sion became uniformly increased in hypertrophic chondrocytes, whereas CD36 expression developed most robustly at sites of the most intense injury. We demonstrated that forced expression of CD36 blocked the capacity of S100A11, TNFalpha and CXC8L2 to induced chondrocyte hypertrophy. CD36 also blocked the capacity of S100A11 to inhibit proteoglycans synthesis. Last, the PPARγ agonist N-(2-Benzoylphenyl)-O-[2-(methyl-2-pyridinylamino)ethyl]-L-tyrosine hydrate induced CD36 expression without affecting RAGE expression in chondrocytes.

Conclusions: Early induction of CD36 expression by chondrocytes occurs at sites of cartilage injury as OA develops experimentally. PPARγ-βagonism, which is chondroprotective, induces chondrocyte CD36 expression in vitro. Chondrocyte CD36 expression suppresses the capacity of not only its ligand S100A11, but also of TNFalpha and CXC8L2 to induce chondrocyte hypertrophy, and CD36 expression promotes preservation of proteoglycans synthesis. We conclude that chondrocyte expression of CD36 is chondroprotective by disengaging responsiveness to inflammation from dysregulated chondrocyte differentiation and function.

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THE “ALARMIN” S100A8: AN ACTIVATOR OF CHONDROCYTE MEDIATED CARTILAGE DAMAGE?

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Purpose: In a previous study we have shown that “alarmins” S100A8 and S100A9 which can form dimers and are predominately produced by myeloid cells in the synovial fluid and synovial membrane are involved in MMP-mediated cartilage destruction during experimental arthritis. S100A8 forms the active part which is stabilized by S100A9 and protects S100A9 from degradation. As MMP mediated cartilage destruction is particularly found around the chondrocyte this prompted us to investigate whether S100A8 and A9 are produced by chondrocytes and whether these “alarmins” are actively involved in MMP-mediated chondrocyte activation.

Methods: S100A8 and A9 proteins were detected in knee joints of murine arthritis and osteoarthritis using immunolocalisation. Murine chondrocyte cell line H14 was stimulated with pro-inflammatory cytokines (100 ng/ml) to investigate rS100A8 and A9 production or by recombinant S100A8 (0.2, 1 and 5 µg/ml) to investigate MMP and cytokine production mRNA and protein levels were measured using RT-PCR and blotanalysis. S100A8/A9 dimers and cytokines were measured in culture supernatant using ELISA and Luminex. Breakdown of aggrecan on the pericellular surface of the chondrocyte was measured using VDIVPEN and NITEGE (MMP and aggrecanase neoepitopes) antibodies and FACS analysis.

Results: Immunolocalisation of inflamed knee joints depicted that S100A8 and S100A9 proteins were abundantly expressed in chondrocytes. Expression was particularly found in the superficial layers of the cartilage surfaces at the margins of the joint. Stimulation of murine chondrocytes by pro-inflammatory cytokines IL-17, IL-18 and IFNγ caused strong upregulation of particularly S100A8 and in lesser extent S100A9 mRNA (S100A8: 24, 48 and 4 fold and S100A9: 4, 4 and 0 fold respectively). Stimulation of chondrocytes by rS100A8 caused a significant autoinduction of S100A8 and in lesser extend upregulation of S100A9 mRNA and protein levels. High concentrations of cytokines IL-6, KC and protein levels were measured using RT-PCR and blotanalysis. High concentrations of cytokines IL-6, KC and RANTES were measured in the culture supernatant whereas TNFα, IL-1α and β were below detection level. S100A8/A9 dimers could not be detected by ELISA confirming that S100A8 is the active component and can directly activate chondrocytes. Moreover MMP’s (-2,-3,-9,-13) and ADAMTS(-4,-5) mRNA levels in the chondrocyte were strongly upregulated (maximal at 1 µg/ml (4, 4, 3, 16, 8 and 4 times respectively). VDIVPEN and NITEGE neoepitopes on the pericellular membrane of chondrocytes were significantly elevated after stimulation with rS100A8 for 24 hours in a concentration (0.2, 1 and 5 µg/ml) dependent manner. (VDIVPEN 17.67,108% and NITEGE 8, 33 and 67% respectively).

Conclusions: The alarmin S100A8 is produced by chondrocytes and directly activates MMP and aggrecanase mediated peri-cellular matrix degradation. S100A8 may be an important mediator of severe cartilage destruction.

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INTERLEUKIN-7 STIMULATES SECRETION OF S100A4 BY ACTIVATING THE JAK-STAT PATHWAY IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: S100A4, a member of the S100 family of calcium binding proteins, has been shown to be increased in OA cartilage and to stimulate chondrocyte RAGE signaling resulting in increased expression of MMP-13. Members of S100 family are known to be secreted into the extra-cellular environment, however the mechanism(s) of secretion is not completely understood. The aim of this study was to determine the pathway involved in secretion of S100A4 in response to cytokines in chondrocytes.

Methods: Human articular chondrocytes isolated from normal ankle cartilage obtained from tissue donors were cultured in high density monolayers in media with 10% serum for 5-7 days. Confluent monolayers were then changed to serum free media 16-18hr prior to treatment with 10ng/ml of IL-7, IL-1α and TNF α. In some experiments, cells were pre-treated with a JAK-3 inhibitor or Brefeldin-A (BFA), a chemical inhibitor that blocks classical protein translocation. Immuno-blotting with phospho-specific antibodies was used to determine the activation of signaling proteins. Secretion of S100A4 was measured in the conditioned media by immuno-blotting with polyclonal antibodies to S100A4.

Results: Chondrocyte secretion of S100A4 was observed after treatment with IL-7 and TNFα but not with IL-1α. Treatment with IL-7 resulted in activation of the JAK/STAT pathway with increased phosphorylation of JAK-3 and STAT-3. IL-7 stimulation of S100A4 secretion was inhibited by pretreatment with a JAK-3 inhibitor. In addition, pretreatment with Brefeldin-A (BFA) didn’t effect the secretion of S100A4 suggesting a pathway independent of Golgi-endoplasmic reticulum (classical pathway).

Conclusions: Our study demonstrates for the first time that IL-7 or TNFα but not IL-1α can stimulate chondrocyte secretion of S100A4 via activation of JAK/STAT signaling. We have recently found that OA chondrocytes produce IL-7 and others have shown TNFα. These cytokines may contribute to cartilage degradation through secretion of S100A4 which can function as an autocrine factor to stimulate MMP-13 production via RAGE.

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INFLAMMATORY GENE EXPRESSION IN EARLY KNEE OSTEOARTHRITIS PATIENTS

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Purpose: Synovial inflammation in osteoarthritis (OA) patients has been associated with increased pain, disability, and more