Microbiome and SpA

OP0098 PREDICTING TREATMENT OUTCOME IN PATIENTS WITH SPONDYLOARTHRITIS USING MICROBIOTA ANALYSIS

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Background: Autoimmune-related rheumatic diseases such as rheumatoid arthritis (RA) and spondyloarthritides (SpA) are caused by an interplay of various factors including genetics, environmental factors and lifestyle. Among them, the intestinal microbiome has been suggested to influence disease initiation and progression. While the pathogenic connection between the microbiome and autoimmunity remains ambiguous, there is evidence that, for instance, microbiota-derived antigens cross-react with autoantibodies triggering an immune response and disease development. However, other hypothesis such as the leaky gut model have been explored. In addition to this potential involvement in disease initiation, microbial signatures have been identified to predict treatment outcome, i.e., as shown for the first-line medication Methotrexate (MTX) in RA patients.

Objectives: Our study aimed to longitudinally compare gut microbiota composition between treatment-naive patients with different forms of rheumatic disorders from disease onset to remission/relapse to identify (i) disease-specific signatures and (ii) their impact on therapeutic responses.

Methods: Patients with new onset of rheumatic disorders as well as their household members were recruited in the RheumaVOR cohort. The patients presented without treatment with steroids or DMARDs. The diagnosis was made by experienced rheumatologists. Faecal samples were collected, and 16S rRNA amplicon sequencing was performed to determine microbiota composition. Patients with non-inflammatory rheumatic disorders served as controls, whereas treatment was initiated in patients with inflammatory rheumatic diseases. Clinical data of patients was recorded including monitoring of medication and treatment response.

Results: A total of 422 faecal samples were analyzed from patients diagnosed with different forms of immune-mediated rheumatic diseases, non-inflammatory rheumatic diseases as well as from household controls (Table 1). Using 16S rRNA amplicon sequencing, we did not detect any clustering of patient groups during disease onset based on the microbiota composition (Figure 1A). However, PsA patients responding to MTX (MTX-R) (=patients in DAPSA remission 3-6 months after MTX initiation or patients showing partial response) and patients not in remission (MTX-NR) differed in microbial diversity (Figure 1B) and specific bacteria were enriched in MTX responders.

Table 1. Overview of RheumaVOR samples analyzed in this study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>MTX response</th>
<th>no response data</th>
<th>Remission not available</th>
<th>Total number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household controls</td>
<td>188</td>
<td>110</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Non-inflammatory rheumatic diseases (NIRD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis (RA)</td>
<td>33</td>
<td>28</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>Psoriatic arthritis (PsA)</td>
<td>47</td>
<td>34</td>
<td>13</td>
<td>91</td>
</tr>
<tr>
<td>Axial spondyloarthritis (axSpA)</td>
<td>35</td>
<td></td>
<td>12</td>
<td>63</td>
</tr>
<tr>
<td>Reactive arthritis (ReA)</td>
<td>9</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>In total</td>
<td>422</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Microbiome analysis of RheumaVOR fecal samples. A: Analysis of beta diversity (PCoA) using Bray-Curtis distances. B: Alpha diversity of PsA patients receiving MTX as first-line medication. P values represent an unpaired nonparametric Mann–Whitney test, *p < 0.05.
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Exploring the origin of inflammation in spondyloarthritis

IL-17A BLOCKADE MODULATES DISEASE SPECIFIC IMMUNE AND STROMAL PATHWAYS IN PERIPHERAL SPONDYLOARTHRITIS SYNOVITIS

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Background: The cellular and molecular mechanisms driving inflammation and structural remodelling in spondyloarthritis (SpA) remain largely unknown, though the IL-23/IL-17 pathway can contribute to synovial inflammation and radiographic progression.

Objectives: To investigate the molecular pathways affected by IL-17A blockade (IL-17Ai) with secukinumab in SpA synovitis, and assess if this response is tissue- and/or treatment-specific.

Methods: Synovial biopsies were obtained from peripheral SpA patients by needle arthroscopy before and after 12 weeks of IL-17Ai with secukinumab (n=12), and analyzed by RNA-sequencing and qPCR. We performed pathway enrichment analysis of the differentially expressed genes (DEGs) to identify pathways modulated after treatment. We compared the synovial tissue response in patients with psoriatic arthritis (PsA) in our cohort (n=7) with open source gene expression data of skin biopsies of psoriasis patients receiving secukinumab (n=22) [1] and of synovial biopsies of PsA patients receiving IL-12p40/IL-23p40 blockade (n=7) [2].

Results: IL-17Ai significantly modulated the expression of 1255 genes (549 up- and 706 down-regulated, FDR 0.1) in the synovium at week 12 compared to baseline (Figure 1). Genes downregulated upon IL-17Ai were significantly enriched in GO terms and KEGG pathways related to immune and inflammatory responses, including neutrophil and monocyte chemotaxis, TNF-mediated, NF-κB, Wnt, and JAK-STAT signalling pathways, and importantly, bone-remodelling responses, such as osteoblast and osteoclast differentiation. Upregulated genes are enriched in JNK-, MAPK-, Wnt-, and PI3K-Akt signalling and negative regulation of osteoblast differentiation. We validated differential expression of selected genes between pathways by qPCR, including: IL1B, p=0.027; CXCL6, p=0.020; ADAMTS4, p=0.002; MMP3, p=0.020; and CHRD2L1, p= 0.039.

Figure 1. Heatmap of differentially expressed genes (DEGs) and pathway enrichment analysis of changes in peripheral SpA synovium 12 weeks after anti-IL-17A treatment (IL-17Ai). A. Hierarchical cluster analysis of the top 100 most significant DEGs (FDR < 0.1) modulated by IL-17Ai separates pre- and post-treated groups. Normalized and scaled log2 gene expression levels are shown. B. Pathway enrichment analysis through DAVID. Enriched (p < 0.05) gene ontology terms from IL-17Ai-induced up- and down-regulated DEGs in peripheral SpA synovium are shown.

To assess if this response is tissue- and/or treatment-specific, we compared changes in gene expression by IL-17Ai in PsA synovium versus psoriatic skin, and in the PsA synovium after IL-17Ai versus IL-12p40/IL-23p40 blockade. While many inflammation-related GO terms and KEGG pathways were over-represented in both tissues and treatments, NF-κB-, JAK-STAT-, and PI3K-Akt-signalling were enriched in DEGs in both skin and synovium after IL-17Ai, whereas JNK cascade, IL-17 signalling pathway and Th17 cell differentiation were over-represented in DEGs after IL-17Ai in the synovium specifically. Remarkably, IL-17Ai, but not IL-12p40/IL-23p40 blockade, modulated multiple bone-remodelling related pathways. Also, IL-17Ai modulated ossification and collagen catabolic process terms in PsA synovium and psoriatic skin in the opposite direction: these terms were over-represented in downregulated genes in synovium, but in upregulated genes in skin. Accordingly, genes upregulated after IL-17Ai were enriched in negative regulation of osteoblast differentiation in the synovium, but in positive regulation of osteoblast differentiation in the skin.

Conclusion: These first in vivo human data provide molecular confirmation of previously reported animal data [3] that demonstrated down-modulation of disease-relevant immune and stromal pathways in the synovium in response to IL-17Ai.

REFERENCES:

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MECHANICAL LOADING-INDUCED BHLHE40 PROMOTES INFLAMMATORY ARTHRITIS

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Background: Force induced microdamage to joint tissue is hypothesized to trigger inflammatory events in the joint leading to arthritis. Patients with inflammatory arthritis, such as rheumatoid arthritis (RA) and spondyloarthritis (SpA), are found to have inflammation in “mechanical hotspots” and mechanical loading in mouse models of these diseases is pro-arthrogenic [1][2][3]. To date, the molecular mechanism involved in converting force to a biological signal that promotes arthritis is not known.

Objectives: This study aims to identify stretch induced genes in synovial fibroblasts, and the effect of these “mechano-sensitive” genes on arthritis.

Methods: Human synovial fibroblasts were stretched in vitro for 4hrs using the FlexCell system and analysed by microarray. Top stretch induced genes were measured in RA, SpA and healthy synovial tissue by qPCR. Patient synovium was further analysed by immunohistochemistry. Bhlhe40 deficient mice were subjected to collagen induced arthritis (CIA) and KBxN serum transfer arthritis (STA). FACS was performed on ankle synovium. uCT was performed on whole ankles, with morphological changes scored by blinded readers, and calcaneus erosions by customs scripts in Fiji.

Results: 600 genes were found to be differentially expressed in stretched synovial fibroblasts (fold change > 2; 25% of these genes, n=151, adjusted p< 0.05). 25% of these genes were found to be transcription factors, which included BHLHE40. BHLHE40 mRNA was elevated in the synovial tissue of RA/SpA vs healthy subjects (1.56 fold change), and BHLHE40 protein was widely detectable in synovial fibroblasts and macrophages (Figure 1). Bhlhe40 deficient mice were completely protected against CIA (incidence: 0% vs 40%, n=30 per group), but Bhlhe40 deficient mice were partially protected against STA (peak clinical score at day 7; 5.2 vs 8.8, n=15 per group), with reduced synovial macrophage (CD11b+Ly6G-F4/80+) and neutrophil (CD11b+Ly6G+) frequency observed in the arthritic Bhlhe40 deficient mice compared to wildtype controls. Bhlhe40 had no impact on bone erosions with STA.

Figure 1. Mechanical strain induced expression of BHLHE40 in human synovial fibroblasts. A. BHLHE40 expression levels are shown. B. BHLHE40 mRNA and protein levels are shown.