INTRACELLULAR IL-1 AND IL-1 RECEPTOR ANTAGONIST IN OSTEOARTHRITIS

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Introduction: Interleukin-1α (IL-1α) in chondrocytes isolated from mildly diseased osteoarthritic cartilage is primarily in the 33 kDa precursor form. Consistent with other cell systems, the 18 kDa mature IL-1 is not detected in cell lysates. Presumably, this is because processing and export of the mature form are closely linked events. The observations that IL-1α precursor accumulates within chondrocytes early in the disease, the intracellular or "intracrine" signaling pathway postulated for other cell types, and the expression of intracellular IL-1 receptor antagonist (IL-1 IRA) in chondrocytes (Weissbach et al., 1998) prompted us to more closely examine the expression of cytokine and intracellular inhibitor in chondrocytes isolated from the cartilage of OA patients. In this study we used reverse transcription-polymerase chain reaction (RT-PCR), western blotting, and ELISA to investigate IL-1α and IL-1 IRA within chondrocytes isolated from human OA cartilage.

Materials and methods: Cartilage was obtained from patients undergoing total knee arthroplasty for the treatment of OA. Articular cartilage was obtained from femoral condyles and tibial plateau, taking care to avoid fibrocartilage. Chondrocytes were isolated by collagenase digestion, the cells were collected by centrifugation, and cell pellets were stored frozen until used for RNA extraction (10 patients) or the preparation of chondrocyte lysates (8 patients). Total RNA was isolated from each cell pellet by guanidinium thiocyanate phenol chloroform method using RNA Stat 60. Total RNA (4 ug) from each sample was reverse transcribed using the First Strand cDNA Synthesis kit (Amersham Pharmacia Biotech) with Not 1-oligo dT as a primer. The cDNA from each sample was amplified by PCR using oligonucleotide primers specific for sequences in IL-1α, IL-1 IRA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs. Primers used for splicing variants of the IL-1 receptor antagonist gene would amplify all known transcripts coding for intracellular forms - the type I (short form) to give a 432 bp product, the type II long form at 495 bp, and the exon 3 IL-1 IRA at 602 bp. Primers were designed to span intron-exon junctions so that amplicons resulting from genomic DNA and mRNA could be distinguished by size. PCR products were separated on 1.5% agarose gels and visualized using ethidium bromide.

Chondrocytes lysates were prepared as described previously (Hung et al., 1997). Protein (100 ug per lane) was resolved by SDS-PAGE and transferred to PVDF for immunodetection using antisera against recombinant human IL-1α (mature form) or the secreted form of recombinant human IL-1 receptor antagonist. Aliquots of lysate protein (40 ug) were analyzed by ELISA for IL-1α and IL-1 IRA using commercial kits from R&D Systems.

Results: IL-1α and IL-1 IRA mRNAs were detected in most specimens of OA cartilage. The only IL-1α amplification product detectable on ethidium bromide-stained agarose gels was the type I transcript. By Southern blot analysis using a 3′-radiolabeled internal oligonucleotide probe common to all IL-1 IRA transcripts and internal to the PCR primers, only the 432 bp type I product was clearly detected (fig. 1). The 279 bp PCR products generated using primers corresponding to GAPDH mRNA demonstrated similar amounts of input RNA (fig. 1, bottom panel).

Western blot analysis demonstrated precursor IL-1α and IL-1 receptor antagonist in lysates of chondrocytes isolated from OA cartilage (fig. 2). The prominent 22 kDa band detected by anti IL-1 receptor antagonist is consistent in electrophoretic mobility with the type I IL-1-IRA. A very weak band in the third lane at approximately 18 kDa may be the product of the alternatively spliced exon-3 transcript, but a degradation product or nonspecific staining are also possible. The major 33 and 31 kDa bands detected with anti-IL-1α correspond to the precursor form, with apparent degradation products detectable in some lysates. We do not know the nature of the protein(s) detected at approximately 46 kDa using either primary antiseraum. Nonspecific staining is the suspected cause. However, a protein in this region is also detected by a mouse monoclonal antibody and another polyclonal IL-1α antiserum, but not anti-IL-1β, suggesting the possibility of a complex or perhaps crossreaction with an unrecognized IL-1-related species.

Agonist and antagonist concentrations in each cell lysate were measured by ELISA. The concentration of antagonist was highly variable, ranging from 0 to 6 pg/ug lysate protein. IL-1α ranged from 0 to 0.7 pg/ug. Often but not always, the level of antagonist exceeded IL-1α by at least ten-fold. IL-1α and IL-1IRA were estimated to be greater than 20pg/ml by western blot analysis, suggesting that some of these polypeptides may exist as complexes with intracellular binding protein(s).

Discussion: Western and RT-PCR results support the notion that type I IL-1 IRA is the predominant intracellular antagonist species in OA chondrocytes. It is of interest that in some specimens of cartilage (lanes 5, 9, and 10 of fig. 1), the agonist and its antagonist are discordantly expressed. It is known that IL-1 induces the expression of both IL-1 genes in chondrocytes. As suggested by Watson et al. (1995) high levels of IL-1 IRA may counteract downstream responses to IL-1, possibly including this autoinduction loop. The role of cytoplasmic IL-1α in chondrocyte metabolism is unknown. The cytokine and antagonist may both be released by dying cells. Precursor IL-1α, in contrast to the β cytokine, binds to cell surface receptors and can send neighboring chondrocytes into a catabolic pathway. The possibility that IL-1α may function through an intracrine pathway to regulate chondrocyte metabolism in OA is under investigation.