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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¹Rik FP Schelbergen, ¹AB Blom, ¹W de Munter, ¹A Sloetjes, ²T Vogl, ²J Roth, ¹WB van den Berg, ¹PLEM van Lent. ¹Dept. of Rheumatology, Radboud Univ. Med. Ctr., Nijmegen, The Netherlands; ²Inst. of Immunology, Univ. of Muenster, Muenster, Germany

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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¹Akilan Krishnamurthy, ¹Nancy Vivar Pomiano, ¹Catia Cerqueira, ¹Elena Ossipova, ¹Karin Lundberg, ²Ulrike Harre, ¹Vivianne Malmström, ¹Per Johan Jakobsson, ³Lars Klareskog, ²Georg Schett, ¹Anca Irinel Catrina. ¹Rheumatology Unit, Karolinska University Hospital, Karolinska Institute, Stockholm, Sweden; ²Institute for Clinical Immunology, University of Erlangen-Nuremberg, Erlangen, Germany; ³Karolinska Institute, Stockholm, Sweden

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.