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Interleukin-1β induces synthesis and secretion of interleukin-6 in human chondrocytes

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Increased concentrations of interleukin-6 (IL-6) have been found in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and crystal-related joint deseases. It is therefore of great interest to identify the cells responsible for the production of IL-6, and to investigate whether IL-6 plays a role in the pathogenesis of degenerative or inflammatory joint diseases. Here we show that human interleukin-1β (IL-1β) induces IL-6 synthesis and secretion in differentiated human chondrocytes. In organ cultures resembling closely the in vivo system 10^6 chondrocytes incubated with 100 units of interleukin-1β per ml of medium led to the release of 6×10^3 units of IL-6 within 24 h. Chondrocytes cultured in agarose or as monolayers similary incubated with IL-1β produced even higher amounts of IL-6: 70×10^3 units per 10^6 cells within 24 h. The induction of IL-6 synthesis by IL-1β was also shown at the mRNA level. IL-6 secreted by stimulated chondrocytes showed heterogeneity upon Western blot analysis.

Cartilage; Chondrocyte; Interleukin-1β; Interleukin-6; Glycosylation; (Human)

1. INTRODUCTION

Chondrocytes are actively involved in the regulation of the dynamic balance between synthesis and degradation of proteoglycans, which besides collagen constitute the major components of the extracellular matrix of cartilage (reviewed in [1,2]). This balance is disturbed in rheumatoid arthritis and other inflammatory or degenerative joint diseases [3,4]. Recent studies from several laboratories have demonstrated that the steady state metabolism of chondrocytes is affected by factors such as insulin-like growth factors IGF-I and IGF-II [5,6], transforming growth factor [7,8], and interleukin-1 (IL-1) [9–15]. Increased concentrations of interleukin-6 have been found in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis, and crystal-related joint diseases [16–18]. Since IL-1 appears to be the major stimulator of interleukin-6 (IL-6) synthesis in many different cell types (reviewed in [19]), we asked the question, whether chondrocytes can also be stimulated to synthesize IL-6.

Here we present data which show that human IL-1 induces IL-6 synthesis and secretion in differentiated human chondrocytes.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

Collagenase CLS II from *Cl. histolyticum* was purchased from Worthington (USA). [6-^{3}H]Thymidine (74 GBq/mmol) was obtained from Amersham International (Amersham, UK). Fetal calf serum (FCS), Opti-MEM 1, and Ultroser G were from Gibco (Eggenstein, FRG). Low-gelling-temperature agarose was from Sigma (Munich, FRG). An IL-1-specific ELISA was obtained from Research and Diagnostics Systems (Minneapolis, USA). RhIL-1 (1.3×10^7 U/mg) was kindly supplied by Dr A. Shaw (Glaxo, Institute for Molecular Biology SA, Geneva, Switzerland).

2.2. *In vitro culture of human chondrocytes*

Cartilage was obtained from the knee joint of a 40-year-old patient, who had died from an oesophageal carcinoma. The cartilage appeared normal by morphological examination. For organ cultures pieces of cartilage of equal size were embedded in 0.75% low-gelling-temperature agarose and cultured in Opti-MEM1 supplemented with 10% FCS, 1% Ultroser G, 1 μg/ml insulin and 50 μg/ml ascorbic acid. For agarose cultures chondrocytes were isolated by collagenase treatment essentially as described [20,21]. Culture medium was the same as described above.

Viability of chondrocytes was measured by the Trypan-blue dye exclusion test. In all experiments the viability was >98%.

2.3. *IL-6 assay*

The IL-6 assay was performed using the murine plasmacytoma cell line B9 kindly provided by L. Aarden (Amsterdam, The Netherlands) [22]. Briefly, 5×10^3 B9 cells were incubated with the supernatants from chondrocytes in the presence of 0.5 μCi [^{3}H]thymidine and incorporation of radioactivity was measured. One B9 unit/ml is the concentration that leads to half-maximal [^{3}H]thymidine incorporation in the assay. Under our assay conditions 150–500 fg/ml of IL-6 can be detected.

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2.4. *Northern blot analysis*

Total RNA was isolated from chondrocytes cultured as monolayers essentially as described [23]. 5 μg of RNA were used for Northern blotting. Hybridization was performed with the 1.1 kb *Eco*RI-fragment of the IL-6 cDNA clone [24] labeled by random priming [25].

2.5. *Western blot analysis*

Western blotting was performed according to [26]. The blot was incubated with a specific rabbit anti-IL-6 antiserum (1 : 1000 dilution). After washing with PBS containing 0.05% Tween 20, blots were incubated with a second antibody (goat anti-rabbit IgG peroxidase conjugated) for 1 h at room temperature. Blots were washed and finally stained with aminoethylcarbazole as a substrate.

3. RESULTS AND DISCUSSION

Various experimental systems have been used for in vitro studies of chondrocyte function. Thus far, monolayer cultures have widely been used in spite of the fact that they represent a rather artificial system [2]. Therefore, other systems have been developed, which reflect more closely the in vivo situation of human chondrocytes in cartilage. In contrast to monolayer cultures chondrocytes embedded in agarose have been described to maintain their differentiated phenotypes [2]. Organ cultures resemble most closely the in vivo system, because the chondrocytes remain in their extracellular matrix.

We have studied the effect of recombinant human IL-1 (rhIL-1) on chondrocytes in organ culture, in agarose culture and as monolayers in respect to IL-6 production. Fig. 1 shows the time-dependent IL-6 synthesis and secretion by chondrocytes in organ culture. Addition of 100 units of rhIL-1 per ml of culture medium resulted in a 4-fold increase in IL-6 concentrations within 24 h. Organ cultures without exogeneous rhIL-1 also showed a slight increase in IL-6 production. We excluded that this increase is due to an IL-1 contamination of FCS by measuring IL-1 concentrations.

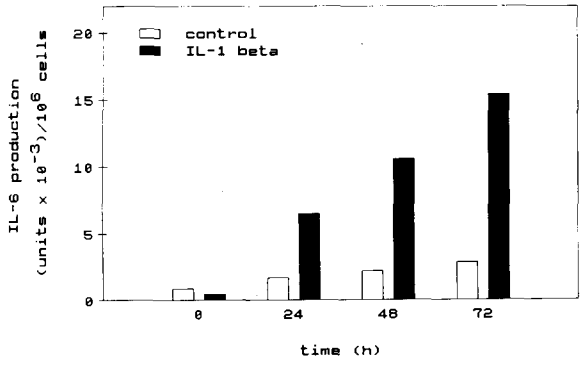

Fig. 1. RhIL-1 induced IL-6 synthesis in human chondrocytes in organ culture. Pieces of 850 mg (control) and 875 mg (rhIL-1) of cartilage (wet weight equivalent to 6.4 $\times$ 10^6 and 6.6 $\times$ 10^6 chondrocytes, respectively) were embedded in 2.5 ml of low-gelling-temperature agarose. Total volume of medium (see section 2) was 7.5 ml. After a preincubation period of 3 days rhIL-1 was added at a final concentration of 100 U/ml. At the times indicated in the figure IL-6 concentrations were determined.

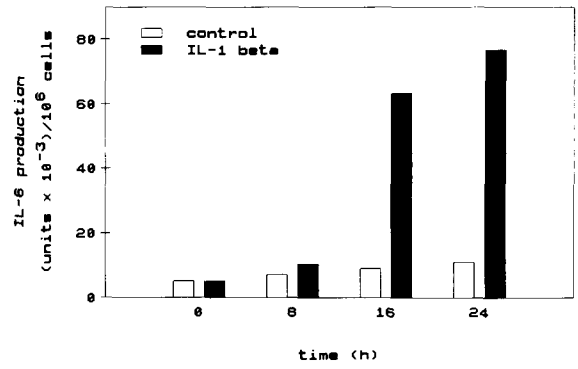

Fig. 2. IL-6 production of human chondrocytes in agarose cultures after stimulation with rhIL-1. Chondrocytes were obtained from cartilage by collagenase treatment (see section 2) and embedded in low-gelling-temperature agarose at a density of 1 $\times$ 10^6 cells per ml of gel. Each dish contained 3 $\times$ 10^6 chondrocytes in 3 ml of agarose overlaid with 5 ml of medium resulting in a total volume of 8 ml medium. After a preincubation period of 10 days 50 U/ml of rhIL-1 were added. At the times indicated medium was taken for IL-6 determinations.

Using an ELISA with a detection limit of 30 pg corresponding to 0.4 units of IL-1 per ml, no IL-1 could be detected in the supernatants. Since lipopolysaccharide (LPS) is a weak stimulator of IL-6 synthesis in human chondrocytes (unpublished results) and since it is well known that most batches of FCS are contaminated by LPS, it is possible that the slight IL-6 induction observed in our controls may be due to the action of LPS.

Compared to organ cultures an even stronger stimulation of IL-6 synthesis by rhIL-1 was observed in agarose cultures. A 7-fold increase in IL-6 concentrations was found after 24 h (Fig. 2). It can be seen from Fig. 2 that the IL-6 release is observed after a lag of 16 h. This lag may be due to the requirement of de novo synthesis of IL-6 and also to the fact that the released IL-6 is primarily trapped within the extracellular matrix and is detectable only after diffusion into the medium.

In monolayer cultures of human chondrocytes from the first passage a 5-fold increase in IL-6 production was measured (Table I). A similar increase was found

Table I

RhIL-1-dependent induction of IL-6 synthesis and secretion in human chondrocytes in monolayer cultures

	IL-6 production ($U \times 10^{-3}/10^6$ cells/24 h)
Control	15.5
RhIL-1 (50 U/ml)	71.1

Chondrocytes were isolated as described for agarose cultures and plated on 9.6 cm^2 dishes at a density of 10^6 cells per dish and 5 ml of medium. After 10 days in culture cells were stimulated with 50 U/ml of rhIL-1 and IL-6 concentrations were measured

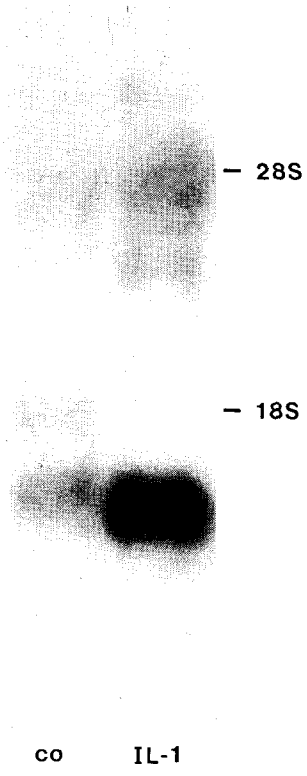

Fig. 3. Northern blot analysis of IL-6 mRNA from human chondrocytes in monolayer culture after stimulation with rhIL-1. Human chondrocytes in monolayer culture were incubated without (co) and with 50 U/ml rhIL-1 for 24 h. Total RNA was isolated and subjected to Northern analysis as described in section 2.

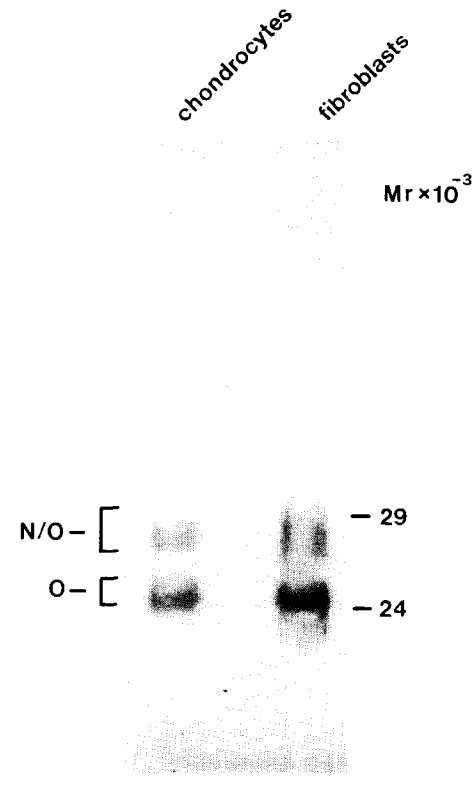

Fig. 4. Microheterogeneity of IL-6 synthesized and secreted by human chondrocytes in monolayer culture after stimulation with rhIL-1. Human chondrocytes in monolayer culture incubated without fetal calf serum and Ultroser G were stimulated with 75 U/ml rhIL-1 for 24 h. One ml of IL-6-containing supernatant was concentrated and subjected to SDS-PAGE and Western blotting. For comparison IL-6 secreted by IL-1 (75 U/ml) stimulated human lung fibroblasts has been included in the figure. N/O- and O- indicate N- plus O- and O-glycosylated forms of IL-6, respectively.

for IL-6 mRNA in IL-1-stimulated chondrocytes (Fig. 3).

When the IL-6 released from chondrocytes in monolayer culture was analyzed by SDS-PAGE and subsequent Western blotting heterogeneity of IL-6 was observed (Fig. 4). We [27,28] and other investigators [29] have recently described such a microheterogeneity for IL-6 secreted by LPS-stimulated human monocytes, rhIL-1-activated human fibroblasts or endothelial cells. By use of deglycosylating enzymes and the N-glycosylation inhibitor tunicamycin, it was shown that the microheterogeneity of IL-6 is the result of N- and O-glycosylation [27–29]. A detailed analysis of the differently glycosylated forms of IL-6 is in progress.

It is evident from the data presented that rhIL-1 is a potent inducer of IL-6 in human chondrocytes in organ cultures, in agarose cultures and in monolayers. Therefore, the increased IL-6 concentrations determined in synovial fluids of patients with inflammatory joint diseases [18] may result from the stimulation of chondrocytes. Although IL-6 can be synthesized by a number of different cells after appropriate stimulation [19,30], endothelial cells, fibroblasts and monocytes/macrophages are presently believed to be the major sources of IL-6 during inflammatory diseases.

From the results of this study it is evident that stimulated chondrocytes are also potent IL-6 producing cells.

It has been shown that IL-1 (a potent inducer of IL-6 synthesis) is found in high concentrations in the synovial fluid of patients with inflammatory joint diseases [31,32]. Chondrocytes respond to IL-1 by the release of hydrolases [33] and in turn an increase of proteoglycan degradation [13,14]. Furthermore, proteoglycan synthesis is impaired [34]. It has to be clarified whether these effects are due to the direct action of IL-1 on the chondrocytes or whether they are mediated by IL-6 involving an autocrine mechanism.

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