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Human chondrocyte culture models for studying cyclooxygenase expression and prostaglandin regulation of collagen gene expression

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Summary

Objective: Since articular chondrocytes and synovial fibroblasts are particularly responsive to interleukin-1 (IL-1) with respect to stimulation of prostaglandin E_2 (PGE_2) biosynthesis, we have used them as models to examine feedback modulatory effects of PGE_2, which blocks or attenuates the direct effects of IL-1β on cell-specific collagen gene expression.

Methods: Immortalized human chondrocytes were developed for studying responses to cytokines and prostaglandins. Regulatory sequences of the type II collagen gene (COL2A1) in reporter gene constructs were analyzed in transient transfection experiments. Endogenous expression of COL2A1 mRNA, as well as aggrecan, biglycan, and decorin mRNAs, and IL-1-inducible cyclooxygenase (COX-2), phospholipase A2 (PLA2), and inducible nitric oxide synthetase (iNOS) mRNAs were analyzed by RT-PCR.

Results: Previous work has shown that IL-1β inhibits, while prostaglandins stimulate, COL2A1 expression. In different immortalized chondrocyte cell lines, the ability to respond to IL-1β with increased levels of COX-2, PLA2, and iNOS mRNAs depends upon expression of the differentiated chrondrocyte phenotype.

Conclusion: Our studies suggest that some IL-1-induced responses in chondrocytes may require differentiation-specific transcription factors that could serve as therapeutic targets for arthritis.

Key words: Cartilage, Cyclooxygenase, Prostaglandin.

A central role for interleukin-1 (IL-1) in rheumatoid arthritis and osteoarthritis involves activation of both synovial fibroblasts and chondrocytes resulting in increased cartilage degradation as well as suppression of normal repair. Previous studies using human articular and costal chondrocyte culture models showed that IL-1 inhibits the synthesis of cartilage-specific collagens (II, IX & XI) and stimulates the synthesis of collagens I & III.[1,2] Since IL-1-stimulated PGE_2 may block or blunt the direct effects of IL-1 itself, incubation with a cyclooxygenase inhibitor such as indomethacin is required to block endogenous prostaglandins (PG) biosynthesis and unmask direct effects of IL-1 on cell-specific collagen gene expression.

Articular chondrocytes and synovial fibroblasts are particularly responsive to IL-1 with respect to stimulation of prostaglandin E_2 (PGE_2) biosynthesis.[2,3] PGE_2 production stimulated by articular chondrocytes and synovial fibroblasts is 20–50-fold greater than that by costal chondrocytes or dermal fibroblasts. However, the magnitude of stimulation above control levels is similar in articular and costal chondrocytes, and the two types of chondrocytes are similarly sensitive to the effects of PGE_2 on collagen synthesis. In contrast, dermal fibroblasts are less responsive to IL-1 than synovial fibroblasts with respect to IL-1-stimulated PGE_2 production. Furthermore, dermal fibroblasts are less sensitive to inhibition of type I collagen synthesis by PGE_2. Feedback modulatory effects of PGE_2, including stimulation of chondrocyte-specific type II collagen and inhibition of fibroblast-specific type I collagen synthesis, occur at least partially at the transcriptional level.[4,5]

In order to analyze molecular events involved in chondrocyte responses to IL-1 and PGs, we examined the expression of type II collagen gene (COL2A1) regulatory sequences transfected in normal and immortalized human chondrocytes.[6] IL-1 alone suppressed COL2A1 (−577/+3426)-CAT expression in human chondrocytes and this suppression was potentiated by indomethacin. Direct dose-dependent stimulatory effects of PGs were observed in the order of potency of $PGE_2 \geq PGF_{2\alpha} >$ misoprostol $\geq PGE_1$. Analysis of deletion constructs indicated that elements responsive to both IL-1 and prostaglandins resided in the upstream promoter region of COL2A1.[6,7]

Our recent work has focused on the development of immortalized human chondrocyte cell lines to use as reproducible models for studying the roles of cytokines and prostaglandins in regulating anabolic and catabolic chondrocyte functions. In earlier studies, we used SV40 large T antigen (TAg) to immortalize juvenile costal chondrocytes,[8] which we had used extensively in primary culture to study chondrocyte-specific matrix gene expression.[1,2,9] These cell lines, designated C-20/A4 and T/C-28a2, have been used to study a variety of chondrocyte functions.[10–17] However, since they were established with a highly proliferative phenotype, it was difficult to maintain them in long-term culture for sufficient periods of time to permit high levels of expression of cartilage-specific matrix proteins. Since articular cartilage is the primary joint tissue requiring replacement or reconstruction after damage in

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arthritic conditions, we have focused our recent work on developing an immortalized human adult articular chondrocyte line, designated T/AC62, using temperature-sensitive SV40 TAg.[18]

Monolayer cultures established after selection with G418 and continuous subculture for >60 passages grew more slowly than the original lines at 32°C, the permissive temperature for proliferation. Transfer to 39°C resulted in loss of TAg detected in cell extracts by Western blotting and complete inhibition of cell proliferation. In alginate culture, the T/AC62 cells were capable of depositing abundant alcian blue-stainable matrix even at 32°C and clumps of cells appeared suggesting that they continued to proliferate in suspension. Type II collagen and aggrecan mRNAs were expressed equally well in monolayer and alginate cultures at 32°C and 37°C, and were decreased by IL-1β in 24–48 h incubations. Collagen II, aggrecan, biglycan and decorin were also detected by Western blotting, and analysis of [^{35}S]sulfate-labeled proteoglycans demonstrated preferential retention of aggrecan and biglycan in the cell-associated matrix and secretion of decorin in the culture medium.[19]

Cyclooxygenase is crucial for the synthesis of PGE_2 through the metabolism of arachidonic acid. While COX-1 is constitutively expressed by a wide variety of cells, COX-2 is transcriptionally induced by proinflammatory cytokines such as IL-1 and restricted to certain cell types, including synovial fibroblasts[20] and chondrocytes.[21] These results suggest the presence of inducible cell-specific transcription factors regulating COX-2. In order to determine the extent to which these responses are dependent upon the differentiated phenotype, PGE_2 synthesis and COX-2 mRNA expression were compared in the immortalized human costal and articular chondrocyte cell lines. The less well differentiated chondrocyte lines, C20/A4 and T/C-28a2, which required culture in alginate beads in order to express markers of differentiation, type II collagen and aggrecan mRNAs, expressed low levels of COX-2 mRNA and synthesized little PGE_2 in response to IL-1β compared to normal primary human chondrocyte cultures.[22] In contrast, the immortalized articular chondrocytes, T/AC62, expressed IL-1-inducible COX-2 mRNA at both 32°C and 39°C and expression was greater in alginate cultures at both temperatures. The T/AC62 cells also expressed low levels of sPLA2 mRNA that were increased by treatment with IL-1β and enhanced by culture in alginate. However, low levels of iNOS mRNA were expressed by the T/AC62 cells only at 39°C in monolayer cultures in response to IL-1β treatment. Since PGE_2 production was increased 10–20 fold after preincubation with the COX-2 substrate arachidonic acid, it is likely that there are additional requirements for these inflammatory responses.

These and previous studies indicate that prostaglandins influence collagen gene expression directly and act as autocrine factors in modulating the effects of IL-1. Our recent work presented here in preliminary form also suggests that the magnitude of expression of IL-1-inducible COX-2 is dependent upon the ability of the chondrocytes to express the fully differentiated phenotype, which is suppressed in proliferating monolayer cultures of immortalized chondrocytes. In the inflammatory process, several responses to IL-1, including increased expression of iNOS, PLA2, and COX-2 are dependent upon expression of the differentiated chondrocyte or synoviocyte phenotype.

The enhanced sensitivity of these cell types to inflammatory cytokines suggests that COX-2 expression relies on cell-specific regulatory factors. An understanding of the unique signal transduction pathways that mediate cell-specific collagen gene transcription may permit targeted therapies that would dissociate the beneficial and detrimental actions of IL-1 and prostaglandins in joint tissues. Furthermore, if differentiation-specific transcription factor(s) are found to be responsible for COX-2 expression in human chondrocytes, they could be targeted for future therapeutic intervention in arthritis.

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