the synovium showed varying responses to the sham and ACL-R surgery. Although no statistically significant differences were detected, the medial and lateral regions exhibited trends for increased synovitis when compared to patellar and posterior side (Fig. 2).

Conclusions: The observations suggest that synovium tissue exhibits heterogeneity with respect to the molecular expression of select inflammatory markers like IL-1β and IL-8. Furthermore, expression of the MMP-13 also followed a trend similar to these IL. However, IL-6 does not mimic this pattern of expression. The synovium tissue does show varied inflammatory response to the ACL-R. As the surgical shams did not exhibit alterations to levels of expression, we conclude that the synovium at the lateral location is sensitive to the inflammatory changes associated with ACL-R.

Figure 1. Images of the four sampling locations: patellar (A), posterior (B), medial (C), and lateral regions (D). Arrows indicate the site of collection of synovium biopsy.

Figure 2. Analysis of mRNA levels at 2 weeks after ACL-R (n = 2) or sham (n = 2) surgery and the control group (n = 2) for IL-1β (A), IL-8 (B), MMP-13 (C), and IL-6 (D).

Figure 3. Aggregate synovitis score at 2 weeks after ACL-R (n = 2) or sham (n = 2) surgery and the control group (n = 2).

821 INSULIN SELECTIVELY SUPPRESSES TNFα/IL-1β-INDUCED CATABOLIC ENZYMES IN OSTEOARTHRITIC FIBROBLAST-LIKE SYNOVICYTES
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Introduction: Obesity is well recognized as a major risk factor for osteoarthritis (OA). Classically, it has been proposed that mechanical overload due to obesity causes cartilage damage leading to OA. Our recently published report, however, established that mice fed a high fat diet, a model of type 2 diabetes mellitus (T2DM), have synovial hyperplasia and accelerated progression of posttraumatic OA that is primarily associated with altered metabolism, and not weight gain. These results indicate that systemic factors, such as the low-grade inflammatory and insulin resistance induced by obesity/diabetes, may be involved via effects on the synovium that are independent of mechanical overload.

The pathogenesis of OA is not fully understood, though some aspects of the molecular mechanism of cartilage degradation are generally accepted. MMP1 and MMP13 are major collagenases that cleave type 2 collagen while aggrecanases, such as ADAMTS4 and ADAMTS5, degrade aggrecan in OA. The expression of these proteinases is induced by pro-inflammatory cytokines such as TNFα and IL-1β. Importantly, obesity and associated T2DM are a chronic inflammatory state involving TNFα and IL-1β. Thus, OA and the metabolic dysfunction of obesity and T2DM share pro-inflammatory features that may explain the accelerated OA progression in the obese population. With these links between OA and T2DM, we questioned whether insulin has an effect on the catabolic response in FLSS. Here we demonstrate that insulin selectively suppresses TNFα/IL-1β-dependent expression of catabolic enzymes in osteoarthritic FLSS.

Methods: Synovial samples: Human FLSS were obtained from 4 OA patients undergoing total knee arthroplasty after obtaining informed consent under approval from the University of Rochester Institutional Review Board.

Pro-inflammatory cytokines and insulin: For gene and protein expression, cells were treated with cytokines and insulin for 24 hr before harvesting.

Quantitative real-time PCR: Total RNA was extracted from FLSS using an RNeasy Mini kit and was converted to cDNA using the iScript kit. Quantitative real-time PCR was performed using iTaq SYBR Green Master Mix.

Western blot analysis: Cell media supernatants were subjected to SDS-PAGE, electrophoresed onto PVDF membranes, and probed with antibodies against MMP1 and MMP13, followed by a HRP-conjugated secondary antibody. Immune complexes were detected with a chemiluminescent reagent using a Bio-Rad image scanner.

Statistical analysis: Comparisons between more than two groups were made by the ANOVA test. Statistical analyses were carried out using Prism statistical software, with p < 0.05 was considered significant.

Results: TNFα and IL-1β markedly induced expression of MMP1, MMP13, and ADAMTS4 in FLSS. Interestingly, the effect of TNFα and IL-1β was selective since expression of ADAMTSS was very modest. Insulin at 100nM inhibited TNFα-dependent induction of MMP1, MMP13, and ADAMTS4, and IL-1β-dependent induction of MMP1, and ADAMTS4 by 50%. In contrast, insulin had no effect on TNFα-dependent expression of ADAMTSS and IL-1β-dependent expression of MMP13 and ADAMTSS.

Next, we evaluated MMP1 and MMP13 at the protein level in cell media supernatants after TNFα/IL-1β and insulin treatment. TNFα/IL-1β-induced MMP1 levels in supernatants while insulin at 100nM partially inhibited the effect of these cytokines. The effect of insulin on TNFα/IL-1β-induced MMP13 in supernatants was even more pronounced with the essential elimination of this protein by insulin. These results demonstrate that insulin suppresses TNFα/IL-1β-dependent release of MMP1 and MMP13 by FLSS into the culture media.

Discussion: TNFα and IL-1β markedly up-regulate expression of the catabolic genes MMP1, MMP13, and ADAMTS4. Additionally, we have made the novel observation that insulin suppresses TNFα/IL-1β-dependent induction of MMP1, MMP13 and ADAMTS4, and IL-1β-dependent
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 hypertrovascularized areas with hypertrophic and hyperaemic villi. A piece of each tissue was frozen for mRNA extraction, fixed in parafomaldehyde 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-17, IL-22, 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. Immunohistochimistry 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 = 0.005, 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-17, TGF-β1 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-17 and 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


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 D and B on the alternative pathway; encoded by C1QA, C1QB, C1QC, C4A, C4D, 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 post-traumatic 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 (Sussc3) 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-transsection. 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 .296, 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