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

Objectives Rheumatoid arthritis (RA) is characterised by chronic synovial inflammation, which results in progressive polyarticular destruction. Immunohistological characterisation of infiltrating cells may help to elucidate the role of specific chemokines and their receptors in pathological processes. In this regard we previously described high expression of chemokine receptor CXCR-3 in synovial tissue. Now we have investigated expression of chemotactic factors IP-10 and Mig, which are ligands for CXCR-3.

Methods Synovial tissue samples were obtained from 5 RA patients who have undergone total joint replacement or synovectomy. Native cryostat tissue sections were examined for CD3, CD4, CD8, CD19, CD14, CD31, CD68, CXCR-3, IP-10 and Mig with mouse anti-human monoclonal antibodies. Two-step visualisation with DAKO EnVision was applied. This visualisation system is based on enzyme-conjugated dextrane polymer, which carries secondary antibody molecules. Negative controls were performed by using isotype-matched irrelevant antibodies.

Results A strong positive reaction for CXCR-3 was detected on lymphocytes and macrophage-like synoviocytes; weak and irregular staining was seen in endothelial cells. IP-10 and Mig were expressed in all tissue samples. The positivity was found mainly in the lymphoid infiltrating cells and the staining also co-localised with CD68 positive synoviocytes. In addition, a stronger expression of IP-10 than of Mig was seen, particularly in tissues with a strong vascularity. Endothelial cells were strongly positive for IP-10 and weakly also for Mig. In general, IP-10 and Mig staining was observed in sporadic aggregates surrounding blood vessels.

Conclusion High expression of IP-10 and Mig on a majority of the infiltrating cells suggests a role for interferon- γ in RA inflammation, as IP-10 and Mig are induced by this cytokine. They may play a role in the attraction of CD4 positive T cells into the synovial environment. Furthermore, a frequent expression of CXCR3 on synovial resident cells propounds a possible role for IP-10 and Mig in their activation, perhaps even in the autocrine fashion. This work was supported by a grant NI/6459 from IGA MZ CR.

THU0107 METHOTREXATE COVALENTLY COUPLED TO HUMAN SERUM ALBUMIN (MTX-HSA) TARGETS TO INFLAMED JOINTS AND IS SUPERIOR TO METHOTREXATE INHIBITING THE DEVELOPMENT OF MURINE COLLAGEN-INDUCED ARTHRITIS (CIA)

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Background Methotrexate (MTX) is a well established drug for the treatment of rheumatoid arthritis (RA). Pharmacological studies have shown, that most of the drug is eliminated by the kidneys. This results in a short plasma half-life and low concentrations of MTX in inflamed joints. In studies with tumour-bearing rats we showed, that due to the pathophysiological conditions in solid tumours and the high demand of tumour cells for amino acids and energy, albumin accumulates in tumours (up to 20% of the initially injected amount) and only a relatively small proportion is found in the liver (about 6%). Covalent coupling of MTX, fluorescent dyes and radioactive labels in a 1:1 molar ratio to albumin leads to conjugates which behave in-vivo like native albumin. In phase I clinical trials with tumour patients MTX-HSA showed promising results and relatively high dosages

were well tolerated over several years. Like tumour cells, synovial cells of patients with RA show a high rate of proliferation and are in an active metabolic state. The plasma protein albumin might therefore be an appropriate carrier to increase drug concentrations in inflamed joints.

Objectives To proof if albumin is a suitable drug carrier to target MTX to inflamed joints in an RA animal model and isolated human RA synoviocytes.

Methods Laser optic, scintigraphic and radiopharmacokinetic experiments with fluorescent and radioactive labelled albumin were performed to study the distribution of albumin in mice with and without CIA (n = 5 each point of the time course). The uptake of fluorescence labelled albumin by human RA synoviocytes was investigated by laser scanning microscopy in-vitro. The pharmacokinetics of MTX in arthritic mice was studied using ³H-labelled MTX. The therapeutic efficiency of MTX-HSA, MTX and a combination of both drugs (injected in different dosages 2x/week) was investigated by its ability to inhibit the development of arthritis (n = 3–35/group).

Results About 3% of the initially injected radioactive albumin was detected in each inflamed paw within a few hours after application, while in healthy paws only 0.3% of the tracer was found (accumulation by a factor of 6 in respect to the weight of the paws). Scintigraphic scans confirmed this results. Only 0.03% of ³H-MTX was measured in the inflamed paws. In the liver only 7% of the radiolabeled albumin but 27% of ³H-MTX was found. Illumination by laser light after injection of fluorescent labelled albumin showed strong fluorescence in arthritic but not in healthy joints of paws affected by CIA. The examination of human synoviocytes in-vitro showed catabolism of albumin in lysosomes. In equivalent dosages (7.5 mg MTX/kg twice a week) MTX-HSA was significantly (p = 0.017) superior to MTX in inhibiting the development of CIA (control: 68% arthritis incidence, MTX: 57%, MTX-HSA 28%). To achieve a comparable result as MTX-HSA almost a five times higher dosage of MTX (35 mg/kg) was necessary (37% arthr. inc.). Both drugs act synergistically (3.75 mg/kg MTX plus 0.8 mg/kg MTX-HSA: 29% arthr. inc.) in respect that 1.6 mg/kg MTX-HSA had no significant effect (57%).

Conclusion Using albumin as drug carrier allows to target drugs such as MTX specifically to inflamed joints. This might increase the therapeutic efficacy of MTX in human RA. Clinical trials to proof this concept are in preparation.

THU0108 ANTIBODIES TO PARVOVIRUS B19 IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS

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Background Parvovirus B19 may cause an acute or chronic polyarthropathy, which in many cases fulfils the criteria of the American College of Rheumatology for the diagnosis of rheumatoid arthritis (RA). Additionally, parvovirus B19 has been implicated in the pathogenesis of RA.

Objectives The aim was to investigate the presence of antibodies to parvovirus B19 in patients with RA and osteoarthritis (OA).

Methods In the study were included 50 patients with RA, 24 patients with OA and 26 healthy controls. In all patients IgG and IgM antibodies to parvovirus B19 were measured by indirect immunofluorescence. In patients found to be positive for IgM