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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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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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

(NCBI). mRNA expression of distinct clones were confirmed by RT-PCR. Moreover, *in situ* hybridization showed that distinct genes, such as Fbx3 and S100A4, were also expressed on synovial tissues from patients with RA but not from non-RA patients, suggesting that these molecules may contribute to the pathogenesis of RA.

Conclusion In this study, a number of molecules, including several novel genes, were isolated from RA-SF using suppression subtraction hybridization. The expression of these molecules may be involved in synovial activation in the pathogenesis of RA. *K. Masuda is supported by Japan Rheumatism Foundation.

OP0023 ADENO-ASSOCIATED VIRUS ENCODING FOR IL-4 INHIBITS COLLAGEN-INDUCED ARTHRITIS

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Background Gene therapy is a promising strategy for rheumatoid arthritis. Adeno-associated virus (AAV) are recently developed vectors with unique set of characteristics like their ability to infect host cells at any time of their division cycle, and their possible weak immunogenicity in mammals. IL-4 is a Th-2 anti-inflammatory cytokine that is efficient if delivered by continuous administration in collagen-induced arthritis (CIA) in mice.

Objectives The present study was undertaken to investigate the possibility to use AAV vectors in CIA.

Methods AAV vectors, expressing lacZ (AAV-lacZ) or IL-4 (AAV-IL-4) cDNA under CMV promotor control, were produced and purified by Génethon (Evry, France). DBA/1 mice were used for all studies. After i.m. injection of 2.5 10^9 AAV-lacZ particles, lacZ expression was examined on tissue cryosection by X-gal staining. IL-4 transgene expression was evaluated *in vivo* different times after i.m. injection of AAV-IL-4 by an IL-4 ELISA on muscles lysates. LacZ quantitative expression was evaluated after i.m. injections of AAV-lacZ by X-gal colorimetric assay on muscle lysates. AAV antibodies levels were measured by a neutralizing assay. CIA was induced by immunisation with bovine type II collagen. For treatment, 1.3 10^{10} AAV-IL-4 were administered i.m. to mice 11 days prior to induction of CIA.

Results Peri-articular injection of AAV-lacZ showed an expression of the transgene localised in the deep muscles cells near the bone. IL-4 *in vivo* expression was detected at least 120 days after AAV-IL-4 injection i.m. Anti-AAV neutralising antibodies were detected in the serum after AAV-lacZ administration *in vivo*, but they didn't altered the transgene expression after readministration of AAV-lacZ. CIA mice treated with AAV-IL-4 were significantly improved in comparison to control, in terms of prevalence of the disease, arthritis scores, and histological evaluation. Locally, a slight inflammation at the point of vector injection was noted.

Conclusion AAV is a powerful vector for gene therapy in CIA. AAV IL-4 prevent the development of CIA. In addition, our data are consistent with a long term persistence of the transgene and the possibility of a readministration.

OP0061 RECOMBINANT OSTEOPROTEGERIN (OPG) REGULATES BONE EROSIONS AND OSTEOCLAST NUMBERS IN A SCHEDULE-DEPENDENT MANNER IN JOINTS OF MALE LEWIS RATS WITH ADJUVANT ARTHRITIS

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Background Our laboratory has shown that bone destruction in the Lewis rat model of adjuvant arthritis (AdA) can be significantly reduced by daily administration of recombinant human osteoprotegerin (rhOPG) if treatment is initiated at the onset of clinical arthritis.¹

Objectives The current study was performed to assess the ability of rhOPG to spare bone using an abbreviated dosing schedule.

Methods AdA was induced in male Lewis rats (8 weeks old, n = 6/group) on Day 0; clinical arthritis (indicated by hind paw swelling) developed on Day 9. Arthritic animals received rhOPG (daily s.c. bolus of 4 mg/kg) by one of four schedules: no rhOPG (Group 1, control for AdA induction); daily rhOPG, Days 9 to 15 (Group 2, control for rhOPG efficacy); early intervention, Days 9 to 11 (Group 3); or delayed intervention, Days 13 to 15 (Group 4). Rats were necropsied on Day 16, and bone mineral density [BMD] of bones in the tibiotarsal (ankle) joint was measured by DEXA (dual X-ray absorptiometry). Next, sections of formalin-fixed, decalcified, paraffin-embedded hind paws were stained by an indirect immunohistochemical method to detect cathepsin K, an osteoclast marker. Histopathologic scores to assess lesion severity (given below as group means $\pm$ standard deviation) were acquired for inflammation, bone erosion, and osteoclast numbers using tiered, semi-quantitative grading scales.

Results Joints of vehicle-treated arthritic animals (Group 1) exhibited measurable loss of BMD as well as extensive inflammation (4.0 ± 0), skeletal erosion (4.5 ± 0.5), and osteoclast production (4.0 ± 0). As noted previously,² rhOPG therapy throughout the 7-day clinical course of AdA (Group 2) preserved BMD and almost completely inhibited erosions (1.2 ± 0.4) and osteoclastogenesis (0.8 ± 0.8) but did not modify the extent of inflammation (3.7 ± 0.5). Early initiation of rhOPG treatment (Group 3) yielded similar retention of BMD as well as reductions in erosion (1.2 ± 0.4) and osteoclast (1.7 ± 0.5) scores. However, in comparison to Groups 2 and 3, delayed rhOPG administration (Group 4) protected BMD and lowered the mean osteoclast score (2.0 ± 0.3) but was significantly less effective in preventing erosions (2.5 ± 1.4).

Conclusion Our data indicate that early intervention with a bone-sparing agent such as rhOPG is needed to obtain the greatest clinical benefit with respect to protecting skeletal integrity in arthritic joints but also show that delayed rhOPG therapy can reduce the loss of joint BMD even in the presence of severe inflammation.

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THU0073 ELASTASE AND LACTOFERRIN AS INDICATORS OF FUNCTIONAL ACTIVITY OF POLYMORPHONUCLEAR CELLS IN RHEUMATOID ARTHRITIS

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