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