(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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