Conclusions Here, we demonstrate that ACPA IgG, isolated from SF of RA patients, have the ability to enhance the RANKL-driven osteocalstogenesis from immature dendritic cells. Our findings suggest that ACPA might have a direct pathogenic effect in RA associated bone destruction.

A8.3 DEFICIT OF S100A4 PREVENTS JOINT DESTRUCTION AND SYSTEMIC BONE LOSS IN hTNFtg MOUSE MODEL

doi:10.1136/annrheumdis-2013-203222.3

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Background Our previous studies demonstrated increased levels of S100A4 protein in sera, synovial fluid and synovial membrane of patients with rheumatoid arthritis (RA) compared to osteoarthritis. S100A4 regulates apoptosis and induces production of matrix metalloproteinases by synovial fibroblasts. Furthermore, S100A4 stimulates synthesis of tumour necrosis factor (TNF)-α by mononuclear cells. The aim of this study was to investigate the effect of loss of S1004 in induction of experimental arthritis in the human TNF transgenic (hTNFtg) mouse model.

Methods We crossed the heterozygous hTNFtg mice with S100A4 knockout ($S100A4^{-/-}$) mice. Mice were clinically assessed for paw swelling, grip strength and body weight weekly from 6th to 14th week of age in a blinded manner. Sections of hind paws and tibias were histologically analysed for synovial inflammation, cartilage loss, bone erosions, osteoclast numbers and bone formation parameters with the OsteoMeasure image analysis system.

Results In the group of hTNFtg; $S100A4^{-/-}$ mice, paw swelling, grip strength and body weight were significantly improved compared to hTNFtg; $S100A4^{+/+}$ ($p < 0.01$ for all parameters). Consistent with the clinical observations, histological analysis of the tarsal joints of hTNFtg; $S100A4^{-/-}$ mice showed reduced pannus formation (area of inflammation decreased by 66 ± 3%, $p < 0.01$) and cartilage destruction (cartilage loss decreased by 63 ± 6%, $p < 0.01$) compared to hTNFtg; $S100A4^{+/+}$ mice. Similarly, osteoclast numbers were decreased by 84 ± 3% ($p < 0.01$) and bone erosions were less severe (area of bone erosion decreased by 81 ± 4%, $p < 0.01$) in hTNFtg; $S100A4^{-/-}$ mice. Furthermore, hTNFtg; $S100A4^{-/-}$ mice were protected from systemic bone loss. Absence of S100A4 completely reversed increased osteoclast formation and bone resorption in hTNFtg mice. hTNFtg; $S100A4^{-/-}$ mice had an increased bone volume per total volume (BV/TV) by 78 ± 20% ($p < 0.05$) and a decrease in trabecular separation by 39 ± 4% ($p < 0.05$), decreased numbers of osteoclasts per bone perimeter (NOc/BPm decreased by 43 ± 2%, $p < 0.01$), decreased bone surface covered by osteoclasts (Oc.S/BS decreased by 52 ± 3%, $p < 0.01$), increased numbers of osteoblasts per bone perimeter (NOb/BPm increased by 129 ± 20%, $p < 0.05$) and increased bone formation rate per bone surface (BFR/BS increased by 112 ± 18%, $p < 0.05$) compared to hTNFtg; $S100A4^{+/+}$.

Conclusions These results suggest that inhibition of S100A4 effectively prevents induction of experimental arthritis via protecting against TNF-induced synovial inflammation, cartilage and bone destruction, and systemic bone loss. Our results support the role of S100A4 in the pathogenesis of RA where an increased S100A4 protein in circulation and locally at sites of inflammation may be linked to the process of aggressive fibroblast behaviour. Thus, S100A4 might represent a novel therapeutic target in RA.

A8.4 Fc-GLYCOSYLATION DETERMINES OSTEOCLASTOGENIC ACTIVITY OF IMMUNE COMPLEXES

doi:10.1136/annrheumdis-2013-203222.4

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Background and Objectives Autoantibodies recognising citrullinated proteins (ACPA) are highly specific for rheumatoid arthritis (RA), precede the clinical onset of the disease by years and are the strongest known risk factor for bone loss. We have recently shown that ACPA specific for citrullinated vimentin directly interact with osteoclast precursors and induce bone loss. In patients with RA, ACPA-containing immune complexes can be detected in synovial fluid and tissue. We hypothesised that (I) immune complexes directly promote osteoclast maturation and, consecutively, bone loss and that (II) the type of IgG-glycan is important for the interaction with osteoclast precursors, since ACPA have been shown to be hyposialylated.

Materials and Methods We differentiated preosteoclasts from human monocytes and stimulated them with artificial immune complexes generated by heat aggregation from pooled human IgG (IVIG). Part of the IgG had been pretreated with neuraminidase or PNGase F to remove sialic acid or the whole Fc glycan, respectively. For in vivo studies we injected murine immune complexes in the knee joints of C57-BL/6 mice.

Results Stimulation of preosteoclasts with immune complexes resulted in their dramatically increased maturation to osteoclasts. This effect was even more pronounced with complexes formed from desialylated IgG. Monomeric IgG and fully deglycosylated immune complexes did not alter osteoclast maturation. qPCR and FACS-analyses revealed that all Fcγ receptors (FcγR) are upregulated during osteoclastogenesis with FcγR I and FcγR III being the most prominent ones. Desialylated immune complexes induced the activation of spleen tyrosine kinase (Syk) and phospholipase Cγ (PLCγ) as well as the upregulation of the transcription factor c-fos in preosteoclasts. Injection of murine immune complexes into the knee joints of C57-BL/6 mice caused accumulation of osteoclasts in the vicinity of the site of injection.

Conclusions Our data show that IgG immune complexes promote osteoclastogenesis. They upregulate the pro-osteoclastogenic transcription factor c-fos, after binding to activating FcγRs on preosteoclasts. This interaction is highly dependent on the absence of sialic acid in the Fc-glycan of the IgG. Altogether, we propose a novel mechanism by which ACPA promote bone loss independent of inflammation.

A8.5 FIBROBLAST ACTIVATION PROTEIN ALPHA IN INFLAMMATORY BONE DESTRUCTION

doi:10.1136/annrheumdis-2013-203222.5

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Background The Fibroblast Activation Protein alpha (FAPα) is an integral membrane serine protease that plays a major role in migration, wound healing, and metastasis. Based on recent studies that have implicated membrane-bound serine proteases in osteoclast migration, we studied the expression of FAPα in rheumatoid arthritis (RA) and analysed its role in osteoclast development under inflammatory conditions.