Background Protein I/II, an adhesin from viridans streptococci, commensal bacteria of the oral cavity, can stimulate different cells including endothelial cells. After binding to its receptor, integrin $\alpha 5\beta 1$, protein I/II promotes release of IL-6 and IL-8 from endothelial cells, which contributes to the recruitment of circulating leukocytes from the blood into tissues. IL-8 has recently by associated with the pathogenesis of various chronic inflammatory disorders such as RA. IL-8 could be produced in response to commensal bacteria which could disseminate through bacteremia.

Objectives To study the interactions of protein I/II with fibroblast-like synoviocytes from RA patients.

Methods

Results

- Protein I/II increased the IL-8 synthesis of fibroblast-like synoviocytes through transcriptional up-regulation of the IL-8 gene (ELISA and RT-PCR).
- IL-8 synthesis was inhibited by the tyrosine kinase inhibitor herbimycin A and by inhibitors of MAPKs such as apigenin and PD 98059, while focal adhesion kinase (FAK) and ERK1/2 phosphorylation was increased by Protein I/II.
- Protein I/II increased activated nuclear translocation of the transcription factor NF- κ B (EMSA with an NF- κ B specific probe). This was confirmed by inhibition with curcumin, an inhibitor of NF- κ B, which blocked IL-8 release from protein I/II-activated synoviocytes.

Conclusion

- After binding to synoviocytes, protein I/II induces IL-8 release by activating the FAK and MAPKs signalling pathways.
- The transcription factor NF- κ B plays a major role in the up-regulation of IL-8 synthesis and release mediated by NF- κ B.
- Synoviocytes activation could be triggered by commensal bacteria, which could spread to the joints through bacteremia in RA and others forms of arthritis.

THU0092 THE L1 MRNA IN RHEUMATOID ARTHRITIS ENCODES A FUNCTIONAL RETROTRANSPOSABLE ELEMENT

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Background L1 retrotransposons have been detected in rheumatoid arthritis (RA) at sites of joint destruction.

Objectives To characterise the L1 mRNA in RA.

Methods RNA was isolated from the synovial tissue of a 35-years old woman with RA. The complete L1 sequence was determined by 3'/5'-RACE and compared with functional L1 retrotransposons in plants, animals and humans using 50% consensus trees of the elements and the neighbour-joining method. Methylation of the genomic 5'UTR was determined by the sodium-disulfite/PCR method. *In situ* hybridization was performed on synovial tissues with riboprobes recognising the ORF1 of this element to detect intact L1 elements.

Results A complete L1 mRNA was obtained with the 5'UTR (including a hypoxia inducing factor-responding element and numerous CpG islands), an ORF1 (encoding the p40/RNA binding protein) and an ORF2 (encoding the p150/endonuclease and reverse transcriptase protein). In RA, the genomic 5'UTR appeared hypomethylated, compared to normal control DNA. *In situ* hybridization showed that the L1 ORF1 transcript is

expressed in the sub-lining of patients with RA. Phylogenetic trees showed that the 5'UTR nucleotide sequence and the ORF1 peptide sequence have high similarities with human L1.39 and L1.25, two functional L1 retrotransposons, and the ORF2 peptide sequence resembled the M22334 L1 insertion in type A haemophilia. The endonuclease region of ORF2 appears to be highly conserved in functional L1s, but as shown by a phylogenetic tree, the small difference appears to be a specific marker for evolution, discriminating L1s in plants, rodents, primates and humans.

Conclusion Intact L1 elements are expressed in the synovial tissue of patients with RA most likely following hypomethylation of their 5'UTR. The L1 mRNA transcript belongs to the functional L1 retrotransposons, including elements producing insertional mutations. Thus, functional L1s could be involved in the transformed phenotype of some synovial cells.

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THU0093 RHEUMATOID SYNOVIA AND ACANTHAMOEBA POLYPHAGA: IMMUNOHISTOCHEMICAL EVIDENCE OF CROSSREACTIVITY

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Background

Objectives The initiation event in rheumatoid arthritis (RA) may be triggered by an infection and an association to various micro-organisms has been discussed. Micro-organisms capable of persistence, such as parvovirus B19 and Epstein-Barr virus, are of special interest. Features which might be expected of any micro-organism associated with RA, such as persistence, are also found in the protozoan *Acanthamoeba polyphaga* (AP). The aim of the present study was by means of immunohistochemical techniques, to search for epitopes in synovial tissue from RA patients and non-RA patients reacting/crossreacting with an AP specific monoclonal antibody.

Methods Patient material: Synovia and pannus was removed from the knee during arthroscopy or open joint surgery on 57 patients in a single surgical unit at Diakonhjemmet Hospital in Oslo. These patients were classified into three groups. One had a clinical diagnosis of RA (n = 30) according to the criteria of the American College of Rheumatology. The other 27 patients had degenerative joint disease (n = 17), or other inflammatory arthropathies (n = 10). The specimens were evaluated by light microscopy and immunohistochemistry. Monoclonal antibodies (MAb) against AP were produced by standard methods. A MAb reactive with the cytoplasm of AP and non-reactive with normal human skin biopsies was selected for use in immunohistochemistry.

Results Synovial membranes from RA patients demonstrated a distinct immunoreactivity whereas the reactivity in non-RA patients was weak. RA patients often demonstrated reactivity in the nuclear membrane in addition to cytoplasm and nucleus. The immunoreactivity in non-RA patients was restricted to cytoplasm and nucleus in endothelial cells, pericytes and fibroblasts.

Conclusion Our findings indicates the presence of an epitope crossreactive between synovia and the protozoan AP. Epitopes

common between micro-organisms and human tissues are possible elements in autoimmunity.

THU0094 EXPERIMENTAL MYCOPLASMA FERMENTANS INFECTION IN RHEUMATOID SYNOVIAL FIBROBLASTS INDUCES M161AG EXPRESSION

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Background Mycoplasma fermentans (Mf) is one of the most likely of the several infectious agents which may be involved in the pathogenesis of rheumatoid arthritis (RA). Mf has been shown to induce arthritis experimentally in rabbits, and several reports have succeeded in detecting it in synovial fluids in RA patients. M161Ag is a 43-kDa surface lipoprotein of Mf, which induces cytokine production in monocytes and activates the host complement system.

Objectives The aim of this study was to investigate whether M161Ag can be induced on rheumatoid synovial fibroblasts by an Mf infected cell line *in vitro*.

Methods Rheumatoid synovial cells were isolated from synovial tissue specimens obtained at operation. Production of Mf infected rheumatoid synovial cells was performed using a coculture system of synovial cells and P39 (+) cells, which were Mf containing human leukaemia cell lines, for three days. PCR with mycoplasma specific primers were done to detect Mf DNA in synovial cells. M161Ag expression was evaluated by an immunohistochemical method with MK5 and MK53 monoclonal anti M161Ag antibodies, and mRNA production was also evaluated by the RT-PCR method.

Results Freshly isolated rheumatoid synovial cells were negative for Mf DNA using PCR with mycoplasma specific primers. DNA of Mf ribosomal RNA was detected in the synovial fibroblasts after coculture of synovial cells and P39 (+) cells by PCR with mycoplasma specific primers. Moreover, the expression of M161Ag was detected only on Mf infected synovial cells using the immunohistochemical method with MK5 or MK53 monoclonal antibodies.

Conclusion Experimental *in vitro* Mf infection in rheumatoid synovial fibroblasts was demonstrated to induce the expression of M161Ag. The significance of this finding requires clarification in further studies.

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THU0095 CIRCULATING AND LOCAL SYNOVIAL FLUID RENIN-ANGIOTENSIN SYSTEM IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS

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Background Recent researches demonstrated presence of local RAS in myocardium, ovary and brain. Angiotensin converting enzyme (ACE) was shown to be produced by synovial membrane macrophages. Angiotensin II is a potent vasoconstrictor, and also can stimulate angiogenesis, fibroblast proliferation and growth factor expression. Hypertrophy and hyperplasia of synovium and angiogenesis are well documented in persistent rheumatoid synovitis.

Objectives In this cross-sectional study we aimed to detect ACE and renin levels in serum and synovial fluid (SF) of patients with RA and osteoarthritis OA.

Methods Sixteen patients with RA (13F, 3M), 13 patients with OA (8F, 5M) and 11 healthy controls (HC) (5F, 6M) were enrolled in the study. The commercially available Angiotensin-Converting Enzyme kit and ACE calibrator were used. Renin assay was carried out with a two-site immunoreactive assay.

Results Median serum ACE levels were 48 U/l, 45 U/l, and 33 U/l in RA patients, OA patients, and HC, respectively. Median serum renin levels were 12.9 pg/ml, 11.8 pg/ml, and 17.9 pg/ml in RA patients and OA patients, respectively. Neither of these results were statistically significant ($p > 0.05$). SF levels of ACE and renin were found to be increased in RA patients (44 U/l and 3.5 pg/ml) compared to OA patients (24 U/l and 1.2 pg/ml) (Table 1). Synovial fluid renin levels in patients with RA was negatively correlated with disease duration.

Abstract THU0095 Table 1

	RA	OA	HControls
No of patients	16	13	11
Serum ACE (U/l)	48 (15–103)	45 (10–82)	33 (30–84)
Serum renin (pg/ml)	12.9 (1.2–67)	11.8 (1.6–39.2)	17.9 (17.9–36.6)
SF ACE (U/l)	44 (14–133)	24 (9–103)	
SF renin (pg/ml)	3.5 (0.48–15)	1.2 (0.5–4)	

Conclusion In this study we demonstrated that ACE and renin levels were elevated in SF of RA patients while serum ACE and renin levels were comparable to those in OA patients and healthy controls. These observations suggest presence of local RAS in RA synovium. Since angiotensin have stimulatory roles in angiogenesis and fibroblast proliferation, local RAS may participate in pannus formation and joint destruction in patients with RA. Negative correlation of synovial renin with disease duration may be compatible with previous observations that joint erosion is most prominent during the first months of the disease. Further studies, including mRNA of ACE and angiotensin II are needed to clarify exact roles of RAS in the pathogenesis of RA.

THU0096 EFFECT OF ANAKINRA (IL-1RA) AND SOLUBLE TUMOUR NECROSIS FACTOR RECEPTOR I (STNF-RI) ON CELLULAR IMMUNE FUNCTION IN RATS

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Background

Objectives This study investigated the potential for combined treatment with inhibitors of IL-1 and TNF- α to affect