OP0037

ABATACEPT AND OTHER DMARDS HAVE COMMON TRANSCRIPTOMIC EFFECTS ON RA SYNOVIAL TISSUE

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Background: Modes of action of DMARDs (disease-modifying antirheumatic drugs) in rheumatoid arthritis (RA) are not completely understood at the level of the synovium. Studying treatment-induced modifications in RA synovial tissue can provide unique insights into the pathways modulated downstream of different DMARDs.

Objectives: Our goal was to assess histological and transcriptomic effects of Abatacept (ABA) on RA synovium, and to compare them with previously published data obtained by our group using the same study design on other DMARDs: Tocilizumab (TCZ), Rituximab (RTX), Methotrexate (MTX) and Adalimumab (ADA).

Methods: Synovial tissue was obtained using ultrasound-guided biopsy from affected joints before (W0) and 16 weeks (W16) after treatment with subcutaneous Abatacept 125mg per week on a MTX background. Paraffin-sections were stained for CD3, CD20 and CD68 and scored by a pathologist for T cell, B cell and macrophage infiltration. Transcriptional profiling was performed using GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix), and analyzed on Genespring GX (Agilent). Pathway analyses were performed on Genespring GX, Metascape (<https://metascape.org/>) and EnrichR (<https://maayanlab.cloud/Enrichr/>). Protein-Protein Interaction (PPI) networks were generated on STRING (<https://string-db.org/>).

Results: 14 RA patients were included (female: 9, ACPA/RF positive: 8, erosive disease: 12, median disease duration in years ($\pm$ SD): 11.7 ($\pm$ 8.1), median DAS28CRP ($\pm$ SD): 4.78 ($\pm$ 1.11)). Median DAS28CRP significantly decreased between W0 and W16, as did US GS score. Evaluation of histological slides (n=11 pairs of samples) showed no significant effect of Abatacept on T cell, B cell or macrophage infiltration. Gene expression analysis (n=10 pairs of samples) identified 304 transcripts differentially expressed (129 downregulated, 175 upregulated) between W0 and W16 ($FC \geq 1.5$ and $p < 0.05$, paired Mann-Whitney). Downregulated genes were significantly enriched for immune processes and included several key T cell regulatory genes (*IL2RA*, *CD28*, *IL7*, *IL7R*), strongly overlapping with data from previous studies on TCZ (n= 12 pairs), RTX (n=12 pairs), MTX (n=8 pairs) and ADA (n=8 pairs). Thus, each treatment shares 31 to 48% of its downregulated genes with the others, with genes downregulated by at least three involved in key RA-associated pathways such as *leukocyte activation*, *NF-kappa B signaling*, *TNF signaling* and *JAK-STAT signaling*. Given their seemingly overlapping effects, data were pooled across these studies, markedly improving power thanks to their paired-design. This revealed that genes downregulated by DMARDs (n=573, Benjamini-Hochberg corrected p-value<0.05, paired Mann-Whitney) were significantly enriched for both *T cell* and *myeloid leukocyte activation* pathways. Interestingly, DMARDs seem to have a coordinate effect on the two pathways (correlation of mean $\log_2 FC$: $r=0.8558$, $p<0.0001$), with a stronger impact ($\log_2 FC_{W16-W0}$) in good responders to therapy (n=17) as compared to moderate (n=20) and to non-responders (n=13) ($p<0.0001$, Mann-Whitney). Finally, Transcription Factor enrichment and PPI network analyses point to a central role for molecules including JAK/STATs as mediators of all studied therapies.

Conclusion: We provide evidence that the effects of five DMARDs on RA synovium culminate in the same pathways (namely, *T cell* and *myeloid leukocyte activation*). This confirms previous studies suggesting the existence of common mediators downstream of DMARDs, independent of their primary targets, and suggests attractive new therapeutic targets.

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Molecular mechanisms in Connective Tissue Disease

OP0038

SPLICEOSOME ALTERATIONS IN LEUCOCYTES FROM APS, SLE AND SLE+APS PATIENTS ARE CLOSELY RELATED TO THEIR MAIN CLINICAL FEATURES

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Background: To date, although multiple molecular approaches have illustrated the various aspects of Primary Antiphospholipid Syndrome (APS), systemic lupus erythematosus (SLE) and antiphospholipid syndrome plus lupus (APS plus SLE), no study has so far fully characterized the potential role of posttranscriptional regulatory mechanisms such as the alternative splicing.

Objectives: To identify shared and differential changes in the splicing machinery of immune cells from APS, SLE and APS plus SLE patients, and their involvement in the activity and clinical profile of these autoimmune disorders.

Methods: Monocytes, lymphocytes and neutrophils from 80 patients (22 APS, 35 SLE and 23 APS plus SLE) and 50 healthy donors (HD) were purified by immunomagnetic selection. Then, selected elements of the splicing machinery were evaluated using a microfluidic qPCR array (Fluidigm). In parallel, extensive clinical/serological evaluation was performed, comprising disease activity, thrombosis and renal involvement, along with autoantibodies, acute phase reactants, complement and inflammatory molecules. Molecular clustering analyses and correlation/association studies were developed.

Results: Patients with primary APS, SLE and APS plus SLE displayed significant and specific alterations in the splicing machinery components in comparison with HD, that were further specific for each leukocyte subset. Besides, these alterations were associated with distinctive clinical features.

Hence, in APS, clustering analysis allowed to identify two sets of patients representing different molecular profile groups with respect to the expression levels of splicing machinery components. Principal component analyses confirmed a clear separation between patients. Clinically, cluster 1 characterized patients with higher thrombotic episodes and recurrences than cluster 2 and displayed a higher adjusted global APS score (aGAPSS). Accordingly, these patients showed higher levels of inflammatory mediators than cluster 2.

Similarly, in patients with APS plus SLE, clustering analysis allowed to identify two sets of patients showing differential expression of splicing machinery components. Clinical and laboratory profiles showed that cluster 2 characterized patients that had suffered more thrombotic recurrences, most of them displaying an aGAPSS over 12 points and expressing higher levels of inflammatory mediators than cluster 1. The incidence of lupus nephropathy was similarly represented in both clusters.

Lastly, in SLE patients, molecular clustering analysis identified two sets of patients showing distinctive clinical features. One cluster characterized most of the patients positive for anti-dsDNA antibodies, further suffering lupus nephropathy, and a high proportion of them also presenting atheroma plaques and high levels of inflammatory mediators.

Correlation studies further demonstrated that several deranged splicing machinery components in immune cells (i.e. SF3B1tv1, PTBP1, PRP8 and RBM17) were linked to the autoimmune profile of the three autoimmune diseases, albeit in a specific way on each disorder. Accordingly, *in vitro* treatment of HD lymphocytes with aPL-IgG or anti-dsDNA-IgG changed the expression of spliceosome components also found altered *in vivo* in the three autoimmune diseases. Finally, the induced over/downregulated expression of selected spliceosome components in leukocytes modulated the expression of inflammatory cytokines, changed the procoagulant/adhesion activities of monocytes and regulated NETosis in neutrophils.

Conclusion: 1) The splicing machinery, profoundly altered in leukocytes from APS, APS plus SLE and SLE patients, is closely related to the activity of these diseases, their autoimmune and inflammatory profiles. 2) The analysis of the splicing machinery allows the segregation of APS, APS plus SLE and SLE, with specific components explaining the CV risk and renal involvement in these highly related autoimmune disorders.

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