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

antibodies to parvovirus B19 results were further confirmed by Western blot.

Results In 28 of 50 patients with RA (56%) and in 7/26 of the controls (26.9%) IgG antibodies to parvovirus B19 were detected (x_2 , $p < 0.05$). In 9 of 24 patients with OA and in 7/26 of controls IgG antibodies to parvovirus B19 were detected (x_2 , $p > 0.05$). In 11 of 50 patients with RA (22%) IgM antibodies to parvovirus B19 were detected and the results were further confirmed by Western blot, while in none of the patients with OA and in none of the controls were IgM antibodies observed (x_2 , $p < 0.001$ and $p > 0.05$, respectively).

Conclusion The prevalence of antibodies to parvovirus B19 was found to be increased in patients with RA as compared to controls. In patients with OA the prevalence of antibodies to parvovirus B19 was found to be comparable to that in controls. These results indicate either that infection with parvovirus B19 may be implicated in the pathogenesis of RA, as previously discussed in the literature, or that some cases of chronic polyarthropathy due to parvovirus B19 may have been misdiagnosed as rheumatoid arthritis.

THU0109 THE DESTRUCTIVE PROCESS OF RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS (RA-SF) IS INDEPENDENT OF P53

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Background

Objectives The aim of this study was to investigate, whether the destructive process of synovial fibroblasts in rheumatoid arthritis is dependent on the expression of p53. Moreover, we searched for the expression of p53 in RA-SF *in vitro* and *in vivo*. In addition, we examined the inducibility of p53 in RA-SF by X-rays *in vitro*.

Methods Paraffin embedded synovial tissues from 14 RA, 3 osteoarthritis (OA), 3 normal synovia and a neurological p53 positive tumour were investigated with DO7 anti-p53 antibodies by immunohistochemistry. RA-SF from passages 2 to 12 ($n = 10$), OA-SF ($n = 2$), normal synoviocytes (N-SF, $n = 1$) and foreskin fibroblasts (FSFB, $n = 1$) from passages 4–8 were grown on chamber slides and analysed with the same primary antibodies utilising immunofluorescence. SV40 transformed RA-SF (HSE) and SV40 transformed N-SF (K4IM) as well as the SV40 transformed bronchial epithelial cell line BEAS 2B served as positive controls. The percentage of positive cells was evaluated by a scoring system. RA-SF and HSE cultured *in vitro* were co-implanted with human cartilage under the renal capsule or the under the skin of SCID mice and kept there for 60 days. Paraffin sections from these SCID mice experiments were stained for p53. In addition, sections from former SCID mouse experiments including retroviral and adenoviral gene transfer studies revealing a strong invasion of synovial cells into the cartilage were investigated for the expression of p53. Moreover, RA-SF ($n = 2$) and controls (FSFB, $n = 1$) were irradiated with 10 Gy (X-rays) and analysed time dependently for the expression of p53 by immunofluorescence.

Results *In vivo*, p53 was not abundantly expressed in RA synovial tissue and in particular not at sites of invasion. In general, less than 5% of the cells in RA and OA synovial tissues stained

positively for p53, while no staining in the normal synovia occurred. In contrast, the positive control revealed a strong nuclear p53 signal in almost 100% of the tumour cells. *In vitro*, the expression rate of p53 in RA-SF was comparable low, while the SV40 transformed cells demonstrated a strong and specific nuclear staining. Furthermore, RA-SF invading the human cartilage in the SCID mouse co-implantation model did not show any expression of p53 at sites of invasion, whereas the implanted SV40 transformed HSE revealed a strong p53 staining indicating that those cells survived at least for 60 days in SCID mice. RA-SF and FSFB cultured *in vitro* responded equally to the irradiation with X-rays by producing transiently p53, demonstrating an adequate and functional response of p53.

Conclusion We suggest that the expression of p53 plays only a limited role in the pathogenesis of RA and particularly not in the invasive process of RA-SF.

THU0110 V-ATPASE INHIBITOR BAFILOMYCIN A1 REDUCES PROTON SECRETION IN FIBROBLASTS

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Background Metabolic activation of synovial fibroblasts (SF,¹), prosthesis loosening fibroblasts (PLF,²) and osteoblasts (OB,³) results in an enhanced acid secretion as determined by cytosensor microphysiometer. In addition, PLF were shown to degrade bone in absence of infiltrating macrophages or osteoclasts.

Objectives We were interested to study if the proton secretion was associated with the activity of specific proton pump, especially with the activity of V-type H⁺ ATPases.

Methods Fibroblasts or osteoblasts were expanded in complete medium containing 10% FCS and antibiotics. For cytosensor analysis cells were seeded at 30 000 cells/ml in microcups, mounted into the cytosensor. For metabolic activation the medium was enriched with cytokines such as TNF-alpha, IL-1 (Roche) or different agents such as ionomycin or PMA (Calbiochem) at different concentrations. To block V-ATPase activity, cells were incubated with Bafilomycin A1 or Amiloride (Calbiochem).

Results Metabolic activation as determined by enhanced acid secretion was observed upon stimulation of the fibroblasts with TNF-alpha, IL-1, PDGF, ionomycin or PMA but not with IL-6 or bFGF. Osteoblasts were activated by TNF-alpha, ionomycin and PMA; bFGF, IL-1, IL-6 or PDGF are not tested yet. Acid secretion occurred as early as 20–30 min. after initial stimulation. Under identical conditions, immortalised synovial fibroblast clones secreted less acid when compared to normal fibroblasts. As metabolic activation stimulates glycolysis, the acidification may be simply a consequence of carbohydrate catabolism generating acetate, lactate or carbonate. Therefore fibroblasts were incubated in medium containing Bafilomycin A1, a specific V-type H⁺ ATPase blocker. Bafilomycin reduced the proton secretion in a time course experiment within 20 min. irreversibly. Interestingly, Amiloride inhibited the proton secretion reversibly.

Conclusion Metabolic activation of fibroblasts or osteoblasts by pro-inflammatory cytokines results in enhanced acid secretion. As pro-inflammatory cytokines enhance the expression of matrix degrading proteases as well, we conclude that the acidification of the pericellular milieu by mesenchymal cells accelerates matrix