effect may be due to either the decreased synthesis of GAG or the enhanced degradation of [^{35}S]sulfate-labeled GAG. The latter possibility may be excluded for the following reasons : 1) the culture medium of IL-1-stimulated cartilage does not contain the sulfatase or glycosidase activities (20, 21) and 2) although the IL-1-stimulated medium contains neutral metalloprotease capable of degrading the proteoglycan, the enzyme is present in a latent form and thus, may not be involved in the GAG degradation (4, 20).

In contrast with the present results, a partially purified human IL-1 has been reported to enhance the incorporation of [^{3}H]glucosamine into proteoglycans in human articular chondrocytes (22). This contradiction may be due to either the species of cells, i.e., human articular and rat costal chondrocytes, or to differences between partially purified and recombinant IL-1. Alternatively, it may be due to the difference between [^{35}S]sulfate and [^{3}H]glucosamine as a labeling isotope. Chondroitin sulfate is a typical sulfated GAG of cartilage, but it has no glucosamine in the repeating disaccharide units. Therefore, [^{35}S]sulfate may be a better labeling isotope for GAG of cartilage.

Little is known concerning the postreceptor events of IL-1 action. It has been reported that IL-1 induces an influx of calcium ion, which then activates membrane phospholipase A_2 to release arachidonic acid from membrane phospholipids (23). It has also been reported that the addition of calcium ionophore A23187, caused an increase in cytoplasmic free Ca^{2+} together with inhibition of proteoglycan synthesis (24). Further experiments will be required to clarify the relationship between IL-1 and Ca^{2+} mobilization.

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