immunocompetence by assessing function of selected cell types involved in host resistance.

Methods Basal NK cell activity, lymphoproliferation following stimulation with anti-CD3, pokeweed mitogen or concanavalin A, and antibody forming cell response (AFC) were determined in splenocytes from female rats (F-344; N = 12 per group) treated with placebo, IL-1ra alone (20 or 200 mg/kg, SC daily), sTNF-RI alone (5 or 50 mg/kg, SC twice weekly) or the dose combinations of the two cytokine inhibitors for 21 days. Satellite groups received positive control agents for the NK cell assay (0.5 mL anti-asialo GM 1 antibody) or the mitogenesis and AFC assay (25 mg/kg cyclophosphamide).

Results Basal NK cell response was increased 21% ($p < 0.05$) over placebo control only at the combination of 200 mg/kg IL-1ra + 5 mg/kg sTNF-RI. No effect was noted with IL-1ra or TNF-RI alone or at higher combination doses. Cells from IL-1ra (200 mg/kg) treated animals showed minimal decreases (30%, $p < 0.05$) on lymphocyte proliferation only in response to anti CD3-mediated proliferation. Statistically significant differences (+10 to -48%) in proliferative activity to all mitogens were observed with sTNF-RI alone or in combination with IL-1ra, but were not dose related. Diminution in the AFC response (29–36%) was noted for sTNF-RI alone (50 mg/kg) or in combination with both IL-1ra doses compared to the control group; however the differences were not statistically significant. IL-1ra alone, the low dose of sTNF-RI (5 mg/kg) alone or in combination with IL-1ra doses had no significant effect on AFC response.

Conclusion Thus, IL-1ra alone has minimal effects on selected functions of the immune system. Effects on immune function noted at 50 mg/kg sTNF-RI were considered minimal and importantly were not exacerbated by the combination of IL-1ra.

THU0097 THE DISCREPANCY BETWEEN MRNA AND PROTEIN EXPRESSION OF THE TUMOUR-SUPPRESSOR GENE MASPIN IN SYNOVIAL TISSUE MIGHT CONTRIBUTE TO SYNOVIAL HYPERPLASIA IN RHEUMATOID ARTHRITIS (RA)

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Background RA is a chronic disease characterised by tumour-like features such as synovial hyperplasia, invasive growth of the synovium and joint destruction. Maspin is a member of the serpin family of protease inhibitors with gene sequence similarities to plasminogen activator inhibitor. Maspin exhibits also tumour-suppressor activity by inhibiting cell motility, invasion and metastasis, and is down-regulated in breast and prostate cancer.

Objectives We investigated the expression of maspin in RA synovial tissue and compared it with the expression in osteoarthritis (OA) and normal synovial tissues (NS).

Methods The expression of maspin was determined in synovial tissue samples obtained during synovectomy or arthroplastic surgery. Using specific primers for maspin, a 237 bp fragment was amplified from cDNA obtained from cultured RA (6), OA (4) and normal (1) synovial fibroblasts (SF) by reverse transcription polymerase chain reaction (RT-PCR). In addition, mRNA expression levels were determined quantitatively by real-time PCR (TaqMan). Using non-radioactive *in situ* hybridization, mRNA expression of maspin was investigated using snap-frozen and

paraffin sections. To identify the cell types expressing maspin, immunohistochemistry was performed on serial tissue sections using monoclonal antibodies against CD3 (T cells) and CD68 (macrophages). SDS-PAGE and Western blotting was performed using anti-maspin antibodies to evaluate the protein expression in total synovial tissue and in cultured synovial fibroblasts (3rd - 4th passage). Protein from normal breast tissue was used as positive control.

Results RT-PCR revealed expression of maspin in all cDNA samples from cultured synovial fibroblasts. By real-time PCR, maspin mRNA was found 2-fold decreased in RA-SF compared to OA-SF and 70-fold in comparison to NS-SF. Using *in situ* hybridization, maspin mRNA was expressed in RA as well as in OA and normal synovial tissue. Whereas in normal and OA samples, expression of maspin mRNA was restricted to synovial fibroblasts in the synovial lining and sublining, in RA, maspin could additionally be observed within perivascular infiltrates in mononuclear and multinucleated cells. Importantly, maspin transcripts were also found at sites of invasion into cartilage and bone. Interestingly, on the protein level, maspin could only be detected in normal breast tissue, but neither in RA synovial tissue nor in RA or OA synovial fibroblasts.

Conclusion Since maspin is not expressed in RA-SF on the protein level, it is hypothesised that the lack of maspin could contribute to the hyperplasia of the synovial tissue in RA.

THU0098 MODIFICATION OF T-LYMPHOCYTES SUBSETS IN RHEUMATOID ARTHRITIS

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Background The role of T-lymphocytes (LT) in the pathogenesis of rheumatoid arthritis (RA) is demonstrated, but the site of T-cell activation is still unknown. The differentiation and maturation of LT took place either in the peripheral blood (PB) and synovial fluid (SF). This process can be followed using the isoforms of the leucocyte common antigen (CD45). Cells expressing CD45RA demonstrate functional characteristics of "naïve cells" and those expressing CD45RO of "memory cells". Following stimulation, naïve cells lose CD45 RA and gain CD45 RO with a transitional stage of dual CD45RA and CD45RO positivity. In RA, the LT from the PB express primarily CD 45RA whereas most LT found in the SF express CD45RO. Expression of HLA-DR is increased on LT found in SF. The population of dual positive LT cells CD45RA+CD45RO+ is expanded in SF, supporting the hypothesis that the LT are activated within the joint.

Objectives Using monoclonal antibodies labelled with fluorochromes and the flow-cytometry technique we tried to evidence the most important modification of lymphocytes subtypes in PB and SF in patients with chronic arthritis.

Methods The study was performed on 29 cases: 19 with RA in the stage II and III of evolution; 8 cases with ankylosing spondylitis and 2 cases with knee osteoarthritis used as control. Paired samples of PB and SF were obtained from all the patients. From these, nucleated cells were prepared (after erythrocyte lysis in PB) and mononuclear cells in SF after density gradient centrifugation. The cells from both sources were labelled with monoclonal antibodies conjugated with fluorochromes in three colour

staining (CD45/CD14/CD3, CD3/CD16⁺56/CD45, CD3/CD19/CD45, CD45RA/CD45RO/CD3, CD45RA/HLA-DR/CD3).

After two washing steps, the cells were analysed within a flow-cytometer (FACSCalibur, BD) using the CellQuest (BD) and Paint-a-Gate (BD) software.

Results From the SF we isolated a greater percent of CD14⁺, CD19⁺ and CD3⁺ cells compared with PB. We found in patients with RA a higher report CD4/CD8 in SF than in the PB; and many of the CD3⁺ CD8⁺ cells showed a cytotoxic phenotype. Among the patients with RA the median percent of cells expressing CD45RA+CD45RO+HLA-DR+ phenotype was 2% in PB respectively 25% in SF; for the CD45RA+CD45RO+HLA-DR- phenotype 25% in PB and 0 in SF; CD45RA-CD45RO+HLA-DR- 24% in PB and 26% in SF; CD45RA-CD45RO+HLA-DR+ 5% in PB and 30% in SF; CD45RA+CD45RO+HLA-DR- 25% in PB and 0 in SF. The HLA-DR expression was 6,1% of LT in PB in RA, predominantly associated with CD45RO expression.

Conclusion Our results confirmed the previous studies reports that described the presence of dual CD45RA+CD45RO+ LT population in PB and SF of patients with RA. The LT dual positive CD45RA and CD45RO population which did not express HLA-DR in PB made up 25% of LT from PB in RA. In SF all dual positive LT coexpress HLA-DR. These results sustained the hypothesis that LT are in a more activated state in SF than in PB.

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THU0099 THE LOCALISATION OF FLICE INHIBITORY PROTEIN (FLIP) IN RHEUMATOID SYNOVIUM

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Background FLICE inhibitory protein (FLIP, I-FLICE) is an inhibitor of caspase 8 (FLICE) and functions as an early mediator of apoptosis. Expression of FLIP is associated with resistance to apoptosis in differentiated macrophages and also in primary B-cells and B-cell lines. The role of FLIP in rheumatoid synovium has not been fully elucidated although it has been shown that FLIP is expressed at sites of bone and cartilage erosion in the rheumatoid joint.

Objectives The aim of this study is to examine the degree of expression of FLIP in rheumatoid synovium.

Methods Samples were obtained intraoperatively from patients with rheumatoid arthritis (n = 17) and osteoarthritis (n = 9). Colon sections from patients with ulcerative colitis were used as biological controls. Immunohistochemistry was carried out using a polyclonal antibody to both the long and short forms of FLIP. Sections of synovium with prominent mononuclear infiltrates were chosen for more detailed analysis of FLIP expression in inflammatory cells. Positive mononuclear cells were counted under a high-power field and the degree of positive staining was graded using the Lindblad and Hedfors scale (see Table 1).

Results FLIP was strongly expressed in the synovial lining of samples from patients with both rheumatoid arthritis and osteoarthritis. There was low expression of FLIP in the rheumatoid mononuclear cells relative to the inflammatory cells in the

osteoarthritis samples. Inflammatory cells in ulcerative colitis showed upregulation of FLIP.

Abstract THU0099 Table 1

	Rheumatoid arthritis (n = 13)	Osteoarthritis (n = 5)
I (	6	0
II (	5	0
III (	1	4
IV (>86% +)	1	1

Conclusion We conclude that the infrequency of lymphocyte apoptosis in RA synovium does not appear to be due to expression of FLIP. There is high expression of FLIP in the synovial lining in both RA and OA however. This may point to differing roles for FLIP in the regulation of apoptosis in synovium and suggests that other apoptotic regulators are involved in the persistence of inflammatory cells in rheumatoid arthritis.

THU0100 INTERLEUKIN-17 INCREASED THE PRODUCTION OF MATRIX METALLOPROTEINASE-3 IN CULTURED RHEUMATOID ARTHRITIS SYNOVIOCYTES

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Background

Objectives To investigate the effect of interleukin-17 (IL-17) on production of matrix metalloproteinase-3 (MMP-3) in rheumatoid arthritis synoviocytes.

Methods Fibroblast-like synovial cell (FLS) were prepared from the synovial tissue of rheumatoid arthritis patients and cultured in the presence of various concentration of IL-17. MMP-3 levels were determined in the culture supernatants by sandwich ELISA according to culturing periods.

Results Stimulation of FLS by IL-17 with 5, 10, 20, 40 ng/ml concentrations increased the production of MMP-3 by 2.7, 2.7, 3, 3.4 fold after 24 h and 8.7, 8.7, 8.1, 10.2 fold after 48 h over the constitutive levels of unstimulated FLS.

Conclusion IL-17, a T cell-derived proinflammatory cytokine produced by rheumatoid synovium, may be involved in the cartilage degradation in rheumatoid arthritis by enhancing the production of MMP-3 from rheumatoid arthritis synoviocytes.

THU0101 HIGHLY ELEVATED LEVELS OF THE DISINTEGRIN METALLOPROTEINASE MDC15 IN RHEUMATOID SYNOVIAL MEMBRANES

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Background MDC15 (ADAM15) belongs to the family of membrane-bound disintegrin metalloproteinases (ADAM or MDC; a