OP0031

SHARED AND DISTINCT GUT MICROBIOME SIGNATURES IN PATIENTS WITH AXIAL SPONDYLOARTHRITIS AND ITS RELATED IMMUNE-MEDIATED DISEASES

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Background: Immune-mediated diseases such as spondyloarthritis (SpA) consistently coincide with dysbiosis of the gut microbiota and frequently present with additional inflammatory pathologies such as Crohn's disease (CD) and acute anterior uveitis (AAU). Deep profiling of gut microbiota may reveal new pathways of how SpA and its related diseases are initiated and perpetuated.

Objectives: To identify the presence of shared and specific gut microbiota signatures for SpA and its related diseases as a whole, as well as for the individual diseases, relative to healthy controls.

Methods: Patients were recruited with a definite diagnosis of axial SpA, AAU or CD and were compared to controls (patients with back pain and previously ruled out SpA/CD/AAU diagnosis). All patients were naïve to or did not receive treatment with biological disease-modifying antirheumatic drugs for at least 3 months before enrollment of the study. Fecal samples were collected and microbiota composition was determined by 16S rRNA gene sequencing, followed by computational analysis referencing the SILVA138 database. Nonparametric Wilcoxon tests were used to calculate differential abundances between binary groups, and the Spearman correlation was used with continuous covariates. Nested linear models and likelihood ratio tests were used to assess confounding with respect to patient characteristics, HLA-B27 expression, inflammatory markers, and the presence of other immune-mediated diseases.

Results: A total of 300 patients were recruited for the study: 111 axial SpA, 110 AAU, and 79 CD patients and were compared to 63 control individuals. Fifty-three of patients were males with an age (mean±SD) of 39.1±12.3 years. The prevalence of HLA-B27 was 63.0% by patients compared to 7.9% by control individuals. A multivariate PERMANOVA test between the groups was significant ($p < 0.001$), revealing a difference in overall composition between the groups.

At the phylum level, patients with axial SpA, AAU and CD contained higher abundances of Proteobacteria, Bacteroidetes and Fusobacteria, and lower abundances of Firmicutes and Actinobacteria compared to the control group. At the genus level, patients (with axial SpA, AAU and CD) displayed a shared gut microbiome signature differing from that of control individuals. Patients samples were strongly depleted in *Blautia* compared to the control group. Many of the differentially abundant taxa also correlated with increased inflammation as measured by C-reactive protein (CRP), including a depletion of *Fusicatenibacter*, *Lachnospiraceae* FCS020 and *Roseburia*, as well as an enrichment of *Lactobacillus* and *Veillonella*. By looking at each separate disease phenotype, CD patients differed significantly from the control individuals with respect to many genera. These primarily consisted of depletions in Clostridiales (*Roseburia*, *Coprococcus*, *Ruminococcaceae*), and enrichments of pathogen-harboring genera such as *Escherichia-Shigella* and *Fusobacterium*. Axial SpA patients were uniquely enriched in *Collinsella* and *Holdemanella* and depleted in *Cupriavidus*; the enrichment of *Lactobacillus* and depletion of *Blautia* observed in all patient groups was also associated to the presence of axial SpA, though confounded by CRP. There were strong taxa associations to the presence of HLA-B27, including enrichment of *Asteroleplasma*, *Coprococcus*, *Faecalibacterium*, *Ruminococcaceae*, *Lachnospiraceae* NK4A136 and *Rikenellaceae*.

Conclusion: There is a robust shared taxonomic signature among related immune-mediated diseases, in addition to individual disease phenotype signatures. Patients frequently exhibited a strong depletion in *Blautia* and an enrichment in *Lactobacillus* as well as pathogen-harboring genera such as *Escherichia-Shigella* and *Fusobacterium*.

Figure 1. Taxa associations within and between the groups resulting from comparing each with the control group and accounting for disease concomitance and patient characteristics (FDR ≤ 0.05). AAU, anterior acute uveitis; CD, Crohn's disease; SpA, spondyloarthritis.

Disclosure of Interests: None declared

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OP0032

SINGLE CELL ANALYSIS OF SPONDYLOARTHRITIS REGULATORY T CELLS IDENTIFIES DISTINCT SYNOVIAL GENE EXPRESSION PATTERNS AND CLONAL FATES

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Background: Regulatory T cells (Tregs) play an important role in controlling inflammation and limiting autoimmunity, but their phenotypes at inflammatory sites in human disease are poorly understood. Whilst the phenotype and transcriptional profile of Tregs have been studied in some immune mediated conditions, they have been little studied (especially at the single cell level) in synovial fluid in the course of inflammatory arthritis. In Spondyloarthritis (SpA), in particular, where pathogenesis and inflammation is driven by dysregulated effector immunity, the role of the regulatory arm of immunity is largely unknown.

Objectives: We aimed to draw an atlas of Tregs in the context of SpA joint inflammation using single cell RNA sequencing of blood and SF Tregs of patients with Ankylosing Spondylitis (AS) and Psoriatic Arthritis (PsA). Functionally distinct specialised Treg subtypes, and specific changes in transcriptional profile occurring in synovial fluid Tregs, providing an insight on Treg adaptation during inflammation. Furthermore, by coupling gene expression analysis with TCR sequencing, we aimed to describe clonally expanded and likely antigen-driven Tregs in the SF.

Methods: Fluorescent activated cell sorting (FACS) was used to isolate 13,400 memory CD3+ CD45RA-ve CD25+127low Tregs from the blood and synovial fluid (SF) of 2 patients with HLA-B27+ AS presenting with active knee arthritis. Single-cell RNA sequencing (scRNA-seq) using 5' V(D)J 10x Genomics technology allowed both transcriptional definition of Tregs, and exploration of their immune TCR repertoire. Findings were compared to >3,000 SF and blood Tregs from 3 patients with oligoarticular PsA¹. Multicolor flow cytometry and in vitro cell-based assays using patient-derived cells were used to confirm and expand, at protein and functional level, the findings that emerged from the gene expression analysis.

Results: We report a large scRNAseq dataset (approx. 17,000 cells) comparing Tregs from SpA blood and joints. We identify multiple Treg clusters with distinct transcriptomic profiles, including, among others, a regulatory CD8+ subset expressing cytotoxic markers/genes, and a Th17-like RORC+ Treg subset characterized by IL-10 and LAG-3 expression. Synovial Tregs show upregulation of interferon signature and TNF receptor superfamily genes, and marked clonal expansion, consistent with tissue adaptation and antigen contact respectively. Individual synovial Treg clones map to different clusters indicating cell fate divergence. Finally, we demonstrate that LAG-3 directly inhibits IL-12/23 and TNF