disintegrin and metalloproteinase).¹ Some MDCs are involved in (patho-) physiological relevant processes such as remodelling of the extracellular matrix as well as shedding of membrane-bound cytokines (e.g. TNF- α).^{2,3} An implication of MDC15 in degenerative processes of the cartilage matrix is supported by a markedly strong up-regulated expression of this proteinase in the chondrocytes of arthritic cartilage.⁴

Objectives In the present study the expression of MDC15 in synovial membranes of patients with Rheumatoid Arthritis (RA) in comparison to osteoarthritic (OA) and normal specimens was analysed.

Methods Using *in situ* hybridization the expression of MDC15 mRNA was investigated in RA synovial tissues of synovectomy samples (n = 25), in OA tissues (n = 55) that was obtained at joint replacement surgery of hip or knee joints and normal synovial tissues (n = 8). On protein level the detection of MDC15 was performed by immunohistochemistry. A specific polyclonal antibody was generated in rabbits by immunisation with a synthetic cyclized peptide corresponding to the disintegrin domain. After affinity chromatographic purification specificity of the antibody was confirmed by Western blot analysis using COS-7 cells transfected with the full-length MDC15 cDNA.

Results By *in situ* hybridization as well as by immunohistochemistry highest expression of MDC15 could be detected in the synovial lining cells in all RA synovial tissues analysed. In comparison to the RA specimens, far lower levels of expression were observed in the synoviocytes of OA specimens that still exceeded the very low expression found in normal synovial tissues.

Conclusion The enhanced expression of MDC15 in the rheumatoid synovial lining layer suggests a potential role in the pathophysiology of cartilage destruction, since the membrane-bound proteinase might be of relevance in the activation of matrix metalloproteinases and/or also be involved in the direct proteolytic degradation of extracellular matrix molecules.

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THU0102 APO(A), A POTENTIAL LINK BETWEEN ATHEROSCLEROSIS AND ARTHRITIS

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Background Patients with rheumatic diseases have an excess in cardiovascular morbidity and mortality, suggesting the presence of common pathogenic abnormalities for both atherosclerosis and arthritis. One particularly atherogenic apolipoprotein, apo (a), an homologue of plasminogen, may be involved by competing with plasminogen for fibrin binding and by interfering with fibrinolysis.

Objectives To study the expression of apo (a) in arthritic joints.

Methods Human studies: synovial fluid and plasma from patients with osteoarthritis (OA, n = 8), rheumatoid arthritis (RA, n = 12) and other types of inflammatory arthritis (n = 12) and synovial tissues from OA and RA patients were collected.

Apo (a) was quantitated by ELISA and in tissues also by RNase protection. Moreover, immunohistochemical analyses of apo (a) and fibrin were performed on synovial tissues.

Animal studies: antigen-induced arthritis (AIA) was induced in genetically modified mice (LDL-receptor deficient (LDL-R $-/-$) mice expressing a human apoB-100 transgene (hApoB $+/+$) and a human apo (a) transgene (apo (a) $\pm$)). Joint inflammation was assessed by technetium scintigraphy and histology.

Results We detected the presence of apo (a) as a full-length glycoprotein in human arthritic joints. The amount of apo (a) in synovial fluid rose in proportion to plasma apo (a) levels and was higher in inflammatory arthritides than in osteoarthritis. In addition, apo (a) immunoreactive material, but not apo (a) transcripts, was detected in arthritic synovial tissues. This data indicated that synovial fluid apo (a) originates from the circulation and that diffusion through synovial tissue is facilitated in inflammatory types of arthritis. In synovial tissues, apo (a) colocalized with fibrin. These observations could be reproduced in a model of antigen-induced arthritis, using transgenic mice expressing human apo (a). In this particular mouse model, the presence of apo (a) did not change joint inflammation.

Conclusion Even if the presence of apo (a) did not change the severity of AIA, we speculate that when levels of apo (a) are sufficiently high to inhibit fibrinolysis in the joints, apo (a) may exacerbate in humans the severity of the disease.

THU0103 THERAPEUTICAL TARGETING OF ANTIGEN-INDUCED ARTHRITIS WITH LIPOSOMAL SECRETORY LEUKOCYTE PROTEASE INHIBITOR (ANTILEUKOPROTEINASE)

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Background Alternative therapeutic principles for rheumatoid arthritis (RA) are still in urgent need. Secretory leukocyte protease inhibitor (SLPI), or antileukoprotease (ALP), is a physiological serine protease inhibitor.¹ Its upregulation in macrophages leads to a reduced LPS response² most likely related to an inhibition of NF- κ B by interference with the proteolytic degradation of its cytosolic inhibitor I κ B β .³ However, the therapeutic use of this principle *in vivo* is hampered by difficulties to target the molecule into a cytosolic compartment. Phosphatidylserine containing liposomes can selectively be taken up by macrophages via Scavenger receptors⁴ thus representing a therapeutic strategy for targeting ALP to the cytosol.

Objectives In this preliminary study we applied a liposomal preparation of ALP in the murine antigen-induced arthritis (AIA) model to enhance intracellular accumulation in inflammatory cells to explore whether ALP can reduce inflammation.

Methods Liposomes were prepared by evaporation of a mixture of phosphatidylserine, phosphatidylcholine and cholesterol in chloroform/methanol and addition of ALP solution or PBS. Antigen-induced arthritis was induced in C57BL/6 mice by intraarticular injection of mBSA after prior immunisation with mBSA. Liposomal ALP was administered intraperitoneally (5 mg/kg/day) between days 1–4 after disease induction and compared with injections of PBS containing control liposomes or non-liposomal ALP. Arthritis was assessed by measuring knee joint diameter and evaluation of histological alterations in knee joint sections.

Results A marked amelioration of arthritis in mice treated with liposomal ALP was observed, already within 24 h after the first

administration, while non-liposomal ALP was only moderately effective and control liposomes did not exhibit any effect. After induction of an arthritis flare on day 28, administration of ALP liposomes following the onset of acute inflammation again led to a marked reduction of joint swelling. Histopathological analysis of knee joints obtained from mice sacrificed at peak flare revealed that signs of acute (cellular infiltrate) and chronic arthritis (synovial proliferation, irregular cartilage surface, cartilage erosions) were exclusively diminished in the group treated with liposomal ALP.

Conclusion Taken together, the data indicate that therapeutic application of liposomal ALP reduces inflammation and consequently cartilage erosion in AIA. Therefore this form of antiproteolytic treatment might be considered as a novel therapeutic option in chronic inflammatory joint disease.

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THU0104 *IN VIVO* AND *IN VITRO* INVESTIGATIONS OF THE TUMOUR SUPPRESSOR P16 IN RHEUMATOID ARTHRITIS

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Background The progression of the cell cycle is tightly controlled by cyclins and cyclin-dependent kinases. The p16Ink4a protein was identified as a specific inhibitor of cdk4. P16 inhibits the cdk4-dependent phosphorylation of the tumour suppressor retinoblastoma protein (Rb) and thereby leads to a G1 arrest. It is suggested that p16 acts as a tumour suppressor. Despite several approaches have been undertaken to inhibit cell growth in rheumatoid arthritis synovial fibroblasts (RA-SF) by gene transfer of p16, its role in RA is poorly understood. Very few data are available concerning the expression of p16 in RA tissue.

Objectives The aim of the study was to investigate whether the tumour suppressor p16 plays a role in RA and to get some basic information about its expression level and pattern. We determined the *in vivo* expression of p16 in tissue sections and its *in vitro* expression in cultured RA-SF. The inducibility of p16 was evaluated by X-ray irradiation.

Methods Anti-human p16Ink4a antibodies (PharMingen, clone G175–1239) were used for immunohistochemistry (IHC) as well as for immunofluorescence (IF). Sections from 13 RA, 1 osteoarthritis (OA) and 2 normal synovia were investigated by IHC. Tissue specimens from normal uterus and pancreas were used as positive controls. RA-SF (n = 6), normal synovial fibroblasts (n = 1) and foreskin fibroblasts (FSFB, n = 1) from passages 3–7 were grown on chamberslides and further analysed by IF. HeLa cervix carcinoma cells (ATCC CCL 2) and the osteosarcoma cell line Saos-2 (ATCC HTB 85) served as positive controls. Six RA-SF and 1 control (FSFB) were irradiated with 10 Gy (X-rays) and analysed after 25 h for expression of p16. Moreover, 2 RA-SF and 1 control were irradiated with the same dose, but kept in culture for 13 days before they were investigated for p16. In both experiments, non-irradiated cultures served as negative controls. The percentage of p16 positive cells on tissue sections and fibroblast cultures was evaluated by a scoring system.

Results P16 was expressed in 10/13 RA specimens in 0–6% of the cells *in vivo*. Less than 1% stained positively for p16 in OA tissue and in normal synovial tissue. However, in three samples derived from patients with RA, p16 was present in 14–23% of the cells. The positive cells were predominantly located in the sublining and in one case also in the lining. *In vitro*, cultured RA-SF and controls did not spontaneously express p16, not even after serum starvation or in senescent cultures (data not shown). When irradiated with 10 Gy (X-rays), no p16 could be detected after 25 h, whereas after 13 days p16 was present in about 30–50% of the cells. No major differences were found between RA-SF and FSFB in terms of response to irradiation.

Conclusion This is, according to our knowledge, the first report investigating the expression of p16 in RA tissue sections by IHC. The *in vitro* experiments demonstrated no constitutive expression of this protein in RA-SF. However, p16 could be induced and detected in irradiated cultures of RA-SF and normal fibroblasts. Based on our *in vivo* data, we suggest that in a subset of patients the expression of p16 might be responsible for the low rate of proliferation of synovial cells in RA.

THU0105 THE EFFECTS OF INTERLEUKIN-17 ON PRODUCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN CULTURED RHEUMATOID ARTHRITIS SYNOVIOCYTES

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Background

Objectives To investigate the effects of interleukin-17 (IL-17) on the production of vascular endothelial growth factor (VEGF) from cultured rheumatoid arthritis synovioocytes.

Methods Fibroblast-like synovial cells (FLS) were prepared from the synovial tissues of rheumatoid arthritis patients and cultured in the presence of IL-17, transforming growth factor- β (TGF- β), tumour necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). VEGF levels were determined in the culture supernatants by sandwich ELISA.

Results Stimulation of FLS by serial concentration of IL-17, TGF- β , TNF- α , IL-1 β increased the production of VEGF by 2.1–2.7, 2.2–3.0, 2.0–2.9, 2.3–3.1 fold over the constitutive levels of unstimulated FLS. Stimulation of FLS by IL-17 with TGF- β , TNF- α or IL-1 β also increased the production of VEGF according to culture periods by 1.6–1.8, 1.1–1.9, 1.5–1.7 fold over the levels stimulated with TGF- β or TNF- α or IL-1 β , respectively. This results indicated that IL-17 increased the effect of TGF- β , TNF- α , IL-1 β on FLS, leading synergistic enhancement of VEGF production.

Conclusion IL-17 may be involved in the neovascularization in rheumatoid synovitis by enhancing the production of VEGF.

THU0106 THE EXPRESSION OF IP-10 AND MIG CHEMOKINES IN RHEUMATOID SYNOVIAL TISSUE

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Background