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Differential effects of IGF-1 and TGFβ-2 on the assembly of proteoglycans in pericellular and territorial matrix by cultured bovine articular chondrocytes

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Summary

Objectives: Knowledge of matrix assembly is necessary to understand the pathogenesis of disease processes and to find solutions for repair of articular cartilage lesions. The influence of growth factors on matrix assembly is largely unknown. We investigated whether, and to what degree, insulin-like growth factor (IGF-1) and transforming growth factor β-2 (TGFβ-2) influence proteoglycan synthesis and accumulation in the cell-associated matrix compartment (consisting of pericellular and territorial matrix) compared to the further-removed matrix compartment (consisting of the interterritorial matrix).

Design: Bovine articular chondrocytes were cultured in alginate beads for day 13. The effect of addition of 25 ng/ml IGF-1 or 25 ng/ml TGFβ-2 during the last 7 days in culture was determined. Cell-associated and further-removed matrix compartments were separated by centrifugation after sodium citrate/EDTA treatment. The amount of DNA, the total amount of proteoglycans and the amount of newly synthesized proteoglycans were analyzed biochemically. Morphometric analysis on electron micrographs was used to calculate the volumes of the cell-associated and further-removed matrix components.

Results: It was demonstrated in control beads that 25 ± 8% of the proteoglycans were laid down in the cell-associated matrix compartment compared with 75 ± 8% in the further-removed matrix compartment. The cell-associated matrix compartment in intact beads could be recognized in electron microscopy by a delineation of dense amorph material. Morphometric evaluation showed a relative volume of the cell-associated matrix compartment of 5.2 ± 0.6% compared with 91.3 ± 0.8% of the further-removed matrix compartment and 3.5 ± 0.3% of the area occupied by cells. Combination of biochemical and morphometric results showed that the concentration of proteoglycans in the cell-associated matrix compartment was 3.63 ± 0.32 mg/ml. By adding IGF-1 or TGFβ-2, the amount of both total accumulated proteoglycans and newly synthesized [^{35}S]proteoglycans at day 13 in culture increased. In addition to an overall rise in proteoglycan content, IGF-1 significantly increased (24%) the percentage of proteoglycans laid down in the cell-associated matrix compartment while not changing the relative volume of this compartment (5.2 ± 0.8%). This leads to a 82% ($P < 0.05$) increase in the proteoglycan concentration in the cell-associated matrix compartment compared to control beads. In contrast, TGFβ-2 significantly decreased (24%) the relative amount of proteoglycans in the cell-associated matrix compartment which was paralleled by a reduction of the relative volume from 5.2 ± 0.6 to 3.6 ± 1.4%. This leads to a significant increase of 87% of the proteoglycan concentration in the cell-associated matrix compartment.

Conclusions: This study demonstrates that both IGF-1 and TGFβ-2 significantly but differently influence proteoglycan synthesis and accumulation in the cell-associated matrix compartment of cultured bovine chondrocytes in alginate. Both growth factors increase the concentration of proteoglycans in the cell-associated matrix compartment. However, addition of TGFβ-2 to bovine articular chondrocytes cultured in alginate beads for 13 days results in a significant reduction of the relative volume of the pericellular matrix compartment compared to controls, indicating differences in assembly of the matrix.

Key words: IGF-1, TGFβ-2, Matrix assembly.

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Introduction

The unique physicochemical and mechanical properties of cartilage depend on the composition of the tissue and its molecular and ultra-structural

organization. The extracellular matrix of cartilage can be considered as a compound structure consisting of a high concentration of proteoglycans (PG) embedded in a dense meshwork of collagen fibres. The predominant PG, aggrecan, is able to interact with hyaluronic acid and forms large PG aggregates, which gives cartilage its ability to resist compressive loads. The assembly of all molecules together determines the chemically and mechanically defined cartilage matrix and provide cartilage with the ability to transmit load and absorb shocks. Knowledge of matrix assembly is necessary to understand disease processes like osteoarthritis and to find solutions for repair of articular cartilage defects in the future. Although significant progress has been made in characterizing novel cartilage molecules, precise morphometric data on specific matrix compartments in articular cartilage and the regulatory influence of growth factors on these compartments are lacking. Recent evidence on the important but differential influence of insulin-like growth factor 1 (IGF-1) and transforming growth factor β (TGFβ) on the expression of chondrocyte integrins and their adhesion to type VI collagen of the surrounding pericellular matrix, points to the importance of these factors in tissue remodelling and repair [1]. Use of TGFβ-2 *in-vivo* has shown to affect cartilage repair in case of full-thickness defects [2] as well as the pericellular proteoglycan content of normal cartilage [3].

When cultured in alginate, chondrocytes have proven to maintain their cartilaginous phenotype and produce typical PG aggregates and mainly collagen type II for a long time [4–6]. Systematic morphometric analysis of cell and matrix volumes of human articular chondrocytes cultured at a density of 8 million cells/ml of alginate for 30 days, revealed a striking similarity to values obtained for age-and sex-matched articular cartilage biopsies from the same topographical area of the joint [7]. In articular cartilage, the pericellular matrix around the chondrocyte and the territorial matrix lying next to it form the cell-associated matrix compartment [8, 9]. Interestingly, the extracellular matrix of chondrocytes cultured in alginate is composed of these two compartments too: the cell-associated matrix (CM), believed to correspond to the pericellular and territorial matrix compartments, previously identified in articular cartilage by electron microscopy [8] and the further-removed matrix (FRM), that is thought to correspond to the interterritorial areas of the articular cartilage [9]. Lohmander [10] was the first to propose that the cartilage matrix contains at least two compartments of proteoglycans, turning over at different rates, a hypothesis supported by his study of guinea-pig costal cartilage. Evidence in support to this contention was recently obtained from a study which compared the rate of turnover of aggrecan molecules present in the cell-associated and further-removed compartments of the matrix formed by mature bovine articular chondrocytes in alginate beads [9]. Because the alginate culture system gives the opportunity to differentiate between the combined pericellular/territorial (= CM) and the interterritorial (= FRM) matrix [5, 7, 9] it provides an important system to study assembly of PG in different matrix compartments.

The important growth factors known to be able to stimulate PG synthesis are IGF-1 and TGFβ [11–17]. However, not much is known about their influence on matrix assembly of PG and other matrix molecules, a fact which according to recent results shown by Loeser [1] is probably much more important than the mere fact, that there is a stimulation of PG synthesis.

In this paper we report our biochemical and morphometrical investigations of the effects of IGF-1 and TGFβ-2 on PG synthesis and accumulation in the two matrix compartments (CM and FRM) of bovine articular chondrocytes cultured in alginate.

Materials and Methods

CHONDROCYTE CULTURE IN ALGINATE

Metacarpophalangeal (MCP) joints of steers (14–18 months old) were obtained from the slaughterhouse. Full-thickness cartilage slices were aseptically collected and washed with physiological saline. In all experiments cartilage of at least two MCP-joints was pooled to reduce potential individual variation. Slices were incubated for 1–1.5 h with pronase E (2 mg/ml; Sigma, St. Louis, MO, U.S.A.) followed by overnight incubation with collagenase B (1.5 mg/ml, 50 ml/joint; Boehringer Mannheim, Germany) in medium with 10% fetal calf serum (FCS). Undigested parts were removed using a 100 μm filter. Isolated chondrocytes were washed with physiological saline. Cell viability was tested using the trypan blue exclusion test.

Isolated cells were encapsulated in alginate beads at a density of 8×10^6 cells/ml of gel. The preparation of chondrocytes in alginate beads was performed as described by Guo *et al*. [4], with slight modifications described by Häuselmann *et al*. [5]. Briefly, the cells were suspended in sterile saline containing 1.2% low-viscosity alginate gel (Keltone LV, Kelco, Chicago, IL, U.S.A.), then slowly

pressed through a 22 gauge needle in a dropwise fashion into a 102 mM $CaCl_2$ solution. After instantaneous gelation the beads were allowed to polymerize further for a period of 10 min in the $CaCl_2$ solution. They were thereafter washed with saline and finally placed in Ham's F12/Delbecco's modified Eagle's medium (DMEM) medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% FCS, 50 μg/ml gentamicin and 25 μg/ml L-ascorbic acid freshly added (Sigma, St. Louis, MO, U.S.A.). Five beads (18 μl/bead) were cultured in 0.5 ml medium in each well of a 24-well plate. The medium was changed daily.

After 6 days one third of the beads remained on medium plus 10% FCS, one third were supplied daily with 25 ng/ml rhIGF-1 (Sandoz, Switzerland) and one third with 25 ng/ml rhTGFβ-2 (Sandoz, Switzerland) and cultured for another week.

RELEASE OF CELLS WITH THEIR CM FROM THE BEADS

Five beads were dissolved by adding 450 μl of 55 mM sodium citrate and 20 mM EDTA in 150 mM NaCl for 10 min at room temperature. The suspension was centrifuged 10 min at 1000 rpm in an Eppendorf centrifuge, to separate the cells surrounded by CM (the pellet) from components originating predominantly from the 'interterritorial' matrix further removed from the cells (FRM; the supernatant) [5]. However, we can not completely rule out the possibility that matrix constituents from the other matrix component were present in smaller amounts.

DETERMINATION OF THE AMOUNT OF DNA

Papain (Sigma, St. Louis, MO, U.S.A.) was added to the sodium-citrate/EDTA dissolved beads to a final concentration of 125 μg/ml in a volume of 900 μl and incubated overnight at 60°C. The amount of DNA in the beads was measured using Hoechst 33258 dye [18] and compared with calf thymus DNA (Sigma, St Louis, MO, U.S.A.). Extinction (365 nm) and emission (440 nm) were measured with a spectrofluorometer (RF-5001 PC, Shimadzu, Japan).

QUANTIFICATION OF GLYCOSAMINOGLYCANS (GAGS) IN DIFFERENT COMPARTMENTS OF THE ALGINATE BEAD

After separation of CM and FRM, GAG was extracted with 4 M GuHCl (in 0.05 M sodium-acetate buffer containing 0.1 M 6-aminocaproic acid, 0.02 M EDTA, 2.5 mM benzamidine HCl, 5 mM NEM, 0.25 mM PMSF) for 48 h at 4°C (total volume 900 μl).

The amount of GAG in CM and FRM was quantified using the Farndale assay [19]. In short, the metachromatic reaction of GAG with dimethylmethylene blue is monitored using a spectrophotometer. The ratio A_{530}:A_{590} is used to calculate the amount of GAG in the samples. Chondroitin sulfate C (Shark; Sigma) was used as a standard. In the standard curve, corrections are made for the possible effects of solubilizing agents, extractants and remnants of alginate.

QUANTIFICATION OF [^{35}S]SULFATE-LABELED PROTEOGLYCANS IN DIFFERENT COMPARTMENTS OF THE ALGINATE BEAD

In order to investigate whether effect of growth factors on the distribution of PG in CM and FRM is caused by different assembly of PG, the distribution of newly synthesized [^{35}S]PG is studied.

In preparation for the 16 h labeling period with 0.74 MBq/ml [^{35}S]sulfate (Du Pont de Nemours, 's-Hertogenbosch, the Netherlands) the beads were placed in new 24-well plates at day 12 of culture, in order to avoid the labeling of a heterogenous cell population due to the formed monolayer at the bottom of wells. After labeling the beads were washed with saline. The matrix was divided in CM and FRM and PG were extracted with GuHCl as described above.

After extraction, PG were precipitated with 0.1% cetylpyridinium chloride (CPC) at 37°C for at least 2 h. The resulting pellet was washed with 0.05% CPC to remove any remaining glycopeptides and unincorporated precursors. To control the efficiency of removal of unincorporated precursor the last wash step had to contain less than 100 cpm. Each pellet was then supplemented with 0.5 ml Luma solve (Hicol, Oud-Beijerland, the Netherlands) and the [^{35}S]PG quantified with liquid scintillation counting.

TRANSMISSION ELECTRON MICROSCOPY

For electron microscopical analysis, beads were prefixed for 24 h with 2% glutaraldehyde and 2% tannic acid in 0.1 M sodium cacodylate (pH 7.4) to which 10 mM $CaCl_2$ was added to prevent disintegration of the beads. After washing in 0.1 M sodium cacodylate buffer, the beads were postfixed with 1% OsO_4 in 0.1 M sodium cacodylate for 1 h, washed, dehydrated in ethanol and embedded in Epon 812 [7]. Thin sections (≈ 60 nm) were stained with 2% ethanolic uranyl acetate and aqueous lead citrate and examined in a Hitachi H7100-B transmission electron microscope.

Morphometrical analysis

Each of the control, IGF-1 and TGFβ-2 treated groups contained five beads for electron microscopy. Ten sections of each bead were analyzed and from each section 10 photomicrographs were taken by a systematic random sampling protocol, and volume densities of matrix compartments determined morphometrically by point counting procedures [20]. The average of the micrographs of the 10 sections were calculated to obtain one value for each bead. Mean and standard deviation of five beads were calculated and presented.

To validate that the two matrix pools by EM and sodium citrate/EDTA extraction map the same pools cells, cells were released from the alginate by sodium citrate/EDTA extraction, pelleted and fixed and stained for EM. These experiments showed that the cells and the CM go along the lines of the fibrous-basket like structures seen with EM on undamaged alginate beads (unpublished results).

CALCULATION OF GAG CONCENTRATION

Using the determined GAG amount from the Farndale assay and the volume of CM and FRM from EM-morphometry, the concentration of GAG was calculated. Shrinkage (20%) due to dehydration and embedding [20] was accounted for to calculate FRM volume. Shrinkage of the cells and CM was negligible.

Based on the distribution of volumes occupied by cells, CM and FRM and knowing the volume of an alginate bead (18 µl), the volume occupied by CM and FRM was determined in microliters. From the Farndale assay, the amount of GAG was known in micrograms. Therefore we could calculate the concentration of GAG in CM and FRM in micrograms per microliter.

STATISTICAL ANALYSIS

For the biochemical measurements, three experiments each consisting of three groups of five beads for measurements were performed. The paired Student's *t*-test was used for statistical analysis. For the morphometrical measurements, we used 5 beads per group from one experiment. The unpaired *t*-test was used for statistical analysis. $P < 0.05$ was considered statistically significant.

Results

GROWTH FACTOR DOSE

Using 0.25, 2.5 and 25 ng/ml of growth factor in a pilot experiment, we could demonstrate a dose response of GAG synthesis and total amount of GAG. For both growth factors 0.25 ng/ml had no effect and the highest responses were found with 25 ng/ml (data not shown). Therefore this concentration was used for further studies.

THE AMOUNT OF DNA

Each bead contained an average of 3.3 µg DNA. According to published values [18] for DNA content per cell (7.7 pg DNA/cell), each bead contains 4.3×10^5 cells at day 13 of the experiment. Comparing this cell number with the initial number of chondrocytes after their suspension in alginate (1.4×10^5 cells/bead), a threefold increase in cell number could be demonstrated during 13 days of culture. This is in agreement with our previous experiments [5]. No significant differences in the amount of DNA could be shown if growth factors were added (Fig. 1), indicating that IGF and TGFβ had no effect on cell proliferation in our system.

THE AMOUNT OF GAG

Control beads contained 15.3 µg GAG after 13 days. The amount of GAG increased by 43% under the influence of IGF-1 and by 62% if TGFβ-2 was added [Fig. 2(a)]. In addition to calculation of the amount of GAG per bead, the amount of GAG per DNA was calculated. The amount of GAG per micrograms of DNA in control beads was 4.8 ± 1.9 µg GAG/µg DNA and this was increased

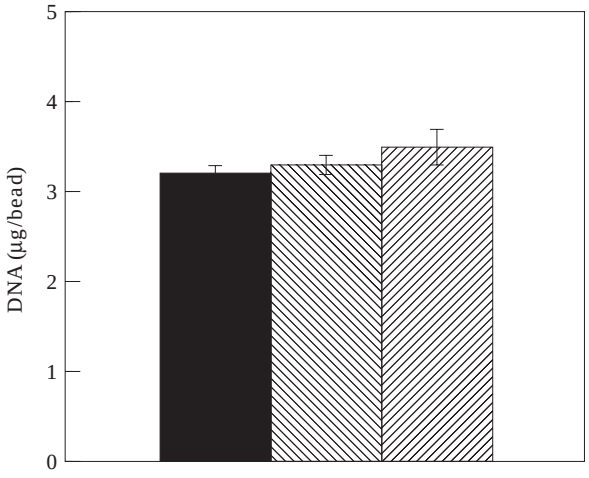

FIG. 1. The amount of DNA (µg) ± S.E.M. per bead after 13 days in culture, the last 7 days in the presence of 25 ng/ml IGF-1 or TGFβ-2. Means of three experiments with five beads are presented. (■) Control; (▧) IGF-1; (▨) TGFβ-2.

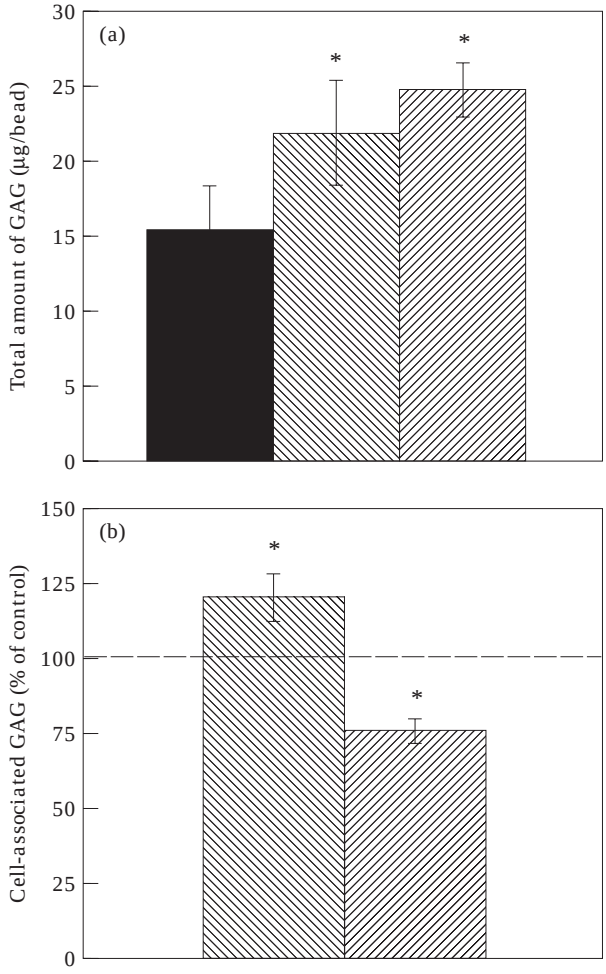

FIG. 2. (a) The total amount of glycosaminoglycan (GAG) (μg) ± S.E.M. per bead after 13 days in culture, the last 7 days in the presence of 25 ng/ml IGF-1 or TGFβ-2. Means of three experiments with five beads are presented. (■) Control; (▧) IGF-1; (▨) TGFβ-2. (b) Percentage of total accumulated GAG in the cell-associated matrix in relation to control cultures. The percentage of GAG in control beads was 25% ± 8. For each of the three experiments the percentage in the control beads is set at 100%. (▧) IGF-1; (▨) TGFβ-2.

by IGF (6.7 ± 1.5 μg/μg DNA) and TGFβ (7.1 ± 0.2 μg/μg DNA).

In control beads, 25% of the GAG was located in the CM and 75% in the FRM compartment. Compared with control beads the percentage of cell-associated GAG was significantly increased with IGF-1 (from 25 to 31%; an increase of 24%) and decreased with TGFβ-2 (from 25 to 19%; a decrease of 24%) [Fig. 2(b)].

SYNTHESIS OF [^{35}S]PG

The same behaviour as demonstrated with the amount of 'cold', non-radioactive accumulated GAG over time in culture, could be demonstrated with regard to the newly synthesized [^{35}S]PG [Fig. 3(a)] at day 13 in culture. IGF-1 increased the percentage of newly synthesized [^{35}S]PG in the CM from 39% in control beads to 47% (21% increase) [Fig 3(b)]. In contrast TGFβ-2 decreased this percentage of [^{35}S]PG in the CM from 39% in the control beads to 29% (26% decrease) [Fig. 3(b)].

With respect to the distribution of newly synthesized [^{35}S]PG in the two compartments of the matrix (CM vs FRM) the percentage of newly synthesized [^{35}S]PG in the CM was somewhat higher than the total accumulated GAG (39 vs 25%).

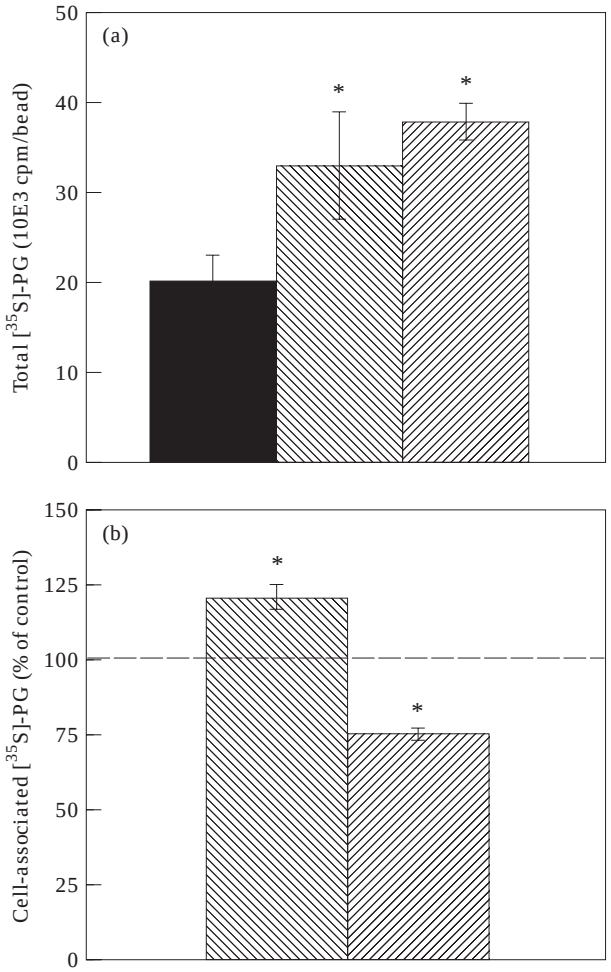

FIG. 3. (a) The amount of newly synthesized [^{35}S]PG during a 16 h pulse at day 13 of culture. The last 7 days of culture the chondrocytes were daily fed with addition of 25 ng/ml IGF-1 or TGFβ-2. Means ± S.E.M. of three experiments with five beads are presented. (■) Control; (▧) IGF-1; (▨) TGFβ-2. (b) Percentage of [^{35}S]PG located in the cell-associated matrix in relation to control cultures. The percentage of [^{35}S]PG in control beads was 39% ± 8. For each of the three experiments the percentage in the control beads is set at 100%. (▧) IGF-1; (▨) TGFβ-2.

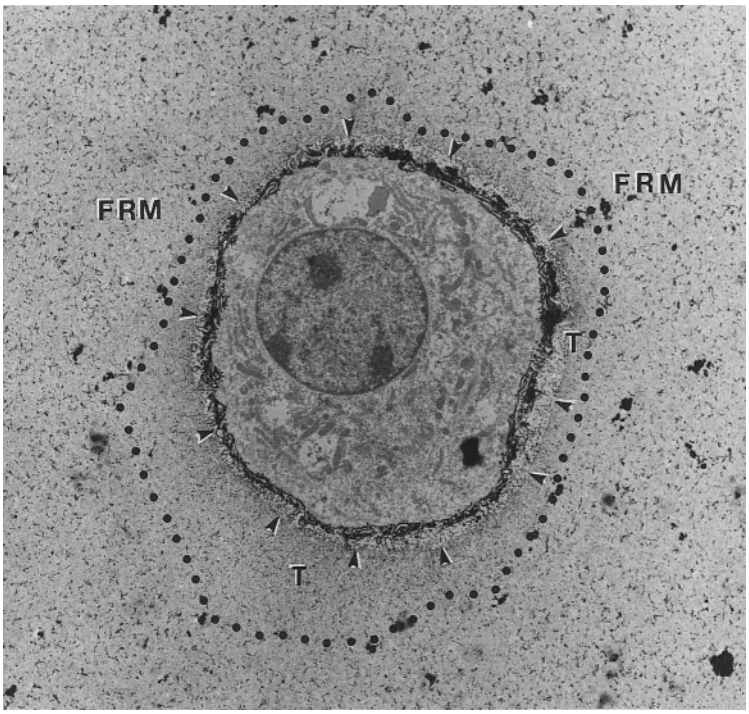

FIG. 4. Electron micrographs of bovine articular cartilage chondrocytes cultured in alginate beads. Control chondrocyte (a), as well as those stimulated with IGF-1 (b), or TGF-β2 (not illustrated), are surrounded by matrix material, which is typically organized into zones: a narrow pericellular mantle (delineated by arrowheads), gives way to a broader territorial coat [(T); outer boundary indicated by solid dots] which, in turn, is surrounded by a 'further-removed' compartment (FRM) that extends almost imperceptibly into the alginate scaffolding. The pericellular matrix bounds in microvilli, protruding from the chondrocyte surface and is also rich in electron-dense clumps of unknown nature. The territorial matrix is characterized by a fine granular appearance, wherase the 'further-removed' one manifests a coarse reticular pattern with scattered electron-dense precipitates (of unknown nature; electron-diffraction negative). Morphometric analysis reveals that the average volumes of the pericellular and territorial matrix compartments surrounding control- and IGF-1 stimulated chondrocytes are larger than those associated with TGFβ-2 treated ones. Magnification: (a): bar = 3 μm. (b): bar = 1 μm.

ELECTRON MICROSCOPY

The cells in alginate showed a spherical morphology in the centre of the bead. Cells of the periphery were more elongated. The beads contained mostly single cells although sometimes small clusters, containing about 3–5 cells, were seen as well. Due to a denser accumulation of amorphous material in the CM compartment a delineation from the FRM is possible (Fig. 4).

Morphological examination revealed [Table 1] that addition of growth factors had no significant effect on the volume occupied by the cells. The compartment of CM however, was significantly smaller around cells treated with TGFβ. The concentration of electron dense

Table I
Relative volumes of cells, CM and FRM and the concentration of PGs in CM and FRM

	Relative volume cells (%)	Relative volume CM (%)	Relative volume FRM (%)	PGs in CM (mg/ml)	PGs in FRM (mg/ml)
Control	3.5 ± 0.33	5.2 ± 0.6	91.3 ± 0.8	3.6 ± 1.0	0.7 ± 2.8
IGF-1	4.2 ± 0.46	5.2 ± 0.8	90.6 ± 1.4	6.6 ± 2.9*	1.0 ± 4.2
TGF-β2	4.2 ± 0.80	3.6 ± 1.4**	92.3 ± 2.4	6.8 ± 1.7*	1.2 ± 3.0

The relative volumes of cells, CM and FRM were quantified using electron microscopy morphometry. The concentration of PG is calculated by combining the results of the Farndale assay and the volumes of CM and FRM.

CM, cell-associated matrix; FRM, further-removed matrix.

*$P < 0.05$; **$P < 0.001$.

material appeared higher. There were no differences visible between control and IGF-1 treated beads.

From the combined biochemical and morphometric data, the concentrations of PG in both the CM and the FRM compartment were determined. Neither IGF-1 nor TGFβ-2 had a significant effect on the concentration of PG in the FRM compartment. However, both growth factors appeared to increase significantly the concentration of PG in the CM compartment [Table 1].

Discussion

In this study we focused on the influence of two important growth factors, IGF-1 and TGFβ-2 on newly synthesized and total PG and their influence on volumes of two different matrix compartments in bovine chondrocytes cultured in alginate for 13 days, using conventional biochemical methodology and exact quantitative morphometrical characterization by electron microscopy. As demonstrated earlier by others and recently by our group using the novel alginate culture system [6, 21–24] the majority of newly synthesized aggrecan molecules will during matrix assembly spend a relatively short time in the cell-associated matrix before moving into the further-removed matrix, where they eventually become incorporated into aggregates. The use of alginate as culture system seems to be advantageous for this type of studies, because of three main reasons. First, the cells maintain their phenotype, resulting in production of collagen type II and aggrecan [4, 6]. Second, the beads can easily be disintegrated using a chelator and after a gentle centrifugation, cells with their pericellular matrix will separate from the further-removed matrix compartment [7]. Third, we have demonstrated previously that human articular chondrocytes, cultured at a density of 8 million cells/ml of alginate for 30 days, revealed cell- and matrix volumes as well as a PG concentration in the CM compartment strikingly similar to values obtained for age- and sex-matched articular cartilage biopsies from the same topographical area of the knee joint [7]. By using the two growth factors IGF-1 and TGFβ-2 in this well characterized model, we were able to study their role in PG synthesis and matrix assembly.

The definition of the effect of IGF-1 and TGFβ-2 on the structure and function of the cartilage matrix as a whole is of critical importance for our understanding of cartilage physiology and potential therapeutic use. In general, IGF is known as anabolic factor and stimulates proliferation and cartilage formation [12, 17, 25]. TGFβ however, has been found to be able to have both stimulating and inhibitory effects [15–17, 25–32]. Differential effects of different TGFβ subtypes are not well known, and Morales [31] described no differences between the effects of TGFβ-1, -2 and -3 on PG synthesis in bovine cartilage. In our study, we could demonstrate that both IGF-1 and TGFβ-2 lead to a stimulation of PG synthesis. These newly synthesized PG were differently laid down in CM or FRM, depending on the growth factor used. The distribution in CM and FRM was comparable for newly synthesized [^{35}S]PG and total PG. This indicates that the differential effect of growth factors is a differential effect on PG assembly and not on PG degradation. Treatment of bovine chondrocytes in alginate for 7 days with 25 ng/ml TGFβ-2, in contrast to IGF-1, not only decreased the relative amount of PG residing in the CM compartment as compared to the FRM compartment, but also unexpectedly lead to a decrease of the matrix volume occupied by the CM compartment, as shown by the corresponding morphometrical analysis. To our knowledge this is the first report demonstrating that the addition of TGFβ to bovine articular chondrocytes cultured in alginate beads for 13 days results in a significant reduction of the relative volume of the pericellular matrix compartment compared to controls.

Cartilage matrix is mainly composed of collagen type II and aggrecan. In addition, small PG (biglycan, decorin, fibromodulin), other glycoproteins (fibronectin), other collagen types (type VI, IX and XI) and non-collagenous matrix proteins (e.g., cartilage oligomeric matrix protein, COMP) are present. All these molecules are important but not well-characterized factors for matrix assembly. The relation between small PG and collagen seems to play a crucial role in matrix assembly. Biglycan is mainly found in the pericellular matrix and is thought to interact with collagen VI which is bound to the cell surface. Decorin is thought to play a role in fibrillogenesis. Roughley *et al*. [33] showed that TGFβ increased the synthesis of biglycan and decreased that of decorin, whereas IGF had no effect. By these effects TGFβ may affect matrix assembly. As published by several groups [1, 7, 34] surface receptors of chondrocytes (CD44, integrins, COMP) bind to several components of pericellular matrix and therefore are important factors for matrix assembly. Recent evidence shown by Loeser [1] points to the importance of growth factors and their differential effects on integrin expression on chondrocytes. In contrast to IGF-1, TGFβ decreased cell surface levels of α1β1 integrins. TGFβ was also able to induce a decreased adhesion of chondrocytes to collagen

type VI, which plays an important role for the assembly of pericellular matrix in cartilage. These results point to a possible inhibitory function of TGFβ regarding the assembly of the pericellular matrix and may explain our findings of a reduced volume of the CM compartment in cultured bovine chondrocytes by TGFβ compared to IGF-1 and control.

In the light of our findings it is worth noting the published results of van Beuningen *et al*. [3] and van den Berg *et al*. [35], who showed an enhanced loss of PG in the middle layers of mouse femoral cartilage after intra-articular injection of TGFβ (but not IGF-1). Further studies are needed to clarify the function and effects of growth factors, and especially TGFβ-2, in matrix assembly and to study whether these effects result in cartilage of different mechanical properties, and a different vulnerability for degradation.

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