MMP1 and ADAMTS4 in FLSs at the gene expression level. Importantly, we also demonstrate that insulin blunts release of MMP1 and MMP13 protein into the culture media, counteracting the effect of TNFα and IL-1β. Thus, insulin appears to play a selective protective role in the diarthrodial joint by suppressing release of catabolic enzymes into the synovial fluid via its effects on FLSs. Further study is needed to determine if insulin resistance in obesity/diabetes impairs this critical role for insulin in protecting cartilage matrix in synovial joints. Although the mechanism of selective suppression of MMP1 and MMP13 by insulin is still unknown, insulin and insulin-sensitizing agents could be new disease-modifying interventions in OA treatment.

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EXPRESSION OF INTERLEUKIN-17 AND INTERLEUKIN-22 IN NON INFLAMMATORY AND INFLAMMATORY SYNOVIAL MEMBRANES FROM OSTEOARTHRITIS PATIENTS

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Purpose: IL-17 and IL-22 are inflammatory cytokines classically involved in chronic inflammation in various diseases. Here, we investigated the expression and the release of IL-17 and IL-22 by OA synovial membranes, in relation to the inflammatory status of synovium.
Methods: Synovial membranes from OA knee patients ($n = 32$) were collected at surgery and inflammatory (Infl) and non-inflammatory (NI) areas were separated according to the macroscopic evaluation of inflammation that appeared as hypervascularized areas with hypertrophic and hyperaemic villi. A piece of each tissue was frozen for mRNA extraction, fixed in paraformaldehyde for histology and incubated in serum free culture medium in order to obtain conditioned media containing soluble factors released by synovial tissues. IL-1β, IL-6, IL-8, IL-18, IL-17, IL-22, IL-23, TNF-α, TGF-β1, myeloperoxidase (MPO) and MMP-9 were analyzed for mRNA expression by quantitative RT-PCR and/or for protein level by ELISA and gelatin zymography. Immunohistochemistry for endothelial cells (CD31) and leukocytes (CD45) among them macrophages (CD68), neutrophils (CD15) and T- and B-lymphocytes (CD3 and CD20, respectively) was performed.
Results: Inflammatory areas of OA synovial membranes were characterized by increased CD45+ inflammatory cell infiltration and vessel area/tissue area as compared to non-inflammatory areas ($p = 0.001$ and $p = 0.009$, respectively). Macrophages were present in the intimal layer of both NI and Infl OA synovial membranes with higher accumulation in Infl as compared to NI. Only the subintimal layer of Infl areas of OA synovial membranes contained macrophages, T- and B-lymphocytes and some neutrophils. Consistently, the inflammatory markers MMP-9 and MPO were released in significantly higher concentrations by Infl than by NI areas ($p = 0.026$ and $p = 0.001$, respectively). IL-17 and IL-22 were both expressed and released by OA synovial membranes. A stronger mRNA expression was found in Infl for both IL-17 and IL-22. Infl also released significantly higher levels of IL-22 than NI ($p = 0.046$). Strong positive correlations were found between IL-17 and IL-22 at mRNA and protein levels. The expression of IL-17 and IL-22 is controlled by a subset of cytokines, including IL-1β, IL-6, IL-8, IL-18, IL-23, TGFβ-1 and TNF-α. With the exception of TNF-α, all were released in significantly higher concentration by Infl as compared to NI areas. IL-12 was not detected in conditioned media of synovial tissues.
Conclusions: Our results show an increased infiltration of lymphocytes as well as an increased release of inflammatory cytokines, including IL-22, in relation to the inflammatory status of OA synovial membranes. Our results support a role for of the immune system in the pathophysiology of OA. This study will be helpful to identify new therapeutic strategies for OA leading to decrease inflammation.

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SYNOVIAL MEMBRANE COMPLEMENT GENE EXPRESSION AFTER ANTERIOR CRUCIATE LIGAMENT TRANSECTION

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Purpose: Complement activation has been described as a pathomechanism of osteoarthritis (OA). It features an abnormally high expression of complement activator genes (i.e. complement C1q, C4 and C2 on the classical pathway of activation and complement factor B and D on the alternative pathway; encoded by C1QA, C1QB, C1QC, C4A, C2, CFB and CFD) in synovial membranes of affected patients. Both pathways lead to complement C3 (C3) activation and deposition of membrane attack complex (MAC, consisting of complement components C5b-C9, encoded by C5, C6, C7, C8A, C8B, C8G and C9) on chondrocytes, affecting their gene expression (i.e. expression of matrix metalloproteinases). Complement mediated OA was observed in mouse models of meniscectomy. However, the role of complement activation after ACL injury, a promising model for posttraumatic OA, is unclear. The aim of this study was to investigate if ACL injury models of OA feature complement activation and therefore are suited to test complement-targeting disease modifying interventions. Specifically, we assessed the changes in gene expression of complement effectors and inhibitors, which are elicited in the synovial membranes early after ACL transection in a porcine model.
Methods: 24 adolescent Yucatan minipigs received unilateral ACL transection after IACUC approval. Synovial tissue was collected after 1, 5, 9 and 14 days (each $n = 6$). Whole transcriptome sequencing was used to quantify mRNA expression in the synovium. In order to control for injury-related changes in gene expression, healthy control tissue was obtained from 6 additional untreated animals ($n = 12$). Individual cDNA libraries were constructed with Illumina TruSeq Kit, multiplexed (8 per lane) and sequenced on multiple lanes of an Illumina HiSeq 2000. Raw reads were mapped to the pig genome (Susscr3) and differential gene expression was calculated with the edgeR subroutine package.
Results: mRNA expression of most complement activators, MAC components and their inhibitors was significantly upregulated 1 day after ACL transection. However, they returned to control levels within 14 days. Specifically, the expression of all assessed classical pathway activators, as well as the classical pathway inhibitors Plasma protease C1 inhibitor (SERPING1) and C4b-binding protein (C4BPA) was significantly increased at day 1 (see table). The increased expression of almost all classical pathway activators and inhibitors returned to control levels within 5 days post-transection. Expression of the alternative pathway activator CFD was significantly elevated at all observed time points (11.1, 7.6, 5.3 and 22.2-fold for day 1, 5, 9 and 14, $p = 5.05E-18$, 6.74E-11, 9.10E-09 and 1.52E-18, respectively), while the expression of the inhibitor complement factor H (encoded by CFH) was increased 1, 5 and 9 days post-transection, before returning to control levels at day 14 (15.9, 3.7, 5.0 and 1.6-fold, $p = 3.12E-20$, 3.98E-05, 1.07E-06 and .266, respectively). In contrast, the more upstream alternative pathway activator CFB was significantly downregulated at day 9, before returning to control levels at day 14 (0.2 and 1.6-fold, $p = 1.86E-07$ and .296, respectively). Expression levels of C3 and all MAC components were significantly elevated at day 1 ($p < 1E-10$, see table), whereas the

Synovial gene expression at day 1 after transection (as fold-change compared to healthy controls)

Complement activators	Classical pathway: C1QA (8.9-fold, $p = 8.50E-15$), C1QB (6.8, $p = 2.85E-11$), C1QC, (8.2, $p = 6.78E-14$), C4A (11.6, $p = 1.25E-16$) and C2 (6.3, $p = 5.18E-11$); Alternative pathway: CFD (11.1-fold, $p = 5.05E-18$), CFB (0.6, $p = 0.185$)
C3 and MAC components	C3 (16.9-fold, $p = 8.05E-22$), C5 (2.4, $p = 0.010$), C6 (30.0, $p = 0.019$), C7 (7.0, $p = 4.21E-10$), C8G (55.0, $p = 3.16E-22$), C8B (1.7, $p = 0.881$), C8A (30.6, $p = 0.887$), C9 (5.9, $p = 4.55E-10$)
Complement inhibitors	Classical pathway: SERPING1 (4.4-fold, $p = 2.29$ E-07), C4BPA (6.3-fold, $p = 2.26E-11$); Alternative pathway: CFH (15.9, $p = 3.12E-20$); MAC inhibitor: CD59 (1.9-fold, $p = 0.031$)