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Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation

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Abstract. Arthritic diseases cause enormous burdens in terms of pain, crippling, and disability. Osteoarthritis (OA), the most common form of arthritis, is characterized by a slow progressive degeneration of articular cartilage. The exact etiology of OA is not known, but the degradation of cartilage matrix components is generally agreed to be due to an increased synthesis and activation of extracellular proteinases, mainly matrix metalloproteinases. Insufficient synthesis of new matrix macromolecules is also thought to be involved, possibly as a consequence of deficient stimulation by growth factors. Although OA is defined as a noninflammatory arthropathy, proinflammatory cytokines such as interleukin-1 have been implicated as important mediators in the disease. In response to interleukin-1, chondrocytes upregulate the production of nitric oxide and prostaglandin E_2, two factors that have been shown to induce a number of the cellular changes associated with OA. The generation of these key signal molecules depends on inducible enzymes and can be suppressed by pharmacological inhibitors.

Key words. Osteoarthritis; cartilage; chondrocyte; inflammation; proteoglycan biosynthesis; alginate; aggrecan; nitric oxide; interleukin-1.

Osteoarthritis (OA) is the most common form of arthritis [1–3]. Among North American and European adults above 30 years of age, symptomatic disease in the knee occurs in approximately 6% and symptomatic hip OA in roughly 3%. Since OA is a disease whose prevalence increases with age, its frequency in the future will increase as the proportion of the population above 70 years of age is expected to rise dramatically. Because of its prevalence and the frequent disability that accompanies the disease in the knee and hip, OA accounts for more trouble with daily life locomotory activities, such as climbing stairs and walking, than any other disease. OA is the most common reason for total hip and total knee replacement. The longevity of working careers and the substantial prevalence of OA in middle-aged persons causes considerable burden in lost working time and early retirement.

Whether OA is a single disease or many disorders with a similar final common pathway is unclear. OA can be defined by symptoms or pathology. The pathology of OA involves the whole joint in a disease process that includes focal and progressive hyaline articular cartilage loss with concomitant changes in the bone underneath the cartilage, including development of marginal outgrowths, osteophytes, and increased thickness of subchondral bone. Soft-tissue structures in and around the joint are also affected. These structures include synovium, which may show modest inflammatory infiltrates, ligaments, which are often lax, and bridging muscles, which become weak. Many people with pathologic and radiographic evidence of the disease have no symptoms. From a clinical perspective, the most compelling definition of a disease is one that combines the pathology with pain that occurs during joint use. Unfortunately, neither the cause of pain nor the exact etiology of OA is known. Most basic research on the pathogenesis of OA has focused on the articular cartilage. As a consequence, we mostly deal with these aspects in this review.

Human articular cartilage is continually remodeled as a consequence of anabolic and catabolic processes. The chondrocytes in normal adult cartilage maintain a balance between synthesis and degradation of extracellular matrix components. In OA, the metabolic activity of the chondrocytes is shifted toward a state where new matrix synthesis is outweighed by breakdown of matrix con-

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stituents. The result is degeneration and gradual loss of articular cartilage.

OA is believed to be caused by a combination of factors. Injury due to mechanical stress, although poorly understood, is considered to play a predominant role during initial stages of the disease. Biochemical and genetic factors are likely to contribute to the further progression. Upregulation of proteinase activities, particularly those of metalloproteinases (MMPs), has been implicated in the disease as the major cause of increased matrix catabolism. The degradation of structural macromolecules like proteoglycans (PGs) and collagens leads to depletion of the most important building blocks of the extracellular matrix. Under these circumstances, the absence of adequate replenishment becomes a main issue. Many questions concerning the control of anabolic processes remain open and these are being given considerable attention in current research.

OA, in contrast to rheumatoid arthritis, is defined as a noninflammatory arthropathy. In OA, classical signs of inflammation are absent, there is no marked infiltration of inflammatory cells into joint tissues, and the synovial fluid usually contains few neutrophils. Nevertheless, much evidence supports the view that inflammatory components are involved in the disease process. Cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α can be detected in synovial fluid from OA patients, and these synovium- and chondrocyte-derived factors have been shown to promote the synthesis of proteolytic enzymes and other catabolic or antianabolic factors. Thus, increased knowledge of the metabolic events that occur within and around chondrocytes in response to proinflammatory cytokines will most likely help to define potential targets for therapeutic intervention.

Role of the chondrocyte in cartilage destruction

Chondrocytes represent the only cell type in hyaline cartilage. These cells are responsible not only for the generation of extracellular matrix during growth and development, but also for the maintenance of tissue homeostasis during adult life. The chondrocyte in mature articular cartilage exhibits virtually no mitotic activity and a very low rate of matrix synthesis and degradation. However, chondrocytes even from old individuals are able to respond to given stimuli by showing increased activity. In early OA, structural changes in the extracellular matrix induce chondrocyte proliferation (clonal growth), stimulated collagen and PG biosynthesis corresponding to a repair attempt, and an increased production of catabolic cytokines and matrix-degrading proteinases.

Growth factors – insulin-like growth-factor-I and the transforming growth factor-β superfamily

The anabolic activity of chondrocytes is enhanced by soluble growth factors. Studies of articular cartilage in organ culture demonstrated that chondrocytes are able to maintain the initial amount of PG and collagen over several weeks if supplied with culture medium containing fetal calf serum [4]. Withdrawal of serum from the system led to decreased biosynthesis and net loss of PG. Insulin-like growth factor (IGF)-I was identified as the serum factor mainly responsible for the stimulation of PG biosynthesis [5]. Further studies revealed that IGF-I can increase PG synthesis also in the presence of IL-1 and TNF-α, and reduce cartilage degradation induced by these cytokines [6]. In addition to its role as an anabolic factor, IGF-I acts as a differentiation factor that promotes the reexpression of cartilage-specific collagen II and aggrecan in chondrocytes that have been growing in a dedifferentiated state [7]. Chondrocytes in human articular cartilage express IGF-I and the type 1 IGF receptor, indicating a local function via autocrine/paracrine mechanisms. IGF-I expression is significantly higher in OA than in normal cartilage, and particularly high levels of expression are shown by cells within areas of more advanced OA lesions [8]. Furthermore, OA chondrocytes possess an increased number of IGF receptors [9, 10], yet appear hyporesponsive to exogenous IGF-I [9, 11]. Changes in cartilage that occur in OA have been suggested to be due in part to a loss of IGF-I effects on articular chondrocytes. A possible mechanism involves the upregulation of IGF-binding proteins [12–14].

Beside IGF-I, the members of the transforming growth factor (TGF)-β family and the related bone morphogenetic protein (BMP) family are the most potent stimulators of chondrocyte biosynthesis. Three isoforms of TGF-β are known to exist in mammals, all of which are expressed in articular chondrocytes [15]. TGF-β induces undifferentiated mesenchymal cells to express a chondrocyte phenotype [16]. Differentiated chondrocytes are further stimulated by TGF-β, as demonstrated by the fact that articular cartilage explants in serum-free culture show increased PG biosynthesis and decreased PG degradation in the presence of the growth factor [17]. In experiments with isolated articular chondrocytes, however, TGF-β may act both as inhibitor and enhancer of biosynthesis, depending on the culture conditions. TGF-β has been shown to inhibit the synthesis of DNA, PG, and collagen in primary chondrocyte cultures, and to stimulate cells subcultured in monolayers with respect to the same parameters [18, 19]. One possible reason for the apparently conflicting results could be that the number of binding sites for TGF-β within the extracellular matrix varies among different culture systems. This view is supported, for example, by a study which showed that the ability of chondrocytes to respond to TGF-β was eliminated in the

presence of pericellular matrix that had formed during preculture in alginate gel, but recovered after enzymatic digestion of the pericellular matrix [20]. Molecules with a potential to bind TGF-β and immobilize the growth factor within the extracellular matrix are the leucine-rich repeat glycoproteins, e. g., decorin and biglycan, that are secreted by chondrocytes, fibroblasts, and other matrix-producing cell types [reviewed in ref. 21]. The possibility that TGF-β and related growth factors are sequestered within cartilage and released upon tissue damage offers an interesting hypothetical mechanism in the modulation of tissue repair activities.

TGF-β counteracts IL-1-mediated resorption in explant cultures of porcine cartilage in vitro [22] and in mice with experimentally induced inflammatory arthritis [23]. Remarkably, transgenic mice with blocked TGF-β signal function, either expressing a functionally inactive TGF-β type II receptor [24] or with disrupted intracellular signaling through Smad3 [25], develop a degenerative joint disease resembling the OA condition in humans. These observations suggest that impaired function of TGF-β within joints is a possible cause of OA. On the other hand, human OA cartilage has proved to be more sensitive to TGF-β than normal cartilage in terms of stimulation of PG synthesis in vitro [26] and TGF-β is relatively abundant in the synovial fluid of OA and rheumatoid arthritis patients [27]. Repeated injections of TGF-β into mouse knee joints result in the formation of osteophytes on the articular surface and cartilage lesions associated with PG depletion as in OA [28]. Thus, the possibility indeed exists that an excessive TGF-β activity is responsible for some of the pathological changes in OA.

The BMP family consists of at least 13 proteins. All of them except BMP-1 belong to the TGF-β superfamily [29]. The original identification of BMPs was based on their ability to promote ectopic bone formation, but BMPs are now known to be involved in many different physiological processes and have regulatory functions in the development and patterning of several different organ systems during embryogenesis [29, 30]. Many BMPs have been given alternative names, such as osteogenic protein (OP) or growth and differentiation factor (GDF). For example, BMP-7 and BMP-8 are identical to OP-1 and OP-2, respectively, BMP-12 is also called GDF-7, and BMP-13 is both GDF-6 and cartilage-derived morphogenic protein 2 (CDMP-2). Some of the BMPs have been found to promote chondrogenesis and at least BMP-2 [31], BMP-3/osteogenin, BMP-4 [32], and BMP-7/OP-1 [33, 34] are potent stimulators of articular cartilage PG synthesis in vitro. BMP7/OP-1 is effective in overcoming the IL-1-induced downregulation of PG biosynthesis of human articular chondrocytes in vitro [35].

Proteinases: MMPs, ADAMTS

The capacity of chondrocytes to degrade constituents of the extracellular matrix depends on their ability to synthesize and secrete proteinases (table 1). Under normal conditions, controlled activity of these enzymes is necessary for appropriate morphogenesis and tissue remodeling. Regulation of proteinase activity occurs at three different levels: synthesis and secretion, activation of latent enzyme, and inactivation by proteinase inhibitors. During degenerative joint diseases, such as OA, the expression and production of proteinases is increased. MMPs are prominent and appear to play several important roles in the pathological destruction of cartilage [36–38]. Analysis of synovial fluid shows higher concentrations of MMPs in OA patients than in healthy controls [39, 40]. The levels of the tissue inhibitor of metalloproteinases (TIMP)-1 are also elevated in OA synovial fluid and correlate with the MMP levels, possibly reflecting chondrocyte attempts to balance excessive proteinase activities.

Collagen in its native state can be cleaved by MMP-1 (interstitial collagenase), MMP-8 (neutrophil collagenase), and MMP-13, and this cleavage is thought to represent the rate-limiting step in collagen degradation. The fragments remaining after the initial cleavage by collagenases become susceptible to degradation by other enzymes, such as MMP-2 (gelatinase A), MMP-9 (gelatinase B), MMP-3 (stromelysin 1), and cathepsin B. Of the three collagenases, MMP-13 has assumed greater importance in OA because it preferentially degrades type II collagen [41] and its expression is markedly increased in OA [36, 37, 42]. Recent studies have shown that the enhanced cleavage of type II collagen in cultured OA cartilage samples is arrested in the presence of a selective collagenase inhibitor against MMP-13 and MMP-8, suggesting that MMP-1 is not involved in cleavage of resident type II collagen in OA cartilage [38, 43].

The enzymes responsible for aggrecan degradation have recently attracted much attention. Amino acid sequence

Table 1. Proteinases involved in the degradation of cartilage matrix.

Metalloproteinases	
Collagenases	MMP-1, MMP-8, MMP-13
Gelatinases	MMP-2, MMP-9
Stromelysins	MMP-3, MMP-7, MMP-10, MMP-11
Membrane type	MMP-14
Aggrecanases	
Disintegrin and metalloproteinase type	ADAM-TS4, ADAM-TS5
Other proteinases	
Elastase	
Cathepsins	cathepsin B, D, G, L
Proteinases of the coagulation system	tPa, uPA, plasmin

analysis of aggrecan fragments in human synovial fluid has defined two major cleavage sites in the aggrecan core protein [44, 45]. Several of the MMPs can cleave at a well-defined site between residues Asn341 and Phe342 [46, 47], whereas cleavage at the second site, between residues Glu373 and Ala374, is a result of 'aggrecanase' activity [44, 48]. Cleavage at the two different sites produces different neoepitopes that can be detected using specific antisera [49]. In chondrocyte cultures treated with the catabolic agents IL-1 or retinoic acid, only neoepitopes resulting from aggrecanase cleavage are detected [48]. However, both MMP and aggrecanase neoepitopes are found in human cartilage from normal, OA, and rheumatoid joints [50]. Aggrecanase 1 and 2 are members of a protein family called ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) which is distinct from the MMP family [51, 52].

Mediators of inflammation in OA

Inflammatory mediators including cytokines, prostanoids and nitric oxide (NO) are generally agreed to induce cartilage degradation in disorders such as rheumatoid arthritis [for reviews see refs. 53, 54]. The inflammation in that case primarily takes place in the synovial membrane and the destruction of cartilage is a secondary event. In non-inflammatory arthropathies, such as OA, synovial cell reactions toward components released from cartilage into the synovial fluid may contribute to disease progression [55, 56], but the major pathogenic processes are localized within the cartilage itself [57, 58]. Chondrocytes in OA-affected cartilage display enhanced and coordinated expression of proinflammatory cytokines and inducible NO synthase (iNOS), the enzyme responsible for NO production [59]. Collectively, evidence is accumulating that mediators of inflammation acting in an autocrine/paracrine fashion within the cartilage play a primary role in the pathogenesis of OA [57–60].

Involvement of cytokines

Proinflammatory cytokines include the ILs, TNFs, interferons, and colony-stimulating factors. Although these factors were identified originally as secreted products of immune cells that modulate the function of other cells of the immune system, many of them have effects on non-immune cells like fibroblasts and chondrocytes. Among the proinflammatory cytokines, IL-1β and TNF-α appear most directly involved in the pathological processes of OA [61]. The net effect of these two catabolic factors depends not only on their absolute concentrations, but also on the influence of modulating cytokines, e.g., IL-6, IL-8, and leukemia inhibitory factor (LIF), that are synthesized in increased amounts when chondrocytes are stimulated with IL-1β or TNF-α [61–63]. Further cytokines, IL-17 and IL-18, have recently been identified as potent inducers of IL-1β and TNF-α as well as MMP-3 and iNOS in human articular chondrocytes [64, 65]. In addition, some cytokines, e.g., IL-4, IL-10, and IL-13, have been classified as antiinflammatory because they inhibit the activity of the proinflammatory cytokines in vitro [66]. Hence, cytokine networks with a considerable degree of complexity appear to influence the metabolic state of chondrocytes. However, the exact roles of inflammatory cytokines other than IL-1β and TNF-α in the activation and modulation of possible cascade reactions have not yet been clearly established. Studies in animal models have been used with the aim to gain insight into the pathogenesis of OA, and the major conclusions so far have been that the cartilage destructive process is mainly IL-1 driven, whereas TNF-α is involved in the onset of arthritis, and that direct generation of IL-1 may also occur in the complete absence of TNF-α [for reviews see refs. 67, 68].

OA cartilage exhibits strong intra- and extracellular staining for IL-1β within the superficial zone when examined by immunohistochemistry [37, 59]. The biological activity of IL-1β in OA tissue is further enhanced by different mechanisms, including an increase in the number of IL-1 type 1 receptors on chondrocytes [69], a relative decrease in the synthesis of the natural receptor antagonist IL-1Ra [70], and an increase in IL-1β-converting enzyme that activates the inactive precursor form of IL-1β [71].

Chondrocyte responses to inflammatory cytokines

As evidence has accumulated that proinflammatory cytokines are important in the pathogenesis of OA, a large number of in vitro studies have been carried out to examine in detail the influence of these factors on chondrocytes. IL-1 has been found to induce catabolic responses in many different ways. Human articular chondrocytes, when stimulated by IL-1, dramatically increase the expression of matrix-degrading proteinases like MMP-1, -2, -3, -7, -8, -9, and -13 [37, 42, 72–74]. Moreover, IL-1 strongly inhibits biosynthesis of cartilage PG and collagens [75, 76]. Relatively little is known about the intracellular events following IL-1 receptor activation in chondrocytes, but the mitogen-activated protein kinase (MAPK) signal transduction pathway is involved. IL-1 activates all three of the MAPK subgroups – ERK, JNK, and p38 [77, 78].

Recent studies have drawn attention to two mediators that are locally produced at sites of inflammation: NO and prostaglandin E_2 (PGE_2). These factors are released if isolated chondrocytes are stimulated with cytokines [79–82]. In response to IL-1β, chondrocytes upregulate the enzymes that are critical for the synthesis of NO and PGE_2, i.e., iNOS and cyclooxygenase-2 (COX-2) [74,

Table 2. Spontaneous release of nitrite by chondrocytes from OA-affected and normal cartilage.

	Organ cultures (nmol NO_2^- per g wet wt.)	Alginate beads (nmol NO_2^- per 10^7 cells)
OA chondrocytes		
Culture day 1	35–220	10–15
Culture day 8	15–70	< 5
Normal		
Culture day 1	15–70	< 5
Culture day 8	5–40	< 5

Articular cartilage from four OA-affected patients and three healthy controls was cultured as 2–3-mm explants in Ham's F12/DMEM medium containing 10% fetal calf serum. In parallel, chondrocytes were isolated from cartilage samples and cultured in alginate gel under the same conditions. Medium was changed daily. NO production was measured as accumulation of nitrite in 24 h-conditioned medium, using modified Griess reaction in microplate format [79].

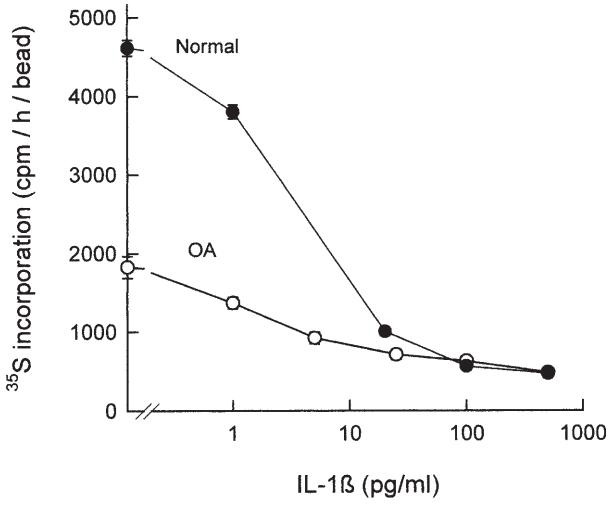

Figure 1. Effect of human recombinant IL-1β on the biosynthesis of sulfated proteoglycan by human articular chondrocytes cultured in alginate beads. Chondrocytes were isolated from macroscopically normal cartilage from organ donors with no history of joint disease, and from cartilage specimens from patients with OA undergoing total joint replacement. Isolated cells were encapsulated in 1.2% alginate at a density of 4×10^6 cells/ml [95]. Incorporation of ^{35}S-sulfate into macromolecules was determined after treatment for 3 days with different doses of hrIL-1β. Each curve represents the results of one typical experiment, with data expressed as the mean ± SD (n = 3) for each concentration. The sulfate incorporation rate of normal chondrocytes in the absence of IL-1β was notably consistent among different experiments. Chondrocytes from four different donors between 30 and 55 years of age, labeled with 50 µCi/ml for 16 h, produced a sulfate incorporation rate corresponding to 4689 ± 72 cpm/h per bead. By contrast, chondrocytes from six different OA cartilage samples showed a high degree of variability, with a mean sulfate incorporation rate corresponding to 2642 ± 1213 cpm/h per bead.

96]. Expression of NOS and COX-2 is significant in OA-affected cartilage but low or absent in normal articular cartilage [59, 83].

As a consequence, OA chondrocytes cultured in vitro spontaneously produce detectable amounts of NO during the initial culture period of time (table 2). Effects of endogenous NO and PGE_2 can be examined in cell or tissue culture systems using synthetic enzyme inhibitors such as N^G-monomethyl-L-arginine (L-NMA) for NOSs and indomethacin for COXs. Hence, endogenous NO has been shown to suppress biosynthesis of proteoglycans [84, 85] and, at least in lapine chondrocytes, inhibits collagen II formation [86]. Furthermore, NO is able to induce chondrocyte apoptosis that occurs in OA [87–89]. In an experimental model of OA in dog, the administration of a selective iNOS inhibitor reduced the degenerative changes, strongly supporting that NO is primarily a catabolic mediator in cartilage [90]. With respect to PGE_2, the net effect on chondrocytes is less clear. On the one hand, catabolic effects like antiproliferative and proapoptotic effects have been reported [91], as have stimulation of MMP-3 synthesis and inhibition of TIMP-1 production [92]. On the other hand, PGE_2 may act in an anabolic fashion to increase production of collagen and PG in cartilage via an autocrine feedback loop involving IGF-I [93, 94].

We have found the alginate suspension cell culture system useful for studying the influence of different factors on chondrocyte properties in general and the synthesis and turnover of PGs in particular [95]. Chondrocytes grown in alginate maintain the phenotype over several months and the cell-associated matrix formed is apparently very similar to native cartilage matrix in its composition [96, 97]. A dose-dependent inhibition of PG biosynthesis in response to IL-1β can be readily demonstrated in this culture system. In isolated OA chondrocytes, the basal proteoglycan biosynthesis rate is lower and more variable than in normal human articular chondrocytes (fig. 1). The reason for this is still elusive. One hypothesis could be that the expression of IL-Ra is lowered in OA chondrocytes. However, data from studies in other culture systems suggest that IL-1β-induced down-regulation of IL-Ra is mediated by NO, and that inhibition of PG biosynthesis by indomethacin does not have an effect on IL-1Ra synthesis [70]. In contrast, we find that the decreased PG biosynthesis in OA chondrocytes is partially restored in the presence of indomethacin, whereas L-NMA inhibition of iNOS has no significant effect (fig. 2).

There is firm evidence that the strong inhibition of PG biosynthesis in response to IL-1β in human chondrocytes is partially dependent on NO production [85]. However, with respect to PG degradation, the role of NO is not that clear. Indeed, we observe a significantly enhanced catabolism of newly synthesized PG if NO synthesis is blocked in both alginate and organ cultures [98]. This effect is possibly a result of iNOS/COX-2 cross-talk in which NO

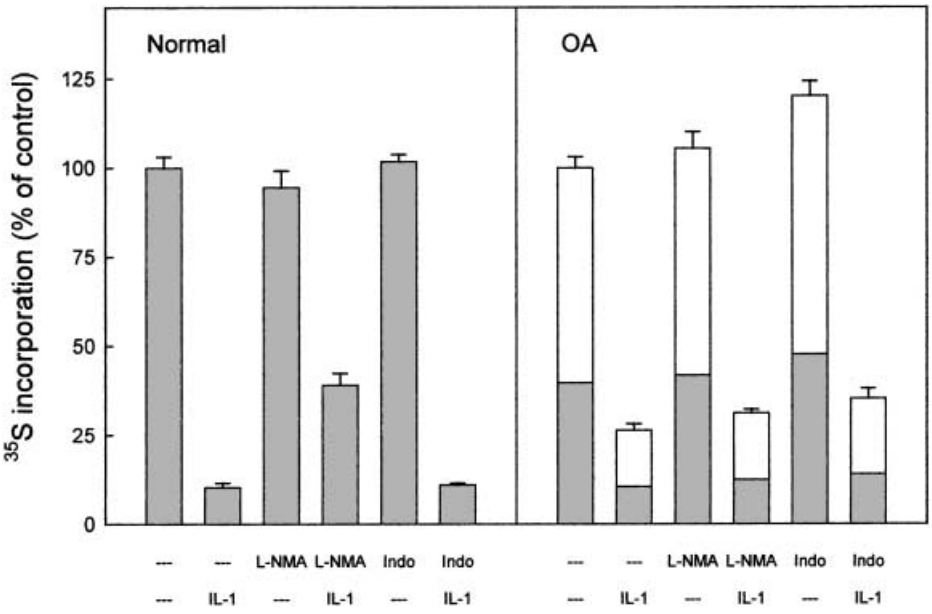

Figure 2. Effect of L-NMA and indomethacin on the biosynthesis of sulfated proteoglycans by human normal and OA chondrocytes cultured in alginate beads in the presence or absence of IL-1β. Chondrocytes were isolated and cultured as described in the legend to figure 1. ^{35}S-sulfate incorporation into macromolecules was determined after culture for 3 days in the presence or absence of hrIL-1β (500 pg/ml), L-NMA (1 mM), and indomethacin (2.8 µM). Data are expressed as percent of control (mean ± SD, n = 3), with the control representing untreated normal chondrocytes (filled bars) or OA chondrocytes (open bars).

suppresses formation of PGE_2 [83]. However, recent studies employing a dog OA model indicate that a synthetic iNOS inhibitor prevents the expression of COX-2 among other inflammatory factors [99]. Most likely, a combination of further in vitro and in vivo studies with iNOS inhibitors will be necessary to gain insight into the complex role of NO in cartilage metabolism and in OA patients.

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1 Felson D. T., Dieppe P. A., Hirsch R., Helmick C. H., Jordan J. M., Kington R. S. et al. (2000) Osteoarthritis: new insights. 1: The disease and its risk factors. Ann. Intern. Med. **133:** 635–646
2 Häuselmann H. J. and Stucki G. (1999) Degenerative Gelenkerkrankungen. In: Thiemes Innere Medizin, pp. 1668–1678, Alexander K., Daniel W. C., Diener H. C., Freund M., Köhler H., Matern S. et al. (eds). Thieme, vol. 2, 2nd edn, pp. 8.11–8.3.16
3 Dequeker J. and Dieppe P. A. (1998) Disorders of Bone, Cartilage and Connective Tissue. In: Rheumatology, Klippel J. H., Dieppe P. A. (eds), Mosby, London
4 Hascall V. C., Handley C. J., McQuillan D. J., Hascall G. K., Robinson H. C. and Lowther D. A. (1983) The effect of serum on biosynthesis of proteoglycans by bovine articular cartilage in culture. Arch. Biochem. Biophys. **224:** 206–223
5 McQuillan D. J., Handley C. J., Campbell M. A., Bolis S., Milway V. E. and Herington A. C. (1986) Stimulation of proteoglycan biosynthesis by serum and insulin-like growth factor-I in cultured bovine articular cartilage. Biochem. J. **240:** 423–430
6 Tyler J. A. (1989) Insulin-like growth factor 1 can decrease degradation and promote synthesis of proteoglycan in cartilage exposed to cytokines. Biochem. J. **260:** 543–548
7 Yaeger P. C., Masi T. L., Ortiz J. L. de, Binette F., Tubo R. and McPherson J. M. (1997) Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. Exp. Cell Res. **237:** 318–325
8 Middleton J. F. and Tyler J. A. (1992) Upregulation of insulin-like growth factor I gene expression in the lesions of osteoarthritic human articular cartilage. Ann. Rheum. Dis. **51:** 440–447
9 Dore S., Pelletier J. P., DiBattista J. A., Tardif G., Brazeau P. and Martel-Pelletier J. (1994) Human osteoarthritic chondrocytes possess an increased number of insulin-like growth factor 1 binding sites but are unresponsive to its stimulation: possible role of IGF-1-binding proteins. Arthritis Rheum. **37:** 253–263
10 Middleton J., Manthey A. and Tyler J. (1996) Insulin-like growth factor (IGF) receptor, IGF-I, interleukin-1 beta (IL-1 beta), and IL-6 mRNA expression in osteoarthritic and normal human cartilage. J. Histochem. Cytochem. **44:** 133–141
11 Loeser R. F., Shanker G., Carlson C. S., Gardin J. F., Shelton B. J. and Sonntag W. E. (2000) Reduction in the chondrocyte response to insulin-like growth factor 1 in aging and osteoarthritis: studies in a non-human primate model of naturally occurring disease. Arthritis Rheum. **43:** 2110–2120
12 Olney R. C., Tsuchiya K., Wilson D. M., Mohtai M., Maloney W. J., Schurman D. J. et al. (1996) Chondrocytes from osteoarthritic cartilage have increased expression of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) and -5, but not IGF-II or IGFBP-4. J. Clin. Endocrinol. Metab. **81:** 1096–1103
13. Tardif G., Reboul P., Pelletier J. P., Geng C., Cloutier J. M. and Martel-Pelletier J. (1996) Normal expression of type 1 insulin-like growth factor receptor by human osteoarthritic chondrocytes with increased expression and synthesis of insulin-like growth factor binding proteins. Arthritis Rheum. **39:** 968–978

14 Chevalier X. and Tyler J. A. (1996) Production of binding proteins and role of the insulin-like growth factor I binding protein 3 in human articular cartilage explants. Br. J. Rheumatol. **35:** 515–522

15 Frazer A., Seid J. M., Hart K. A., Bentley H., Bunning R. A. and Russell R. G. (1991) Detection of mRNA for the transforming growth factor beta family in human articular chondrocytes by the polymerase chain reaction. Biochem. Biophys. Res. Commun. **180:** 602–608

16 Seyedin S. M., Thomas T. C., Thompson A. Y., Rosen D. M. and Piez K. A. (1985) Purification and characterization of two cartilage-inducing factors from bovine demineralized bone. Proc. Natl. Acad. Sci. USA **82:** 2267–2271

17 Morales T. I. and Roberts A. B. (1988) Transforming growth factor beta regulates the metabolism of proteoglycans in bovine cartilage organ cultures. J. Biol. Chem. **263:** 12828–12831

18 Galéra P., Rédini F., Vivien D., Bonaventure J., Penfornis H., Loyau G. et al. (1992) Effect of transforming growth factor-beta 1 (TGF-beta 1) on matrix synthesis by monolayer cultures of rabbit articular chondrocytes during the dedifferentiation process. Exp. Cell Res. **200:** 379–392

19 Kraan P. van der, Vitters E. and Berg W. van den (1992) Differential effect of transforming growth factor beta on freshly isolated and cultured articular chondrocytes. J. Rheumatol. **19:** 140–145

20 Osch G. J. van, Veen S. W. van der, Buma P. and Verwoerd-Verhoef H. L. (1998) Effect of transforming growth factor-beta on proteoglycan synthesis by chondrocytes in relation to differentiation stage and the presence of pericellular matrix. Matrix Biol. **17:** 413–424

21 Hocking A. M., Shinomura T. and McQuillan D. J. (1998) Leucine-rich repeat glycoproteins of the extracellular matrix. Matrix Biol. **17:** 1–19

22 Harvey A. K., Hrubey P. S. and Chandrasekhar S. (1991) Transforming growth factor-beta inhibition of interleukin-1 activity involves down-regulation of interleukin-1 receptors on chondrocytes. Exp. Cell Res. **195:** 376–385

23 Glansbeek H. L., Beuningen H. M. van, Vitters E. L., Kraan P. M. van der and Berg W. B. van den (1998) Stimulation of articular cartilage repair in established arthritis by local administration of transforming growth factor-beta into murine knee joints. Lab. Invest. **78:** 133–142

24 Serra R., Johnson M., Filvaroff E. H., LaBorde J., Sheehan D. M., Derynck R. et al. (1997) Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. J. Cell Biol. **139:** 541–552

25 Yang X., Chen L., Xu X., Li C., Huang C. and Deng C. X. (2001) Tgf-beta/smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. J. Cell Biol. **153:** 35–46

26 Lafeber F. P., Roy H. L. van, Kraan P. M. van der, Berg W. B. van den and Bijlsma J. W. (1997) Transforming growth factor-beta predominantly stimulates phenotypically changed chondrocytes in osteoarthritic human cartilage. J. Rheumatol. **24:** 536–542

27 Schlaak J. F., Pfers I., Meyer Zum Buschenfelde K. H. and Marker-Hermann E. (1996) Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies. Clin. Exp. Rheumatol. **14:** 155–162

28 Beuningen H. M. van, Glansbeek H. L., Kraan P. M. van der and Berg W. B. van den (2000) Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections. Osteoarthritis Cartilage **8:** 25–33

29 Hogan B. L. (1996) Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev. **10:** 1580–1594

30 Reddi A. H. (1998) Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol. 16: 247–252

31 Sailor L. Z., Hewick R. M. and Morris E. A. (1996) Recombinant human bone morphogenetic protein-2 maintains the articular chondrocyte phenotype in long-term culture. J. Orthop. Res. **14:** 937–945

32 Luyten F. P., Yu Y. M., Yanagishita M., Vukicevic S., Hammonds R. G. and Reddi A. H. (1992) Natural bovine osteogenin and recombinant human bone morphogenetic protein-2B are equipotent in the maintenance of proteoglycans in bovine articular cartilage explant cultures. J. Biol. Chem. **267:** 3691–3695

33 Chen P., Vukicevic S., Sampath T. K. and Luyten F. P. (1993) Bovine articular chondrocytes do not undergo hypertrophy when cultured in the presence of serum and osteogenic protein-1. Biochem. Biophys. Res. Commun. **197:** 1253–1259

34 Flechtenmacher J., Huch K., Thonar E. J., Mollenhauer J. A., Davies S. R., Schmid T. M. et al. (1996) Recombinant human osteogenic protein 1 is a potent stimulator of the synthesis of cartilage proteoglycans and collagens by human articular chondrocytes. Arthritis Rheum **39:** 1896–1904

35 Huch K., Wilbrink B., Flechtenmacher J., Koepp H. E., Aydelotte M. B., Sampath T. K. et al. (1997) Effects of recombinant human osteogenic protein 1 on the production of proteoglycan, prostaglandin E2, and interleukin-1 receptor antagonist by human articular chondrocytes cultured in the presence of interleukin-1beta. Arthritis Rheum. **40:** 2157–2161

36 Shlopov B. V., Lie W. R., Mainardi C. L., Cole A. A., Chubinskaya S. and Hasty K. A. (1997) Osteoarthritic lesions: involvement of three different collagenases. Arthritis Rheum. 40: 2065–2074

37 Tetlow L. C, Adlam D. J and Woolley D. E. (2001) Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes. Arthritis Rheum. **44:** 585–594

38 Billinghurst R. C., Dahlberg L., Ionescu M., Reiner A., Bourne R., Rorabeck C. et al. (1997) Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. J. Clin. Invest. **99:** 1534–1545

39 Clark I. M., Powell L. K., Ramsey S., Hazleman B. L. and Cawston T. E. (1993) The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum. **36:** 372–379

40 Ishiguro N., Ito T., Ito H., Iwata H., Jugessur H., Ionescu M. et al. (1999) Relationship of matrix metalloproteinases and their inhibitors to cartilage proteoglycan and collagen turnover: analyses of synovial fluid from patients with osteoarthritis. Arthritis Rheum. **42:** 129–136

41 Knauper V., Lopez-Otin C., Smith B., Knight G. and Murphy G. (1996) Biochemical characterization of human collagenase-3. J. Biol. Chem. **271:** 1544–1550

42 Reboul P., Pelletier J. P., Tardif G., Cloutier J. M. and Martel-Pelletier J. (1996) The new collagenase, collagenase-3, is expressed and synthesized by human chondrocytes but not by synoviocytes: a role in osteoarthritis. J. Clin. Invest. **97:** 2011–2019

43 Dahlberg L., Billinghurst R. C., Manner P., Nelson F., Webb G., Ionescu M. et al. (2000) Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). Arthritis Rheum. **43:** 673–682

44 Sandy J. D., Flannery C. R., Neame P. J. and Lohmander L. S. (1992) The structure of aggrecan fragments in human synovial fluid: evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the Glu 373-Ala 374 bond of the interglobular domain. J. Clin. Invest. **89:** 1512–1516

45 Fosang A. J., Last K. and Maciewicz R. A. (1996) Aggrecan is degraded by matrix metalloproteinases in human arthritis: evidence that matrix metalloproteinase and aggrecanase activities can be independent. J. Clin. Invest. **98:** 2292–2299
46 Fosang A. J., Last K., Knauper V., Neame P. J., Murphy G., Hardingham T. E. et al. (1993) Fibroblast and neutrophil collagenases cleave at two sites in the cartilage aggrecan interglobular domain. Biochem. J. **295:** 273–276
47 Stracke J. O., Fosang A. J., Last K., Mercuri F. A., Pendas A. M., Llano E. et al. (2000) Matrix metalloproteinases 19 and 20 cleave aggrecan and cartilage oligomeric matrix protein (COMP). FEBS Lett. **478:** 52–56
48 Lark M. W., Gordy J. T., Weidner J. R., Ayala J., Kimura J. H., Williams H. R. et al. (1995) Cell-mediated catabolism of aggrecan: evidence that cleavage at the 'aggrecanase' site (Glu373-Ala374) is a primary event in proteolysis of the interglobular domain. J. Biol. Chem. **270:** 2550–2556
49 Hughes C. E., Caterson B., Fosang A. J., Roughley P. J. and Mort J. S. (1995) Monoclonal antibodies that specifically recognize neoepitope sequences generated by 'aggrecanase' and matrix metalloproteinase cleavage of aggrecan: application to catabolism in situ and in vitro. Biochem. J. **305:** 799–804
50 Lark M. W., Bayne E. K., Flanagan J., Harper C. F., Hoerrner L. A., Hutchinson N. I. et al. (1997) Aggrecan degradation in human cartilage: evidence for both matrix metalloproteinase and aggrecanase activity in normal, osteoarthritic, and rheumatoid joints. J. Clin. Invest. **100:** 93–106
51 Tortorella M. D., Burn T. C., Pratta M. A., Abbaszade I., Hollis J. M., Liu R. et al. (1999) Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. Science **284:** 1664–1666
52 Abbaszade I., Liu R. Q., Yang F., Rosenfeld S. A., Ross O. H., Link J. R. et al. (1999) Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family. J. Biol. Chem. **274:** 23443–23450
53 Arend W. P. (2001) Physiology of cytokine pathways in rheumatoid arthritis. Arthritis Rheum. **45:** 101–106
54 Häuselmann H. J. (1997) Mechanisms of cartilage destruction and novel nonsurgical therapeutic strategies to retard cartilage injury in rheumatoid arthritis. Curr. Opin. Rheumatol. **9:** 241–250
55 Smith M. D., Triantafillou S., Parker A., Youssef P. P. and Coleman M. (1997) Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J. Rheumatol. **24:** 365–371
56 Pelletier J. P., McCollum R., Cloutier J. M. and Martel-Pelletier J. (1995) Synthesis of metalloproteases and interleukin 6 (IL-6) in human osteoarthritic synovial membrane is an IL-1 mediated process. J. Rheumatol. Suppl. **43:** 109–114
57 Amin A. R., Attur M. and Abramson S. B. (1999) Nitric oxide synthase and cyclooxygenases: distribution, regulation, and intervention in arthritis. Curr. Opin. Rheumatol. **11:** 202–209
58 Moos V., Fickert S., Muller B., Weber U. and Sieper J. (1999) Immunohistological analysis of cytokine expression in human osteoarthritic and healthy cartilage. J. Rheumatol. **26:** 870–879
59 Melchiorri C., Meliconi R., Frizziero L., Silvestri T., Pulsatelli L., Mazzetti I. et al. (1998) Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. Arthritis Rheum. **41:** 2165–2174
60 Towle C. A., Hung H.. H, Bonassar L. J., Treadwell B. V. and Mangham D. C. (1997) Detection of interleukin-1 in the cartilage of patients with osteoarthritis: a possible autocrine/paracrine role in pathogenesis. Osteoarthritis Cartilage **5:** 293–300
61 Westacott C. I. and Sharif M. C. (1996) Cytokines in osteoarthritis: mediators or markers of joint destruction? Semin. Arthrtis. Rheum. **25:** 254–272
62 Lotz M., Blanco F. J., Kempis J. von, Dudler J., Maier R., Villiger P. M. et al. (1995) Cytokine regulation of chondrocyte functions. J. Rheumatol. Suppl. **43:** 104–108
63 Henrotin Y. E., De Groote D. D., Labasse A. H., Gaspar S. E., Zheng S. X., Geenen V. G. et al. (1996) Effects of exogenous IL-1 beta, TNF alpha, IL-6, IL-8 and LIF on cytokine production by human articular chondrocytes. Osteoarthritis Cartilage **4:** 163–173
64 Shalom-Barak T., Quach J. and Lotz M. (1998) Interleukin-17-induced gene expression in articular chondrocytes is associated with activation of mitogen-activated protein kinases and NF-kappaB. J. Biol. Chem. **273:** 27467–27473
65 Olee T., Hashimoto S., Quach J. and Lotz M. (1999) IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. J. Immunol. **162:** 1096–1100
66 Alaaeddine N., Di Battista J. A., Pelletier J. P., Kiansa K., Cloutier J. M. and Martel-Pelletier J. (1999) Inhibition of tumor necrosis factor alpha-induced prostaglandin E2 production by the antiinflammatory cytokines interleukin-4, interleukin-10, and interleukin-13 in osteoarthritic synovial fibroblasts: distinct targeting in the signaling pathways. Arthritis Rheum. **42:** 710–718
67 Van den Berg W. B. (1997) Lessons for joint destruction from animal models. Curr. Opin. Rheumatol. **9:** 221–228
68 Goldring M. B. (1999) The role of cytokines as inflammatory mediators in osteoarthritis: lessons from animal models. Connect Tissue Res. **40:** 1–11
69 Martel-Pelletier J., McCollum R., DiBattista J., Faure M. P., Chin J. A., Fournier S. et al. (1992) The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type I receptor and analysis of binding kinetics and biologic function. Arthritis Rheum. **35:** 530–540
70 Pelletier J. P., Mineau F., Ranger P., Tardif G. and Martel-Pelletier J. (1996) The increased synthesis of inducible nitric oxide inhibits IL-1ra synthesis by human articular chondrocytes: possible role in osteoarthritic cartilage degradation. Osteoarthritis Cartilage **4:** 77–84
71 Saha N., Moldovan F., Tardif G., Pelletier J. P., Cloutier J. M. and Martel-Pelletier J. (1999) Interleukin-1beta-converting enzyme/caspase-1 in human osteoarthritic tissues: localization and role in the maturation of interleukin-1beta and interleukin-18. Arthritis Rheum. **42:** 1577–1587
72 Borden P., Solymar D., Sucharczuk A., Lindman B., Cannon P. and Heller R. A. (1996) Cytokine control of interstitial collagenase and collagenase-3 gene expression in human chondrocytes. J. Biol. Chem. **271:** 23577–23581
73 Chubinskaya S., Huch K., Mikecz K., Cs-Szabo G., Hasty K. A., Kuettner K. E. et al. (1996) Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1 beta in human cartilage from knee and ankle joints. Lab. Invest. **74:** 232–240
74 Ohta S., Imai K., Yamashita K., Matsumoto T., Azumano I. and Okada Y. (1998) Expression of matrix metalloproteinase 7 (matrilysin) in human osteoarthritic cartilage. Lab. Invest. **78:** 79–87
75 Benton H. P. and Tyler J. A. (1988) Inhibition of cartilage proteoglycan synthesis by interleukin I. Biochem. Biophys. Res. Commun. **154:** 421–428
76 Goldring M. B., Birkhead J., Sandell L. J., Kimura T. and Krane S. M. (1988) Interleukin 1 suppresses expression of cartilage-specific types II and IX collagens and increases types I and III collagens in human chondrocytes. J. Clin. Invest. **82:** 2026–2037
77 Geng Y., Valbracht J., and Lotz M. (1996) Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes. J. Clin. Invest. **98:** 2425–2430
78 Mengshol J. A., Vincenti M. P., Coon C. I., Barchowsky A. and Brinckerhoff C. E. (2000) Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear

factor kappaB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum. **43:** 801–811
79 Stadler J., Stefanovic-Racic M., Billiar T. R., Curran R. D., McIntyre L. A., Georgescu H. I. et al. (1991) Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J. Immunol. **147:** 3915–3920
80 Palmer R. M., Hickery M. S., Charles I. G., Moncada S. and Bayliss M. T. (1993) Induction of nitric oxide synthase in human chondrocytes. Biochem. Biophys. Res. Commun. **193:** 398–405
81 Geng Y., Blanco F. J., Cornelisson M. and Lotz M. (1995) Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. J. Immunol. **155:** 796–801
82 Attur M. G., Patel R. N., Abramson S. B. and Amin A. R. (1997) Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. Arthritis Rheum. **40:** 1050–1053
83 Amin A. R., Attur M., Patel R. N., Thakker G. D., Marshall P. J., Rediske J. et al. (1997) Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage: influence of nitric oxide. J. Clin. Invest. **99**: 1231–1237
84 Taskiran D., Stefanovic-Racic M., Georgescu H. and Evans C. (1994) Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem. Biophys. Res. Commun. **200:** 142–148
85 Häuselmann H. J., Oppliger L., Michel B. A., Stefanovic-Racic M. and Evans C. H. (1994) Nitric oxide and proteoglycan biosynthesis by human articular chondrocytes in alginate culture. FEBS Lett. **352:** 361–364
86 Cao M., Westerhausen-Larson A., Niyibizi C., Kavalkovich K., Georgescu H. I., Rizzo C. F. et al. (1997) Nitric oxide inhibits the synthesis of type-II collagen without altering Col2A1 mRNA abundance: prolyl hydroxylase as a possible target. Biochem. J. **324:** 305–310
87 Blanco F. J., Ochs R. L., Schwarz H. and Lotz M. (1995) Chondrocyte apoptosis induced by nitric oxide. Am. J. Pathol. **146:** 75–85
88 Hashimoto S., Ochs R. L., Komiya S. and Lotz M. (1998) Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum. **41:** 1632–1638
89 Heraud F., Heraud A. and Harmand M. F. (2000) Apoptosis in normal and osteoarthritic human articular cartilage. Ann. Rheum. Dis. **59:** 959–965
90 Pelletier J. P., Jovanovic D., Fernandes J. C., Manning P., Connor J. R., Currie M. G. et al. (1998) Reduced progression of experimental osteoarthritis in vivo by selective inhibition of inducible nitric oxide synthase. Arthritis Rheum. **41:** 1275–1286
91 Miwa M., Saura R., Hirata S., Hayashi Y., Mizuno K. and Itoh H. (2000) Induction of apoptosis in bovine articular chondrocyte by prostaglandin E(2) through cAMP-dependent pathway. Osteoarthritis Cartilage **8:** 17–24
92 Yamada H., Kikuchi T., Nemoto O., Obata K., Sato H., Seiki M. et al. (1996) Effects of indomethacin on the production of matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 by human articular chondrocytes. J. Rheumatol. **23:** 1739–1743
93 Di Battista J. A., Dore S., Morin N., He Y., Pelletier J. P. and Martel-Pelletier J. (1997) Prostaglandin E2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: possible mediation by Ca(++)-calmodulin regulated processes. J. Cell Biochem. **65:** 408–419
94 Goldring M. B. and Berenbaum F. (1999) Human chondrocyte culture models for studying cyclooxygenase expression and prostaglandin regulation of collagen gene expression. Osteoarthritis Cartilage **7:** 386–388
95 Häuselmann H. J., Aydelotte M. B., Schumacher B. L., Kuettner K. E., Gitelis S. H. and Thonar E. J. (1992) Synthesis and turnover of proteoglycans by human and bovine adult articular chondrocytes cultured in alginate beads. Matrix. **12:** 116–129
96 Häuselmann H. J., Masuda K., Hunziker E. B., Neidhart M., Mok S. S., Michel B. A. et al. (1996) Adult human chondrocytes cultured in alginate form a matrix similar to native human articular cartilage. Am. J. Physiol. **271:** C742–C752
97 Häuselmann H. J., Fernandes R. J., Mok S. S., Schmid T. M., Block J. A., Aydelotte M. B. et al. (1994) Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. J. Cell Sci. **107:** 17–27
98 Häuselmann H. J., Stefanovic-Racic M., Michel B. A. and Evans C. H. (1998) Differences in nitric oxide production by superficial and deep human articular chondrocytes: implications for proteoglycan turnover in inflammatory joint diseases. J. Immunol. **160:** 1444–1448
99 Pelletier J. P., Lascau-Coman V., Jovanovic D., Fernandes J. C., Manning P., Connor J. R. et al. (1999) Selective inhibition of inducible nitric oxide synthase in experimental osteoarthritis is associated with reduction in tissue levels of catabolic factors. J. Rheumatol. **26:** 2002–2014