in SSc patients with pulmonary fibrosis compared to other SSc patients ($p < 0.05$). There was a significant correlation between sE-selectin level and DLCO ($r = -0.536$, $p < 0.01$).

Abstract THU0046 Table 1

	sE-selectin (ng/ml)	sL-selectin (ng/ml)	sP-selectin (ng/ml)
RA	43.7 ± 30.9 ^a	378 ± 168 ^{b, c}	383 ± 198 ^c
SSc	44.9 ± 22.3 ^d	552 ± 224	246 ± 163
Healthy subjects	24.9 ± 12.9	672 ± 140	292 ± 199

^a $p < 0.05$ vs. healthy subjects; ^b $p < 0.001$ vs. healthy subjects; ^c $p < 0.01$ vs. SSc; ^d $p < 0.01$ vs. healthy subjects.

Conclusion These results suggest that the measurement of levels of serum soluble selectins may provide a useful additional marker for disease activity in RA patients and for pulmonary involvement, especially sE-selectin, in SSc patients.

THU0047 MATRIXINS, CYTOKINES AND ADHESIVE IMMUNOGLOBULINS WITHIN OSTEOARTHRITIC JOINT

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10.1136/annrheumdis-2001.844

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Background The most abundant joint disease is osteoarthritis. Recently it was classified as cartilagenous, synovial or osseous but the unique forms may be combined. The main feature of the disease is imbalance between synthetic and degradative processes within joint tissues. Executive part in degradative processes play matrix metalloproteinases, so called matrixins. *In situ*, they are induced by many stimuli, namely by cytokines and by interaction of different adhesive molecules with their ligands. Having been secreted into the extracellular space, they are activated by serine proteinases, or by other matrixins and inhibited by tissue inhibitors of matrix metalloproteinases, TIMPs.

Objectives In the sera of OA patients we found matrixins MMP-9, MMP-2 and MMP-3 and TIMP-1 together with cytokines IL-8 and TNF α and adhesive molecules ICAM-1 and VCAM-1. To distinguish between the forms of osteoarthritis, we search for these molecules in all joint tissues that is in cartilage, synovium, granulous tissue within subchondral bone, synovial fluid and blood serum. The joint tissues and fluids we obtained from surgery during total hip or knee replacement.

Methods The joint tissues were derived from 22 patients. The patients were 17 female and 6 male, 67,5 ± 9,6 years old. The metalloproteinase spectrum in the body fluids and extracts of joint tissues were evaluated by zymography. The concentration of metalloproteinases and TIMP-1 were assessed by ELISA (Bio-trak, Amersham Pharmacia Biotech), the concentration of adhesive immunoglobulins by Bender ELISA (MedSystems Diagnostics GmbH), and the concentration of IL-8 and TNF α were determined on Immulite (DPC, USA).

Results We found that the most abundant matrixine within cartilage and synovium is either gelatinase A (MMP-2) or stromelysin-1 (MMP-3). On the other hand, within granulous tissue, blood sera and synovial fluid, the prevailing matrixine is gelatinase B or stromelysin-1. In cartilage, synovial fluid and blood serum TIMP-1 is abundant over sum of matrixins, whereas in synovium and granulous tissue the sum of matrixins is abundant over TIMP-1. There is about 2 to 10 times greater concentration of IL-8 in the cartilage than in other joint compartments.

We found significant correlation between levels of sICAM-1 and sVCAM-1; between TNF α and MMP-3, TIMP-1, sICAM-1, sVCAM-1, and IL-8; and between IL-8 and sICAM-1 in all solid joint tissues. In the synovial fluid and blood serum the correlation was less pronounced.

Conclusion There is a great degradative potential within all compartments of osteoarthritic joint due to presence of matrixins, namely that of stromelysin-1. Within cartilage and synovial fluid the proteolytic potential is equilibrated by TIMP-1 whereas within synovium and granulous tissue is not. Synovium and granulous tissue are highly probably the propellers of degradative process in osteoarthritis.

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THU0048 ADDITION OF THE RGD MOTIF IN THE FIBRE KNOB OF AN ADENOVIRAL VECTOR IMPROVED THE TRANSFECTION EFFICIENCY AND WHEN USED FOR OVEREXPRESSION OF IL-1RA RESULTED IN A MORE EFFECTIVE INHIBITION OF ARTHRITIS

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10.1136/annrheumdis-2001.845

Background Adenoviral (Ad) vectors expressing anti-inflammatory cytokines are used for therapy in experimental arthritis. Cell recognition and entry is dependent on the initial recognition of the coxsackievirus and adenovirus receptor (CAR) on cells. Recently introducing an Arg-Gly-Asp (RGD) motif in the HI loop of the fibre knob modified the tropism of the virus.¹ The RGD motif is capable of mediating a CAR-independent cell entry via interactions with the α v β 3 and α v β 5 integrins.

Objectives In this study we explored the transfection efficiency of the RGD modified viruses and investigated the effectiveness of intra-articular IL-1Ra overexpression in the collagen-induced arthritis (CIA) model mediated by the RGD modified vector compared to wildtype viruses.

Methods 10e7 pfu of the AdLuc, AdLucRGD, AdmIL-1Ra, or AdmIL-1RaRGD were intra-articularly injected in all experiments. The CIA model was used to compare the wild type and the RGD viruses on their ability to downregulate arthritis. DBA/1 mice were immunised with collagen type II, and boosted 22 days later. Four days after the booster the adenoviral vectors were injected into both knee joints of the mice. Clinical scores of the knee joints were on a scale of 0–3.

Results Twenty-four hours after intra-articular injection of 10e7 pfu virus into normal knee joints, luciferase production in AdLucRGD injected joints was around 22 times higher than in AdLuc injected joints. On day 26 of the CIA model, 10e7 pfu of AdLuc, AdLucRGD, AdmIL-1Ra, or AdmIL-1RaRGD were injected into the knee joints. Onset of the CIA was significantly inhibited in the AdmIL-1RaRGD group (clinical score in the knee joints at day 38 was 0.82 ± 0.24) compared to the other groups (clinical scores were 1.57 ± 0.24 for the AdmIL-1Ra group and 1.86 ± 0.21 for the AdLucRGD group).

Conclusion Transduction of the synovial lining cells in normal knee joints was up to 30 times more efficient when the RGD mediated pathway was used. CIA was significantly better

inhibited when mIL-1Ra was overexpressed using the RGD adenoviruses for infection compared to the wild type adenovirus.

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THU0049 PROTEIN KINASE INHIBITION ENHANCES STEADY STATE MRNA LEVELS ENCODING IL -16 BUT REDUCES IL-18 ENCODING MESSAGE LEVELS IN FIBROBLASTS

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10.1136/annrheumdis-2001.846

Background In previous studies we have shown that synovial fibroblasts from rheumatoid arthritis (RA) patients transcribe elevated levels of IL-16 encoding mRNA. In addition, in RA synovial fluid high concentrations of IL-16 were detected. The chemotactic activity to CD4⁺ cells was associated with IL-16. In osteoarthritis synovial fibroblasts, IL-16 was not found.

Objectives Therefore we initiated experiments to study the activation pathway regulating IL-16 transcription in synovial versus skin fibroblasts.

Methods OA synovial fibroblasts (n = 3), skin fibroblasts (n = 6) and neonatal preputial fibroblasts (n = 3) were expanded (less than 4 passages). Cells were incubated in complete medium (10% FCS) supplemented with MAS-7, ocadaic acid (ODA), ionomycin, cAMP, forskolin, staurosporin, phorbol ester, H7 and an unrelated peptide as mock control. Untreated cells served as controls. After 6 to 48 h of incubation, mRNA was extracted from experimental samples and controls. The mRNA yield was determined by spectrophotometry and, cDNA was generated from 1 µg RNA using the oligo (dT) priming procedure. Steady state transcript amounts encoding IL-16 or IL-18 were determined by real time quantitative RT/PCR (LightCycler, Roche) using the SYBR green method. The PCR products were visualised by EtBr fluorescence after gel electrophoresis. Quantitative GAPDH RT/PCR served as internal standard.

Results mRNA encoding IL-16 and IL-18 was detected in all fibroblasts analysed. Incubation of the fibroblasts for 24 to 48 h with staurosporin or forskolin enhanced IL-16 steady state mRNA levels two to three fold. Incubation with ODA or H7 reduced IL-16 mRNA three-fold and ten-fold, respectively. PMA reduced IL-16 signals to about 70%. Interestingly, IL-18 signals were not reduced in ODA treated fibroblasts whereas H7 reduced IL-18 signals about ten-fold. Staurosporin and forskolin did not enhance IL-18 encoding signals but reduced the IL-18 signals two to three fold. IL-18 encoding mRNA amounts were elevated in ionomycin treated cells. All other components did not show considerable activation or reduction of the IL-16 or IL-18 RT/PCR signals. In addition, there was no remarkable difference in IL-16 or IL-18 transcriptional responses to all agents analysed so far.

Conclusion Staurosporin is a broad protein kinase inhibitor and activated IL-16 transcript levels whereas phorbol ester PMA activates protein kinase C and reduced IL-16 mRNA levels. We conclude that IL-16 transcription is regulated by kinase/phosphatase signal transduction pathways. In addition, a cAMP dependent enhancement of IL-16 encoding steady state mRNA levels was

observed. By contrast, IL-18 RT/PCR signals are reduced upon kinase inhibition but not activated after PMA incubation. Raising intracellular calcium by ionomycin induced IL-18 encoding mRNA. Therefore the IL-16 and IL-18 gene activities are regulated differently. Since in RA synovial fibroblasts IL-16 mRNA is elevated when compared to OA fibroblasts and blocking kinase activity activated IL-16 mRNA levels in OA synovial or skin fibroblasts, we hypothesise that a reduced kinase activity may be associated with enhanced levels of IL-16 message in RA synovial fibroblasts.

THU0050 NOVEL DESCRIPTION OF FUNCTIONAL CHEMOKINE RECEPTORS ON FIBROBLAST LIKE SYNOVIOCYTES

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10.1136/annrheumdis-2001.847

Background The relevance of chemokines (CK) in rheumatoid arthritis (RA) has been mainly focused on their chemotactic properties to direct selective migration of leukocytes. However, demonstration of chemokine receptors (CKR) on various non-haematopoietic cells is expanding the knowledge of their roles in biology.

Objectives To explore the presence of CKR and their functional status on cultured fibroblast-like-synoviocytes (FLS) obtained from synovial tissues of RA and osteoarthritis (OA) patients.

Methods

Results Flow cytometry analysis with monoclonal antibodies detected expression of CCR2 (MCP-1,2,3 R) on 28 ± 11% FLS, and yielded a wide range of expression of CCR5 (MIP-1a, -1b, RANTES R), CXCR4 (SDF-1a R) and CXCR3 (IP-10, Mig R) on FLS (8–18%) both from RA and OA. CXCR4 mRNA and, to a lower extent, CCR2 mRNA, were detected by RNase protection assays on RA FLS. To assess the functional ability of these CKR in response to putative ligands we performed assays of CKR engagement that typically lead to pertussis toxin-sensitive PLC activation. After stimulation with CK, FURAred-loaded FLS were screened for changes in cytosolic calcium concentration by measuring related fluorescence changes in a FACScan. SDF-1a induced a significant cytosolic calcium influx, as did positive control stimulation with calcium ionophore A23187. A consistent effect from IP-10 or MCP-1 was not detected.

Assays of synoviocyte chemotaxis using two-compartment trans wells showed significant increments in FLS transmigration induced by SDF-1a (224 ± 134%, p < 0,05) and IP-10 (252 ± 31%, p < 0,05) compared to MCP-1, RANTES, MIP-1b or unstimulated FLS (100%). This enhanced migration could be inhibited in the presence of pertussis toxin (57–77% inhibition).

Conclusion This is, to our knowledge, the first description of the presence of CKR on FLS, notably CXCR4. Our data demonstrate that these receptors are functional as it is shown by CK promotion of both calcium mobilization and cell migration. As occurs with leukocytes, CKR could guide synoviocytes through the tissue, likely following a hierarchy of chemotactic signals and therefore, CK might enhance invasive properties of pannus FLS into the cartilage and bone.