OP0293

A FIRST-IN-CLASS METABOLIC REPROGRAMMING AGENT, MBS2320, SELECTIVELY MODULATES IMMUNE CELL FUNCTION AND IMPROVES OSTEOID FORMATION AND BONE PROTECTION VERSUS ETANERCEPT IN THE MOUSE COLLAGEN-INDUCED ARTHRITIS MODEL

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Background: Despite the availability of several treatment options, some Rheumatoid Arthritis (RA) patients do not reach low disease activity. There thus remains a need for differentiated new therapies. Prior studies identified a series of biphenylsulphonamides with bone protecting and anti-inflammatory activity^{1,2} which might meet this need.

Objectives: To evaluate the selectivity of a novel small molecule, MBS2320, in myeloid and lymphoid cells, characterise its selectivity for osteoclasts versus osteoblasts and characterise its effects on synovitis and osteoprotection compared to an anti-TNF α agent in a therapeutically administered collagen-induced arthritis (CIA) mouse model.

Methods: Viability, proliferation or cytokine production were assessed in human primary monocytes, lymphocytes or a Mixed Lymphocytes Reaction (MLR). Human primary osteoclasts (OCs) were differentiated from CD14⁺ monocytes with M-CSF and RANK-L. Mature OCs were stained with tartrate-resistant acid phosphatase (TRAP) and quantified by light microscopy. Osteolytic activity was assessed on mineral-coated surfaces. Osteoblasts were derived from mesenchymal stem cells by differentiation in the presence of MBS2320; mineralisation was assessed by Alizarin Red S staining. CIA was induced in DBA/1J mice by collagen immunisation. MBS2320 and etanercept were dosed once daily after onset of disease. Arthritic Index (AI) was scored throughout the dosing period after which serial paw sections were assessed for inflammation, synovitis, stromal cavity osteolysis, pannus hyperplasia, osteoid layering and bone resorption foci.

Results: MBS2320 reduced the production of cytokine from monocytes and inhibited T-cell proliferation, and cytokine production in a MLR. MBS2320 also reduced primary OC differentiation and function *in vitro* to a greater degree than alendronate but showed no effect on the differentiation of primary osteoblasts. MBS2320 (0.3 mpk/d) and etanercept (10 mpk/d) inhibited the onset and severity of CIA arthropathy, and synovitis, pannus infiltration and osteolysis, with equivalent efficacy. In addition MBS2320 showed anatomically appropriate osteoid layering with conservation of the tide mark, and the bone marrow showed no cell atypia, with all progenitor classes present although reduced in number and distribution. In contrast, osteoid formation in the etanercept group was multi-focal with an often erratic tide-mark morphology and was localised to areas of reduced osteoclastic resorption and osteolysis with erratic osteocyte distribution.

Conclusion: MBS2320 selectively inhibits myeloid and lymphoid activity/differentiation, whilst sparing mesenchymal cells, *in vitro*. In murine CIA, MBS2320 treatment led to the formation of anatomically appropriate osteoid layering indicating an ongoing process of osteogenesis conditioned by biomechanics. By contrast osteoid formation due to etanercept was more 'reactive' and secondary to suppression of inflammation. The data suggest that MBS2320 offers equivalent anti-inflammatory activity, but a broader spectrum of osteoprotective efficacy in CIA compared to TNF α inhibition and may offer an alternative therapeutic approach to improving bone quality in RA.

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OP0294

TRANSCRIPTIONAL PROFILING OF RA PATIENTS SYNOVIAL TISSUE REVEALS TARGETS FOR PRECISION MEDICINE

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Background: We were the first in the United States to demonstrate the applicability of performing transcriptional profiling of macrophages cells isolated from ultrasound guided synovial biopsies on RA patients (Mandelin et al, *A&R* 2018¹). While the numbers of macrophages are known to correlate with response to therapy in RA patients, little is known about macrophage heterogeneity or dendritic cell (DC) involvement in disease.

Objectives: Here, we performed single cell RNA seq and bulk population RNA-seq on RA synovium and peripheral blood (classical monocytes, non-classical monocytes, and DCs) from RA patients in order to compare their genome-wide transcriptional profile within and across individuals.

Methods: We obtained blood samples and ultrasound-guided minimally invasive synovial biopsy tissue from RA patients with active disease. Using Fluorescence-Activated Cell Sorting (FACS), we isolated classical monocytes (MHCII+CD14⁺+CD16⁻), non-classical monocytes (MHCII+CD14⁺+CD16⁺), and dendritic cells (MHCII+CD1c⁺) from blood. After processing synovial tissue for single-cell suspension, we isolated CD45⁺ cells for single cell RNA seq and macrophages (MHCII+CD14⁺+CD11b+CD206⁺) and dendritic cells (MHCII+CD1c⁺) by FACS. We extracted RNA from these cell populations and prepared libraries for RNA-seq. These libraries were sequenced on an Illumina NextSeq 500 and assessed for quality of RNA, sequencing, and gene detection.

Results: For each cell population, we assessed the variability of gene expression across patients. We identified 5 different populations of macrophages that differ in their origin, response to methotrexate, differentiation status, and activation status. Additionally, as expected, the bulk population of DCs were highly variable across individuals, which this is likely due to the heterogeneity of subtypes within this population as shown in the single cell RNA seq for macrophages. In circulating monocytes, we observed varying levels of common cytokines and chemokines, such as TNF and CCL1. We also compared gene expression across cell populations to characterize transcriptional signatures that were distinctive to a given cell population. In addition to the genes previously known to be unique to dendritic cells vs. monocytes/macrophages in health, we also identified potential pathogenic factors that varied in their expression across cell types. Finally, to explore the relationship between circulating and tissue cell populations, we asked whether there were pathways that were turned on in the blood prior to extravasation into the synovium. For example, we identified genes that maintained their expression across monocytes and synovial macrophages in RA patients supporting the differentiation of the former into the latter.

Conclusion: Together, these results provide a survey of myeloid cells in the blood and synovial tissue of RA patients. We aim to understand how these cells vary across patients and what clinical variables and medication status influence their transcriptional profile across individuals. Our long-term goal is to use these studies to better understand the underlying mechanisms of pathogenesis and response to current treatments of RA as well as to identify potential targets for future therapies.

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OP0295

N-LINKED GLYCANS IN THE VARIABLE DOMAIN OF ACPA-IGG IN THE DEVELOPMENT OF RHEUMATOID ARTHRITIS

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Background: Anti-citrullinated protein antibodies (ACPA) are disease-specific biomarkers in rheumatoid arthritis (RA). Recently, we described that more than

90% of ACPA-IgGs harbour N-linked glycans in the antibody variable (V) domain. The corresponding N-glycosylation sites in the amino acid backbone of ACPA V-regions result from somatic hypermutation, a T cell-dependent process. Notably, both genetic evidence and data obtained from the analysis of serum ACPA indicate that T-cells drive the maturation of the ACPA-response prior to the onset of arthritis.

Objectives: We investigated whether ACPA-IgG carry V-domain N-glycans prior to the development of arthritis and whether the occurrence of such glycans predicts the transition from pre-disease autoimmunity to overt RA.

Methods: Two independent sets of serum samples were obtained from RA patients and from ACPA-positive first-degree relatives (FDR) of RA-patients (n=126) of an Indigenous North American (INA) population with high incidence rates of ACPA-positive RA. These samples comprised cross-sectional and longitudinal samples of individuals who did or did not transition to inflammatory arthritis. Serum ACPA-IgG were affinity-purified and subjected to enzymatic glycan release and UHPLC-based glycan analysis.

Results: ACPA-IgG V-domain glycosylation could be detected in RA patients and in FDR of RA patients. In both datasets, FDR-derived ACPA-IgG displayed markedly lower levels of V-domain glycans (<50%) compared to ACPA-IgG from RA-patients. Notably, FDRs that later developed RA showed extensive V-domain glycosylation before the onset of arthritis. Moreover, the degree of ACPA-IgG V-domain glycosylation in FDRs was strongly associated with future development of RA (HR: 6.07 [95% CI: 1.46-25.2]; p=0.013).

Conclusion: Glycosylation of the ACPA-IgG V-domain can be detected prior to the onset of disease. Extensive glycosylation is present in a subset of predisposed FDRs of INA RA patients. The presence of this feature substantially increases the risk of RA development. These observations fit well with a pivotal role for T cells in the selection and expansion of ACPA-expressing B cells, possibly by facilitating the introduction of N-glycosylation sites in ACPA-IgG V-domains. Moreover, glycosylation of the ACPA-IgG V-domain represents a predictive marker for RA development in ACPA-positive individuals and may serve to better time and target preventive therapeutic interventions.

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OP0296 COMPARISON OF TRANSCRIPTOMIC PROFILES BETWEEN PAIRED JOINT BIOPSIES FROM RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid Arthritis (RA) is a chronic and heterogenous condition characterized by inflammatory involvement of the synovial membrane in multiple joints. Synovial biopsies are used in research setting in order to identify diagnostic and theranostic markers. Many studies have shown a high degree of heterogeneity in histological and transcriptomic profiles between patients.

Objectives: We wanted to explore histological and transcriptomic profile of synovial biopsies across pairs of joints in the same patients to assess heterogeneity at the individual level.

Methods: Synovial biopsies were performed simultaneously in one small and one large joint per patient using needle-arthroscopy for the knee and US-guided needle biopsy for the hand or foot. Synovium from individuals with osteoarthritis (OA) were used as control. Paraffine-embedded samples were stained for CD3, CD 20, CD 68 and CD138. Whole-tissue RNA was extracted and hybridized on GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). The samples were tested for RNA integrity by Bioanalyzer (Agilent). Normalization and statistical analyzes were performed on Genespring. Pathway analysis were performed using DAVID.

Results: 10 RA patients were included (females: 10/10, ACPA/RF positivity: 8/10, mean age (± SEM): 54.4 (± 4.4) years, mean disease duration (± SEM): 13.3 (± 3.7) years, mean DAS28CRP (± SEM): 5.01 (± 0.34), mean HAQ (± SEM): 1.7 (± 0.28)).

Quantification of histological markers did not show differences in population of macrophages, plasmocytes, T and B Cells, across pairs of joints.

After correction for multiple comparisons, no transcripts were differentially expressed between large and small joints. Similarly, we did not find any significant difference in the expression of transcripts involved in pathways (TCR-activation and cell-division) specifically overexpressed in RA compared to OA synovial tissue.

In order to increase our ability to observe pair-wise differences in gene expression profiles, we studied correlations between transcripts significantly overexpressed in RA compared to OA joints with a fold change > 2 (n = 581) and clinical or biological markers of disease activity (DAS28-CRP, CRP, Physician Global Assessment of disease activity). Similar patterns of correlations indicated that disease activity was not driven by different pathways in small versus large joints.

Conclusion: This study is an important methodological milestone in the field of synovial biopsies, as it indicates that cellular and molecular alterations occurring in RA synovitis are similar across small and large joints from the same patient. Hence, biopsy of a single joint is representative and can be used to explore pathogenic processes or potential biomarkers in RA.

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OP0297 ABERRANT ADENOSINE TO INOSINE RNA EDITING IN ACTIVE RHEUMATOID ARTHRITIS

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Background: Adenosine to inosine (A-to-I) RNA editing is a widespread post-transcriptional RNA modification mainly located in repetitive *Alu* elements and mediated by the enzyme adenosine deaminase acting on RNA-1 (ADAR1). A-to-I RNA editing controls various aspects of RNA metabolism, which may affect tissue-specific gene expression. Although deregulation of RNA editing has been previously reported in various human diseases including cardiovascular disease and cancer (Stellos et al., *Nat Med*, 2017; Liu et al., *Nat Med*, 2019 and Ishizuka et al., *Nature*, 2019), its role in autoimmune diseases and especially in rheumatoid arthritis (RA) remains unknown.

Objectives: To study whether A-to-I RNA editing is involved in the pathogenesis of RA and to determine the impact of anti-rheumatic treatment on RNA editing.

Methods: We first analysed the expression of ADAR1 in 185 RA synovial tissues versus 76 healthy/osteoarthritic synovia derived from 4 independent RNA-sequencing and microarray datasets. We validated the findings in peripheral blood mononuclear cells (PBMCs) derived from 19 patients with active RA vs 14 controls and performed an additional ADAR1-isoform analysis (ADAR1p110/ADAR1p150) by RT-qPCR. Further, we studied in single nucleotide level the A-to-I RNA editing levels of the pro-inflammatory gene cathepsin S (CTSS) 3'-untranslated region (3'UTR), a matrix degradation enzyme which is a well-established target of ADAR1, by *Alu* Sanger sequencing and RNA editing analysis. Last, we examined the effect of anti-rheumatic treatment on RNA editing.

Results: Expression of the RNA editor ADAR1 was significantly increased in RA synovium compared to healthy or osteoarthritic synovium. Similarly, a significant increase of ADAR1, mainly due to an increase of the interferon-inducible ADAR1p150 isoform, was observed in PBMCs from active RA. Next, we studied the RNA editing levels in PBMCs from active RA patients before and after 12-week treatment versus controls. RNA editing of CTSS 3'UTR *AluSx*⁺ was increased in active RA (6-47% increase in editing rate of 8 individual adenosines, all P<0.05). Increased CTSS mRNA expression in RA PBMCs was associated with both ADAR1p150 expression (r=0.623, P=0.004) and RNA editing rate of 12 individual adenosines (r=range 0.45-0.72, P<0.05 for all) located within the CTSS 3' UTR *AluSx*⁺. The correlation between CTSS and ADAR1 was also observed in synovial tissue. Notably, ADAR1p150 expression and RNA editing rate reached control levels after 12-week treatment with methotrexate ± corticosteroids and/or biologics in patients with good clinical response (EULAR responders) but remained unchanged in the EULAR moderate/non-responders.