Cytokine-mediated down-regulation of BMP expression in human neocartilage

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Introduction
Osteoarthritis (OA) is characterized by a loss of joint cartilage that appears to result from an imbalance in the anabolic and catabolic activity of resident chondrocytes. Nitric oxide (NO) is an important mediator of cartilage destruction in both inflammatory arthritis and osteoarthritis (1). NO synthesis can be induced in chondrocytes by exogenous treatment with IL-1, TNF, IL-17, IL-18, and bacterial LPS (2). It is established that these cytokines inhibit S-GAG and collagen synthesis. The purpose of this study was to determine whether cytokine treatment alters chondrocyte BMP expression in an in vitro model of experimentally induced arthritis.

Materials & methods
Chondrocytes derived from human postnatal articular cartilage were enzymatically isolated and seeded into 12 well culture plates for neocartilage production as described previously (3). Day 200 cultures were transferred to 6 well plates and treated either with IL-1β (1.0 ng/ml) + TNFα (0.5 ng/ml) or IL-1β + TNFα + NIL (100 μM, Alexis Biochemicals). NIL is a selective inhibitor of inducible nitric oxide synthetase (iNOS). Spent media were collected and the neocartilage discs harvested for tRNA extraction at 12, 24, and 48 h post cytokine addition as described (4). NO production was measured via the Griess reaction, using sodium nitrite as standard (5). BMP-1, 3, 4, 5, 6, CDMP-1 & 2 and BMPR-IA, IB & II mRNA expression were evaluated by semiquantitative RT-PCR using published primers as described (4). Parallel cultures were formalin fixed and paraffin embedded for immunohistochemical detection of BMP synthesis, and these patterns were compared to normal (7 yr) and grade-3 OA (58 yr and 75 yr) human articular cartilage. Polyclonal goat and chicken antibodies to human BMP-3, CDMP-1 and -2 were obtained from Santa Cruz Biotechnology and Pfizer Research, respectively.

Results
Incubation of human neocartilage with IL-1β and TNFα resulted in both a 40- and greater than 60-fold increase in NO production at 24 and 48 h, respectively. RT-PCR analysis of specific BMP and BMP receptor mRNA transcripts endogenously expressed by neocartilage chondrocytes revealed that cytokine activation caused selective down-regulation of BMP-3 and CDMP-1 mRNA expression at each of the time points examined (Fig 1). However, BMP-1, 4, 5, 6, CDMP-2 and BMPR-IA, IB & II mRNA expression was unaffected by cytokine treatment. Because down-regulation of BMP-3 and CDMP-1 mRNA expression was associated with a marked increase in nitrite levels, we next examined whether selective inhibition of iNOS could restore basal BMP-3 and CDMP-1 expression. Treatment with 100 μM NIL inhibited cytokine-induced NO synthesis by 43±5% at 24 h and 67±9% at 48 h, respectively. More importantly, NIL restored CDMP-1 mRNA expression to basal levels. In contrast, BMP-3 mRNA expression remained unchanged following NIL treatment. Immunohistochemical staining for CDMP-1 confirmed that protein synthesis was markedly reduced relative to untreated controls, and that treatment with NIL restored pericellular staining of CDMP-1 (Fig 2). Immunohistochemical staining of BMP-3 and CDMP-1 native human tissue demonstrated positive reactivity in the superficial and mid-layer of normal articular cartilage, while the superficial layer of OA tissue showed weak immunoreactivity.

Discussion
The inhibition of proteoglycan synthesis during cartilage destruction in advanced OA has been well documented. In vitro studies have implicated IL-1β as a key mediator of cartilage destruction. Furthermore, IL-1-mediated inhibition of chondrocyte proteoglycan synthesis and proliferation, appears to depend, at least in part, on chondrocyte derived NO synthesis (6, 7). In the current model, NO synthesis was markedly increased by treatment with IL-1β and TNFα. While neocartilage BMP-1, 4, 5, 6, CDMP-2, BMPR-IA, IB, II mRNA expression appeared to be unaffected by cytokine activation, mRNA transcripts for BMP-3 and CDMP-1 were significantly reduced. Treatment with NIL resulted in partial inhibition of iNOS, which was associated with rescued expression of CDMP-1. It is established that BMPs and their receptors act as key regulators of bone and cartilage growth and maintenance. In previous reports, BMP-3 was shown to stimulate and maintain the chondrocyte phenotype in vitro, playing a role in embryonic cartilage development (8). In addition, CDMP-1 has been localized to the precartilagenous regions of the developing limb and epiphyseal cartilage, and its primary role in skeletal development is believed to promote proliferation and differentiation of cartilage during joint morphogenesis (9). A recent report by Erlacher et al, 1998 demonstrated that exogenous CDMP-1 can replenish S-GAG synthesis in matrix-depleted cartilage explants (10). Thus, cytokine-mediated down-regulation of chondrocyte CDMP-1 and BMP-3 mRNA expression and synthesis may contribute in part to the destructive processes accompanying inflammatory arthritis and OA.

References

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