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OSTEOARTHRITIS and CARTILAGE

SHORT COMMUNICATION

Decrease of cartilage transforming growth factor-β receptor II expression in the rabbit experimental osteoarthritis—potential role in cartilage breakdown

Keywords: TGF-β receptors, Cartilage, Osteoarthritis.

Introduction

OSTEOARTHRITIS (OA) is characterized by cartilage destruction in which the cytokine interleukin-1 (IL-1) plays a central role, by its ability to induce expression of cartilage degrading enzymes and to depress the synthesis of major matrix components [1–4]. Interestingly, it has been reported that OA chondrocytes are hyper-responsive to stimulation by IL-1 and display upregulation of IL-1 receptor [5].

However, OA animal models have revealed an activation of chondrocytes and enhanced proteoglycan (PG) synthesis in early stages of the process [6, 7], and even at late stages of OA, the remaining chondrocytes quite often show enhanced PG synthesis, as if they were attempting to replenish the depleted matrix. Among the local growth factors that could be involved in this repair potentiality, TGF-β (transforming growth factor-β) may play a pivotal role as it has been shown to enhance matrix production and to modulate cell proliferation [reviewed in 8]. TGF-β can also counter the IL-1-induced effects on expression of metalloproteases and matrix molecules and down-regulate the expression of IL-1 receptor in articular chondrocytes [8]. Furthermore, repeated intra-articular injections of TGF-β in the murine knee joint caused upregulation of chondrocyte PG synthesis and development of osteophytes [9].

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Articular chondrocytes express at least the three major TGF-*β* receptors, type I, type II and type III (betaglycan). It is now established that only types I and II are implicated in the signal transduction. TGF-β binds to type II receptor and then forms an heterotrimer with type I which is transphophorylated and transmits the signal through a serine-threonine kinase mechanism [10].

So far, it is not known if the expression of TGF-β and its receptors is altered in osteoarthritic cartilage. Our hypothesis was that changes in TGF-β expression could explain the enhanced metabolic activity of OA cartilage, as well as the decreased metabolic activity of the tissue observed at the end stage of cartilage destruction [7]. Cruciate ligament section in the rabbit knee is known to induce an initial stage of joint hypertrophy including an increased cartilage thickness and lately a stage of cartilage destruction similar to that observed in OA human joint at the time of joint replacement [6, 7]. Thus, the model allows the study of factors regulating both the repair and the destruction of articular cartilage. Here we show that the expression of TGF-β1 and TGF-β2 was slightly decreased in advanced stages of the rabbit OA (fibrillated cartilage) whereas the message for TGF-β receptor II was dramatically reduced as early as the hypertrophic stage. These alterations could result in the reduced sensivity of articular chondrocytes to TGF-β during development of the osteoarthritic process. This would explain, at least partially, that repair potential of cartilage becomes progressively unable to balance the erosive process.

Methods

Twenty-five male rabbits (1.8–2.0 kg), were subjected to transection of either the anterior cruciate ligament (ACL) or of both the ACL and the posterior cruciate ligament (PCL) [7]. Both

knees of the same animal were subjected to the same surgery. The animals were divided into (1) a 'control group' of five unoperated animals which were killed 4 weeks later, (2) an 'early' group of five rabbits with section of the ACL ligaments and were killed 1 week after surgery, (3) an 'hypertrophic' group of six rabbits with section of the ACL and killed after 4 weeks, (4) a 'late' group of nine animals which had section of both cruciate ligaments and were killed 8 weeks after surgery.

Each of the six articular surfaces of the knee joint (patella, femoral groove, medial and lateral femoral condyles, medial and lateral tibial plateaux) was examined with a magnifying-glass and graded as follow: 0 = normal, 1 = hypertrophic cartilage with intact surface and osteophytes; 2 = grade 1 + roughening or minimal fibrillation of the articular surface; 3 = grade 1 + a region of deep cartilage erosion; 4 = grade 1 + a small area of bone exposure; 5 = large area of bone exposure (total score range: 0–30). Only the grossly normal cartilage of both the early and hypertrophic groups was collected and fibrillated areas were carefully avoided. Inversely, only the fibrillated cartilage of the late group was collected while the remaining normal cartilage was excluded. Cartilage slices were frozen into liquid nitrogen and stored at –70°C. The samples were subsequently pulverized into powder in the presence of liquid nitrogen and total RNA was then extracted (RNAXEL protocol, Eurobio, France). To avoid interference with aggrecans, the extracts were further purified in a 4-ml cushion of 5.6 M CsCl, by centrifugation for 24 h at 32 000 rpm and 18°C (Beckman SW 41 Ti rotor). The concentration of RNA was determined by measuring the OD_{260}. The OD_{260}/OD_{280} ratios were greater than 1.8.

Total RNA (0.1 μg) was reverse transcribed into cDNA according to the Life Technologies PCR kit instructions. The reaction was performed at 42°C for 1 h. Amplification of generated cDNA was performed in an Omni E Hybaid thermocycler with specific primers of TGF-β1, TGF-β2, TβR-I, TβR-II and β-actin. Thirty-five cycles were chosen as the appropriate number deduced from preliminary amplification curves, with the following conditions: 95°C, 30 s/55°C, 30 s/72°C, 1 min. Then, an additionnal step at 72°C for 10 min was included. Products were analyzed on a 2% agarose electrophoresis gel, using ethidium bromide staining, and photographied with 665 Polaroid film. The specificity of the transcripts was verified by Southern blotting using human cDNA probes. Bands were quantified by densitometric scanning (Image-QuaNt software, Molecular Dynamics) and normalized to β-actin expression.

Results/Discussion

In this study, special attention was paid to the sampling method, taking care to collect only small areas of normal and hypertrophic cartilage and leaving apart the fibrillated zone in the first two groups of operated animals and inversely taking only fibrillated areas in the last group. This approach permits to isolate well-defined cartilage states but it has the disadvantage of material scarcity so that the samples from the same group of animals must be pooled. However, a preliminary experiment revealed that the variation between animals was low and did not cause unacceptable scatter. RNA levels were sufficient to allow semi-quantitative analysis of four to six different genes and competitive PCR could not be applied as it requires at least five-times as much RNA. In our experiments the expression of actin was included in all the assays as a reference gene and its expression proved to be approximately constant in all the samples. Furthermore, the differences observed in the messages, specially in the case of receptor II were so large that precise quantification by competitive PCR would have been of limited additional value.

We found that both the mRNA levels for TGF-β1 and TGF-β2 were significantly decreased in late stages of OA, i.e., in cartilage samples showing evidence of destructive lesions [Fig. 1(a) and 1(b)]. Moreover, at this time and in the earlier hypertrophic stage, the expression of receptor type II was dramatically reduced to almost undetectable levels. Given that TβR-II is absolutely required to bind TGF-β, form an heteromeric complex with TβR-I and transmit the signal [10], we may suggest that this down-regulation results in reduced sensitivity of the articular chondrocytes to TGF-β during development of the osteoarthritic process. That the responsiveness of OA chondrocytes diminishes would explain, at least partially, that repair potential of altered cartilage is no longer capable of balancing the erosive process and that unreversible degradation takes place.

It may seem surprising that down-regulation of the TβR-II expression was observed as early as the hypertrophic stage of the OA process, a time phase where the chondrocytes are metabolically more active, as it has been reported that cartilage from OA lesions shows immediate sensitivity to TGF-β and a greater stimulation of PG synthesis than normal cartilage [11]. However, the hyperactivity of chondrocytes during the hypertrophic phase may result from a previous exposure to TGF-β during the early stages preceeding this period. It

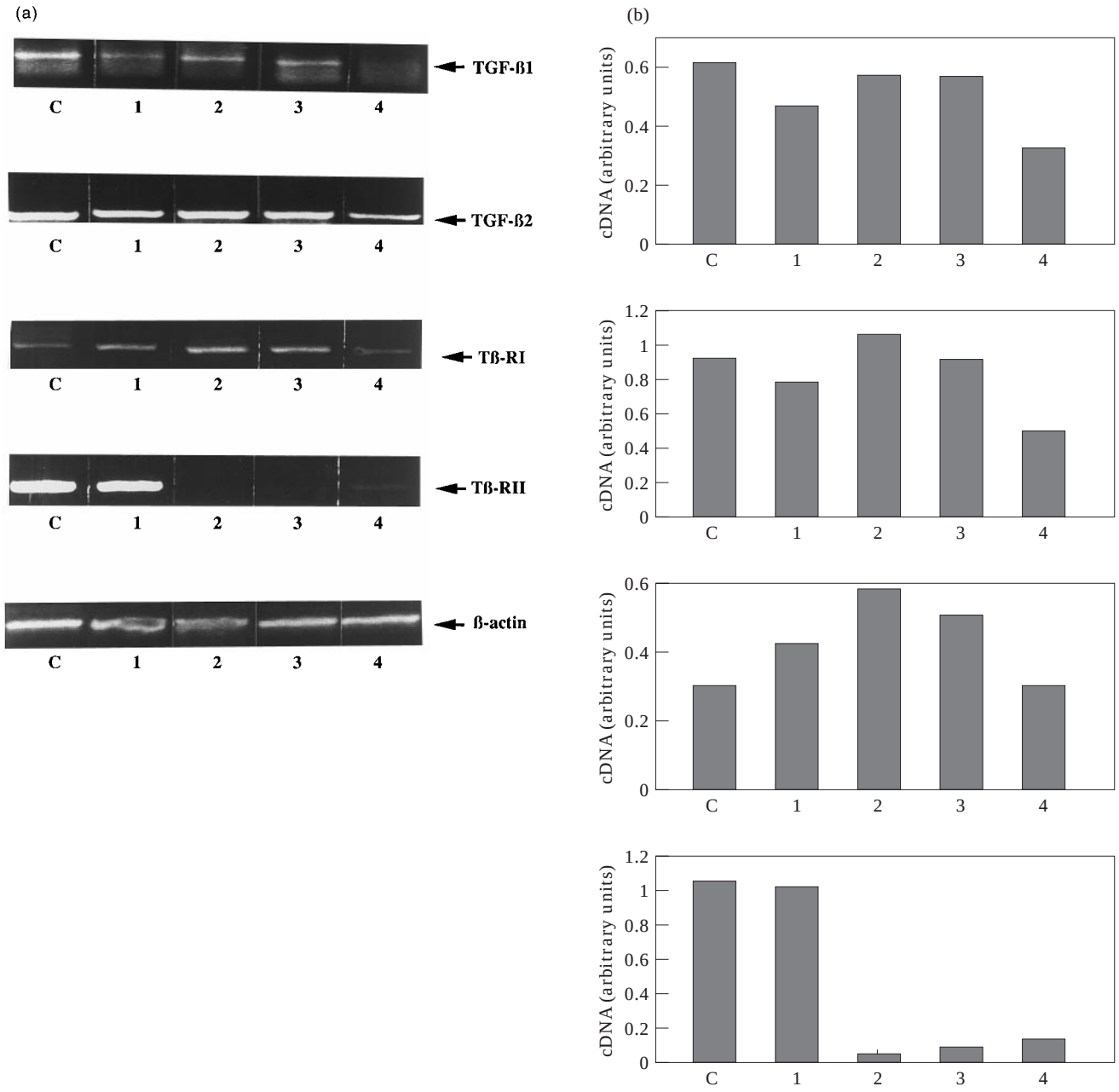

FIG. 1. Semiquantitative analysis of TGF-β and receptor mRNA levels. RT-PCR reactions were performed using the following primers: TGF-β1: sense 5'-GCCCTGGACACCAACTATTGC-3', antisense 5'-GCTGCACTTGCA-GGAGGGCAC-3'; TGF-β2: sense 5'-GCTTTGGATGCGGCCTATTGC-3', antisense 5'-GCTGCATTTGCAAGACTTTAC-3'; TβR-I: sense 5'-CTGCTCCCGGGGGCGACGGCGTTACAGTGTTTCTGC-3', antisense 5'-TAAGTCTGCAATACAGCAAGTTC-CATTCTT-3';TβR-II: sense 5'-CGCTTTGCTGAGGTCTATAAGGCC-3', antisense 5'-GATATTGGAGCTCTTGAGGTC-CCT-3'; β-actin: sense 5'-GTGGGGCGCCCCAGGCACCA-3', antisense 5'-CTCCTTAATGTCACGCACGATTTC-3'. (a) Ethidium bromide staining; (b) Densitometric scanning of the signals, normalized to β-actin expression. (c) Control (unoperated rabbits). 1, Early stage of OA (1 week after section of cruciate ligaments). 2 and 3, Two different batches of hypertrophic stage (4 weeks after section). The amount of material being greater at this stage, two pools of three samples each were studied separately. 4, Late stage, fibrillated cartilage (8 weeks after section).

would also be possible that cartilage samples were collected at the end of the hypertrophic stage, at a transient stage in which cartilage would no longer be able to produce TGF-β. This stage of cartilage repair failure would be responsible for the final destruction of the tissue. Work is in progress to monitor the expression of aggrecan in parallel to that of TGF-β and its receptors in the same experimental OA model as an attempt to clarify this point.

The altered expression of TGF-β receptor types may have profound consequences on the functionnal activity of the chondrocytes and the properties of cartilage matrix. Evidences have been recently provided that the ratio of type I to type II receptors modulates responsiveness to TGF-β in several cell types, including chondrocytes [12–14]. Interestingly, we previously demonstrated that rabbit articular chondrocytes in culture express different affinity for TGF-β1 as a function of the cell-cycle phases [8, 14]. Recently, we could provide evidence that the ratio of type I to type II receptor expression levels decreased in S-phase chondrocytes, compared to G_0/G_1 cells, as a result of down-regulation of type I (Boumediene *et al*., in preparation). Therefore, differential phenotype of TGF-β receptors may play a great role in the proliferation of articular chondrocytes in OA since we know that after the initial cartilage lesion these cells leave their quiescent state and start proliferate to form clones. This stimulus may at this time induce the quiescent chondrocytes to express a receptor profile that predisposes them to be stimulated by TGF-β and proliferate or that facilitates the activation by other growth factor such as BMPs. Later on, there could be changes in this profile as the disease progresses and chondrocytes become less active.

In addition to transcriptional controls, several potential mechanisms may control the availability and biological effects of TGF-β molecules in the cartilage: processing and activation of precursors, interaction with other cell-surface binding proteins including betaglycan and specific binding to matrix components such as decorin and biglycan. To this respect, it is of interest that the synthesis of cartilage small dermatan sulfate proteoglycans (decorin, biglycan and fibromodulin) is increased in the hypertrophic phase of canine experimental OA [15].

Despite that our findings are preliminary and based on a single model, they suggest that new perspectives in OA treatment should therefore tend to stimulate the expression of TGF-βs and their receptors in articular cartilage.

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