Conclusions: Several studies reported the potential of BIO withdrawing, spacing or reducing in the patients with RA who reached remission^{3, 5}. All cases in spacing and withdrawing group have shown DAS28-4ESR<2.6 in this study, however, 50% (5/10 cases) in withdrawing group have re-flare and shown the radiological joint destruction. Spacing or reducing of BIO may have potential to maintain the remission of RA and prevent the joint destruction. Withdrawing of BIO after even sustained deeper remission may be difficult to keep real remission.

REFERENCES:

- [1] O'Dell JR. Arthritis Rheum 2002;46:283–85.
- [2] Smolen JS, et al. Ann Rheum Dis 2017;76:960–77.
- [3] Tanaka Y, et al. Ann Rheum Dis 2015;74:389–95.
- [4] Takeuchi T, et al. Ann Rheum Dis 2014;73:536–43.
- [5] Fautrel B, et al. Ann Rheum Dis 2016;75:59–67.

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Spondyloarthritis – etiology, pathogenesis and animal models

FRI0148 A STUDY OF MICROBIAL TRANSLOCATION IN AN ANIMAL MODEL OF SPONDYLOARTHRITIS

M. Asquith¹, M. Schleisman¹, S. Davin¹, L. Karstens², J.T. Rosenbaum¹. ¹Division of Arthritis and Rheumatic Diseases; ²Department of Medical Informatics and Clinical Epidemiology, Oregon Health and Science University, Portland, USA

Background: The intestinal microbiota is believed to have a central role in SpA pathogenesis. However, the mechanism through which enteric microbes contribute to peripheral inflammation remains enigmatic.

Objectives: The primary objective of this study was to determine whether microbial translocation can be observed to extra-intestinal tissues in the HLA-B27/β2m transgenic rat – a foremost translational model of spondyloarthropathy. Moreover, since an arthritis phenotype only presents in less than half of HLA-B27/β2m transgenic animals, we could examine whether microbiota composition between transgenic animals with and without arthritic disease.

Methods: Intestinal tissue (cecal contents), mesenteric lymph nodes (MLN), spleen, serum, liver, lung, ankle joint and eye were collected from age matched (20–24wk old) HLA-B27/β2m transgenic rats with or without arthritis and WT controls (n=20–45 per group). DNA was extracted and the 16 s rRNA V4 region amplified according to the standard Earth Microbiome Project protocol. Extraction blanks were run with each tissue to control for environmental contamination. Sequencing data (generated by Illumina MiSeq) was first processed using the SourceTracker algorithm to identify and remove contaminant sequences. Remaining reads were run through the DADA2 pipeline implemented in QIIME2.

Results: Our study of microbial translocation revealed a number of striking observations.

Firstly 16 s rRNA was detected at all tissue sites examined. Second, rather than observing a limited number of species, a highly polymicrobial and intestinal DNA signature was observed in all tissue sites examined. This observation was independent of genotype or disease state. The number of total reads in each tissue was highest in cecum as anticipated (approx. 1 00 000 reads) with the yield from other tissues roughly an order of magnitude lower.

The most abundant species in joint tissue included *Prevotella spp.* *Prevotella shahii* and *Prevotella stercorea*, *Roseburia faecis* and *Muribaculum intestinale*. The microbe *Blautia obeum*, a close relative of [*Ruminococcus*] *gnavus* within the same genus was also found in joint tissue. This of interest since this microbe has recently been associated with disease activity in SpA patients.

Interestingly an arthritis phenotype was strongly associated with a loss of intestinal bacterium *Eubacterium Oxidoreducens*. This is a flavone metabolising bacterium and supports previous metabolomic studies in which we have shown flavone compounds are greatly over-represented in the HLA-B27/β2m transgenic rats vs WT controls.

Conclusions: We propose translocation of microbes/microbial products from the gut to extra-intestinal tissues may be a contributory mechanism to SpA pathogenesis, although alone is not sufficient to elicit inflammatory disease. Specific changes in microbial community DNA profile in the gut or elsewhere may serve as useful biomarkers of disease state in either patient populations or disease models. This approach may yield useful candidates for further study such as *Eubacterium Oxidoreducens*. Future studies will verify our findings using PCR-independent methods.

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Disclosure of Interest: None declared

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FRI0149 INFLAMMATION INTENSITY-DEPENDENT EXPRESSION OF OSTEOINDUCTIVE WNT PROTEINS IS CRITICAL FOR ECTOPIC NEW BONE FORMATION IN ANKYLOSING SPONDYLITIS

H. Liu, X. Li, J. Wang. Spine Surgery, The first affiliated hospital of Sun Yat-sen University, Guangzhou, China

Objectives: To investigate the molecular mechanism underlying the inflammation-related ectopic new bone formation in ankylosing spondylitis (AS).

Methods: Spinal tissues and sera were collected from patients or normal volunteers to detect the expression of Wnt proteins. An *in vitro* cell culture system mimicking the local inflammatory microenvironment of bone-forming sites was established to study the relationship between inflammation and Wnt expression, the regulatory mechanism of inflammation-induced Wnt expression and the role of Wnt signalling in new bone formation. A modified collagen-induced arthritis (mCIA) and a proteoglycan-induced spondylitis (PGIS) animal model were used to confirm the key findings *in vivo*.

Results: The levels of osteoinductive Wnt proteins were obviously increased in the sera and spinal ligament tissues of patients with AS. Only constitutive low-intensity TNF-α stimulation, but not short-term or high-intensity TNF-α stimulation, induced persistent expression of osteoinductive Wnt proteins and subsequent bone formation through NF-κB (p65) and JNK/AP-1 (c-Jun) signalling pathways. Furthermore, inhibition of either Wnt/β-catenin or Wnt/PKCδ pathway significantly suppressed new bone formation. The increased expression of Wnt proteins was confirmed in both mCIA and PGIS models. A kyphotic and ankylosing phenotype of the spine was observed during long-term observation in mCIA model. Inhibition of either Wnt/β-catenin or Wnt/PKCδ signalling pathway significantly reduced the incidence and severity of this phenotype.

Conclusions: Inflammation intensity-dependent expression of osteoinductive Wnt proteins is a key link between inflammation and ectopic new bone formation in AS. Activation of both canonical Wnt/β-catenin and noncanonical Wnt/PKCδ pathways is required for inflammation-induced new bone formation.

Abstract FRI0149 – Figure 1. (A) IHC staining of Wnt proteins in spinal tissues from patients with AS and DS. (B) ELISA analysis of Wnts expression in RAW cells subject to different pattern of TNF-α stimulation. (C) Alizarin red staining of MC3T3 cells stimulated with CM produced from Raw cells transfected with Wnt siRNAs. (D) Site-directed mutagenesis analysis of the Wnt3a promoter. (E) MicroCT images of the spines and hind paws of the mCIA mice. (F) Immunohistological staining of Wnt3a, Wnt5a and Wnt7b in

lumbar spine specimens from mCIA mice. (G) A schematic diagram illustrating the theory in the current study.

Disclosure of Interest: None declared

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FRI0150

MTOR BLOCKADE BY RAPAMYCIN DECREASES ARTHRITIS AND SPONDYLITIS DEVELOPMENT AND SEVERITY IN HLA-B27 TRANSGENIC RATS

S. Chen¹, M. van Tok¹, D. Pots¹, J. Taurog², M. van de Sande¹, D. Baeten¹, L. van Duivenvoorde¹. ¹Clinical Immunology and Rheumatology, Amsterdam Medical Center, Amsterdam, Netherlands; ²Internal Medicine, Rheumatic Diseases Division, UT Southwestern Medical Center, Dallas, USA

Background: HLA-B27 misfolding is thought to play an important role in the pathogenesis of spondyloarthritis (SpA), possibly through triggering of ER stress and the unfolded protein response. One of the mechanisms that regulates the unfolded protein response is autophagy. Autophagy is a process that degrades proteins, cytoplasmic particles and organelles in lysosomes and is regulated by protein kinases, mechanistic target of rapamycin (mTOR) and AMP activated protein kinase.

Objectives: To study whether blockade of mTOR will affect spondyloarthritis development and/or severity in the *Mycobacterium tuberculosis* (*M. tub*) induced disease HLA-B27 tg rat model.

Methods: 6 weeks old, female or orchiectomized male HLA-B27/Huβ2m transgenic rats were immunised with 60–90 µg heat-inactivated *M. tub* in IFA. Rats were prophylactically or therapeutically treated three times a week intra-peritoneally with 1.5 mg/kg rapamycin or vehicle. Clinical measurements included weight, clinical scores for spondylitis and arthritis, and hind paw swelling measured by plethysmometry. After 5 weeks of treatment rats were sacrificed; axial and peripheral joints were isolated for histology and metacarpophalangeal joints, spleen and lymph nodes were isolated for RNA isolation.

Results: In the prophylactic experiment 72.7% (8/11) and 18.2% (2/11) rapamycin treated rats developed arthritis and spondylitis compared to respectively 100% (13/13; p=0.0225) and 92.3% (12/13; p<0.0001) control animals. Also severity of arthritis and spondylitis was significantly decreased in rapamycin treated animals compared to control treated animals; mean arthritis severity of diseased rats was respectively 0.45 versus 7.15 on a scale from 0–12 (p<0.0001) and mean spondylitis severity was respectively 0.18 versus 2.07 on a scale from 0–3 (p<0.0001). Clinical findings were confirmed by histology with a significant decrease of inflammation (p<0.0001), bone- and cartilage destruction (p=0.0021) and new bone formation (p=0.0010) in peripheral joints of rapamycin treated rats compared to vehicle treated rats and a similar trend was observed in spinal joints. Also in a therapeutic setting rapamycin treatment decreased arthritis severity (mean score of 6 compared to 8.8 in controls; p=0.0317) and spondylitis severity (mean score of 1.23 compared to 2.8 in controls; p=0.0159). Histology for the therapeutic experiment is currently being performed as well as RNA analyses for autophagy genes and pro-inflammatory cytokines, like IL-17A and TNF.

Conclusions: mTOR blockade significantly suppressed arthritis and spondylitis in the *M. tub* induced disease HLA-B27 transgenic rat model of SpA.

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FRI0151

PROTEIN INHIBITOR OF ACTIVATED STAT3 PREVENTS PERIPHERAL ARTHRITIS AND GUT INFLAMMATION BY REGULATING TH17/TREG CELL IMBALANCE VIA STAT3 SIGNALLING IN MICE MODEL OF SPONDYLOARTHRITIS

E. Kwon¹, H.K. Min¹, M.Y. Kim¹, J. Choi², S.-Y. Lee², H.-B. Seo², K. Jung³, H. S. Na², J.-G. Ryu², S.-K. Kwok¹, M.-L. Cho², S.-H. Park¹. ¹College of Medicine, The Catholic University of Korea; ²Catholic Research Institute of Medical Science, The Catholic University of Korea; ³Impact Biotech, Seoul, Korea, Republic Of

Background: Spondyloarthritis (SpA) is inflammatory arthritis, and interleukin (IL) –17 is crucial on pathogenesis of SpA. Type 17 helper T cell (Th17) is one of major IL-17 secreting cells. Signal transducer and activator of transcription (STAT)–3 signalling induces Th17 cell differentiation. Present study investigated the effect of protein inhibitor of activated STAT3 (PIAS3) on SpA pathogenesis.

Methods: Curdlan was injected to SKG ZAP-70^{W163C} mice for SpA induction. Then PIAS3 or Mock vector was inserted to mice for 10 weeks. Clinical score and histologic scores of paw, spine, and gut were evaluated. Expressions of IL-17, tumour necrosis factor-α (TNF-α), STAT3, bone morphogenic protein (BMP) were

measured. Confocal stain and flow cytometry were used to assess helper T cell differentiation.

Results: PIAS3 significantly diminished the histological scores of paw and gut. PIAS3 group displayed lesser expression of IL-17, TNF-α, and STAT3 in the paw, spine, and gut. BMP-2/4 expressions were lower in spine of PIAS3 group. Helper T cell differentiation was polarised toward upregulation of regulatory T cell (Treg) and downregulation of Th17 in the PIAS3 mice.

Conclusions: PIAS3 showed preventive effects in mice with SpA by suppressing peripheral arthritis and gut inflammation. Proinflammatory cytokines and Th17/Treg differentiation were controlled by PIAS3. Additionally BMPs were decreased in spine of PIAS3 mice. These findings suggest that PIAS3 could be a potential therapeutic choice of SpA treatment.

REFERENCES:

- [1] Smith JA, Colbert RA: Review: The interleukin-23/interleukin-17 axis in spondyloarthritis pathogenesis: Th17 and beyond. *Arthritis & rheumatology* 2014, 66:231–41.
- [2] O'Shea JJ, Lahesmaa R, Vahedi G, Laurence A, Kanno Y. Genomic views of STAT function in CD4+ T helper cell differentiation. *Nature reviews Immunology* 2011;11:239–50.
- [3] Miossec P, Korn T, Kuchroo VK: Interleukin-17 and type 17 helper T cells. *The New England journal of medicine* 2009;361:888–98.
- [4] Ruutu M, Thomas G, Steck R, Degli-Esposti MA, Zinkernagel MS, Alexander K, Velasco J, Strutton G, Tran A, Benham H, Rehaume L, Wilson RJ, Kikly K, Davies J, Pettit AR, Brown MA, McGuckin MA, Thomas R. beta-glucan triggers spondylarthritis and Crohn's disease-like ileitis in SKG mice. *Arthritis and rheumatism* 2012;64:2211–22.

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FRI0152

INFLAMMASOMES ACTIVATION OCCURS IN THE INFLAMED TISSUES OF AS PATIENTS AND DRIVES IL-23 EXPRESSION

F. Ciccia¹, A. Rizzo², G. Guggino¹, R. Alessandro¹, S. Raimondo¹, F. Macaluso¹, S. Peralta¹, S. Milling³, D. Elewaut⁴. ¹University of Palermo; ²Azienda Ospedaliera Ospedali Riuniti Villa Sofia Cervello, Palermo; ³University of Glasgow, Glasgow, Italy; ⁴University of Ghent, Ghent, Belgium

Background: A growing body of evidences indicate that the aberrant activation of innate immune systems, occurring in genetically predisposed patients, drives inflammatory processes in Ankylosing Spondylitis (AS).¹

Objectives: Aim of this study was to evaluate the activation and the functional relevance of inflammasome pathways in patients with AS.

Methods: Intestinal, synovial and bone marrow expression of inflammasome pathways, pyroptosis and IL-1b and IL-18 was evaluated in AS patients. Organic acid extraction was performed on ileal samples as previously described on. The expression of the metabolite-sensing receptors GPR43 and GPR109A involved in the regulation of the intestinal inflammasome was also assessed. The role of intestinal dysbiosis in modulating inflammasome activation was also studied in AS patients and HLA-B27 transgenic rats. Inflammasome activation was evaluated in isolated peripheral AS monocytes. The role of LPS, PGE2 and nicotine in inducing monocyte inflammasome activation and the role of inflammasome in modulating IL-23 production was also evaluated.

Results: Activation of inflammasomes was observed in the inflamed gut, synovial and bone marrow samples of AS patients and associated with an increased expression of caspase-1, IL-1b and IL-18. In AS, AIM2 expression was observed in the context of tuft cells and of adherent ileal bacteria. Inflammasome activation in AS gut, was associated with the occurrence of dysbiosis and increased pyroptosis as demonstrated by the membrane localization of Gasdermin D. Isolated intestinal bacteria from AS ileal samples, significantly modulated inflammasome activation in isolated monocytes. Reduced Short-chain fatty acids concentrations and increased expression of GPR43 and GPR109 were demonstrated in the AS ileal samples. Inflammasome activation was also observed in the inflamed gut of HLA-B27 TG rats and suppressed by antibiotics treatment. Increased expression of NLRP3, NLRC4 and AIM2 was confirmed in AS isolated peripheral monocytes. Serum levels of IL-1b and IL-18 were increased in AS patients, especially in smoker patients, and directly correlated with the ASDAS-CRP. In *in vitro* studies, LPS and nicotine strongly activated NLRP3, NLRC4 and AIM2 pathways in AS monocytes. The CC genotype of PTGER4 SNP rs6896969 was associated with a significantly increased activation of inflammasome in AS. Finally, inflammasome activation in AS monocytes was required for the induction of IL-23p19 expression in an IL-1b-dependent way.

Conclusions: Inflammasome activation occurs in AS patients being modulated by a plethora of different stimuli. Inflammasome drives IL-23 production in an IL-