AB0145 REGULATION OF OSTEOBLASTS BY ALKALINE PHOSPHATASE IN ANKYLOSING SPONDYLITIS

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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

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

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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.

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AB0147 FAECAL MICROBIOTA STUDY IDENTIFIES DYSBIOSIS IN ANKYLOSING SPONDYLITIS PATIENTS

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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.

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SLE, Sjögren's and APS – etiology, pathogenesis and animal models

AB0148

EXTRACELLULAR VESICLE-MEDIATED DELIVERY OF EBV SMALL RNA (EBER1) ACTIVATES LUPUS NEPHRITIS RELATED ANTIVIRAL IMMUNITY IN TUBULAR EPITHELIAL CELLS VIA TLR3

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Background: In lupus nephritis (LN), genetic and environmental factors drive the chronic activation of antiviral defenses leading to immune complex-mediated glomerular and tubular damage. Increasing evidence suggests the involvement of extracellular vesicles (EVs) in autoimmune disease. Currently a role for EVs in the pathogenesis of lupus nephritis has not been proposed.

Objectives: To investigate the role of EVs in the pathogenesis of LN.

Methods: To determine the presence of EVs in kidneys, biopsies from LN patients and IgA- nephropathy and Focal Segmental Glomerulosclerosis control patients were used. Serum samples from SLE patients and from RA patients as controls were used to determine the presence of circulating EVs. Primary renal tubular epithelial cells (TEC) were cultured, and Kidney injury molecule-1 (KIM1) expression was assessed by FACS. Exosomes were analyzed by electron microscopy and western blot. mRNA analysis was performed by qPCR. TLR3 inhibition was performed with TLR3/dsRNA complex inhibitor and with hydroxychloroquine.

Results: We show that EVs deliver virus-derived small RNA and activate TEC via toll-like receptor 3 (TLR3). Highly specific stem-loop RT-PCRs revealed Epstein Barr Virus (EBV)-encoded small RNAs in LN biopsies while quantitative EBV-DNA PCR, sensitive to a single copy was negative. In situ hybridization failed to detect nuclear EBV-EBER1 (i.e. EBV-infected cells) in LN biopsies. However, we observed atypical EBER signal in the cytoplasm of TECs in LN but not in disease control biopsies, suggestive of uptake of extra-renal EBER. Consistent with this, we detected EBER1 in circulating EVs of SLE sera. The LN tissues express strongly elevated levels of TLR3, Interferon induced transmembrane-1 and -3, and TNF α . Primary TEC cultured in vitro endocytose EBER1-EVs secreted by EBV-infected B cells via phosphatidylserine receptors such as KIM-1. Importantly, EV-EBER1 uptake by TEC triggers antiviral immunity and pro-inflammatory cytokine secretion in a Toll-like receptor 3 (TLR3)-dependent manner. Treatment with hydroxychloroquine (HCQ) or a small molecule inhibitor that blocks TLR3-RNA interactions strongly reduced the pro-inflammatory effects of EBER1.

Conclusions: We propose that small RNA-loaded EVs exacerbate pre-existing autoimmunity in SLE patients by engaging tubular epithelial TLR3, supporting the rationale for TLR3-blockade as therapeutic strategy in the treatment of lupus nephritis.

Disclosure of Interest: None declared

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AB0149

DECREASED MICRORNA-130A EXPRESSION DRIVES ACTIVATION OF CLASSICAL DENDRITIC CELLS FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Background: Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and dryness of mouth and eyes. Classical dendritic cells are very potent antigen presenting cells known to induce strong T-cell proliferation and cytokine production.

Objectives: Considering the critical role of microRNAs (miRNAs) in regulation of gene expression, we investigated miRNA expression in circulating CD1c⁺ dendritic cells (cDCs) of patients with pSS.

Methods: Two independent cohorts consisting of pSS patients and healthy controls were established: a discovery cohort (15 pSS, 6 HC) was used to screen the

expression of a large panel of 758 miRNAs. An independent validation cohort (14 pSS, 11 HC) was used to test the reproducibility of the results. cDCs were isolated from peripheral blood using MACS and miRNA profiling of 758 targets was performed using the OpenArray platform in the discovery cohort. A selection of 16 differentially expressed miRNAs was measured in the validation cohort using a custom-made array. Isolated cDCs from HC were stimulated with a panel of Toll-like receptor (TLR) ligands and the expression of miR-130a and miR-708 was measured by qPCR. The effect of transfection with miR-130a on protein synthesis was analysed by using the pulsed stable isotope labelling by amino acids in cell culture (pSILAC) method (quantitative mass spectrometry-based technique) in a HEK-293T cell-line.

Results: A total of 24 miRNAs was downregulated in pSS patients versus HC in the discovery cohort ($p < 0.05$, with a difference between the groups of $> \log_2$). Of the 16 miRNAs that were selected for replication, the decreased expression of miR-130a and miR-708 in pSS was validated. Activation of cDCs via TLR3 and TLR7/8 induced downregulation of both miRNA-130a and miRNA-708. Transfection with a miR-130a mimic resulted in downregulation of several proteins with a seed match for the miRNA. These proteins are known to be involved in membrane trafficking and cell activation through CREB/NF- κ B signalling.

Conclusions: miR-130a and miR-708 are significantly downregulated in cDCs of patients with pSS. We show that the expression of these miRNAs is decreased upon cDC activation and that upregulation of miR-130a decreases the expression of proteins involved in the CREB/NF- κ B pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of circulating cDCs from pSS patients.

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AB0150

DIMINISHED EXPRESSION OF PD-L1 BY ACTIVATED B CELLS IS CHARACTERISTIC OF SLE

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Background: Programmed cell death 1 (PD-1) and its ligands PD-L1 and PD-L2 are known to play an important role in immune response regulation via a co-inhibitory signal during T cell activation. Genome-wide association studies in humans and the fact that PD-1 knock-out mice develop a lupus-like pathology point toward the involvement of the PD-1 pathway in the pathogenesis of systemic lupus erythematosus (SLE).^{1,2} However, little is known regarding the role of this pathway in B cells from patients with SLE.

Objectives: This study addressed the kinetics of PD-1, PD-L1 and PD-L2 expression on B cells from patients with SLE.

Methods: Blood samples were obtained from healthy donors (HD) and SLE patients. PBMCs from HD and SLE patients were stimulated with IL2/IL10, aBCR (IgG/IgM/IgA), CpG oligodeoxynucleotides (CpG) and CD40L alone or in combination. The membrane expression of PD-1, PD-L1 and PD-L2 on CD19⁺CD20⁺B cells was measured by FACS at baseline and after 48 hour stimulation.

Results: At baseline, the expression of PD-1, PD-L1 and PD-L2 on B cells between HD and SLE patients was similar. Notably, 48 hour stimulation of B cells with IL2/IL10, aBCR, CpG and CD40L gave rise to twofold reduced expression of PD-L1 by SLE compared to HD B cells. Interestingly, PD-L1 expression by SLE B cells was increased only in presence of CD40L, whereas in HD the mix of CpG, aBCR, and IL2/IL10 led also to a significant increase in PD-L1 expression.

Abstract AB0150 – Figure 1. Diminished PD-L1 expression in activated B cells may explain increased activation of T cells in SLE. (A) CD40L activated HD B cells upregulate PD-L1 that suppress activated T cells via PD-1. (B) CD40L activated SLE B cells fail to upregulate PD-L1 leaving activated T cells unchecked