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# Effects of growth factors and cytokines on proteoglycan and collagen synthesis by chondrocytes in guinea pigs with spontaneous osteoarthritis

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**Abstract** We examined the effects of various growth factors and cytokines on proteoglycan (PG) and collagen synthesis by chondrocytes isolated from osteoarthritic and normal articular cartilage of Hartley strain guinea pigs. The guinea pig represents a useful animal model of spontaneous osteoarthritis (OA). Cartilage tissue samples were obtained from the knee joints of under-3-month-old guinea pigs (control group) as well as 5- to 8-month-old guinea pigs with OA changes (OA group). Chondrocytes were isolated enzymatically and maintained in suspension culture. Growth factor addition groups were then prepared from both the OA group and the control group, using the factors TGF-β, bFGF, and IGF-1 (1.25 ng/ml each). Cytokine addition groups were also prepared using IL-1α and IL-1β (10 ng/ml each). An addition group was also prepared for sodium hyaluronate (HA) (500 μg/ml). In each group, $^{35}S$ was added as a PG metabolic marker, $^{3}H$-proline was added as a collagen metabolic marker, and the groups were cultured. Next, $^{35}S$ and $^{3}H$-proline uptake was measured by a liquid scintillation counter. The results revealed that (1) both PG synthesis and collagen synthesis were promoted significantly more in OA chondrocytes than in normal chondrocytes; (2) with the addition of growth factors, PG and collagen synthesis was enhanced in OA chondrocytes; and (3) PG synthesis and collagen synthesis were inhibited in both normal and OA chondrocytes with the addition of IL-1α and -β. This result suggests that the repair function is activated more in OA chondrocytes than in normal chondrocytes, thereby promoting the synthesis of the cartilage matrix by chondrocytes. This synthesizing capability is enhanced and acts to effectively repair degenerative articular cartilage further through the addition of growth factors.

**Key words** Chondrocyte · Growth factor · Cytokine · Guinea pigs · Osteoarthritis

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## Introduction

Many studies in recent years have investigated the effects of various growth factors and cytokines on normal cultured chondrocytes. However, the effects of these factors on chondrocytes at diseased sites of osteoarthritis (OA) remains to be clarified. Spontaneous OA is known to occur in guinea pigs, and as such they are considered to be a useful model of human OA. In the present study, we examined the effects of various growth factors and cytokines on proteoglycan (PG) and collagen synthesis using isolated chondrocytes taken from the knee joints of guinea pigs.

## Materials and methods

### Animal model

Hartley strain guinea pigs have been reported to be optimal models of human OA, given that they are relatively large and develop OA as they grow, the cartilage lesions that develop are similar to those in humans, and the OA lesions progress relatively slowly, with uniform initial sites and period of onset.[1–3] The OA group consisted of the knee joints of female Hartley strain guinea pigs aged 5–8 months that were observed macroscopically or histopathologically to have spontaneous OA (*n*, 20 joints). Normal knee joints from female Hartley strain guinea pigs under 3 months of age (*n*, 20 joints) served as controls. The guinea pigs were killed under deep ether anesthesia, and their legs were promptly amputated and the knee joints extracted together with the periarticular tissue.

### Macroscopic and histological examinations

The extracted knee joints were exposed and examined. The 5- to 8-month-old animals were observed macroscopically to detect any OA changes. Specimens were prepared, stained with hematoxylin and eosin, safranin-O, or alcian blue, and evaluated using the histopathological classification of Mankin et al.[4]

### Culture chondrocyte

The knee joints were aseptically exposed, and articular cartilage tissue samples without subchondral bone were surgically excised. Based on the method of Treadwell et al.,[5] cartilage tissue samples of both the OA and control groups were cultured for 12 h in modified Ham's F-12 (Gibco, Grand Island, NY, USA; supplemented with 10% fetal calf serum [FCS] and antibiotics) containing 0.1% clostridium collagenase type 1 (Sigma, St. Louis, MO, USA). The chondrocytes were then separated. The chondrocytes were washed thoroughly in phosphate-buffered saline (PBS) to inhibit any effect of collagenase. The cells were then subjected to cell suspension in a culture medium for 24 h, after which they were again cultured in modified Ham's F-12 inside a test tube placed in an incubator with 5% $CO_2$ and 95% air. All procedures were performed aseptically on a clean bench.

### Experimental system

The number of cells in the culture liquid was calculated using a Thoma's counting chamber, and cells were placed in test tubes so that each group contained $1.0 \times 10^5$ cells (Fig. 1). Next, 1.25 ng/ml transforming growth factor-β (TGF-β; Promega, Madison, WI, USA), basic fibroblast growth factor (bFGF; Mallinckrodt, St. Louis, MO, USA), insulin-like growth factor-1 (IGF-1; Pepro Tech, Rocky Hill, NJ, USA), and 10 ng/ml interleukin-1α and -β (IL-1α and IL-1β; Pepro Tech) were added. Next, 500 μg/ml hyaluronic acid (HA; Seikagaku Kogyo, Tokyo, Japan), which is frequently used clinically, was added to each group, and the reactions were observed. HA has an approximate molecular weight of $9.0 \times 10^5$ and a purity of roughly 99%. As indicators of chondrocyte PG synthesis and collagen synthesis, $2.22 \times 10^5$ Bq of $H_2{}^{35}SO_4$ and $1.85 \times 10^5$ Bq of $^3$H-proline, respectively, were added and the samples were further cultured for 12 h. The samples were then centrifuged for 20 min at 700 *g* to separate the supernatant and the cell pallet. The supernatant was subjected to dialysis for 12 h in 0.05 M sodium acetate buffer (0.01 M EDTA and 0.005 M benzamidine HCL). The free $H_2{}^{35}SO_4$ and $^3$H-proline were removed, and the remaining $H_2{}^{35}SO_4$ and $^3$H-proline were measured using a liquid scintillation counter (Aloka, Tokyo, Japan).

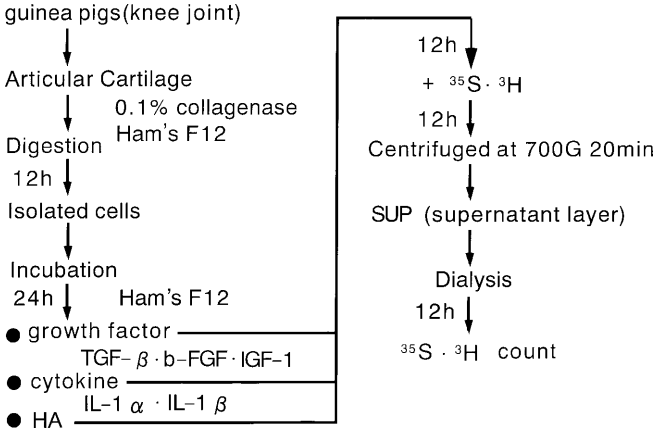


**Fig. 1.** Experimental methods

### Statistical analysis

Values were determined to be significant at $P < 0.05$ using Student's *t*-test.

## Results

### Macroscopic findings

Macroscopic findings for all knee joints of our 5- to 8-month-old Hartley strain guinea pigs showed reduced luster in cartilage at weight-bearing sites on the femur and medial condyle of the tibia compared to the lateral condyle. Slight proliferation of the synovial membrane was also noted, but no retention of synovial fluid was observed. In all guinea pigs under 3 months of age, the surface of the articular cartilage had remained shiny and no proliferation of the synovial membrane had occurred (Fig. 2).

### Histopathological findings

In guinea pigs aged 5–8 months, hematoxylin and eosin staining showed thinning of the cartilage layer, fissuring from the surface, and irregular chondrocyte arrangement. As seen by safranin-O staining, there was reduced coloring in the areas of strong degeneration. In addition, alcian blue staining showed a nonuniform color. According to the grading of Mankin et al.,[4] the structure grade was 1, the cell grade was 1, safranin-O staining grade was 1, and tide mark integrity grade was 0 (Fig. 3).

### Changes in PG synthesis in the non-additive group

Compared to the $^{35}$S count in the control group, which was determined to be 1.00, the stimulation index in the OA group was significantly higher at 1.21 ($P < 0.05$) (Fig. 4).

### Changes in collagen synthesis in the non-additive group

Compared to the $^3$H-proline count in the culture supernatant in the control group, which was determined to be 1.00,

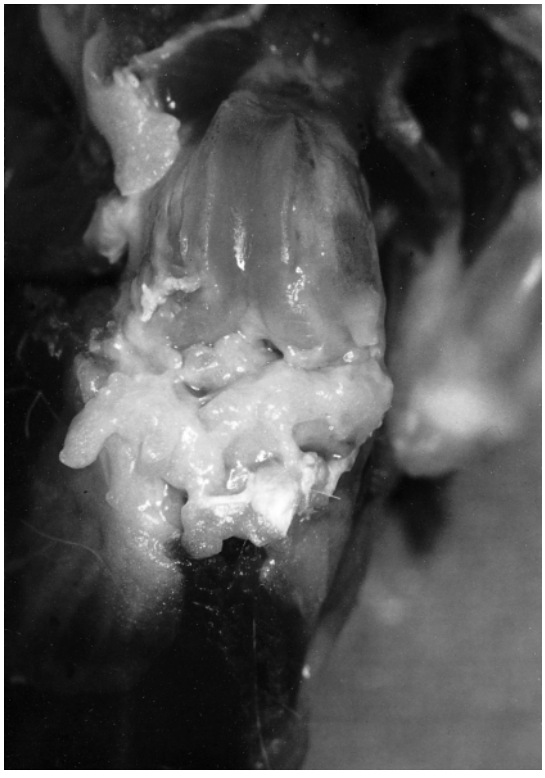

**Fig. 2.** Macroscopic findings in the knee joints of 8-month-old Hartley strain guinea pig with spontaneous osteoarthritis (OA)

the stimulation index in the OA group was significantly higher at 1.32 ($P < 0.05$) (Fig. 5).

Changes in PG synthesis caused by addition of growth factors and HA

The addition of each of the growth factors increased $^{35}S$ in the supernatant in the OA group; this increase was significant with TGF-β, bFGF, and IGF-1, but was marked but not significant with IGF-1 (TGF-β and bFGF, $P < 0.05$; IGF-1, $P < 0.01$) (see Fig. 4). However, in the control group, counts increased by approximately 20% in response to addition of all growth factors, but there were no significant differences when compared to the non-additive group. Although the $^{35}S$ count in the control group increased following the addition of HA, the increase was not significant. In the OA group, however, the count did increase significantly ($P < 0.05$).

Changes in collagen synthesis caused by the addition of growth factors and HA

The $^{3}H$-proline count increased significantly in the OA group in response to addition of each growth factor, and changes were marked in the group in which IGF-1 was added ($P < 0.01$) (see Fig. 5). Counts also increased in the control group with the addition of growth factors, but the increases were not significant for any factor. The $^{3}H$-proline count increased in both the OA group and the control group when HA was added but did so significantly only in the OA group ($P < 0.05$).

Changes in PG synthesis caused by cytokine addition

The $^{35}S$ count decreased but not significantly in both the OA group and control group following the addition of IL-1α and IL-1β (Fig. 6). There were no significant differences between the two groups.

Changes in collagen synthesis caused by cytokine addition

The $^{3}H$-proline count decreased but not significantly in both the OA group and control group following the addition of IL-1α and IL-1β (Fig. 7). There were no significant differences between the two groups.

## Discussion

Osteoarthritis (OA) is a chronically progressive articular disease that is characterized by both degeneration (destruction) of cartilage and proliferative (or regenerative) changes in bone and cartilage. Human studies are of limited use in investigating the pathology of this disease, and for this reason research has been conducted using experimental animal models. OA can be induced experimentally by either surgery or a systemic/intraarticular injection of chemicals, and in some animals OA develops spontaneously. We used Hartley guinea pigs in this study because they develop primary OA spontaneously, and as such experimental procedures are not required to induce OA. Given that it is not necessary to account for differences in the onset rate of experimentally induced OA, Hartley guinea pigs are the most suitable animal model when investigating OA from its early to terminal phases.

However, when comparing human OA and animal OA it is important to recognize that certain differences exist in the characteristics of cartilage lesions. The results of macroscopic and histopathological studies have shown that Hartley guinea pigs do indeed represent an appropriate animal model of human OA. Bendele et al.[6–8] observed cartilage degeneration in areas of the medial tibial articular cartilage that were not covered by the meniscus. Tokuda et al.[1–3] reported the following features as being common between guinea pig and human OA: fibrillation of the surface layer of cartilage, cluster formation of chondrocytes, reduced safranin-O staining, and abnormal tide mark. They also determined that the following features were not present in guinea pig OA: eburnation, sclerosis of subchondral bone, and proliferative changes in synovial cells.

In recent years, there have been a number of reports of histological and biochemical studies of early-phase OA articular chondrocytes using experimental OA models.[9–12] However, these reports were primarily done on articular cartilage tissue cultures. On the other hand, Green documented that even when cartilage cells were cultured as iso-

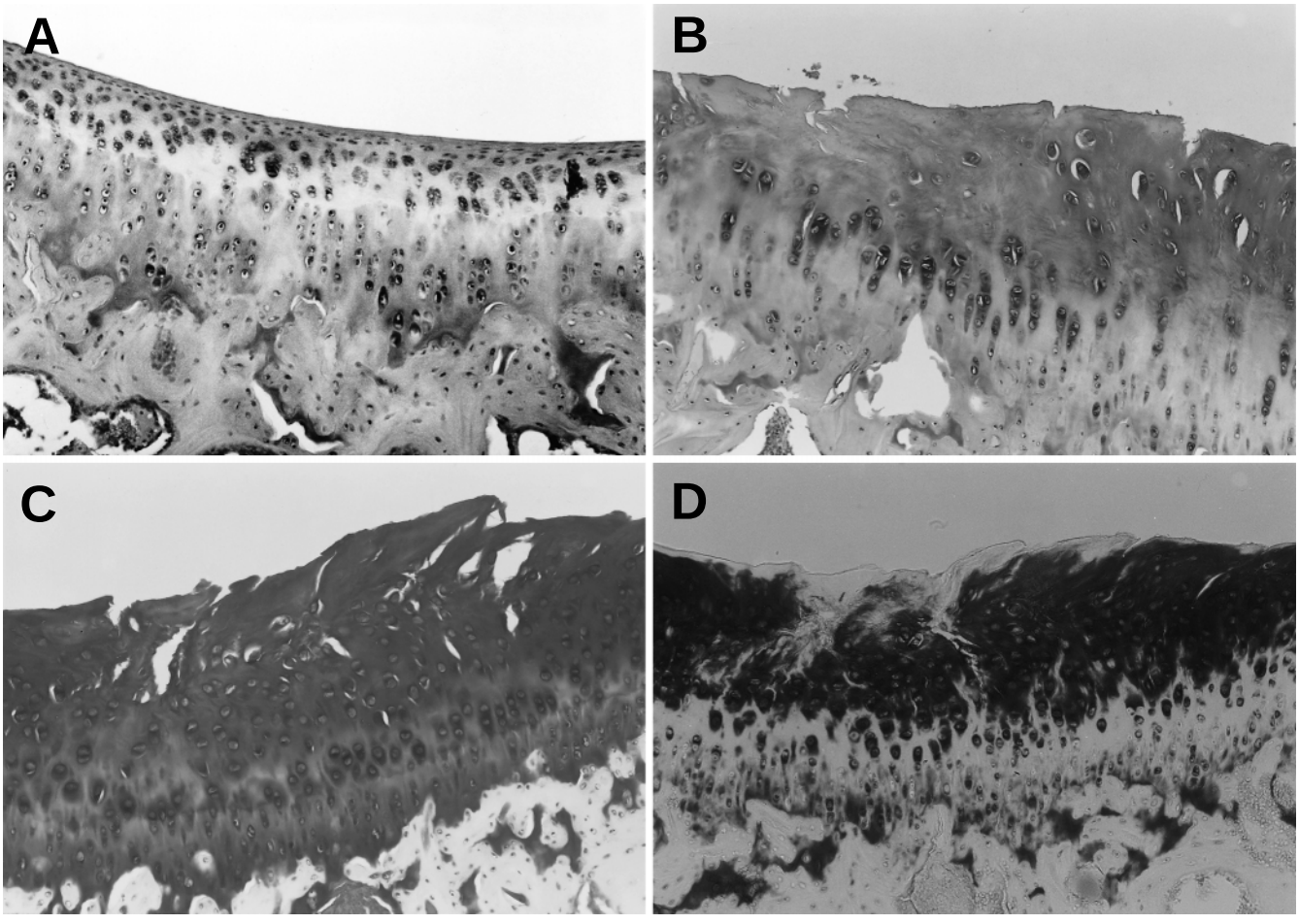


**Fig. 3A–D.** Histopathological findings in the control group (**A**). Hematoxylin and eosin. (Original magnification ×40). Histopathological findings in the OA group (**B–D**). **B** Hematoxylin and eosin stain showed thinning of the cartilage layer, fissuring from the surface, and irregular chondrocyte arrangement (Original magnification ×40). **C** Safranin-O showed reduced staining in the areas of strong degeneration (Original magnification ×40). **D** Alcian blue staining showed nonuniformity in color (Original magnification ×40)

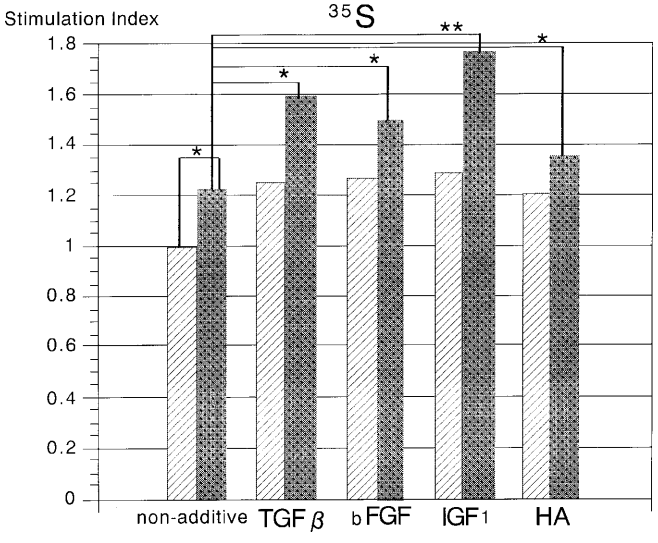


**Fig. 4.** Prostaglandin (PG) synthesis in the presence of growth factors and sodium hyaluronate (HA). The count increased in $^{35}S$, was significantly increased with TGF-β, bFGF, and IGF-1, and was marked with IGF-1. In the control group, counts increased by about 20% with the addition of all growth factors, but there was no significant difference with the non-additive group. Control, *hatched bar*; OA, *dotted bar*. **, $P < 0.01$; *, $P < 0.05$

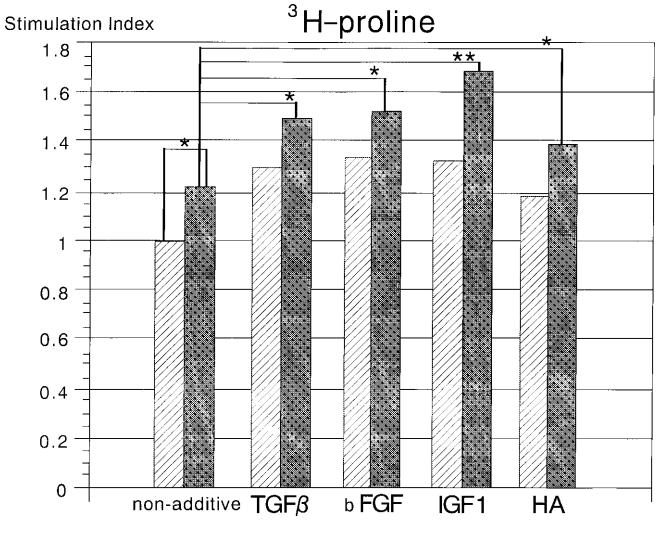


**Fig. 5.** Collagen synthesis in the presence of growth factors and HA. The counts increased significantly in the OA group with the addition of each growth factor, and changes were marked in the group in which IGF-1 was added. In the control group, counts also increased with the addition of growth factors, but the increase was not significant for any factor. **, $P < 0.01$; *, $P < 0.05$

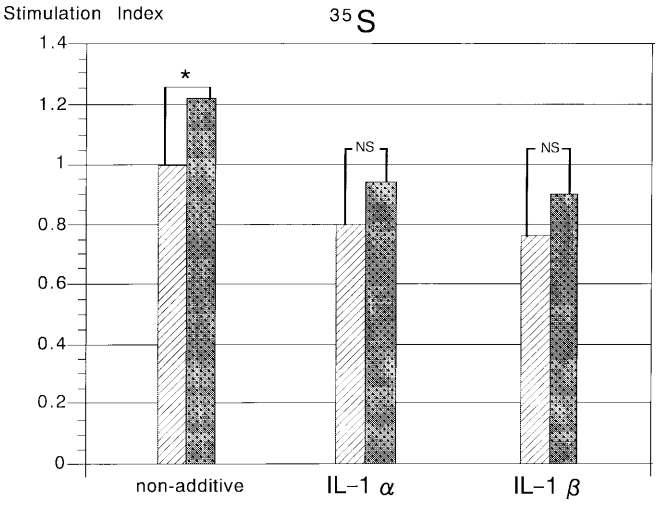


**Fig. 6.** PG synthesis in the presence of cytokines. The counts decreased in both the OA group and control group with the addition of IL-1α and IL-1β, but there was no significant difference between the two groups. The count reduction rate was greater in the OA group than in the control group. **, $P < 0.01$; *, $P < 0.05$

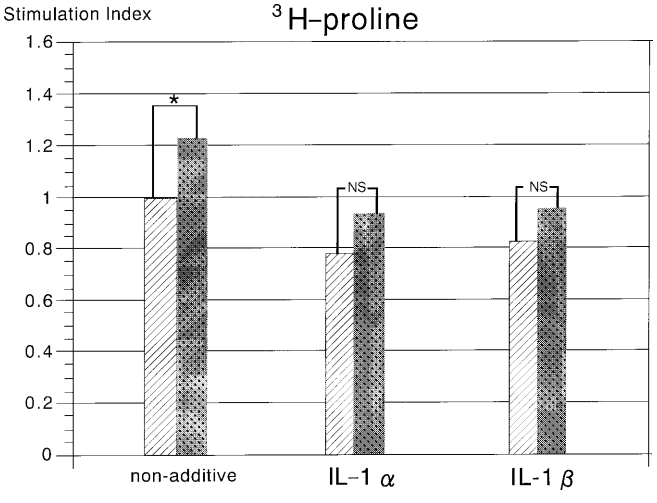


**Fig. 7.** Collagen synthesis in the presence of cytokines. The counts decreased in both the OA group and control group with the addition of IL-1α and IL-1β, but there was no significant difference between the two groups. The rate of count decrease was greater in the OA group than in the control group. **, $P < 0.01$; *, $P < 0.05$

lated cells, their in vivo characteristics could be maintained through the use of an appropriate culture medium and incubation for a short period of time.[13]

Thus, because Hartley guinea pigs spontaneously develop OA, we used them in the present study involving a macroscopic and histopathological experimental assessment of OA. Isolated OA chondrocytes (cartilage affected by OA at the age of 5–8 months) and free normal cartilage cells (cartilage not affected by OA at the age of less than 3 months) were harvested and subjected to suspended cell culture to compare PG and collagen syntheses. Mankin et al. reported that although the levels of PG in areas affected by OA and their surrounding regions were reduced, PG synthesis in chondrocytes was elevated.[4] In addition, under the same experimental conditions, Ono et al.[14] reported that the PG synthesis of chondrocytes was higher in articular cartilage affected by OA when compared to healthy articular cartilage.

The present results revealed that PG and collagen synthesis in OA chondrocytes was significantly higher than that in normal chondrocytes, suggesting active regeneration of OA chondrocytes in response to damage to articular cartilage. Thus, the effects of growth factors (TGF-β, bFGF, and IGF-1) and cytokines (IL-1α and IL-1β) on OA and normal chondrocytes were then investigated.

Growth factors are responsible both for the proliferation and differentiation of various tissues and cells as well as for accelerating the proliferation of chondrocytes that subsequently elevate the synthesis of matrix components such as PG and collagen. Various studies on the effects of these growth factors on chondrocytes have been carried out,[10,15–17] and the results have shown that the addition of TGF-β, bFGF, and IGF-1 to chondrocyte culture medium accelerates PG and collagen syntheses.

In the present study we observed that synthesis of PG and collagen was accelerated by the addition of TGF-β, bFGF, and IGF-1 to isolated chondrocyte culture medium. Furthermore, the levels of PG and collagen syntheses were higher in OA chondrocytes when compared to normal chondrocytes. We therefore concluded that OA chondrocytes had reacted strongly to external stimuli, and that when the articular cartilage of the knee was damaged, the regenerative property of OA chondrocytes activated PG and collagen synthesis. These findings suggest that growth factors accelerate PG and collagen synthesis in OA chondrocytes and thus contribute to the repair of degenerative articular cartilage.

The present findings demonstrated that, of the different growth factors, IGF-1 was the most effective in accelerating PG and collagen synthesis in chondrocytes, in spite of the fact that the action of this compound when not acting together with other growth factors is not potent.[18] Based on the results of our previous study using female piglets, the concentration of all growth factors was set at 1.25 ng/ml in the present study. Setting one standard level may not have been the most effective way of expressing individual growth factors.

We used IL-1α and IL-1β to investigate the suppression of cartilage metabolism in OA chondrocytes because these cytokines are one of the cytokines involved in cartilage matrix metabolism and are deeply involved in articular cartilage destruction. Aydelotte et al.[19] utilized an isolated chondrocyte culture system using pig articular cartilage and reported that IL-1α and IL-1β suppressed PG and collagen synthesis in a concentration-dependent manner; the action of IL-1α was stronger than that of IL-1β. In general, the action of IL-1α is stronger than that of IL-1β in animal articular cartilage[12,19] whereas IL-1β is stronger in human articular cartilage.[20] In the present study, when only IL-1 was added, PG and collagen synthesis was suppressed in both OA and normal chondrocytes. There were no

significant differences between the two cell groups or between IL-1α and IL-1β. Given that the effect of IL-1 on cartilage matrix synthesis varies depending on animal species and concentration, future research should determine the optimal concentration and clearly identify any and all animal species differences.

Clinically, HA is used as an intraarticular injection for the treatment of OA. As is the case with HA, growth factors may be clinically useful for OA therapy and cytokines for anticytokine therapy.

## Conclusion

We studied the effects of growth factors and cytokines on PG and collagen synthesis in free chondrocytes using guinea pigs showing spontaneous osteoarthritis. Results showed that significantly more PG and collagen synthesis was induced in OA chondrocytes than in healthy chondrocytes. Moreover, the promotion of PG and collagen synthesis was enhanced via the addition of growth factors more in OA chondrocytes than in healthy chondrocytes. Hence, PG and collagen syntheses in OA chondrocytes responded well to growth factors. PG and collagen syntheses were similarly inhibited in both OA chondrocytes and normal chondrocytes by the addition of IL-1.

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