FUNCTIONAL TOLL-LIKE AND NOD-LIKE RECEPTORS IN PROSTAGLANDIN D2 INHIBITS INTERLEUKIN-1-
HISTONE DEACETYLASE INHIBITORS SUPPRESS S90 of these pathways may have therapeutic utility in the treatment of OA.

Methods: Human articular cartilage was obtained with informed consent and was used under the approval of the institutional review board (IRB). Cartilage explants were incubated with LPS, MDP, iE-DAP, or their respective controls for up to 10 days, and conditioned media was removed and replaced with fresh treatments every 1–4 days. Media was analyzed for total aggrecan degradation based on glycosaminoglycan (GAG) levels using the DMMB colorimetric assay, and for ARGSSVII-aggrecan content by Western analysis. To measure induction of aggrecanase, isolated human articular chondrocytes were grown on a streptavidin-coated 96-well plate containing an immobilized aggrecanase peptide substrate. Induction of aggrecanase resulted in specific cleavage of the peptide substrate that is detected by a highly sensitive, specific monoclonal antibody in an ELISA. Monolayer cultures of articular cartilage were also used to examine the effects on gene expression by Q-PCR and cytokines/chemokine levels were determined by Bioplex.

Results: LPS induced aggrecanase activity in a chondrocyte-cell based assay, and this activity was blocked by a broad spectrum Aggrecanase inhibitor. Interestingly, this activity, as well as LPS-induced nitric oxide and cytokine/chemokine production, was inhibited by TLR antagonist CRX-526. LPS also induced aggrecanase activity in human cartilage explants as measured by an increase in aggrecan fragments carrying the N-terminal ARGSSVII-neoepitope, and CRX-526 was able to block generation of these fragments induced by LPS. These data support a role of TLRs in the induction of aggrecanases by LPS. CRX-526 was not effective in blocking aggrecanase induction by IL-1 and OSM, nor the generation of ARGSSVII-aggrecan fragments in the absence of stimulus in human cartilage explants, suggesting that these pathways do not involve TLRs. Since LPS may also induce cytokines that result in the induction of the aggrecanases, these data suggest that the TLR contribution is 'upstream' of cytokine induction of proteolytic enzymes by LPS. The effect of the classical NLR ligands, iE-DAP (NOD-1) and MDP (NOD-2, NLPR3, etc.) was also evaluated in this system. Only MDP was effective in inducing a concentration-dependent induction of aggrecanase activity that appeared to reach a plateau between 1–10 μg/ml. MDP was also effective in inducing aggrecanase-mediated aggrecan degradation based on the production of the ARGSSVII-neoepitope. Gene expression analysis demonstrated the expression of several NLRs in chondrocytes, including NOD-1, NOD-2, NLR-3, suggesting a potential role of these NLRs in OA.

Conclusions: These data suggest that aggrecanase activity and aggrecan degradation are inducible by ligands of both TLR and NLR pathways, and suggest that these receptors may be involved in cartilage degradation in OA. The observation that a TLR-2,4 antagonist was effective in blocking aggrecanase-mediated aggrecan degradation suggests that modulation of these pathways may have therapeutic utility in the treatment of OA.

HISTONE DEACETYLASE INHIBITORS SUPPRESS INTERLEUKIN-1(II)-INDUCED mPGES-1 EXPRESSION IN HUMAN CHONDROCYTES

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Purpose: Micromosomal prostaglandin E synthase-1 (mPGES-1), catalyzes the terminal step in the biosynthesis of PGE2, which plays an important role in the pathogenesis of osteoarthritis (OA). In the present study, we investigated the effect of trichostatin A (TSA), butyric acid (BA), and valproic acid (VA), three histone deacetylase (HDAC) inhibitors, on IL-1-induced mPGES-1 protein and mRNA expression in human chondrocytes. We also investigated the expression and DNA binding activity of Egr-1, a key transcription factor in mPGES-1 expression.

Methods: Chondrocytes were stimulated with IL-1 in the absence or presence of increasing concentrations of TSA, BA or VA. The expression of mPGES-1 protein and mRNA were evaluated using Western blotting and real-time reverse transcriptase-polymerase chain reaction (RT-PCR), respectively. Electrophoretic mobility shift assay (EMSA) was utilized to analyze the DNA binding activity of Egr-1.

Results: TSA, BA and VA prevented IL-1-induced mPGES-1 protein and mRNA expression, implying that a deacetylase activity is required for the induction of mPGES-1 expression. Furthermore, we demonstrated that the induction of Egr-1 expression and its DNA binding activity by IL-1 were not impaired upon treatment with histone deacetylase inhibitors.

Conclusions: These data indicate that HDAC inhibition prevents IL-1-induced mPGES-1 expression without interfering with Egr-1 expression or DNA-binding activity. They also suggest that HDAC inhibitors could be of potential interest in the treatment of OA.

INTERLEUKIN-4/INTERLEUKIN-4 RECEPTOR SYSTEM ACTIVITY IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: In osteoarthritis (OA), the final result of chondrocyte metabolic modification is the enhancement of cartilage matrix catabolism, not adequately counterbalanced by anabolic events. Interleukin-4 (II-4)/interleukin-4 receptor (II-4R) system has a pivotal role in chondrocyte anabolic response to mechanical stimulation which in turn is an essential condition to maintain cartilage homeostasis. Previous studies reported a modification of mechanotransduction response in OA chondrocytes suggesting an impaired efficacy of the II-4/II-4R system protective effect and underline the importance of disordered mechanical signalling in cartilage breakdown pathways.

We aim to investigate chondrocyte response induced by II-4/II-4R system, focusing on modulation of peculiar pathways involved in cartilage breakdown.

Methods: Articular cartilage specimens were obtained from OA patients undergoing joint replacement surgery. Chondrocytes were isolated from articular cartilage by sequential enzymatic digestion. Chondrocyte micro-mass and hyperconfluent cultures were set up with II-4 alone or with pro-inflammatory (II-1) and anti-inflammatory cytokites (II-13). Culture supernatants were analysed for chemokinesis production, CXCL8 (II-8), CXCL1 (GROalpha), CCL5 (RANTES), by enzyme-linked immuno-sorbent assay (ELISA). RNA were extracted from chondrocytes cultured in described conditions and real time PCR analysis were performed. mRNA expression of chemokines (II-8, GRO alpha, RANTES), matrix degrading enzymes (metalloprotease-13/MMP-13, aggrecanases ADAMTS-4 and ADAMTS-5) and tissue inhibitors of metalloproteinases (TIMP-1, -3, -4) were investigated.

Results: Data obtained from ELISA quantification of chemokine production by OA chondrocyte cultures showed that II-4 presence significantly inhibits RANTES release induced by II-1. On the other hand, II-4 did not affect GROalpha and II-8 production induced by II-1. These data were also confirmed by overlapping pattern of chemokine mRNA expression, mRNA modulation analysis of matrix degrading enzymes and their inhibitors shows that II-4 down modulated also MMP-13, ADAMTS-4, -5 and TIMP-1, -3 mRNA expression induced by II-1. On the contrary, TIMP-4 mRNA expression appears to be inhibited by II-1 and strongly induced by II-4.

Conclusions: These data underline the complexity of II-4/II-4R activity and suggest the involvement of multiple signal transduction pathways of cellular response. Actually, preliminary results suggest that II-4 can selectively inhibit nuclear translocation of some members of the NF-kB transcription factor family.

PROSTAGLANDIN D2, INHIBITS INTERLEUKIN-1(II)-INDUCED MATRIX METALLOPROTEINASE-1 AND -13 PRODUCTION BY HUMAN CHONDROCYTES VIA ITS DP1 RECEPTOR AND CAMP/PKA PATHWAY


Purpose: To investigate the effects of prostaglandin (PG) D2 on interleukin-1(II)-induced matrix metalloproteinase (MMP)-1 and MMP-13 expression in human chondrocytes and the intracellular signalling pathways involved in the effects of PGD2.

Methods: Chondrocytes were stimulated with II-1±PGD2 and MMP-1 and MMP-13 protein expression was evaluated by ELISA. mRNA expression and promoter activity were analyzed by real-time RT-PCR and