Background: Autoantibodies (Aab) are frequent in systemic sclerosis (SSc) [1]. Recently, it has been shown that immunoglobulins (IgG) from SSc patients can promote proinflammatory and profibrotic phenotype in monocyte culture [2]. Fibroblasts (FB) are key effector cells in SSc and data on FB proteins secreted in the presence of IgG from SSc patients are lacking. While recognized as potent biomarkers, the pathogenic role of Aab is much more debated.

Objectives: To explore the FB secretome in the presence of purified IgG from SSc patients

Methods: Normal dermal FB were cultured in the presence of purified IgG from patients with diffuse cutaneous SSc (dcSSc), limited cutaneous SSc (lcSSc) or healthy controls (HC). After 72 h of culture, the cell supernatants were collected, centrifuged and passed through a filter to remove the cells. After hierarchical clustering were used to identify proteins responses patterns.

Results: Proteomic identified and quantified 1268 proteins, among them 377 were significant after ANOVA. SSC and HC secretome appeared distinct. Hierarchical clustering on significant proteins after ANOVA showed 3 distinct groups of patients secretome: a first group including mostly dcSSc anti-topoisoamerase-I (ATA) positive (dcSSc ATA+) patients, a second group including mostly dcSSc ATA negative (dcSSc ATA-) patients, a third group more heterogeneous including the majority of HC, lcSSc anti-centromere positive (lcSSc ACA+) patients and dcSSc ATA+ patients (Figure 1A). The comparison of FB secretome in the presence of purified IgG from dcSSc ATA+ vs HC revealed 203 differentially expressed proteins (DEP) (Figure 1B). The enriched Gene Ontology (GO) terms upregulated in IgG dcSSc ATA+ were involved in endocytosis vesicle lumen, vesicle lumen, extracellular matrix or glycosaminoglycan binding. The comparisons of IgG dcSSc ATA+ vs IgG HC and IgG lsSsc ACA+ vs IgG HC revealed 85 and 15 DEP, respectively. Follistatin, amyloid beta A4 protein, myosin-9 and calreticulin were overexpressed in the condition dcSSc ATA+ (Figure 1C). Volcano plot represented the comparison IgG dcSSc ATA+ vs IgG HC (B). Venn diagram representing the proteins in common and exclusive of the following comparison: IgG dcSSc ATA+ vs IgG HC and IgG lsSsc ACA+ vs IgG HC revealed 85 and 15 DEP, respectively. Follistatin, amyloid beta A4 protein, myosin-9 and calreticulin were overexpressed in the condition dcSSc ATA+. Among them, collagen alpha-1(VI) chain, galectin-3-binding protein, desintegrin and metalloproteinase domain-containing 10, low affinity immunoglobulin gamma Fc region receptor III-A, CD59 glycoprotein, growth arrest-arrest specific protein and clusterin were overexpressed in the condition dcSSc ATA+ (Figure 1C).

Conclusion: Using sensitive proteomic approach, we identified that purified IgG from SSc patients modified FB secretome in a serotype dependent manner. The data support the pathogenic role of Aab in SSc.

REFERENCES

Disclosure of Interests: None declared.

Figure 1. Heatmap representing the 377 differentially expressed proteins after ANOVA in all samples. Cluster analysis identified 3 groups of proteins and 2 different clusters of protein expression (A). Volcano plot represented the comparison IgG dcSSc ATA+ vs IgG HC (B). Venn diagram representing the proteins in common and exclusive of the following comparison: IgG dcSSc ATA+ vs IgG HC and IgG lsSsc ACA+ vs IgG HC revealed 85 and 15 DEP, respectively. Follistatin, amyloid beta A4 protein, myosin-9 and calreticulin were overexpressed in the condition dcSSc ATA+ (Figure 1C).
**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 spondyloarthritis (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, microbial-derived antigens cross-react with autoantibodies triggering an immune response and disease development1. However, other hypothesis such as the leaky gut model have been explored2. 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 patients3. 

**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 and 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 Rheuma-VOR cohort. The patients presented without treatment with steroids or DMARDs. The diagnosis was made by experienced rheumatologists. Native and stabilized fecal 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 fecal 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>total number of samples</th>
<th>MTX response</th>
<th>no response data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household controls</td>
<td>188</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-inflammatory rheumatic</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diseases (NID)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis (RA)</td>
<td>33</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Psoriatic arthritis (PsA)</td>
<td>47</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>Axial spondyloarthritis (axSpA)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Reactive arthritis (ReA)</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In total</td>
<td>422</td>
<td></td>
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</tr>
</tbody>
</table>

**Conclusion:** While our study did not reveal global differences in the bacterial composition of the gut microbiota between patients diagnosed with RA and SpA as well as control groups, we identified changes in microbial diversity and the abundance of specific bacteria between MTX responders and non-responders in PsA patients. Further investigations are needed to clarify the complex interactions between the microbiome, disease onset in rheumatic disorders and DMARD treatment response to ameliorate disease progression and improve therapeutic outcome.

**REFERENCES:**


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The role of the synovial microenvironment during onset and progression of arthritis

**OP0099**

**INCREASED FREQUENCY OF CD4+ AND CD8+ FOLLICULAR HELPER T CELLS IN HUMAN LYMPH NODE BIOPSY DURING THE EARLIEST STAGES OF RHEUMATOID ARTHRITIS**

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**Background:** Follicular T helper (Tfh) cells provide key help for B cell differentiation into plasma and memory B cells and are essential in germinal center formation and (auto) antibody generation (1).

**Objectives:** To gain more insights into the role of Tfh cells in RA development, we assessed whether Tfh cells have an altered frequency, phenotype and cytokine profile in peripheral blood, lymphoid and synovial tissues during the earliest stages of RA development.

**Methods:** Using flow cytometry, we analyzed the phenotype, frequencies and cytokine profile of Tfh cells and B cells in peripheral blood and lymph node biopsies of healthy controls (HCs), autoantibody positive individuals at risk for developing RA (RA-risk individuals), and early RA patients. Using immunofluorescence we confirmed the presence of Tfh cells and follicles of lymph nodes and synovial tissue biopsies of RA patients.

**Results:** In blood, the frequency of Tfh cells did not differ between study groups. In lymphoid and synovial tissue, Tfh cells were localized in B-cell areas, and their frequencies correlated strongly with the frequency of CD19+ B cells. Compared to lymphoid tissue of healthy controls, that of RA patients and RA-risk individuals, showed more CD19+ B cells and more CD4+CXCR5+ and CD8+CXCR5+ follicular T cells. Of note, compared to healthy controls, specifically lymph node Tfh cells of RA-risk and early RA patients produced less IL-21 upon ex-vivo stimulation.

**Conclusion:** Tfh cells are localized in B-cell rich areas in lymphoid and synovial tissues of early RA patients. The analysis of lymph node tissue in early RA patients showed increased frequencies of Tfh cells, where they clearly associate with B cells. Interestingly, IL-21 production is already aberrant in the very early at risk phase of the disease. Our data suggest that Tfh cells may present a novel rationale for therapeutic targeting during the preclinical stage of RA to prevent further disease progression.

**REFERENCES:**


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