We showed that our ELISA was able to demonstrate immune ence in binding of serum EM-IgA levels in kOA patients in comparison with serum EM-IgM levels relative to normal sera: 66% AII (m ≤ 222 vs 705 ± 222; p < 0.0007). No significant difference in binding of serum EM-IgA levels in kOA patients in comparison with that in control. No correlations were found to conventional parameters for pain and function with expression of EM-IgA, EM-IgM.

Conclusions: We showed that our ELISA was able to demonstrate immune responses to each of the 4 type specific self-antigens in kOA patients. EM represent a group of novel self-antigens which are targeted by NA from kOA patients. Serum NA profiling is a promising approach for early detection and diagnosis of KOA. This results show a specific dysbalance of Ig content in kOA patients. Circulating BK-IgG in the sera has been proposed as a sensitive and specific marker of diagnosing KOA at early stages of the disease. Our results have potential applications for controlling unwanted angiogenesis, inflammation, infection, pain and future response to therapy in kOA patients.

051 CONTRIBUTION OF HIGH MOBILITY GROUP BOX 1 TO SYNOVITIS IN OSTEARTHROPATHS

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Purpose: Extracellular release of High Mobility Group Box 1 (HMGB1) may exert pro-inflammatory activity in rheumatoid arthritis. However, the role of HMGB1 during the progression of synovitis in osteoarthopathic (OA) remains unclear. We have recently demonstrated that IL-1β induces HMGB1 secretion by OA synoviocytes, suggesting the contribution of HMGB1 to the pro-inflammatory effects elicited by IL-1β. In this study, we have evaluated the effects of HMGB1 in OA synoviots and the signaling pathways involved.

Methods: Synovial tissue samples were obtained from 15 OA patients undergoing total knee joint replacement and 3 normal donors. HMGB1 expression was determined in both normal and OA human synovium by immunohistochemistry and confocal microscopic procedures. OA synoviocytes were treated with human recombinantHMGB1 (15-25 ng/ml) in presence or absence of IL-1β (10 ng/ml). Gene expression was analyzed by quantitative PCR and protein expression by Western Blot and ELISA. MMP activity was determined by fluorometric procedures and activation of NF-κB by magnetic transfection of the reporter construct NF-κB-luc.

Results: Despite HMGB1 distribution in both normal and OA synovium was similar (lining, sublining and vascular wall cells), the number of HMGB1 positive cells was higher in OA, though it was also present in infiltrated cells. Regarding to HMGB1 intracellular localization, we observed that HMGB1 was mostly found in the nucleus of normal synovium cells, whereas in OA the localization was markedly cytoplasmic. Treatment of synoviocytes with 15 or 25 ng/ml of HMGB1 did not modify the production of IL-6, IL-8, CCL2, CCL20 and matrix metalloproteinases (MMP)-1, 3 and 13. However, the concomitant treatment of HMGB1 with IL-1β significantly enhanced, in a dose dependent manner, both mRNA and protein expression of these mediators, as well as MMP-activity. In addition, HMGB1 potentiated the phosphorylation of Akt, ERK 1/2 and p38 induced by IL-1β as well as NF-κB activation.

Conclusions: Significant HMGB1 expression is higher in OA, with a marked cytoplasmic localization. HMGB1 can act in synergy with IL-1β, with the consequent increase in the production of several pro-inflammatory and degenerative mediators, thus contributing to synovitis progression during OA.
m and degenerative meniscal tears. Despite proposed differences in etiology, both patterns are associated with development of osteoarthritides (OA). In established OA, synovitis is associated with pain and progression, but a relationship between synovitis and symptoms in isolated meniscal disease has not been previously investigated. The present studies were undertaken to characterize synovial pathology in patients with traumatic meniscal injuries and determine the relationship between inflammation, meniscal and cartilage pathology, and symptoms.

Methods: Thirty-three patients without evidence of OA undergoing arthroscopic menisectomy for traumatic meniscal injuries were recruited. Pain and function were assessed preoperatively using three different outcome scores: the Lysholm score (a knee-specific metric of pain and disability), the SF-12, and a visual analog pain scale. Meniscal and cartilage abnormalities were documented at the time of surgery. Synovial inflammation was assessed in synovial biopsies and associations between inflammation and clinical outcome scores determined. Gene expression analysis using microarray technology was performed comparing patients with and without inflammation, to identify gene products that contribute to development of inflammation. Microarray results were validated using quantitative real-time PCR.

Results: Synovial inflammation was present in 42% of the patients and was associated with worse pre-operative pain and function scores, independent of age, gender, or cartilage pathology. Microarray analysis and real-time PCR revealed a chemokine signature in synovial biopsies with increased inflammation scores.

Conclusion: In patients with traumatic meniscal injury undergoing arthroscopic menisectomy without clinical or radiographic evidence of OA, synovial inflammation was a frequent finding and was associated with increased pain and dysfunction. Synovial inflammation was associated with increased expression of chemokines involved in lymphocyte recruitment. These chemokines could have implications for development of synovial inflammation in patients with meniscal pathology and represent potential therapeutic targets to reduce inflammatory symptoms.

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THE INFRAPATELLAR FAT PAD SHOULD BE CONSIDERED AS AN ACTIVE OSTEOARTHRITIC JOINT TISSUE

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Purpose: Osteoarthritis (OA) of the knee joint may be caused by inflammation, in combination with biomechanical alterations. It is characterized by a loss of articular cartilage, synovial inflammation and subchondral bone sclerosis. Considerable evidence indicates that the menisci, ligaments, perichondral muscles and the joint capsule are also involved in the OA process. The purpose of this study is to provide a theoretical framework for investigating the infrapatellar fat pad as an additional joint tissue involved in the development and progression of knee-OA by performing a narrative review of literature. To reinforce this framework, we aim to demonstrate that macrophages are present in the human infrapatellar fat pad and that the production of cytokines by the infrapatellar fat pad can be stimulated in vitro in an explant culture model.

Methods: A literature search was performed in PubMed considering publications from 1948 until October 2009 with keywords infrapatellar fat pad, Hoffa fat pad, intraarticular adipose tissue, knee, cartilage, bone, cytokine, adipokine, inflammation, growth factor, arthritis, osteoarthritis. Explants of the infrapatellar fat pad were obtained from 2 OA patients that underwent total knee arthroplasty. Explants were cultured in DMEM-high glucose and ITS with or without 10 ng/ml IL-1β as a pro-inflammatory stimulus. Gene expression was analyzed for MCP-1, IL-6, II-10 and TNF-α and immunohistochemical analysis of explants with CD68 and CD206, markers for macrophages, was performed.

Results: The infrapatellar fat pad is situated intracapsularly and extrasynovially in the knee joint. Besides adipocytes, the infrapatellar fat pad contains macrophages, lymphocytes and granulocytes, which are able to contribute to the inflammatory process of knee-OA. Furthermore, the infrapatellar fat pad contains nociceptive nerve fibers that could in part be responsible for anterior pain in knee-OA. These nerve fibers secrete substance P, which is able to induce inflammatory responses and cause vasodilation, which may lead to infrapatellar fat pad edema and extravasation of the immune cells. The infrapatellar fat pad secretes cytokines, interleukins, growth factors and adipokines that influence cartilage by upregulating the production of matrix metalloproteinases, stimulating the expression of pro-inflammatory cytokines and inhibiting the production of cartilage matrix proteins. The infrapatellar fat pad may also stimulate the production of pro-inflammatory mediators, growth factors and matrix metalloproteinases in synovium. The infrapatellar fat pad may be influenced by inflammatory cytokines in the synovial fluid of osteoarthritic joints, since we were able to stimulate the gene expression of MCP-1 (p < 0.001), II-6 (p < 0.001), TNF-α (p < 0.01) and II-10 (p < 0.10) in explants from the infrapatellar fat pad by the addition of 10 ng/ml II-1β. Immunohistochemical analysis of infrapatellar fat pad explants revealed the presence of CD68+ and CD206+ cells.

Conclusions: These data are consistent with the hypothesis that the infrapatellar fat pad is an active osteoarthritic joint tissue capable of modulating inflammatory and destructive responses in knee-OA. It should be further investigated in search for new therapeutic strategies.

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SYNOVIAL EXPRESSION OF CANONICAL WNT INDUCES CHONDROCYTE PHENOTYPE CHANGE AND OA-LIKE CARTILAGE DAMAGE

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Purpose: Although damage of cartilage and bone are the main pathological features in osteoarthritis (OA), a significant involvement of the synovium has been described in a large proportion of OA-patients.

The consequences for this synovial involvement in OA pathology are not yet known. OA incidence is associated with polymorphisms of genes from the wnt/p-catenin pathway, a pathway that is involved in cartilage development. In addition, TGFβ-signaling is critical for cartilage integrity. The aim of the present study is to investigate the contribution of the synovium to OA pathology via the production of wnts, and whether synovial wnt-production may lead to skewing of TGFβ-signaling from the protective to hypertrophy-inducing.

Methods: Wnt-expression was determined in 2 murine models for OA, one with clear synovial involvement, collagenase induced (CIAO), and spontaneous OA in Str/ort mice, which shows less synovial involvement. CIAO was generated by intra-articular injection of collagenase, which induces joint instability. Targets were selected for the generation of adenoviral vectors to overexpress specific genes. To study the effect of these genes on chondrocytes, human chondrocytes were isolated from cartilage that was obtained from joint replacement surgery and stimulated with wnt3a and/or TGFβ. Western Blot analysis was performed to detect phosphorylation of smad1,5,8, which can drive chondrocytes to terminal differentiation. In this way, these targets were determined by intra-articular injection of the viral vectors and determining joint pathology by histology at several time points after injection.

Results: Strong upregulation of the canonical wnt16 (up to 256-fold) and wnt2b (up to 90-fold) was found in both models. Induction of expression of wnt-proteins was solely found in the synovium, not in cartilage. However, clear intracellular accumulation of β-catenin was found in both synovium and cartilage, which indicates the activation of wnt/p-catenin in both tissues. Wnt-1 induced signaling protein (WISP1), a protein downstream canonical wnt signaling, was highly expressed in both tissues, again indicating activation of this pathway. To determine whether canonical wnt expression in the synovium can cause cartilage damage, wnt8a was overexpressed specifically in the synovium by intra-articular injection of an adenoaviral vector. At day 1 and 3, no changes in the cartilage were observed. Remarkably, at day 7, a strong induction of cartilage pathology was observed at the medial margin of the medial tibial plateau, a preferential site for the start of cartilage damage in our models. Incidence of these lesions was 17% (n=12) in joints with control virus, and 92% (n=12) in joints that overexpressed canonical wnt8a. This shows that expression of canonical wnt in the synovium causes cartilage degeneration. Due to their size, wnt proteins and WISP1 can reach the chondrocytes in the cartilage matrix and may alter the chondrocyte phenotype. Overexpression of wnt8, wnt16 and WISP1 in human chondrocytes led to a significant increase within 14 days of collagen type I, and a significant decrease of collagen type II expression, suggesting loss of the chondrocyte phenotype. Preincubation of chondrocytes with canonical wnts led to the prolonged phosphorylation of smad1,5,8 after TGFβ-stimulation which indicates a change in TGFβ-signaling from protective to hypertrophy-inducing.