were given for the following events: Initial contact, peak knee flexion and toe-off. In addition knee and hip excursion (ROM) were calculated. Moments were given as internal moments.
Results: Fifty-two patients, 33 women and 20 men, with a mean age of 60.3 (range 39.9–80.6) years were included. Of these, 12 had bilateral hip OA. Mean MJS in the affected hip joints was 2.01 (SD 1.14) mm, and mean HHS score 78.3 (SD 8.0). There were no significant differences between the PE and the PE+ET group at baseline. Significant differences were established in gait characteristics between the affected and unaffected hip joints at baseline, in that knee and hip excursion (ROM) were decreased, and hip and knee extension moments at peak knee flexion were reduced in the affected joints ($p < 0.05$). There were no significant differences between the two intervention groups at follow-up. However, a significant improvement was found in knee extension moment ($p = 0.046$) on the affected side, but not the unaffected, in both groups.
Conclusions: Gait characteristics during the stance phase of gait were significantly altered in the affected joints already at the stage of hip OA when patients have mild to moderate pain. There were no significant differences between the two intervention groups at follow-up.

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ELUSIVE ROLE FOR TOLL LIKE RECEPTOR 2 IN JOINT PATHOLOGY DURING EXPERIMENTAL OSTEOARTHRITIS

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Purpose: There has been a huge interest in the involvement of Toll-Like Receptors (TLR) in human and experimental arthritis. Several endogenous ligands for TLR-2, like biglycan, hyaluronan or HMGB-1, are present in osteoarthritic joints. However, the involvement of this receptor in osteoarthritis (OA) has not been studied extensively. The present study was performed to evaluate the role of TLR-2 in experimental OA pathology.
Methods: Three different models for OA were used, collagenase induced OA (CIOA), destabilization of the medial meniscus (DMM) and spontaneous OA in IL-1ra$^{-/-}$ murine knee joints. CIOA was induced by 2 intra-articular injections of 1 Unit collagenase into murine knee joints. Due to the instability that results from these injections, OA-like pathology develops within 42 days. At day 42, knee joints are dissected and processed for histological examination. DMM was induced by surgical transsection of the ligament that stabilizes the medial meniscus, which results in an OA-like phenotype within 56 days. OA-like changes in the cartilage of 4 joint surfaces were scored using a modified Pritzker score. Five sections of each joint were scored. The role of TLR2 was studied by induction of CIOA and DMM OA in TLR2$^{-/-}$ mice ($n = 16$) and C57BL/6 controls ($n = 15$), and by backcrossing of TLR-2$^{-/-}$ mice to IL-1-ra$^{-/-}$ mice. At different time points during OA development, synovial tissue was isolated to study TLR-2 expression in the wild type (WT) mice by Q-PCR.
Results: Synovial activation, indicated by thickening of the synovial lining layer, was studied in the three OA models. During CIOA and to a lesser extend in the IL-1ra$^{-/-}$ mice, synovial activation was clearly present during the whole course of the disease. However, no synovial activation was observed in the DMM model. At day 7 after induction of CIOA in WT mice, synovial TLR-2 levels were upregulated up to 56 fold, probably a reflection of cellular influx at this time point. At day 21 TLR2 levels were up 2-fold and 42 after induction, TLR-2 levels in the synovium were down to levels found in naïve mice. In the DMM model, TLR-2 was not regulated at any of these time points.During CIOA in TLR2$^{-/-}$ mice, OA cartilage pathology increased from 10.0 in the wildtype controls to 15.5 ($p < 0.02$). Changes were observed in all surfaces, but were strongest in the lateral compartment of the joint, both in the femur and tibia. Incidence of severe cartilage damage was increased in the medial femur, medial tibia, lateral femur and lateral tibia respectively from 13% to 44%, from 27% to 69%, from 60% to 81% and from 53% to 81%. In knee joints of IL-1ra$^{-/-}$ mice, damage was less severe compared to CIOA WT mice with a mean score of 2.2 and showed an increase in the IL-1ra$^{-/-}$/TLR2$^{-/-}$ up to a score of 4.1. In contrast, cartilage pathology during DMM OA in TLR2$^{-/-}$ was not changed in the medial compartment of the joints, compared to WT mice, respectively 13.7 and 14.7. Severe cartilage damage did not differ between WT and TLR2$^{-/-}$, 30% and 40% respectively. In all models, synovial activation in TLR-2$^{-/-}$ mice was comparable to the WT, indicating no direct role for TLR-2 in synovial activation.
Conclusions: In contrast to our expectations, these studies indicate that in TLR2 deficient mice OA pathology is more severe in experimental OA that involves synovial activation. This suggests a protective role for this receptor in OA, in stead of the inducing roles that were anticipated. This protective role was abolished in the absence of synovial activation, as was found in the DMM model. The mechanisms via which TLR2 exerts this effect remain unclear, although stimulation of TLR2 by endogenous ligands such as biglycan, hyaluronan or HMGB-1 has been shown to induce IL-10, TGFβ and HGF, which are protective mediators for OA. Although further research is needed, these results indicate that during OA the TLR-2 pathway protects cartilage from developing OA and that this effect is mediated by the synovium. These findings may have important implications for future therapies.

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SYNOVIAL FLUID FROM PATIENTS WITH OSTEOARTHRITIS AND MENISCAL INJURY MODULATES THE RESPONSE OF FIBROBLAST-LIKE SYNOVIOCYTES TO TLR-2 AND TLR-4 LIGANDS VIA SOLUBLE CD14

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Purpose: Synovial inflammation is detectable in osteoarthritis (OA) patients as well as patients with meniscal injuries, and this synovitis is associated with symptoms. Inflammation in OA is thought to be triggered in part by products of tissue degradation via stimulation of innate pattern-recognition receptors such as the Toll-like Receptors (TLRs). Many endogenous ligands of Toll-like receptors have been described, some of which are present in the OA and injured joint. We tested whether a TLR-2 or TLR-4 stimulating factor could be detected in synovial fluid (SF) from early knee OA patients, and lead to inflammatory activation of synoviocytes.
Methods: SF was obtained from patients with early OA undergoing arthroscopic meniscal procedures. HEK293 cells transfected with either TLR-4, or TLR-4 + MD2, or transfected with CD14 or TLR-2 + CD14 were used to screen SF for the ability to induce IL-8 production. Primary cultures of fibroblast-like synoviocytes (FLS) were established from post-mortem tissue donors, and also stimulated with a TLR-4 stimulus (LPS), a TLR-2 stimulus (Pam3Cysk4) SF alone, or SF in combination with LPS or Pam3CysK4. Interleukin-8 (IL-8) in culture supernatants was measured by ELISA. In blocking experiments, SF was pre-incubated with anti-CD14 (clone MEM-18) prior to using as stimuli for FLS cultures.
Results: SF from these patients did not generally stimulate HEK transfectants. However, the addition of SF (0.09–25%) in combination with TLR-2 or TLR-4 ligands resulted in significant augmentation (up to 100-fold) of cytokine production from both the HEK transfectants and from primary FLS. Levels of sCD14, a co-receptor for both TLR-2 and TLR-4, were measured in SFs from early OA patients and found at levels comparable to advanced OA and Rheumatoid Arthritis. Treatment with anti-CD14 abolished the ability of SF to augment IL-8 production by FLS in response to LPS, and diminished Pam3CysK4 responses.
Conclusions: In vitro, SF augments the response of FLS to both TLR-2 and -4 ligands. This effect appeared to be largely due to sCD14, particularly in response to the TLR-4 ligand LPS. As synoviocytes are expected to be in contact with SF in vivo, these results suggest that SF sCD14 in the setting of OA and meniscal injury can sensitize FLS to respond to inflammatory stimuli such as TLR ligands. We speculate that this priming of FLS inflammatory responses may have relevance to symptomatic “flares” seen clinically in OA patients.

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SOLUBLE PROTEINS DERIVED FROM INFRAPATELLAR FAT PAD ADIPOCYTES OF OSTEOARTHRITIS PATIENTS MODULATE CD4+ T CELL CYTOKINE PRODUCTION

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Purpose: As shown in previous studies, the infrapatellar fat pad (IFP) of OA patients has an inflammatory phenotype and could contribute to the biological processes in the OA knee joint. We have previously characterized the immune cell infiltrate in the IFP and have shown the presence of CD4$^+$ T cells. Because several studies have shown that the phenotype of CD4$^+$ T cells in adipose tissue is changing with the

adiposity of the individual, we hypothesized that this effect could be due to the modulation of infiltrating T cells by the tissue-resident adipocytes. Therefore, we investigated whether adipocytes from IFP can modulate $CD4^+$ T cell cytokine production.

Methods: $CD4^+$ T cells were purified from peripheral blood mononuclear cells isolated from buffycoats obtained from healthy volunteers. The purity of the $CD4^+$ T cells isolated using magnetic beads coated with anti-human CD4 was above 95%. Plate-bound anti-CD3 and soluble anti-CD28 antibodies were used to activate T cells. Adipocytes were isolated from IFP of OA patients by collagenase digestion and were either cultured with purified $CD4^+$ T cells or were cultured in vitro for 24 hours in DMEM/F12 medium supplemented with 0.5% bovine serum albumin to generate adipocyte-conditioned medium (ACM). Cytokine production was measured by intracellular cytokine staining (ICS), ELISA or cytokine multiplex. Separation of the protein and lipid fractions was performed using the Bligh and Dyer method, followed by precipitation of the protein fraction and reconstitution in culture medium.

Results: $CD4^+$ T cells produced increase levels of IFNγ when activated in the presence of adipocytes. This effect is mediated by soluble mediators, as shown in transwell and ACM transfer experiments. Moreover, separation of the protein and lipid fractions from the ACM medium showed that soluble factors present in the protein fraction can mediate this effect. Measurement of ten different cytokines known to be secreted by T cells upon activation revealed that, besides IFNγ, also TNFα, IL-17 and IL-5 are modulated by IFP-derived ACM.

Conclusions: Soluble mediators secreted by IFP of OA patients can modulate cytokine production of $CD4^+$ T cells. Although the precise mechanism involved in this modulation is still unknown, protein/lipid fractionation studies indicated that molecules present in the protein fraction can mediate this effect.

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TENASCIN-C LEVELS IN SYNOVIAL FLUID ARE ELEVATED AFTER HUMAN JOINT INJURY AND CORRELATE WITH MARKERS OF MATRIX DEGRADATION AND INFLAMMATION

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Purpose: Tenascin-C (TNC) is a modular, multifunctional extracellular matrix glycoprotein associated with the development of articular cartilage that decreases during maturation of chondrocytes, and almost disappears in adult cartilage. It is highly reexpressed in the cartilage and synovium of diseased joints [1], and stimulates the production of pro-inflammatory cytokines in human macrophages and synovial fibroblasts through activation of Toll-like receptor-4 (TLR4) [2]. We have shown elevated levels of TNC in joint fluid in animal models of joint disease [3], and shown the capacity of TNC to induce inflammatory mediators and matrix degradation in vitro in human joint cartilage [4]. The objective of this study was to determine the release of TNC into synovial fluid of patients in joint disease and after joint injury, and to correlate levels of TNC with markers of cartilage matrix degradation and inflammation in the same fluids.

Methods: Human knee synovial fluids (n = 160) were obtained without lavage from a cross-sectional convenience cohort. Diagnosis was by arthroscopy, radiography, assessment of joint fluid and clinical examination. Diagnostic groups were knee healthy reference (REF n = 8), anterior cruciate ligament rupture, with or without concomitant meniscus lesions (ACL n = 56), isolated meniscus injury (MI n = 69), acute inflammatory arthritis (AIA n = 16) and osteoarthritis (OA n = 11). TNC was measured using TNC Large ELISA (IBL®), with anti-TN-C 19C4MS mab against the FNIII-C domain for capture, and HRP conjugated anti-TN-C 4F10TT mouse mab against the EGF domain for detection. ARG-aggrecan, MMP1 and MMP3 ELISAs were performed as described [5,6].

Results: TNC in synovial fluids varied widely between 44 and 2430 ng/ml (Table). Significantly higher levels of TNC compared to REF subjects were observed in all disease groups: 7, 5, 10, and 4-fold higher in ACL, MI, AIA, and OA, respectively. TNC levels of ACL and MI patients decreased over time following joint injury. Synovial fluid levels of TNC were significantly correlated with aggrecanase-dependent ARG-aggrecan fragment release (r = 0.46) and MMP1 (r = 0.51) and MMP3 protein (r = 0.46).

Conclusions: We show strongly elevated levels of TNC in human knee joints immediately after injury or onset of disease, associated with markers of inflammation and matrix degradation. Due to the complex function of TNC we can only speculate on the consequences of the increased TNC release into joint fluid on cartilage matrix function and disease development. TNC has a role in the assembly of the chondrocyte matrix and as a soluble mediator of chondrocytes; deficiency of TNC delays articular cartilage repair in mice [7]. The induction by TNC of TLR4, inflammatory pathways, and matrix degradation in articular cartilage is consistent with the correlation observed in the current study between TNC levels and ARG-aggrecan fragments, MMP1, and MMP3 in human synovial fluids. TNC may serve as a marker of joint tissue injury.

	ACL			MI					
Study Group	0–12 w	12–52 w	>52 w	0–12 w	12–52 w	>52 w	AIA	OA	REF
Mean sampling time w (range)	1 (0–6)	25 (13–48)	279 (57–1115)	3 (0–12)	24 (13–40)	418 (52–1926)	32 (0–510)	189 (0–481)	-
N	26	8	22	27	22	20	16	11	8
Mean age (range)	25 (14–50)	31 (17–50)	31 (14–53)	34 (18–59)	35 (16–55)	45 (28–70)	68 (30–91)	67 (39–86)	28 (17–42)
Mean OA grade (range)	1 (1–3)	2 (1–3)	3 (1–6)	2 (0–5)	2 (0–3)	3 (1–8)	3 (0–9)	8 (3–9)	1 (0–1)
% male	62	63	68	93	86	70	63	45	75
Mean TCN (ng/ml) (range)	631 (196–1888)	533 (215–1032)	339 (101–1405)	405 (96–903)	327 (47–1062)	284 (12–1170)	939 (196–2430)	354 (107–817)	91 (44–218)
TNC 95% CI	459, 803	327, 739	212, 466	323, 487	207, 447	165, 402	587, 1290	236, 472	50, 132
Mean fold /REF	6.9	5.9	3.7	4.5	3.6	3.1	10.3	3.9	-
p-value	0.0009	0.0005	0.0156	0.0001	0.0152	0.0298	0.0016	0.0011	-

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"ALARMINS" S100A8 AND A9 ARE EXPRESSED IN THE SYNOVIUM OF EARLY OA PATIENTS AND ARE INVOLVED IN DRIVING SYNOVIAL ACTIVATION AND JOINT DESTRUCTION DURING EXPERIMENTAL OSTEOARTHRITIS

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Purpose: "alarmins" S100 A8 and A9 are prominent proteins released by activated phagocytes Evidence is accumulating that synovial tissue activation contributes to OA cartilage pathology The aim was to evaluate the presence of S100A8/S100A9 in synovia of patients with early OA and to explore active involvement of S100A8/A9 in cartilage destruction in experimental osteoarthritis models that differ in degree of synovial activation.

Methods: Arthroscopic biopsies were taken from 30 early OA patients. mRNA levels (RT-PCR) and immunolocalisation was determined and related to joint destruction (Kellgren Lawrence score). Experimental OA was either induced by transsection of the medial anterior meniscotibial ligament which leads to destabilisation of the medial meniscus (DMM) or by injection of collagenase into murine knee joints, which causes overall ligament damage and broad instability. Collagenase-induced-osteoarthritis involves chronic synovial activation in contrast to DMM. Synovial expression of S100A8 and S100A9 was measured using immunolocalisation. Both models were induced in $S100A9^{-/-}$ deficient mice (myeloid cells also lack S100A8 at the protein level). Primary chondrocytes were stimulated with S100A8 and A9 and MMP levels were measured using RT-PCR.

Results: mRNA and protein levels of S100A8 and A9 were significantly higher in synovial biopsies of early OA patients when compared to control joints. S100 was predominantly found in synovial macrophages. Of great interest, high levels correlated to increased joint destruction (KL score). The function of S100A8 and S100A9 was further studied in experimental OA models. Kinetic studies show that in surgically induced DMM model, S100A8 and A9 was marginally expressed within the synovium, only evident at day 7 after induction and consistent with limited synovial thickening. The degree of OA cartilage pathology was similar in $S100A9^{-/-}$ and WT mice at day 42 after induction of DMM.

In contrast, during the course of collagenase-induced osteoarthritis, S100A8 and S100A9 was strongly upregulated in synovium at day 7 and remained high at days 14, 28 and 42. Expression of these proteins