trabecular separation of MK3 deficient, MK2 deficient and MK2/3 deficient mice compared with wild type. MK3 deficient bones have lower trabecular number and higher trabecular separation than MK2 deficient bone while MK2/3 deficient bones showed the same phenotype than MK2 deficient bones. Number of osteoclasts was reduced in MK3 deficient, MK2 deficient and MK2/3 deficient bones in vivo compared with wild type. Number of osteoclasts was higher in MK3 deficient bones than in MK2 bones, MK2/3 deficient bones showed the same number of osteoclasts than MK2 deficient bones. Ex vivo osteoclast differentiation assay showed reduced osteoclasts number using MK3, MK2 and MK2/3 deficient cells compared with wild type cells.

Conclusions MK3 deficient mice showed increased trabecular bone volume than wild type mice, but trabecular volume was less increased than in MK2 deficient mice. MK2/3 deficient mice showed no additional effect compared to MK2 deficient mice. Increased trabecular volume was associated with reduced number of osteoclasts due to impaired osteoclast differentiation. Thus MK3 regulated osteoclast differentiation and bone homeostasis but there is no additional effect to MK2.

A8.11 RANKL EXPRESSION IS LOWER ON T AND B LYMPHOCYTES AND RANKL$^+$ CELLS TEND TO ACCUMULATE IN CIRCULATION OF RHEUMATOID ARTHRITIS PATIENTS TREATED WITH TNF BLOCKERS

doi:10.1136/annrheumdis-2013-203222.11

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Background and Objectives Rheumatoid arthritis (RA) is characterised by bone resorption and joint destruction. The receptor activator of NF-kB ligand (RANKL) plays a major role in bone loss because it is responsible for osteoclast differentiation and it is known that hyperactive immune system cells express surface RANKL. Several therapies commonly used for RA treatment have been shown to stop RA joint destruction. One of the hypothetical mechanisms explaining this effect could be an interference with the RANKL system.

The aim of this work was to assess the effects of RA therapies in RANKL surface expression in different leukocyte populations by flow cytometry.

Methods Forty-nine patients diagnosed with RA were recruited for this study. Seventeen patients were naïve to any therapy, 14 were under methotrexate (MTX) – 8 of them at baseline of treatment with TNF blockers – and 18 patients were treated with TNF blockers. Blood was collected and total leukocytes were used for flow cytometry staining with anti human-CD66b for neutrophils, CD3 for T lymphocytes, CD19 for B lymphocytes and RANKL.

Results There were no differences regarding gender distribution, age, disease activity, C-reactive protein (CRP) levels or erythrocyte sedimentation rate (ESR).

Patients treated with MTX or TNF blockers have reduced RANKL expression in neutrophils, T and B lymphocytes ($p = 0.0027$, $p = 0.0003$ and $p = 0.0032$, respectively) when compared to untreated patients. However the number of circulating RANKL$^+$ T and B lymphocytes was increased in patients treated with TNF blockers when compared to naïve patients ($p = 0.0070$ and $p = 0.0183$ respectively). No differences were found between groups regarding circulating number of leukocytes. We found no correlation of the studied parameters with CRP, ESR or DAS28.

Conclusions RANKL surface expression on T and B lymphocytes decreases and RANKL$^+$ cells tend to accumulate in the circulation of patients treated with TNF blockers. The reasons for this effect are not clear but might be related to disturbances induced by TNF blockage in gene expression, cell activation and migration.

A8.12 SMALL UBIQUITIN RELATED MODIFIER-1 (SUMO-1) REGULATES OSTEOCLASTOGENESIS

doi:10.1136/annrheumdis-2013-203222.12

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Background and Objectives Rheumatoid arthritis (RA) is a common autoimmune disease characterised by the hyperplastic transformation of synovium, its infiltration with different inflammatory cells and by stimulation of bone resorption through osteoclast activation leading to joint destruction. Posttranslational modification of proteins by SUMO has been shown for a number of target molecules including transcription factors and is involved in a variety of cellular processes, including protein localisation, transcriptional regulation, protein stability, cell survival and death. Previously, we have shown that the increased expression of SUMO-1 contributes to the inflammatory response in RA. Here, we investigated the role of SUMO-1 in osteoclastogenesis and studied the skeletal phenotype of *SUMO-1*$^{-/-}$ mice under physiological conditions.

Materials and Methods For all in vitro experiments, bone marrow macrophages were isolated from *SUMO-1*$^{-/-}$ mice and wild type (WT) controls and were cultured in the presence of macrophage colony-stimulating factor and receptor activator of nuclear factor k-B ligand. Osteoclast differentiation was verified by tartrate-resistant acid phosphatase (TRAP) staining. Using real time PCR mRNA levels of DC-STAMP and Cathepsin K were analysed. Proliferation of preosteoclasts was determined using CyQuant proliferation assay. Osteoclast resorption capacity was analysed using a calcium phosphate bone resorption assay. The skeletal phenotype of 8-week old mice was investigated by μCT-analysis of trabecular bone in the lumbar spine and femora. The vertebral bodies L5 from each animal were dehydrated and embedded nondecalcified into methylmetacrylate for sectioning. Sections were stained using van Kossa and for TRAP activity.

Results In PCR analyses, we found decreased expression of DC-STAMP and Cathepsin K in *SUMO-1*$^{-/-}$ mice compared to wt mice during osteoclast differentiation. Proliferation of preosteoclasts was not affected by loss of SUMO-1. In osteoclast formation assays, the loss of SUMO-1 was associated with impaired osteoclast differentiation and with impaired bone resorption capacity. In addition, histological analyses revealed a reduced number of osteoclasts in *SUMO-1*$^{-/-}$ mice. At 8-weeks old, *SUMO-1*$^{-/-}$ mice had a 20% higher trabecular bone volume fraction compared with wt mice. Moreover, trabecular thickness was higher and trabecular separation was lower in *SUMO-1*$^{-/-}$ mice.

Conclusions In our study, we found that *SUMO-1*$^{-/-}$ mice have high bone mass owing to a decrease in number, size and function of osteoclasts. Furthermore, osteoclast markers contributing to osteoclast fusion and to osteoclast resorption capacity were decreased. These data suggest that SUMO-1 is involved predominantly in the regulation of bone mass by osteoclast formation and activity, and therefore may be an interesting target for treating diseases associated with bone loss.

A8.13 SYNDECAN-4 FUNCTION IS ESSENTIAL FOR MATRIX REMODELLING UNDER INFLAMMATORY CONDITIONS, BUT DISPENSABLE DURING EMBRYOGENESIS

doi:10.1136/annrheumdis-2013-203222.13

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