IL-1 and IL-1 Receptor Antagonist in Chondrocytes in Human Osteoarthritis

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by the loss of articular cartilage, remodeling of subchondral bone, osteophyte formation, and limited synovial inflammation. One role of chondrocytes is to maintain the cartilage extracellular matrix. In osteoarthritis, the finely tuned balance of anabolic and catabolic processes is disturbed resulting in a loss of cartilage. Cytokines such as interleukin-1 (IL-1) promote cartilage degeneration by stimulating the synthesis of proteolytic enzymes, cytokines, nitric oxide, prostaglandins, and other mediators and effectors of tissue destruction.[1] IL-1α and ß are potent proinflammatory molecules that exert their effects by binding to high-affinity receptors on a variety of target cells including articular chondrocytes. It is generally accepted that IL-1s are important mediators in the cartilage destruction seen in inflammatory joint disease, but the role of these cytokines in the pathogenesis of OA is less clear.

The discovery in our laboratory that chondrocytes express IL-1 genes raised the possibility that IL-1s produced by chondrocytes, rather than by synovial cells, play a role in OA pathogenesis. Subsequent studies from this laboratory have demonstrated that IL-1 is expressed in OA cartilage and that IL-1 proteins accumulate within chondrocytes early in the development of OA.[2-5] The concept that IL-1s are important in OA pathogenesis is further supported by work showing that the natural IL-1 inhibitor IL-1receptor antagonist (IL-1ra) ameliorates degenerative change in a lapine surgical instability model of OA.[6]

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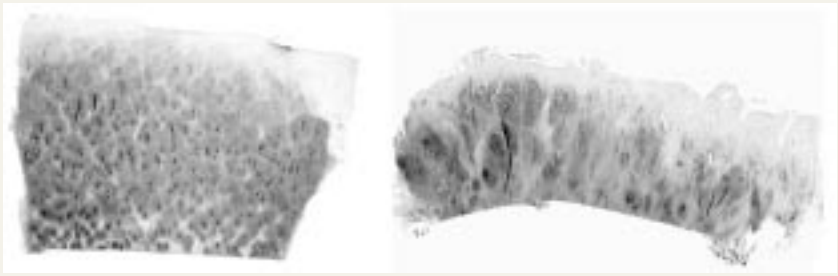
IL-1 BIOLOGY

The IL-1 family includes three known genes encoding ligands that bind to IL-1 receptors. Two of the proteins are the agonists IL-1α and ß, and the third is the competitive antagonist IL-1ra.[7] The IL-1s are synthesized as precursor molecules, and precursor IL-1 accumulates within the chondrocytes of osteoarthritic patients. Like certain other regulatory molecules such as fibroblast growth factors, IL-1s lack signal peptides to direct nascent polypeptides into the secretory pathway. The route(s) that these "signal peptide-less" polypeptides travel as they exit the cell remain controversial, but possibilities include transit through the damaged plasma membrane on cell death, release of endolysosomes, and shedding of plasma membrane microvesicles.[8-10]

IL-1 and IL-1ra compete for binding to IL-1 receptors on the cell surface, thus providing a mechanism for titrating the cellular response.[11] The single IL-1ra gene yields four known IL-1ra transcripts derived by alternative splicing. One mRNA variant encodes a polypeptide (sIL-1ra) that is secreted by the classical pathway, while three variants predict polypeptides that lack signal peptides and thus are termed intracellular (icIL-1ra).[12-17]

STUDIES OF IL-1 AND IL-1RA IN OSTEOARTHRITIC CARTILAGE

Because the IL-1 cytokines accumulate within chondrocytes in the cartilage of osteoarthritic patients early in the progression of disease, the relative expression of IL-1 and icIL-1ra by chondrocytes may influence the localized activity of IL-1 in cartilage. In order to investigate this relationship, samples of osteoarthritic cartilage were obtained from patients undergoing knee or hip arthroplasty. Clinical data were reviewed to exclude secondary OA and inflammatory joint diseases such as rheumatoid arthritis. Cartilage was excised taking care to avoid fibrocartilage, adjacent tissue and osteophytes. Specimens of cartilage were taken from locations that macroscopically appeared to be typical in the extent of degenerative change for the preparation of histological sections. Safranin O-stained sections were examined for features such as staining intensity, cellularity, and integrity of the articular surface, and specimens were scored for OA severity using the histological-histochemical grading system of Mankin et al.[18] Specimens of OA cartilage were classified based on the Mankin score as either early or advanced OA. Light micrographs of safranin O-stained sections of typical early and advanced OA cartilage specimens



A B

Figure 1. Safranin O stained histological sections of representative specimens of early (A) and advanced (B) OA cartilage.

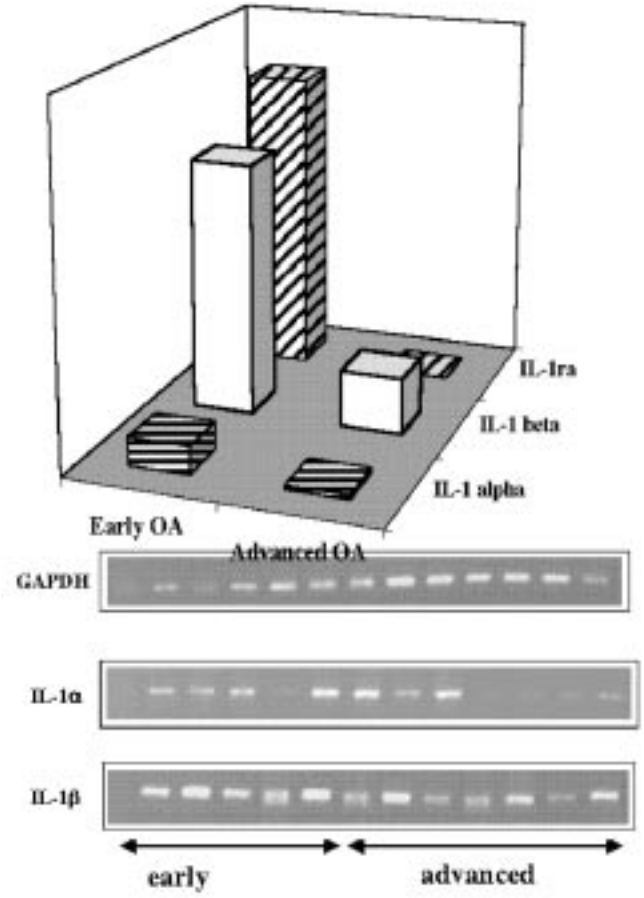


Figure 2. ELISA and RT-PCR to evaluate IL-1 and IL-1ra expression in relation to Mankin score for the extent of degenerative change. A) Relative Elisa data compare IL-1 and IL-1ra concentrations in chondrocyte lysates. B) RT-PCR products amplified from RNA extracted from OA chondrocytes. Bands represent products of RT-PCR on ethidium bromide-stained agarose gel displayed in order of increasing grade.

are shown in Figure 1.

Chondrocytes were isolated from cartilage specimens for mRNA and protein analyses. IL-1α, IL-1β, and intracellular IL-1ra mRNAs were assessed by RT-PCR, and chondrocyte lysates were analyzed by ELISA for the respective proteins. IL-1α and IL-1ß mRNA and protein were detected in most specimens of OA cartilage. However, in advanced OA both IL-1 agonist proteins decreased, correlating with a decrease in IL-1α and ß mRNA (Figure 2). Both IL-1ß and IL-1ra protein levels in chondrocyte lysates generally exceeded IL-1α levels by one order of magnitude. The exception was cases of moderate OA (grades 4-5), where antagonist levels were only slightly higher than IL-α levels (Figure 3).

Signaling through IL-1 receptors is exquisitely efficient, and binding of agonist to only a few receptors per cell is sufficient for full activation. In order to prevent IL-1 from binding to enough cell surface receptors to activate the cell, IL-1ra levels must exceed agonist levels by two to three orders of magnitude.[7] OA chondrocytes contain IL-1ß, presumably in the inactive precursor form, at levels10 times greater than those of IL-1α and cytoplasmic IL-1ra. The presence of active IL-1β converting enzyme (ICE) in human articular cartilage was recently demonstrated, with greatly increased levels in OA tissue.[19] Mature, biologically active IL-1ß may be released from chondrocytes after ICE clipping. Because icIL-1ra is released together with IL-1 in response to stimulus or upon trauma or cell death, it would be better localized to antagonize IL-1 effects than the readily diffusible secreted IL-1ra. However, in osteoarthritic chondrocytes that are stimulated to release the "signal peptide-less" IL-1s, IL-1ra levels are not high enough to prevent agonist binding to cell surface receptors. Thus, IL-1 exported from OA chondrocytes may locally overwhelm inhibition by IL-1ra to promote the degenerative changes.

Unconventional intracellular pathways may exist for signaling by IL-1α that is neither processed nor secreted, since pro-IL-1α is competent to bind to and activate IL-1 receptors.

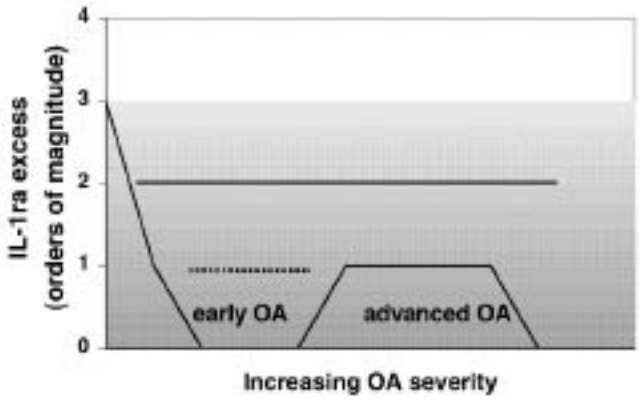


Figure 3. IL-1α and IL-1ra in chondrocyte lysates. The magnitude of the excess of IL-1ra over IL-1α is displayed across the range of OA severity (Mankin score 1 to 8). In all but one specimen (grade 1), IL-1ra exceeded IL-1α by less that the 100-fold that is required to block signaling through cell surface receptors (solid line). IL-1ra was only in slight excess over IL-1α in early OA specimens near the transition to advanced OA (dotted line). Note that in the two highest grade OA cases, IL-1α was zero, and the ratio is not meaningful.

Signaling pathways may be activated by binding of pro-IL-1α to receptors internalized from the cell surface, or perhaps to as yet unidentified intracellular IL-1 binding proteins. Intracellular IL-1ra may also regulate intracellular signaling. IL-1ra, which is believed to be an important endogenous regulator of IL-1 signaling, was only in slight excess over IL-1α in chondrocytes from cartilage approaching the transition to advanced OA. Furthermore, antagonist levels exceeded IL-1α levels by the two orders of magnitude required to block signaling through cell surface receptors in only one case (Mankin score of 1). Particularly in these cases of moderate OA, intracellular antagonist may not be sufficiently abundant to block postulated intracellular functions of precursor IL-1α. Thus we postulate that intracellular and/or localized extracellular IL-1 signaling modulates cartilage metabolism at the critical transition from early to more advanced OA.

The mechanisms regulating IL-1 gene expression in OA cartilage are unknown, but IL-1 and other cytokines, various matrix degradation products such as fibronectin fragments that appear to increase catabolic activity via IL-1, and physical forces within the joint are likely culprits. In the cartilage of OA patients the large proteoglycan aggrecan, which is essential for cartilage biomechanical function, is depleted, and the additional mechanical stress under load may promote IL-1 gene expression. Recent data from this laboratory have implicated IL-1 in the anti-anabolic effects of mechanical compression in bovine articular cartilage.[20] Static mechanical compression decreases proteoglycan synthesis in human OA cartilage, as has been reported for bovine cartilage (Figure 4). Mechanical forces associated with load bearing may induce IL-1 gene expression (*manuscript in preparation*), trigger the release of IL-1 and icIL-1ra,[9] and perturb the cell membrane leading to clustering and internalization of IL-1 receptors.[21] It has been postulated that precursor IL-1α stored within cells is accessible to internalized IL-1 receptors and competent to activate signaling,[22] but pathways for IL-1 release and receptor internalization in "compressed" chondrocytes remain undefined. We postulate that in early OA cartilage that is weakened by the loss of aggrecan, the mechanical forces on load bearing activate IL-1 signaling in chondrocytes.

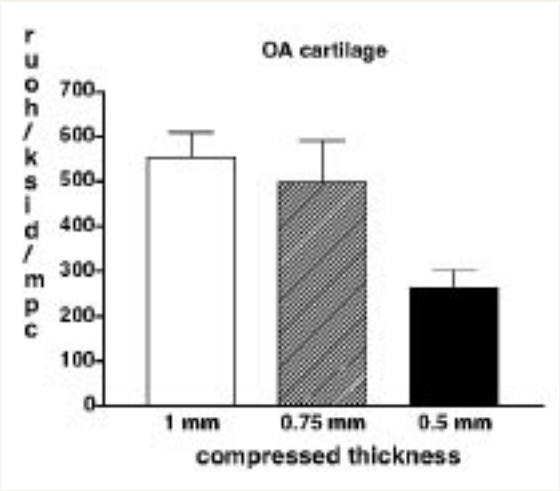


Figure 4. Mechanical compression decreases proteoglycan synthesis in OA cartilage. Articular cartilage (superficial region) from OA patients was maintained at the original cut thickness (1 mm) or compressed 25% or 50% (0.75 mm or 0.5 mm) for 4 hours. Proteoglycan synthesis, estimated from the incorporation of [^{35}S] sulfate into macromolecules, is expressed as counts per minute per disk per hour.

SUMMARY

IL-1α and IL-1ß genes are expressed in osteoarthritic cartilage, and the cytokines accumulate in the chondrocytes in the early phase of the disease. The osteoarthritic chondrocyte may release its stored IL-1 in response to stress or other stimulus, and thus serves as a powderkeg of stimulatory molecules. There is a decrease in the levels of both IL-1 forms in advanced OA, which correlates with decreased IL-1α and ß mRNA. In moderately degenerated cartilage, levels of intracellular IL-1 antagonist may not be sufficient to block intracrine or autocrine/paracrine signaling by the IL-1 agonists. The stored IL-1 may exacerbate the effects of mechanical load in the weakened articular cartilage, predisposing the osteoarthritic cartilage to mechanical damage. Our results support the concept that chondrocyte-derived IL-1α and IL-1ß may overwhelm inhibition by IL-1ra to promote early degenerative changes in OA.

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