1b-dependent mechanism in AS patients supporting the auto-inflammatory nature of the disease and possibly representing a future therapeutic target in AS.

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FRI0153 ROLE OF ROR GAMMA T INHIBITION IN THE IL-23 MINICIRCLE MODEL AND SPA PATIENT TISSUE CULTURES

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Background: Retinoic acid receptor related Orphan Receptor gamma t (RORγt) is a nuclear hormone receptor expressed in a subset of pathogenic T cells and innate lymphoid cells. RORγt regulates the transcription of key pro inflammatory cytokines such as IL-17 and IL-22 in response to multiple activation signals including cytokines and T cell receptor engagement. Antagonism of RORγt is hypothesised to block transcription of these pathogenic cytokines resulting in reduced tissue inflammation and aberrant joint remodelling.

Spondyloarthropathy (SpA) is characterised by a peripheral oligoarthritis and enthesitis. A key feature of SpA is the imbalance between bone resorption and formation leading to aberrant bone formation and ankylosis. The contribution of the IL-23/IL-17 axis to the pathogenesis of SpA is supported by several lines of evidence. However, the specific role of RORγt in regulating the cellular and molecular pathways contributing to enthesal and synovial inflammation remains incompletely understood.

Objectives: The goal of this study was to determine the role of RORγt antagonism in both an IL-23 minicircle *in vivo* model and *ex vivo* tissue culture model.

Methods: Model 1: Mice were administered an IL-23 minicircle via hydrodynamic injection to induce systemic IL-23 expression on day 1. Starting on day 2, mice were dosed p.o. for 28 days. Mice were monitored daily for signs of arthritis. Upon sacrifice tissue was collected for mRNA biomarker analysis and histologic assessment.

Model 2: Patients undergoing total hip replacement were recruited into 2 groups: SpA (either psoriatic arthritis or ankylosing spondylitis) or OA (osteoarthritis). Enthesal and synovial tissues were collected and cultured ±RORγt inhibitor and cytokine stimulation (hTNFα/hIL23) for 24 and 48 hours and then analysed for gene expression.

Results: Model 1: Overexpression of IL-23 induced SpA like phenotype including enthesitis, synovitis and aberrant bone formation. RORγt inhibition resulted in significant reduction of inflammation and arthritic score, histologic parameters of bone remodelling, and tissue biomarkers associated with the IL-23 axis (IL17A, IL17F, IL22).

Model 2: Both enthesal and synovial cultures from SpA patients expressed increased Th17 axis genes relative to OA after cultures. RORγt inhibition reduced the expression of these Th17 axis genes (IL17A, IL17F, IL22) as well as reducing tissue remodelling related genes (FN1, MMP1/3, BMP5), in alignment with the results seen in model 1.

Conclusions: IL-23 induced Th17 axis gene expression induced a SpA-like phenotype in mice, including arthritis and aberrant bone formation, that was reversed upon RORγt inhibition. Similarly, SpA patient explants showed increased expression of Th17 axis cytokines and tissue remodelling related genes that were decreased with RORγt inhibition. Together, these complementary approaches support the hypothesis that RORγt treatment could block inflammation as well the underlying pathologies associated with SpA.

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FRI0154

ADDED VALUE OF BIOMARKERS COMPARED TO ROUTINE CLINICAL PARAMETERS FOR THE PREDICTION OF RADIOGRAPHIC SPINAL PROGRESSION IN AXIAL SPONDYLOARTHRITIS

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Background: Structural damage in the spine determines the functional status and spinal mobility in axial spondyloarthritis (axSpA). Already present syndesmophytes, elevated C-reactive protein, cigarette smoking, and to a lesser extent male sex are routine clinical parameters predicting radiographic spinal progression. In the last years, several biomarkers with a predictive value for radiographic spinal progression were identified. It is, however, not known, if biomarkers have a meaningful added value over clinical parameters in prediction of radiographic spinal progression in axSpA.

Objectives: The objective of the study was to examine whether adding biomarkers to the routine clinical parameters would improve prediction of radiographic spinal progression in axSpA.

Methods: Altogether 117 patients with ankylosing spondylitis who completed a 2 year clinical and radiographic follow-up in the ENRADAS trial were included. Radiographic spinal progression was defined as a worsening of the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) by ≥ 2 points after 2 years. A clinical prediction model for radiographic spinal progression was constructed out of already present syndesmophytes, elevated CRP, cigarette smoking, and male sex. Serum biomarkers measured at baseline included: matrix metalloproteinase-3, vascular endothelial growth factor (VEGF), calprotectin, leptin, high molecular weight adiponectin (HMW-APN), osteoprotegerin, sclerostin, N-terminal telopeptide, procollagen type II N-terminal propeptide, and serum amyloid A.

Results: Repeated cross-validation analyses revealed the following biomarker combination with added predictive value for radiographic progression compared to the clinical model (syndesmophytes, smoking, elevated CRP, and sex): Leptin + HMW-APN + VEGF. A combination of these biomarkers resulted in an Area Under the Curve (AUC) of $AUC_{\text{Biomarkers}} = 0.731$ (95%CI 0.614–0.848), thus numerically superior to the clinical model ($AUC_{\text{Clinical}} = 0.665$ (95%CI 0.553–0.776)). A combination of both models resulted in improvement of the predictive value reflected by the $AUC_{\text{Clinical+Biomarkers}} = 0.768$ (95%CI 0.666–0.871); though this improvement was not statistically significant compared to the clinical model in the permutation test ($p=0.051$). However, when only considering the part of receiver operating characteristic (ROC) curves with a specificity of $\geq 75\%$, the improvement becomes statistically significant (partial $AUC_{\text{Clinical+Biomarkers}} = 0.119$ versus partial $AUC_{\text{Clinical}} = 0.053$; $p=0.010$).

Abstract FRI0154 – Figure 1. ROC curve analyses of biomarker and clinical model alone versus the combined model for the prediction of radiographic spinal progression (mSASSS worsening ≥ 2 after 2 years) in ankylosing spondylitis. Area under the curves (AUC) and respective 95% confidence intervals (CI) shown. *Leptin + HMW-APN + VEGF.

Conclusions: Biomarkers are able to improve prediction of radiographic spinal progression in axSpA, especially if used in addition to the clinical parameters, but the added value seems to be rather small.

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FRI0155

GENES REGULATING BONE HOMEOSTASIS ARE DIFFERENTIALLY EXPRESSED AT PERIPHERAL VERSUS AXIAL ENTHELIAL SITES IN AN ANIMAL MODEL OF SPONDYLOARTHRITIS

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Background: In spondyloarthritis (SpA), mechanisms of enthesial bone formation are not well understood. Currently, no treatment can reverse this process, although TNF inhibitors may modify bone formation if initiated early. SKG mice exhibit features of SpA with both peripheral joint and axial inflammation after induction by curdlan via the Dectin-1 pathway residing upstream of TNF α , IL-23 and IL-17.

Objectives: The goal of this study was to identify genes and pathways that regulate enthesial bone formation. SKG mice were previously reported to develop axial bone formation within 12 weeks of curdlan injection, as well as enthesial bone formation around peripheral joints¹.

Methods: 9 week-old SKG or control BALB/c mice were injected IP with curdlan. Histology and microCT scanning were performed at serial time points. Laser capture microscopy was performed on formalin-fixed paraffin-embedded (FFPE) tissue sections from enthesial sites around the ankle and tail vertebrae 3 weeks after curdlan injection. RNA was extracted using the Qiagen RNeasy FFPE kit and whole transcriptome analysis was performed using Affymetrix gene array MTA 1.0.

Results: Inflammation at axial entheses developed earliest at the tail base. Contrary to previous reports, we did not observe axial bone formation, but rather saw significant erosion of vertebral bodies, throughout the spine. The absence of axial bone formation was noted as late as 24 weeks post curdlan. In contrast, exuberant enthesial bone formation occurred around ankles at predictable sites of tendon/ligament insertions into bone. We identified distinct expression of genes involved in bone resorption and formation at ankle when compared to spine enthesial sites. IPA upstream regulator analysis identified significant gene expression of upstream regulators of TGF-beta, known to recruit early osteoblast precursors to bone (z-value 2.83), and WNT3A, a WNT signalling pathway agonist that promotes osteoblast differentiation and function (z-value 2.54), in the ankle, but not the spine. These findings support the histologic observation of bone formation at ankle, but not spine, enthesial sites. In addition, significant increased gene expression of upstream regulators of the aryl hydrocarbon receptor (AhR) (z-value 2.14) was found at spine, but not ankle, enthesial sites (z-value -1.78). AhR is a ligand-activated transcription factor that is a positive regulator of osteoclastogenesis via c-fos-mediated RANKL signalling.

Conclusions: Whole transcriptome analysis revealed increased expression of upstream regulators of genes in anabolic pathways, including WNT3A and TGF-beta, at ankle enthesial sites where bone formation occurs, but not at spine enthesial sites where bone erosions persist without bone formation. These pathways may thus contribute to peripheral enthesial bone formation. Significantly increased gene expression of upstream regulators of AhR, a positive regulator of osteoclastogenesis, was found at spine enthesial sites. AhR is thus a candidate regulator of continued bone resorption at spine enthesial sites in this murine model of SpA.

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FRI0156

AN ANTIMICROBIAL MECHANISM, NETOSIS, IS POTENTIALLY INVOLVED IN ANKYLOSING SPONDYLITIS PATHOGENESIS

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease, of unknown etiology, that mainly affects the axial skeleton and the sacroiliac joints in the pelvis. Neutrophils are critical actors in innate immunity and their activation has been involved in the progression of AS symptoms. Recently, NETosis has been suggested to play a central role in several pathologic states, including rheumatology diseases. Nevertheless, this process has not been described yet in AS patients

Objectives: 1) To evaluate and characterise the presence of NETosis in AS patients. 2) To explore the relationship among NETosis markers and clinical characteristics of this disease

Methods: Thirty patients with AS and 32 healthy donors (HD) were included in the study. Disease activity was determined by BASDAI index and, CRP and ESR levels; in parallel, inflammatory markers were determined in plasma by ELISA kits. Spinal mobility of AS patients was measured by the BASMI index, and structural damage was calculated by the mSASSS index. *Ex vivo*, spontaneous NETosis generation in purified neutrophils from AS patients and HDs were measured by fluorescence (n=6) and scanning electron (n=3) microscopy after 6 hour of incubation. PMA, known to promote NETosis, was used as positive control. DNA extrusion was analysed by fluorescence microscopy and fluorimetry after SYTOX staining, whereas elastase percentage (NE) was analysed by fluorescence microscopy after staining of neutrophils with NE antibody. *In vivo*, mieloperoxidase (MPO) and NE protein expression were measured by flow cytometry (FACSCalibur), whereas extracellular DNA was examined in plasma using fluorimetry after SYTOX staining

Results: Compared to HDs, AS neutrophils showed spontaneous extracellular release of a meshwork of DNA nuclear and granule proteins (NETs), as demonstrated by fluorescence microscopy, fluorimetry, and scanning electron microscopy. Indeed, analysis of DNA fibres staining by SYTOX revealed that NETosis rate was above baseline levels after 6 hour of *ex vivo* AS neutrophil incubation as compared to those from HDs (p<0.05). Furthermore, higher cell free-DNA levels were observed between AS patients and HDs at 6 hour (p<0.05). Increased spontaneous NETs production in this pathology was additionally corroborated by the observation of an enhanced percentage of NE-staining cells after 6 hour of incubation (p<0.05). *In vivo*, higher intracellular NE levels (p=0.036), and plasma DNA concentrations (p=0.021) were also found in AS patients as compared to HDs. Correlation studies showed that plasma DNA levels positively correlated with inflammatory markers (i.e. CRP, ESR and TNF α), and with the spinal mobility index BASMI. In addition, a positive correlation was found between intracellular NE levels and plasma IL-1 β concentration

Conclusions: 1) NETosis is increased in AS patients. 2) Raised NETosis in AS is associated with several markers of inflammation and mobility. Thus, NETosis might act as a key mediator in the etiopathogenesis of AS. Funded by JA PI-0314-2012, SER, ISCIII (RIER RD16/0012/0015)

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FRI0157

ALTERED EXPRESSION OF THE SPLICEOSOME COMPONENTS IN LEUKOCYTES SUBSETS FROM PATIENTS WITH ANKYLOSING SPONDYLITIS: ASSOCIATION TO DISEASE PATHOGENESIS AND THERAPEUTIC RESPONSE

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Background: Ankylosing spondylitis (AS) is a chronic inflammatory disease for which the etiopathogenesis has not yet been fully clarified. Splicing is a post-