Results The addition of TNF- α and M-CSF was sufficient to induce RA synovial macrophage- and monocyte-osteoclast differentiation in the absence of OPGL. This was evidenced by the formation of tartrate-resistant acid phosphatase (TRAP), vitronectin receptor, cathepsin K-positive multinucleated cells on glass coverslips and lacunar resorption pits on dentine slices. When IL-1 α was added to this cultures together with TNF- α , the number of lacunar resorption pits were increased. It was found that anti-TNF- α inhibited osteoclast formation by RA synovial macrophages and monocytes and that although the number of TRAP-positive multinucleated cells was not changed, inhibition of pit formation was seen when anti-IL-1 α was added to RA macrophage and monocyte cultures. OPG, however, showed no effect on osteoclast formation and activation induced by TNF- α and IL-1 α .

Conclusion This study has shown that TNF- α induces RA synovial macrophage- and monocyte-osteoclast differentiation and that IL-1 α activates osteoclasts to resorb bone. This osteoclast formation and activation is independent of OPGL signalling system. The new mechanism, inflammatory bone resorption, may represent an important role in the RA joint destruction and osteoporosis.

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OP0021 EFFECTS OF TNF- α , IL-1 BETA AND IL-15 ON THE PRODUCTION/EXPRESSION OF OPG AND RANKL BY SYNOVIAL FLUID MONONUCLEAR CELLS AND FIBROBLAST-LIKE SYNOVIOCYTES ISOLATED FROM PATIENTS WITH RHEUMATOID ARTHRITIS (RA)

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10.1136/annrheumdis-2001.946

Background The receptor activator of nuclear factor κ B (RANKL), its receptor RANK and osteoprotegerin (OPG) are key regulators of bone remodelling that influence immune functions as well. RANKL is responsible for the development and activation of osteoclasts. OPG, a soluble decoy receptor for RANKL, competes with RANK for RANKL binding. Proinflammatory cytokines (TNF- α , IL-1b and IL-15) play critical role in the pathogenesis of RA and bone erosions.

Objectives To compare the effects of TNF- α , IL-1b and IL-15 on the production/expression of OPG and RANKL by synovial fluids mononuclear cells (SFMC) and fibroblast-like synoviocytes (FLS) isolated from RA patients.

Methods SFMC and FLS were obtained from 8 RA patients. The isolated SFMC and FLS (cultured *in vitro* for 2–5 passages) were stimulated with recombinant human TNF- α , IL-1b or/and IL-15. The concentrations of OPG in culture supernatants were measured by a specific ELISA and the expression of surface RANKL and OPG was tested using flow cytometry.

Results SFMC and FLS from RA patients spontaneously produced considerable levels of OPG. The production of OPG by FLS was strongly enhanced by TNF- α and IL-1b, but not by IL-15. In contrast, the production of OPG by SFMC was only

slightly enhanced in the presence of TNF- α , IL-1b or IL-15. Interestingly, additive effects of IL-15 and IL-1b or IL-15 and TNF- α on OPG production by SFMC were observed. In addition to the soluble form, high levels of surface expressed OPG on CD33⁺/SFMC, but not on CD3⁺/SFMC, was observed. The high expression of RANKL was found on CD33⁺/SFMC, intermediate on CD3⁺/SFMC and low on FLS. Tested cytokines modulate the expression of RANKL and OPG, in a complex, cell type and time dependent manner.

Conclusion TNF- α , IL-1b and IL-15 affecting balance between RANKL and OPG influence inflammation and bone destruction in RA patients.

OP0022 MOLECULAR PROFILE OF RHEUMATOID ARTHRITIS-SYNOVIAL FIBROBLASTS DURING PROLIFERATION

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10.1136/annrheumdis-2001.947

Background Synovial fibroblasts (SF) play an important role in chronic inflammation and joint destruction in rheumatoid arthritis (RA). While many anti-apoptotic mechanisms in RA-SF have been demonstrated, various molecules promoting the proliferation of RA-SF were also believed to participate in the pathogenesis of RA.

Objectives The aim of this study was to investigate the expression profile of RA-SF during proliferation and to explore the molecular mechanisms of synovial proliferation in RA.

Methods Cultured RA-SF in passage 6 from one patient were used for cDNA subtraction and RT-PCR. Total RNA was extracted from two culture conditions, 4 days after replating (low-density cells; LD) and 14 days after replating (high-density cells; HD), respectively. One μ g of total RNA from each culture was used for cDNA synthesis with the SMART PCR cDNA Synthesis Kit (CLONTECH), then suppression subtraction hybridization was performed with the PCR-Select cDNA Subtraction Kit (CLONTECH). Forward (LD-specific) and reverse (HD-specific) subtracted cDNA was subcloned into PCR-Script Amp SK (+) cloning vector (STRATAGENE). The nucleotide sequences of these clones were analysed by commercial sequencing (Microsynth), then BLAST searches (NCBI) were performed to identify each clone. mRNA expression of distinct clones in RA-SF were confirmed by RT-PCR with primers specific for each gene. The expression and distribution of distinct genes were examined on paraffin-embedded sections of synovial tissues from 7 patients with RA and 3 non-RA patients by *in situ* hybridization with digoxigenin-labelled RNA probes specific for each gene.

Results 44 clones were upregulated in LD cells and 44 clones were upregulated in HD cells, respectively. 46 of 88 clones were identical to the sequences that have been already identified and characterised, including the sequences of enzymes for metabolic pathways (i.e., aldose reductase, glutamine synthetase), cytoskeletal proteins (i.e., CDC10, EPLIN), proliferation-associated molecules (i.e., S100A4, NAP-1 related protein), matrix proteins (i.e., EFEMP1, versican), and ribosomal proteins. On the other hand, 29 clones were identical to cDNAs that have been already identified, but with unknown functions so far. 13 gene fragments did not show significant homology to any sequence in the GenBank