AB0145 REGULATION OF OSTEOBLASTS BY ALKALINE PHOSPHATASE IN ANKYLOSING SPONDYLITIS

S. Jo¹, J. Han², Y.L. Lee¹, T.-H. Kim¹. ¹Rheumatology, Hanyang University Hospital for Rheumatic Disease; ²Gencurix, Inc, Seoul, Korea, Republic of Ireland

Background: Ankylosing spondylitis (AS) is characterized by excessive spinal ankylosis and bone formation. Alkaline phosphatase (ALP) activity is reported to be high in AS, but little is known about the molecular relationship between ALP and AS.

Objectives: The aims of this study were to investigate the relevance of ALP to AS and the role of ALP in the regulation of osteoblast differentiation in AS.

Methods: The high-throughput data were downloaded from Gene Expression Omnibus with accession numbers GSE73754 and GSE41038. We retrospectively collected the ALP levels of male patients with AS, to compare with those of gender and age-matched healthy controls (HC) and rheumatoid arthritis (RA) patients. Total ALP and ALP activity in patient's sera were measured in AS and RA groups. ALP gene expression and intracellular ALP activity were analyzed in microarray data of primary bone-derived cells (BdCs) and in In Vitro experiments. Furthermore, the effect of ALP knockdown and inhibitor were performed in primary BdCs and human osteoblasts cells, respectively.

Results: ALP level was increased in AS compared with RA and HC. Increased ALP level in AS was associated with radiograph progression. ALP expression was also enriched in bone tissue of AS patients. Furthermore, AS BdCs exhibited elevated ALP activity, leading to accelerating osteoblastic activity and differentiation. Intriguingly, suppression of ALP expression and activity inhibited expression of osteoblastic genes, which are critical for osteogenic differentiation.

Conclusions: A multifaceted analysis showed ALP was highly expressed in AS patients. ALP may be involved in the ankylosis of AS and a therapeutic target for preventing ankylosis.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6048

AB0146 APREMILAST POTENTLY INHIBITS IL-12/IL-23P40 PRODUCTION IN HUMAN ARTHRITIC EX VIVO MODELS

T.W. Kragstrup^{1,2}, M. Adams³, S. Lomholt¹, M. Nielsen¹, L. Heftdal¹, P. Schafer³, B. Deleuran^{1,4}. ¹Department of Biomedicine, Aarhus University, Aarhus C; ²Department of Internal Medicine, Randers Regional Hospital, Randers, Denmark; ³Department of Translational Development, Celgene Corporation, Summit, USA; ⁴Department of Rheumatology, Aarhus University Hospital, Aarhus C, Denmark

Background: Apremilast (Otezla) is a phosphodiesterase 4 (PDE4) inhibitor approved for the treatment of psoriasis and psoriatic arthritis (PsA), but the reason why apremilast shows clinical effect in PsA is not fully understood.

Objectives: The objective of this study was to study the downstream effects of apremilast on cells of the inflamed joint in *ex vivo* models of immune mediated inflammatory arthritis. First, we tested the effect of apremilast on the secretion of several cytokines, chemokines and growth factors by synovial fluid mononuclear cells (SFMCs). Then, we tested whether apremilast affect factors involved in structural changes by studying fibroblast-like synovial cells (FLSs), osteoclasts, synovial macrophages, and osteoblasts.

Methods: Synovial fluid was obtained from a study population consisting of patients with active rheumatoid arthritis (RA), psoriatic arthritis (PsA) or peripheral spondyloarthritis (SpA) with at least one swollen joint (n=18). Synovial fluid mononuclear cells (SFMCs) cultured for 48 hours were used to study the effect of apremilast on secretion of a large panel of cytokines, chemokines and growth factors using the Olink Proseek Multiplex interferon I panel. These effects were compared with the effects of the tumour necrosis factor alpha (TNF α) inhibitor adalimumab. Further, fibroblast-like synovial cells (FLSs) were used to study metalloproteinase secretion, SFMCs cultured for 21 days were used to study inflammatory osteoclastogenesis and macrophage differentiation, an osteoclast pit formation assay was used to study osteoclast activity, and a mineralization assay was used to study new bone formation.

Abstract AB0146 – Figure 1

Results: In SFMCs cultured for 48 hours apremilast decreased the production of IL-12/IL-23p40 (the shared subunit of IL-12 and IL-23) ($p < 0.00001$), colony stimulating factor 1 ($p = 0.009$), CD6 ($p = 0.03$), CD40 ($p = 0.04$), and MCP-1 ($p = 0.02$), and increased the production of C-X-C motif chemokine 5 ($p = 0.003$) dose-dependently. In sub-analyses, the apremilast induced decrease in cytokine production was greater in cultures with a high lymphocyte count and in cultures from patients with a low C-reactive protein level. Further, apremilast had a very different response signature compared with adalimumab, e.g. with a much greater inhibition of IL-12B ($p = 0.01$) and less inhibition of IL-8 ($p = 0.0001$) (see Figure). In SFMCs cultured for 21 days apremilast increased the secretion of IL-10 ($p = 0.04$) and in FLS cultures apremilast decreased MMP3 production ($p = 0.005$). Apremilast decreased osteoclast pit formation but did not change mineralization by human osteoblasts.

Conclusions: This study reveals the downstream effects of apremilast in *ex vivo* models of arthritis with a strong inhibition of IL-12/IL-23p40. Our findings could explain some of the efficacy of apremilast seen in IL-12/IL-23 driven immune mediated inflammatory diseases such as psoriasis and psoriatic arthritis.

Disclosure of Interest: T. Kragstrup: None declared, M. Adams: None declared, S. Lomholt: None declared, M. Nielsen: None declared, L. Heftdal: None declared, P. Schafer Shareholder of: Celgene, Employee of: Celgene, B. Deleuran: None declared

DOI: 10.1136/annrheumdis-2018-eular.2348

AB0147 FAECAL MICROBIOTA STUDY IDENTIFIES DYSBIOSIS IN ANKYLOSING SPONDYLITIS PATIENTS

Z. Chen, J. Qi, X. Zheng, X. Wu, X. Li, J. Gu. Rheumatology, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Background: Ankylosing spondylitis (AS), a prototype of spondyloarthritis (SpA), is a chronic inflammatory disorder with diverse clinical phenotypes. It is widely accepted that AS is genetically determined and trigger of environmental factors is common. Accumulating research have indicated that gut microbiota may play a role in the pathogenesis of AS.

Objectives: We conduct this study to characterise and investigate differences in the gut microbiome between patients and healthy donors.

Methods: 41 patients with AS fulfilled the modified New York criteria for AS and 19 healthy controls (HCs) were recruited in this study. Fresh faecal samples were collected and microbial DNA were extracted by faecal DNA extraction kit according to the manufacture's instruction. V4 hypervariable region of the 16S ribosomal RNA (16S rRNA) was amplified and sequenced on an IlluminaHiSeq2500 platform. The resulting sequencing data were analysed through an in-house bioinformatics pipeline, including removing the barcodes and primers, merging forward and reverse reads, filtering tags, removing chimaeras, clustering and annotation. The mean species diversity for each sample and the differentiation among those samples are captured by alpha and beta diversity, respectively. Principle Coordinate Analysis (PCoA) was performed to get principal coordinates and visualise from complex, multidimensional data.

Results: 16S rRNA community profiling of the faecal sample yielded high sequencing depth, with quality-filtered and connexion to generate a mean number of Taxon_tag being 66398±5606 versus 63061±5474 in AS and HCs group respectively. The gut microbial communities were significantly different and more diverse in AS patients when compared with the HCs by calculating metrics (Chao1) of a diversity ($p < 0.001$). PCoA analysis shown that gut microbiota was able to predicted samples to be AS or HCs. At phylum level, the profile of gut microbiota were dominated by *Bacteroidetes* (56.15% vs 53.31%), *Proteobacteria* (11.19% vs 9.97%) and *Firmicutes* (30.10% vs 34.22%) in both AS and HCs groups respectively. However, difference of distribution were appeared between two groups, such that *Bacteroidetes* and *Proteobacteria* were more common in AS group, while *Firmicutes* was more common in HCs group. At genus level, both group were dominated by *Bacteroides* and *Prevotella-9*. Abundance of *Prevotella-9* were great different between AS patients and HCs with relative abundance of 16.07% and 2.86%, respectively.

Conclusions: These results suggested that faecal microbiota of patients with AS differed significantly from that of healthy controls. Further analysis and larger cohort replication will be helpful to identified specific microbial marker of AS.

REFERENCES:

- van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984;27(4):361–68.
- Chao A, Chazdon RL, Colwell RK, et al. Abundance-based similarity indices and their estimation when there are unseen species in samples. *Bio-metrics* 2006;62(2):361–71.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6877