Methods We performed SNP genotyping for *IRF5*(Rs20024640), *IRF7*(Rs1061502), *TYK2*(Rs2304256) genes using the Taqman Assay in 4 large cohorts comprising of North-American Caucasian, Dutch, Italian and Spanish samples totaling to 2,091 SSc patients and 1,434 race-matched controls. All SSc patients fulfilled ACR criteria or had at least 3 of the 5 CREST features. HWE testing, chi-square, logistic regression(LR) were used for statistical comparisons. Mesoscale assays were used for cytokine detection.

Results LR analysis after controlling for gender and cohorts the association was confirmed in all cohorts {*IRF5*: $P < 0.0001$, OR(CI)–0.68(0.6–0.8); *IRF7*: $P = 0.006$, OR(CI)–0.80(0.7–0.9); *TYK2*: $P = 0.05$, OR(CI)–0.85(0.7–0.99)}. The association was stronger with anti-centromere subset {*IRF5*: $P = 0.0002$, OR(CI)–0.59(0.5–0.8); *IRF7*: $P = 0.0008$, OR(CI)–0.69(0.6–0.9); *TYK2*: $P = 0.04$, OR(CI)–0.79(0.6–0.9)}.

The data was modelled based on mode of inheritance for the 3 SNPs and LR analysis performed controlling for gender and cohorts and revealed an extremely protective effect for the combination of mutations versus the wildtype {*IRF5*^{MM}/*IRF7*^{MM}/*TYK2*^{MM} versus *IRF5*^{WT}/*IRF7*^{WT}/*TYK2*^{WT}: $P < 0.0001$; OR(CI)–0.39(0.3–0.6)}.

In the *IRF5*^{WT}/*IRF7*^{WT}/*TYK2*^{WT} group, the SSc patients had increased levels of TNF- α and IL-6 as compared to controls and there was no difference amongst the patients and controls in the *IRF5*^{MM}/*IRF7*^{MM}/*TYK2*^{MM} group.

Conclusions We demonstrate association of *IRF5*, *IRF7* and *TYK2* SNPs with SSc.

We demonstrate a gene-gene interaction in SSc between three non-linked loci- *IRF5*, *IRF7* and *TYK2*.

The 3 gene-SNPs have a protective effect in SSc patients and the presence of the 3 mutations simultaneously has the most protective effect.

Plasma TNF- α and IL-6 levels were increased in the SSc patients wildtype for the 3 SNPs versus controls, whereas there was no difference in TNF- α and IL-6 levels in the SSc patients having mutations for the 3 SNPs versus controls.

In summary, *IRF5*, *IRF7*, *TYK2* SNPs have a protective effect in SSc which is stronger when there are polymorphisms on all of the genes as compared to each of them alone.

This suggests an important role of interferon pathway polymorphisms in susceptibility to SSc and the exact role of these interactions and their function in SSc susceptibility needs to be elucidated experimentally.

8. Bone/cartilage biology

A8.1 ALARMIN S100A8/A9 CAUSE OSTEOPHYTE FORMATION IN EXPERIMENTAL OSTEOARTHRITIS WITH HIGH SYNOVIAL INVOLVEMENT

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Background and Objectives Osteophyte formation is an important hallmark of osteoarthritis (OA) causing limited joint movement and pain. There is increasing belief that synovial activation contributes to OA pathology. As shown recently in our lab, alarmins S100A8 and S100A9 (major products of synovial macrophages) are involved in cartilage degradation and synovial activation during human and murine OA.

In the current study, we explored the involvement of S100A8/A9 in osteophyte formation in experimental OA.

Methods Experimental OA was elicited in C57Bl/6 (WT) mice and S100A9^{-/-} mice, which also lack functional S100A8. Collagenase induced OA (CIOA) was induced by two times intra-articular

injection of 1U collagenase, DMM was induced by transection of the medial anterior meniscotibial ligament leading to destabilisation of the medial meniscus (DMM). Osteophyte size was assessed by a blind observer using Leica Application Suite (LAS) imaging software. Chondrogenesis was induced by bringing human foetal mesenchymal stem cells (MSCs) in pellet and stimulating for 5 days with BMP-2 and TGF β 1, with or without human recombinant S100A8. Proteoglycan content was quantified using the LAS imaging software on SaFO stained sections.

Results First, we measured osteophyte size in S100A9^{-/-} mice at day 42 of CIOA. Synovial activation is high in CIOA and this is significantly reduced in S100A9^{-/-} mice. Osteophyte size was dramatically reduced in the S100A9^{-/-} compared to WT in the medial collateral ligament (92.5% reduction) but also significantly at the medial side of both tibia and femur (68.2% and 64.6% reduction) (n = 10).

One explanation for the reduced osteophyte size in S100A9^{-/-} mice may be a direct effect of S100-proteins on chondrogenesis. To investigate this, we stimulated MSCs in pellet culture with BMP-2 and TGF β 1, supplemented with 1 and 5 μ g/ml S100A8. Proteoglycan deposition as measured by redness in SaFO staining was increased 27% and 71% respectively, indicating that S100A8 stimulates chondrogenesis.

Finally, we determined osteophyte size in the DMM model, in which synovial involvement is very low. At day 56, we observed no significant differences in osteophyte size between the S100A9^{-/-} and WT at the medial femur and tibia (105% and 136% of WT, n = 8). This confirms the importance of the synovium in the S100-effect on osteophyte development.

Conclusions S100A8/S100A9 play a crucial role in osteophyte formation in an OA model that shows clear synovial involvement, probably by stimulating chondrogenesis.

Considering also the deleterious effect of S100A8/A9 on joint destruction in OA, targeting these alarmins during OA may be very promising.

A8.2 ANTI CITRULLINATED PROTEIN ANTIBODIES FROM SYNOVIAL FLUID OF RHEUMATOID ARTHRITIS PATIENTS ENHANCE OSTEOCLASTOGENESIS

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Background/Purpose Presence of anti CCP2 antibodies identifies a subgroup of RA patients that are more prone to develop bone erosions. We hypothesised that anti CCP2 IgG might have a direct effect on bone, and thus investigated the effect of anti CCP2 IgG isolated from synovial fluid (SF) of RA patients on osteoclastogenesis and bone destruction in an in vitro system.

Methods IgG were isolated on Protein G columns from SF of 26 RA patients and applied on CCP2 affinity columns. Pools of the purified anti-CCP2 and flow through IgG fractions were tested for the ability to influence osteoclastogenesis (TRAP positive multinucleated cells) and bone destruction (% of resorption area on osteologic discs). To do this immature dendritic cells derived from CD14+ cells from peripheral blood of healthy individuals were cultured in the presence of RANKL and M-CSF, with or without CCP2 IgG or flow through IgG (at a final concentration of 100 ng/ml).

Results The CCP2 IgG pool induced a significant mean $\pm$ SEM of 1.5 ± 0.1 fold increase in the number of osteoclasts formed from immature dendritic cells in the presence of RANKL, while no such effect was observed with flow through IgG fractions. Osteoclasts cultured in the presence of the CCP2 IgG induced a significant mean $\pm$ SEM of 3.4 ± 1.3 fold increase of bone resorption while no such effect was observed for the flow through fractions.

Conclusions Here, we demonstrate that ACPA IgG, isolated from SF of RA patients, have the ability to enhance the RANKL-driven osteoclastogenesis 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

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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 S100A4 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 $66 \pm 3\%$, $p < 0.01$) and cartilage destruction (cartilage loss decreased by $63 \pm 6\%$, $p < 0.01$) compared to hTNFtg; S100A4^{+/+} mice. Similarly, osteoclast numbers were decreased by $84 \pm 3\%$ ($p < 0.01$) and bone erosions were less severe (area of bone erosion decreased by $81 \pm 4\%$, $p < 0.01$) 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 $78 \pm 20\%$ ($p < 0.05$) and a decrease in trabecular separation by $39 \pm 4\%$ ($p < 0.05$), decreased numbers of osteoclasts per bone perimeter (NOc/BPm) decreased by $43 \pm 2\%$, ($p < 0.01$), decreased bone surface covered by osteoclasts (Oc.S/BS) decreased by $52 \pm 3\%$, ($p < 0.01$), increased numbers of osteoblasts per bone perimeter (NOb/BPm) increased by $129 \pm 20\%$, ($p < 0.05$) and increased bone formation rate per bone surface (BFR/BS) increased by $112 \pm 18\%$, ($p < 0.05$) 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

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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 Fc γ receptors (Fc γ R) are upregulated during osteoclastogenesis with Fc γ R I and Fc γ R III being the most prominent ones. Desialylated immune complexes induced the activation of spleen tyrosine kinase (Syk) and phospholipase C γ (PLC γ) 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 Fc γ Rs 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

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Background The Fibroblast Activation Protein alpha (FAP α) 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 FAP α in rheumatoid arthritis (RA) and analysed its role in osteoclast development under inflammatory conditions.