Transforming growth factor β stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes

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The effect of transforming growth factor β (TGF-β) on the production of matrix macromolecules was studied in cultures of rabbit articular chondrocytes. A 24 h exposure to TGF-β at concentrations of 0.1, 1 and 10 ng/ml markedly stimulated the synthesis of collagen and non-collagen protein. Similar increases of glycosaminoglycan production was observed in the same experimental conditions. The distribution of these newly synthesized macromolecules between cell layer and medium was not altered by treatment with TGF-β. The factor slightly enhanced the proliferation of chondrocytes in these experiments but its potent effect on matrix synthesis was independent of this growth stimulation. These results indicate that articular chondrocytes are target cells for TGF-β and suggest that this growth factor could play a role in the repair process of cartilage during osteoarticular diseases.

Transforming growth factor β; Collagen; Glycosaminoglycan; Extracellular matrix; Articular chondrocyte

1. INTRODUCTION

Osteoarticular diseases such as rheumatoid arthritis and osteoarthritis are characterized by an alteration of the cartilage extracellular matrix leading to irreversible lesions. Although degradation of the main components of that tissue (collagen, proteoglycans) represents the predominant features in these pathologic situations [1], it has also been reported that synthesis and deposition of the matrix macromolecules could be affected [2,3]. A modification of collagen and proteoglycan metabolism directly implicates the articular chondrocyte in the basic pathogenesis of these disorders since this cell is the producer of such components in cartilage. The precise nature of the mechanisms regulating chondrocyte metabolism is not fully understood. However, during the last few years, evidence has emerged indicating that a number of mediators such as Interleukin-1 (Il-1) released by immune and inflammatory cells have the ability to interact with the chondrocyte and thereby modulate its functional behaviour [4–6]. Recent studies have also shown that transforming growth factor β (TGF-β), a 25 kDa protein derived from normal tissues and transformed cells (rev. in [7]), stimulates the production of fibronectin and collagen by cultured fibroblasts from several origins [8,9] and the synthesis of proteoglycan in arterial smooth muscle cells [10]. TGF-β is present in a high concentration in platelets [11] but it is also expressed in activated macrophages [12] and lymphocytes [13] which are the principal cellular elements of chronic inflammatory tissues. Therefore, it was of interest to determine whether TGF-β could interact with articular chondrocytes as target cells and modulate their matrix production. To date, there is but a single report indicating that TGF-β was able to decrease synthesis of glycosaminoglycans and collagen by rabbit articular chondrocytes [14]. In that study, the cells were cultured in soft agar in three dimensions.

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In contrast with these results, we report here that TGF-β is a potent stimulant of the synthesis of these matrix molecules in monolayer cultures of rabbit articular chondrocytes, suggesting that this factor could play a role in the cartilage metabolism during osteoarticular diseases.

2. MATERIALS AND METHODS

2.1. *Chondrocyte culture*

Articular cartilage slices were taken from the shoulders and the knees of 1–2 month old rabbits. Chondrocytes were obtained by enzymatic dissociation [15] and cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with glutamine, penicillin-streptomycin-fungizone and 10% FCS (fetal calf serum). The cells were grown at 37°C in a 5% CO_2 environment and experiments were performed on primary confluent cultures.

2.2. *Source of TGF-β*

Purified TGF-β from human platelets (hTGF-β_1) was supplied by R & D Systems, Inc., Minneapolis, USA.

2.3. *Experimental conditions*

For the experiments, the cells were seeded in 9.6 cm^2 Petri dishes. After reaching confluency, the cultures were incubated for 24 h in 1.5 ml DMEM containing 2% FCS, β-aminopropionitrile (100 μg/ml) and ascorbic acid (100 μg/ml). Then the medium was removed and 1.5 ml of serum free-medium supplemented with ascorbic acid, β-aminopropionitrile, 2 μCi/ml [^{3}H]proline (CEA, France, 20–30 Ci/mmol) or 2 μCi/ml [^{3}H]glucosamine (CEA, France, 11 Ci/mmol) was added to the dishes, together with various concentrations of TGF-β (0.1–10 ng/ml). After 24 h radiolabeled collagen and proteoglycan were assayed in the culture medium and in the cell layer. Each experiment was performed in triplicate.

2.4. *Assay of collagen and non-collagen protein*

At the end of the experiment, the medium from each well was collected and the cell layers scraped with a rubber policeman and disintegrated by ultrasonication for 30 s. Radiolabeled collagen in medium and cell layer was estimated separately after digestion with highly purified bacterial collagenase (Advance Biofactures Corp., Lynbrook, NY, USA) [16]. The amount of non-collagen was estimated as the difference between total protein and collagen respective radioactivities.

2.5. *Glycosaminoglycan assay*

After incubation with [^{3}H]glucosamine, the medium was removed, the cell layer was washed twice with phosphate-buffered saline (PBS) at 37°C, and the washes were added to the medium. The cell layer and 1 ml of the medium sample were digested with pronase (5 mg/ml, Sigma) for 24 h at 55°C. Glycosaminoglycans were isolated by cetylpyridinium chloride (1%, w/v) and ethanolic (5 vols) precipitations as previously described [17]. The final pellet was dissolved in 1 ml of 0.075 M NaCl and the radioactivity of an aliquot was assayed by liquid scintillation counting.

2.6. *Cellular protein assay*

The protein content of cell layers was determined on parallel dishes incubated in the same conditions. After two rinses with PBS, the cells were dissolved in 0.2 M NaOH (1 ml) and the amount of protein assayed according to Hartree [18].

3. RESULTS

3.1. *Effect of TGF-β on proliferation of rabbit articular chondrocytes*

The experiments described in this report were performed on primary cultures to avoid dedifferentiation of the chondrocytes. In such conditions, we have previously shown that rabbit articular chondrocytes still produce mainly type II collagen with only a small amount of type I collagen [5]. The cultures were confluent to minimize any effect of TGF-β on cell proliferation which could have masked an action on collagen synthesis per se. However, it was found that TGF-β at the concentrations used (0.1, 1 and 10 ng/ml) increased the cell number over the 24 h period of experiment, as reflected by the cell protein amount (table 1). The increase was about 15% for the highest concentration of TGF-β. Therefore, the levels of collagen and glycosaminoglycan synthesis were expressed as incorporated radiolabeled per μg of cell protein.

3.2. *Effect of TGF-β on protein and collagen synthesis*

To determine the effect of TGF-β on [^{3}H]proline

Table 1

Effect of TGF-β on the amount of cellular protein

	Cellular protein	
	μg/well	% of control
Control	568	
TGF-β (0.1 ng/ml)	595	+5
TGF-β (1 ng/ml)	641	+13
TGF-β (10 ng/ml)	654	+15

Confluent cultures of rabbit articular chondrocytes in 9.6 cm^2 dishes were preincubated for 24 h in 1.5 ml of DMEM containing 2% FCS, β-aminopropionitrile (100 μg/ml) and ascorbic acid (100 μg/ml). Then the medium was removed and 1.5 ml of the same medium without FCS was added, in the presence or absence of TGF-β. After a 24 h incubation, the amount of total cell layer associated protein was estimated as described in section 2. Values are the mean of duplicates. Increases are indicated in brackets as per cent of controls

incorporation and collagen biosynthesis, three concentrations of TGF-β were tested. The results shown in fig.1 (A and B) demonstrate that TGF-β caused a dose-dependent stimulation of both collagen and non-collagen protein production. This stimulation was observed with as little as 0.1 ng/ml (30–33% greater for both total synthesis) and reached about 80–100% for 10 ng/ml. The amounts of medium and cell layer associated protein were similarly increased so that no change could be detected in the distribution of newly synthesized collagen and non-collagen protein. These experiments clearly showed that the stimulative effect of TGF-β was not selective for collagen.

3.3. *Effect of TGF-β on glycosaminoglycan production*

Treatment of rabbit articular chondrocytes with

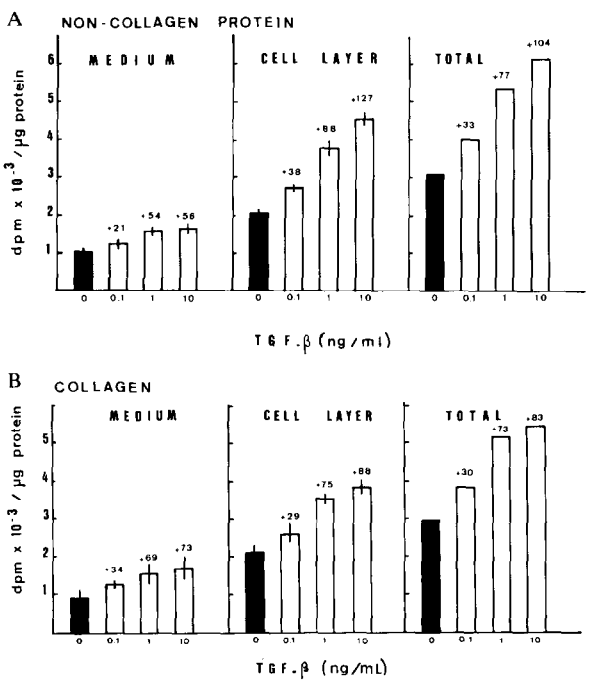

Fig.1. Effect of TGF-β on production of non-collagen (A) and collagen (B) protein. Experimental conditions were the same as for table 1, except that 2 μCi/ml [^{3}H]proline were added during the 24 h incubation period. At the end of the experiment, the amount of radiolabeled collagen was estimated in both medium and cell layer as collagenase-digestible radioactivity (cf. section 2). Non-collagen protein content of the same pools was deduced from the difference between the value of total protein radioactivity and that of collagen. Values are the mean ± SE of triplicate dishes. Increases are indicated as per cent of controls.

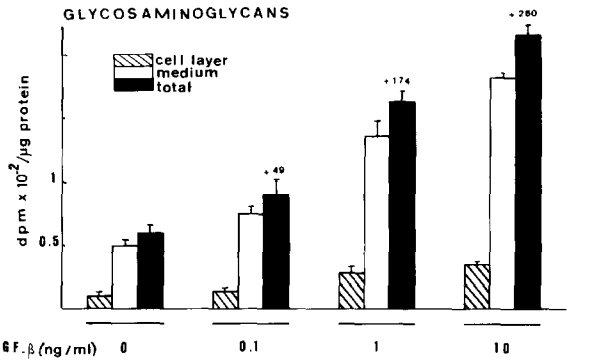

Fig.2. Effect of TGF-β on production of glycosaminoglycan. Experimental conditions were the same as for table 1, except that 2 μCi/ml [^{3}H]glucosamine were added during the 24 h incubation period. At the end of the experiment, the amount of radiolabeled glycosaminoglycans was assayed in both medium and cell layer as described in section 2. Values are the mean ± SE of triplicate dishes. Increases are indicated for the total amount of glycosaminoglycan as per cent of controls.

TGF-β over a 24 h period resulted in a significant increase of [^{3}H]glucosamine incorporation into GAG (fig.2). The radioactivities of cellular and medium pools from cultures exposed to 0.1, 1 and 10 ng/ml TGF-β were, respectively, 1.5, 2.7 and 3.6-fold greater than in controls. The amount of GAG present in the medium of control cultures was approx. 5-fold higher than that of cell layer. This relative proportion was not significantly affected by TGF-β.

4. DISCUSSION

TGF-β has been reported to exhibit a wide spectrum of regulatory activities that affect both normal and neoplastic cells [19]. This factor exerts either growth-stimulating or growth-inhibiting effects depending on the cell type. Recent studies have also shown that TGF-β stimulates matrix formation and inhibits matrix degradation by a dual effect: increased synthesis of protease inhibitors and reduced synthesis of proteases themselves (review [7]). The functional properties of this molecule are highly relevant to inflammation and tissue repair and therefore could be involved in osteoarticular diseases.

The results presented here demonstrate that cultured chondrocytes which could control the metabolism of cartilage matrix are sensitive to

TGF-β. They respond to this factor by an increased production of collagen and glycosaminoglycans. With reference to collagen synthesis, previous studies have reported a stimulating effect of TGF-β on other cell lines, such as fibroblasts [8,9] and osteoblasts [20]. In contrast to the data presented by others on fibroblasts [9], suggesting a selective effect on collagen production, no difference in the extents of stimulation was observed between synthesis of collagen and non-collagen protein.

Our results clearly indicate for the first time that TGF-β also exerts a stimulatory effect on glycosaminoglycan synthesis by articular chondrocytes. Interestingly, it has been recently reported that TGF-β could enhance synthesis of proteoglycan in human arterial smooth muscle cells [10]. However, our results are not in agreement with those of Skantze et al. [14] describing an inhibition of TGF-β of collagen and GAG synthesis in cultured rabbit articular chondrocytes. As a possible explanation for that discrepancy, it must be noted that the experimental procedure used by these authors was very different from ours. In that particular case, the cells were cultured in soft agar, i.e. anchorage independent growth conditions, the incubation period was seven days in the presence of TGF-β followed by seven more days without factor (total: 14 days) and fetal calf serum (10%) was present in the culture medium all along. In contrast with transformed or dedifferentiated cells which grow in soft agar or suspension, chondrocytes in these conditions keep their phenotype and still produce type II collagen [21] as they did in our primary monolayer cultures [5]. However, these agarose cultures are composed of clones of separated spherical chondrocytes whereas our monolayer cultures contain confluent polygonal cells. In fact, biochemical differences associated with cell shape modulation have been reported. For example, Wittelsberger et al. [22] have shown that RNA and protein synthesis in normal diploid cells was rapidly inhibited when these cells were placed in suspension cultures. Therefore, even if chondrocytes are well differentiated in agarose culture, expressing type II collagen and specific proteoglycans, both cell shape and lack of intercellular contact may have some influence on the response to TGF-β that could account for the discrepant results. Another possible explanation may come from the presence of serum in the experiments of Skantze et al. [14], a critical point for the demonstration of the modulatory activity of a particular factor [23]. For example fetal calf serum contains several growth factors which could interfere with the response of cells to TGF-β. Moreover, it cannot be ruled out that TGF-β may induce in the cultured chondrocytes the expression of other factors susceptible of modulating matrix production, especially over so long an incubation period.

As only the GAG fraction of the proteoglycan released by articular chondrocytes has been assayed in our system, it remains to determine whether synthesis of the proteoglycan core protein is also affected by TGF-β. Work is in progress to characterize further the mechanism whereby TGF-β stimulates the production of the main matrix components by articular chondrocytes. Nevertheless, this preliminary report suggests that TGF-β may play a role in the repair response of cartilage to destruction generally observed in osteoarticular diseases. It is possible that this factor contributes to pathogenesis by inducing an excessive inflammatory response at the level of synovial tissue and produces at the same time a repair process in articular cartilage. The above described growth-promoting effect and stimulation of collagen and glycosaminoglycan synthesis are all functional properties of TGF-β which could be of some therapeutic benefit for repair of cartilage matrix by chondrocytes in diseases such as osteoarthritis.

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