Conclusions: Several studies reported the potential of BIO withdrawing, spacing or reducing in the patients with RA who reached remission. All cases in spacing and withdrawing group have shown DIAS29–4ESR-2.6 in this study, however, 50% (5/10 cases) in withdrawing group have re-flare and showed 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:

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Spondyloarthritis – etiology, pathogenesis and animal models

A STUDY OF MICROBIAL TRANSLLOCATION IN AN ANIMAL MODEL OF SPONDYLOARTHITIS
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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 in extra-intestinal tissues in the HLA-B27*02 transgenic rat – a foremost translational model of spondyloarthritis. Moreover, since an arthritis phenotype only presents in less than half of HLA-B27*02 transgenic animals, we could examine whether microbiota composition between transgenic animals with and without arthritic disease.

Methods: Intestinal tissue (cecral contents), mesenteric lymph nodes (MLN), spleen, serum, liver, lung, ankle joint and eye were collected from age matched (20–24wk old) HLA-B27*02 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. 1 000 000 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 stenoreca. Roseburia faecis and Mmbaculum intestinale. The microbe Blautia obeum, a close relative of [Ruminococcus] gravis 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 bacte- rium and supports previous metabolomic studies in which we have shown flavone compounds are greatly over-represented in the HLA-B27*02 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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INFLAMMATION INTENSITY-DEPENDENT EXPRESSION OF OSTEOSTIMULATORY WNT PROTEINS IS CRITICAL FOR ECTOPIC NEW BONE FORMATION IN ANKYLOSING SPONDYLITIS
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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 osteostimulatory 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 osteostimulatory 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/PKCβ3 pathway significantly suppressed new bone formation. The increased expression of Wnt proteins was confirmed in both mCIA and PGIS models. A kyphtic and ankylosing phenotype of the spine was observed during long-term observation in mCIA model. Inhibition of either Wnt/β-catenin or Wnt/PKCβ3 signalling pathway significantly reduced the incidence and severity of this phenotype.

Conclusions: Inflammation intensity-dependent expression of osteostimulatory 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/PKCβ pathways is required for inflammation-induced new bone formation.

Abstract FR0149 – 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) Immunohistochemical staining of Wnt3a, Wnt1a and Wnt7b in

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

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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 rats.

Methods: 6 weeks old, female or orchiectomized male HLA-B27/HuI2m 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=0.0022) 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 – 8 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 development and/or severity in the Mycobacterium tuberculosis (M. tub) induced disease HLA-B27 tg rat model of SpA.

Disclosure of Interest: None declared


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

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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).

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 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-ESR. In vitro studies, LPS and nicotine strongly activated NLRP3, NLRC4 and AIM2 pathways in AS monocytes. The CC genotype of PTGER4 SNP rs6898969 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-