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.

REFERENCE

- 1 Neidhart M, et al. *Arthritis Rheum.* 2000;**43**:2634-47

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