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

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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

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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

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Objective The heparan sulphate proteoglycan syndecan-4 (Sdc4) has been associated strongly with osteoarthritis, a disease that mimics key aspects of early cartilage remodelling during endochondral ossification, but its role in embryonic and adult bone formation remains unclear. Therefore, we used *Sdc4*$^{-/-}$ mice to analyse the distribution and functional role of Scd4 in endochondral ossification of mouse embryos and in adult fracture repair, which recapitulates endochondral ossification, but like osteoarthritis, involves an inflammatory component.

Methods *Sdc4* promoter activity was analysed in *Sdc4*$^{-/-}$*/LacZ knock-in* animals using β-galactosidase stainings. E16.5 embryos were used for histological (alcian blue/alizarin red) and immunohistological (PCNA, Col10a1, ADAMTS-4, BC-3, Sdc2) staining and the calcified bone area was quantified using whole mount staining of these embryos. Histological (Masson-Goldner, alcian blue) and immunohistological (Col10a1, Sdc2, PCNA) staining at day 7, 14 and 28 fracture calli were performed. These experiments were repeated with anti-TNF treatment during fracture healing. Callus size and cartilage area were quantified using image J Chondrocytes were isolated from neonatal knee joints and embryonal cartilage. Proliferation was investigated using MTT assay. Gene expression analysis for Sdc-2, Sdc-4 with and without stimulation using TNFα and WNT3a was performed using quantitative RT-PCR.

Results In *Sdc4*$^{-/-}$*/LacZ knock-in* animals, *Sdc4* promoter activity was detectable in all stages of chondrocyte differentiation during embryogenesis. *Sdc4* deficiency inhibited chondrocyte proliferation both in vivo and in vitro, but this did not lead to a growth phenotype at birth. In contrast to embryogenesis, fracture healing in adult mice was markedly delayed in *Sdc4*$^{-/-}$ animals and accompanied by increased callus formation. Analysing the discrepancy between the mild embryonic and the severe adult phenotype, we found a compensatory up-regulation of *Sdc2* in the developing cartilage of *Sdc4*$^{-/-}$ mice that was absent in adult tissue. Stimulation of chondrocytes with Wnt3a in vitro, led to an increased expression of *Sdc2*, while stimulation with TNFα resulted in an up-regulation of *Sdc4* but a decreased expression of *Sdc2*. In consequence treatment with a blocking anti-TNF antibody during fracture healing abolished the difference in callus size between wildtype and *sdc4*$^{-/-}$ mice.

Conclusions We conclude that Sdc4 is functionally involved in endochondral ossification and that the loss of Sdc4 impairs adult fracture healing due to the inhibition of compensatory mechanisms under inflammatory conditions.

A8.14 THE ANTI-PROLIFERATIVE FUNCTION OF RSK2 IN SYNOVIAL FIBROBLASTS PROTECTS AGAINST TNF-α-INDUCED JOINT DESTRUCTION IN INFLAMMATORY ARTHRITIS

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Background/Objectives The pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNF-α) directly activates the ribosomal S6 kinase RSK2 in vitro. We recently demonstrated the protective effect of RSK2 against TNF-induced bone loss. Interestingly, we found an increased activation of RSK2 in the joints of arthritis patients as well as in the inflamed joints of mice overexpressing the human TNF-α (*hTNF*tg). These observations prompted us to investigate the function of RSK2 in the development of TNF-α-induced inflammatory arthritis.

Materials and Methods *hTNF*tg mice were crossed with RSK2-deficient (*Rsk2*$^{y/-}$) mice. Clinical scoring and histomorphometry of the joints were assessed. We compared the levels of circulating pro-inflammatory cytokines as well as the cellularity of myeloid lineages in the spleen. The expression of cytokines and mesenchymal markers in the joints was determined via QPCR. Bone marrow transfer of *Rsk2*$^{y/-}$ and wild-type littermates into *hTNF*tg mice was performed and clinical scoring as well as histomorphometry of the joints was assessed. Primary fibroblast-like synoviocytes (FLS) from *hTNF*tg and *hTNF*tg; *Rsk2*$^{y/}$ mice were isolated to analyse their expression of inflammatory cytokines and metalloproteinases as well as their proliferation and apoptosis in vitro.

Results RSK2 deficiency in *hTNF*tg mice resulted in an early onset of clinical signs of arthritis as well as a drastic exacerbation of inflammation, increased cartilage destruction and increased local bone destruction. Increased levels of circulating pro-inflammatory cytokines and the increased proportion of all myeloid lineages in the spleen confirmed the enhanced inflammation in the *hTNF*tg mice lacking RSK2. Increased activation of synovial fibroblasts and macrophages in the joints of *hTNF*tg; *Rsk2*$^{y/-}$ mice was demonstrated by the locally increased expression of pro-inflammatory cytokines and matrix metalloproteinases (MMPs). Importantly, the phenotype could not be transmitted by the transfer of *Rsk2*$^{y/-}$ bone marrow into *hTNF*tg mice that demonstrated the essential role for RSK2 expression in mesenchymal cells driving the pathogenesis. In agreement, although no difference in the expression of pro-inflammatory cytokines or MMPs nor a change in apoptosis was detected in synovial fibroblasts isolated from *hTNF*tg; *Rsk2*$^{y/-}$, these cells displayed an increased proliferation rate.

Conclusions The anti-proliferative function of RSK2 controls a cell autonomous negative feed-back against the activation of synovial fibroblasts by TNF-α, therefore limiting joint destruction in arthritis. Thus, activation of RSK2 is a potential target for the treatment of both local and systemic bone destruction in RA.

A8.15 THE FOCAL CONTACT PROTEIN LASP-1 MODULATES THE MIGRATION CAPACITY OF SYNOVIAL FIBROBLASTS

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Background and Objectives RA synovial fibroblasts (SF) have been suggested to contribute to the spreading of disease through their ability to leave cartilage destruction sites, migrate via the bloodstream and re-initiate the destructive process at distant articular cartilage surfaces. In this context, the actin-crosslinking protein Lasp-1 is of interest, because it is localised at leading edges of migrating cells and regulates metastatic dissemination of different tumours. Therefore, it is particularly important to investigate the role of Lasp-1 in SF migration and its effects on RA.

Materials and Methods To identify different Lasp-1 expression levels in the hind paws of wt and hTNFtg mice, an established model for human RA, Western- blot analyses were performed. In parallel, Lasp-1 expression and its sub-cellular distribution was investigated in SF from wt and hTNFtg mice by Western-blot analyses and immunofluorescence. The migratory capacity of SFs derived from wild-type, Lasp-1$^{-/-}$, hTNFtg and Lasp1$^{-/-}$/hTNFtg mice was studied in a modified scratch assay as well as in live cell imaging