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.

REFERENCES

- 1 Beverley PC. CD45 isoform expression: Implication for recirculation of naïve and memory cells. *Immunol Res.* 1991;**196**
- 2 Cush J, Lipsky PE. Cellular basis for rheumatoid inflammation. *Clin Orthop.* 1991;**265**:9
- 3 Lee WT, Vitetta ES. Changes in expression of CD45R during the development of Th1 and Th2 cell lines. *Eur J Immunol.* 1992;**22**:1455

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

¹S Busted, ¹MW Bennett, ¹M Stone, ¹C Molloy, ¹J O'Connell, ¹F Shanahan, ²MG Molloy. ¹Medicine; ²Rheumatology, Cork University Hospital, Cork, Ireland

10.1136/annrheumdis-2001.976

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

¹K Sung, ¹K Ihm Soo, ¹R Ha Yeon, ²K Yu Sun. ¹Internal Medicine, College of Medicine; ²Medical Institute, Pusan National University Hospital, Pusan, Korea

10.1136/annrheumdis-2001.977

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

¹B Böhm, ²T Aigner, ³CP Blobel, ¹JR Kalden, ¹H Burkhardt. ¹Internal Medicine III, Clinical Immunology and Rheumatology, Erlangen, Germany; ²Institute of Pathology, Cartilage Research Group, Erlangen, Germany; ³Memorial Sloan Kettering Cancer Institute, New York, USA

10.1136/annrheumdis-2001.978

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

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.

REFERENCES

- 1 Primakoff P, Myles DG. *Trends Genet.* 2000;**16**:83–7
- 2 Chubinskaya S, Cs-Szabo G, Kuettner KE. *J Histochem Cytochem.* 1998;**46**:723–30
- 3 Patel IR, Attur MG, Patel RN, Stuchin SA, Abagyan RA, Abramson SB, Amin AR. *J Immunol.* 1998;**160**:4570–79
- 4 Böhm BB, Aigner T, Gehrsitz A, Blobel CP, Kalden JR, Burkhardt H. *Arthritis Rheum.* 1999;**42**:1946–50

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

¹N Busso, ¹J Dudler, ¹R Salvi, ¹V Pédat, ²R Darioli, ³P Nicod, ¹A So, ³V Mooser. ¹Laboratory of Rheumatology; ²Medical Policlinic; ³Department of Medicine, CHUV, Lausanne, Switzerland

10.1136/annrheumdis-2001.979

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)

¹C Schneider, ²S Söder, ¹B Sehnert, ¹RE Voll, ¹JR Kalden, ¹H Burkhardt. ¹Department of Internal Medicine III, Institute for Clinical Immunology, Erlangen, Germany; ²Institute of Pathology, Cartilage Research Group, Erlangen, Germany

10.1136/annrheumdis-2001.980

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