GENE EXPRESSION PROFILES RETRIEVED FROM SINGLE CELL RNA SEQUENCING REVEAL PHENOTYPIC TRAITS IN PATIENTS WITH PSORIATIC ARTHRITIS INITIATING TUMOUR NECROSIS FACTOR ALPHA INHIBITOR AND INTERLEUKIN-17 INHIBITOR

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Background: Patients with Psoriatic Arthritis (PsA) suffer from heterogeneous debilitating symptoms caused by immune-mediated inflammation. However, great diversity in clinical response to available medical therapies targeting the immune response in PsA complicates treatment decision-making.

Objectives: The primary objective of the study was to evaluate gene expression profiles at baseline in responders versus non-responders of Tumour Necrosis Factors alpha inhibitor (TNFi) and Interleukin-17 inhibitor (IL-17i), respectively, to examine the foundation for future improved PsA patient stratification.

Methods: Single cell RNA sequencing (scRNAseq) was included to evaluate the transcriptome in responders versus non-responders of Tumour Necrosis Factor alpha inhibitor (TNFi) and Interleukin-17 inhibitor (IL-17i). Peripheral blood mononuclear cells (PBMCs) were isolated for scRNAseq and Disease Activity Score in PsA (DAPSA) retrieved in 40 PsA patients; 20 initiating TNFi and 20 initiating IL-17i. Responders and non-responders were stratified based on 50% improvement after 4-months (DAPSA50). The BD Rhapsody Whole Transcriptome Approach [1] was applied to prepare cDNA libraries sequenced to a depth of approximately 50,000 reads per cell on the Illumina NovaSeq 6000. The Seurat pipeline [2] was implemented for the analysis of gene expression on single cell level.

Results: A total of 273,515 cells were retrieved after initial quality control. Comparing patient demographics and characteristics at baseline revealed no difference between groups (Table 1). Dimensionality reduction with principal component analysis and additional clustering analysis indicated different phenotypic traits of responders versus non-responders to TNFi and IL-17i (Figure 1). The results were limited by the DAPSA50 stratification only requiring 50% improvement at 4-month. Nevertheless, it is believed that scRNAseq can be included to improve PsA patient stratification.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>All (n=40)</th>
<th>TNFi responders (n=12)</th>
<th>TNFi non-responders (n=8)</th>
<th>IL-17i responders (n=8)</th>
<th>IL-17i non-responders (n=12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, female</td>
<td>23 (57.5%)</td>
<td>7 (58.3%)</td>
<td>4 (50.0%)</td>
<td>8 (66.7%)</td>
<td>5 (41.7%)</td>
<td>0.653</td>
</tr>
<tr>
<td>Age</td>
<td>51.9 ±12.1</td>
<td>48.4 ±10.9</td>
<td>54.7 ±18.6</td>
<td>49.6 (17.5)</td>
<td>52.7 ±9.9</td>
<td>0.796</td>
</tr>
<tr>
<td>Disease duration</td>
<td>8.1 ±7.4</td>
<td>6.7 ±6.8</td>
<td>10.1 ±10.1</td>
<td>10.4 ±7.9</td>
<td>13.7 ±6.4</td>
<td>0.903</td>
</tr>
<tr>
<td>DAPSA</td>
<td>36.5 ±18.5</td>
<td>34.4 ±23.2</td>
<td>34.1 ±13.8</td>
<td>50.5 ±17.3</td>
<td>38.5 ±17.9</td>
<td>0.176</td>
</tr>
</tbody>
</table>

Patient characteristics were presented as number with corresponding percentage or mean with corresponding standard deviations. P-value < 0.05 was considered statistically significant. TNFi; Tumour Necrosis Factor alpha inhibitor (TNFi) and Interleukin-17 inhibitor (IL-17i). Peripheral blood mononuclear cells (PBMCs) were isolated for scRNAseq and Disease Activity Score in PsA (DAPSA) retrieved in 40 PsA patients; 20 initiating TNFi and 20 initiating IL-17i.

Conclusion: High-dimensional data, retrieved from scRNAseq combined with bioinformatical data modelling and clinical knowledge, is important and should possibly be implemented to explore PsA disease heterogeneity and for better stratification of PsA patients prior to initiating available medical therapies.

REFERENCES:


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EXPANDED CD8+ T CELL CLONES FROMHLA-B*27-POSITIVE PATIENTS WITH SPONDYLOARTHRITIS SHOW SIGNS OF ANTIGEN-EXPERIENCE

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Background: The pathogenesis of Spondyloarthritis (SpA) remains unknown but its strong association with some alleles of HLA-B*27 is peculiar. The arthritogenic antigen hypothesis assumes the existence of specific peptides presented by risk-conferring HLA-B*27 alleles to antigen-specific CD8+ T cells, which then initiate or sustain autoimmune reactions. Several studies analyzing T cell receptor (TCR) repertoire found preferred variable TCR chains and motifs in the hypervariable complementary determining region (CDR) 3, but analyzed only TCR β-chains in bulk analyses.

Objectives: To analyze full sequence information of TCR including matching α- and β-chains from single CD8+ T cells and characterize the transcriptional expression of expanded and non-expanded clonotypes in synovial fluid (SF) of SpA patients.

Methods: We included 17 patients with active gonarthritis: 10 patients with HLA-B*27 positive (B27 poz) SpA, 4 with HLA-B*27 negative (B27 neg) SpA and 3 rheumatoid arthritis (RA) patients. Antigen-experienced CD8+ T cells were sorted out of SF by flow cytometry. Single cell sequencing was performed for all patients to

Figure 1. Gene expression profiles of DAPSA50 responders and non-responders to IL-17iHeat maps revealing differential gene expression of DAPSA50 responders and non-responders to IL-17i implying the possibility of improved stratification of PsA patients.

REFERENCES:

analyze matching TCR α- and β-chains. For 7 patients (3 B27pos SpA, 2 B27neg SpA, 2 RA), additionally whole transcriptome analyses were performed. 

**Results:** We found strong biases when analyzing α- and β-chains of TCR Variable regions and CDR1 and CDR2 sequences (Figure 1 a,b): AV21, AV12-2, and AV17 were highly enriched in B27pos SpA as compared to B27neg subjects. Amongst the highest expressed clones, we could confirm enrichment for previously described TRBV genes as BV19, BV5-1 and BV6-2. We examined TCR α/β combinations and focused on those detected in at least three different B27pos SpA but not in any of the B27neg patients (Figure 1 c-f). The combinations TRBV19/TRAV21 and TRBV6-2/TRAV21 were most likely specific for B27pos SpA but not in any of the B27neg patients (Figure 1 c-f). The combinations and focused on those detected in at least three different B27pos SpA and might reflect interaction of these TCR chains with HLA-B*27.

Sequences of CDR3 loops, which predominantly interact with HLA-bound antigenic peptides, revealed striking common structural motifs in α- and β-chains. Focusing on the most prominent TRAV21 chains pairing with TRBV19 (6-1) and 6-2 chains, revealed identical sequences in different patients and striking common structural motifs in α- and β-CDR3 sequences in other patients. Such marked similarities in the antigen-recognition loops of the β-chains associated with TRAV21 suggest common or highly similar antigens. Gene expression levels provided evidence that expanded cell populations had tissue resident memory (TRM) phenotypes (elevated expression of activation, migration and tissue retention markers, downregulated genes characteristic for T cell egress), while this phenotype was not very pronounced in non-expanded cells. Furthermore, markers for T cell exhaustion and apoptosis were elevated in expanded cells of B27pos SpA patients.

**Conclusion:** Analysis of single antigen experienced CD8+ T cells from SF of B27pos SpA patients revealed significant clonal expansions and common motifs in the CDR loops. Two of the four CDR1 and CDR2 loops were highly homologous suggesting that these loops interact with α-helices of HLA-B*27. Common motifs in CDR3 loops of expanded clonotypes suggest recognition of a limited set of antigenic peptides presented by HLA-B*27. Many of the expanded clonotypes showed a TRM phenotype, were exhausted and on the way to become apoptotic, which suggests that these clones had sustained contact to specific antigens.

**REFERENCES:**

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**Figure 1.** Distinct TRAV V chain usage in expanded clones from HLA-B27 positive SpA patients. A,B Mean number of all productive TRAV (A) and TRBV (B) genes used in expanded, antigen-experienced C8D8 T cell clones (>1% of all cells) from SF of 10 B27pos SpA, 4 B27neg SpA and 3 B27neg RA patients. C-F TRAV chains paired with TRBV19 (C), TRBV5-1 (D), TRBV6-2 (E), or TRBV chains paired with TRAV21 (F) with corresponding TRAV sequences recommended (frequency ≥2) from all 10 B27pos SpA. Number of chains are 1250 (C), 866 (D), 1220 (E), and 4006 (F).