MULTI-OMICS ANALYSIS REVEALS COX5A AS A BIOMARKER OF DISEASE ACTIVITY AND ORGAN DAMAGE OF LUPUS

Keywords: Systemic lupus erythematous, Biomarkers

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Background: Systemic Lupus Erythematosus (SLE) is a heterogeneous systemic autoimmune disease with protean clinical manifestations[1]. Although the long-term outlook of SLE patients with SLE has been greatly improved, increased organ damage is associated with poor prognosis in a number of patients. The type I interferon signature, a hallmark of SLE, is not an ideal treatment target or outcome predictor[2, 3], suggesting other critical immunological pathways might contribute to disease pathogenesis.

Objectives: To explore key immunological pathways and gene markers in SLE more precisely, we performed a systematic analysis of transcriptional data from 27 immune cells in the blood and from single cells in the skin and kidney.

Methods: We included a large RNA-seq data from a total of 64 SLE and 62 control with 27 immune cells. Integrated analyses were conducted to find key pathways and driver genes in SLE pathogenesis. The expression of COX5A between SLE phenotypes was compared in two independent cohorts. Single-cell RNA sequencing (scRNA-seq) data from skin and kidney were used to further determine the association of COX5A expression with organ damage.

Results: We found lymphocytes in SLE showed an overall active immunometabolic state when compared to healthy controls, and oxidative phosphorylation (OXPHOS) is the most significant metabolic pathway that differs between SLE and HC, especially for effector T cells. Besides, the OXPHOS enrichment score was significantly correlated with IFN response molecular signature across various T cell subtypes. Particularly, we identified an OXPHOS hub gene, COX5A, as a key driver in SLE T cells. COX5A expression was significantly higher in effector T cells than those in naive T cells and showed associations with SLE clinical phenotypes including disease activity index, flare, and organ damage. Furthermore, we revealed that high expression of COX5A in T cells contributes to skin and kidney involvement of SLE through scRNA-seq analysis.

Conclusion: Our results identified OXPHOS signature is a prominent feature in SLE T cells. The key gene of OXPHOS, COX5A, showed associations with IFN response molecular signature, severity, skin, and kidney involvement of SLE, which supported that COX5A as a potential candidate biomarker of severity and organ damage of SLE.

REFERENCES:

Acknowledgements: I have no acknowledgments to declare.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.4250

ENTEROCOCCUS GALLINARUM IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS.

Keywords: Descriptive studies, Outcome measures, Systemic lupus erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is associated with epithelial defects and disrupted intestinal barrier, risking bacterial translocation and promoting systemic inflammation, known as dysbiosis, which is associated with increased disease activity[1]. Enterococcus gallinarum has been previously linked to gastrointestinal autoimmune diseases like autoimmune hepatitis and primary sclerosing cholangitis[2]. However, little is known about E. gallinarum prevalence in SLE.

Objectives: To describe the prevalence of E. gallinarum in SLE stool samples, as well as clinical and laboratory characteristics.

Methods: A cross-sectional, descriptive study was conducted at the University Hospital “Dr. José Eleuterio González”, in northern Mexico. We included adult patients who met current criteria for SLE and had recent (<3 months) paracinal routine tests, including acute phase reactants. Patients with other autoimmune other chromatin components, like histones and nucleosomes. These antibodies are known to characterize a SLE subgroup with early disease onset and increased occurrence of nephritis.

Objectives: We investigated how anti-nuclear autoantibody (ANA) specificities associate with pro-inflammatory cytokines in two ethnically different cohorts of SLE patients, from Sudan and Sweden.

Methods: We included 93 Sudanese and 480 Swedish SLE patients. Serum levels of autoantibodies against dsDNA. Sm, the Sm/U1RNP complex, U1RNP, SSA/Ro52, SSA/Ro60, SSB/La, ribosomal P, PCNA and histones were quantified with a bead-based multiplex immunoassay; with positive reactions determined as above the 98th percentile among respective national controls. In the Swedish cohort another bead-based multiplex immunoassay including anti-nucleosome antibodies was also used. Relative levels of 73 plasma biomarkers were determined with Proximity Extension Assay technique except for Interferon gamma-induced protein (IP-10) in the Swedish cohort that was quantified by ELISA. Adjusted p values were considered significant when <0.05.

Results: Among Sudanese patients, levels of 5/7 biomarkers showed significant associations to ANA-associated antibodies. Anti-histone antibodies showed the strongest positive correlations with interferon-inducible factors (monocyte chemoattractant protein [MCP]-1 and IP-10), monocyte chemoattractant protein-3 (MCP-3) and S100 calcium-binding protein A12 (S100A12), and negative correlation with stem cell factor (SCF). F(6) values were 0.15(0.04), 0.18(0.008), 25(0.001), 0.13(0.04) and 0.31(-0.0001) respectively. Biomarker associations remained significant for anti-histone antibody after adjustment for age and sex. Also, anti-dsDNA antibodies associated with MCP-3 (0.13(0.04)), IP-10 (0.13(0.03)) and S100A12 (0.13(0.04)), but when combining with anti-histone in the same regression model, anti-dsDNA associations were lost while anti-histone antibodies remained. Positive associations with lower F(6) values were found also for anti-ribosomal P antibodies with MCP-1, MCP-2 and C-C motif ligand 19 (CCL-19), and for anti-Sm with IP-10. Validation analysis among Swedish patients for MCP-1, IP-10, SA100A12 also demonstrated significantly stronger associations to anti-histone and anti-nucleosome antibodies compared to anti-dsDNA and other ANA specificities, and in combined regression models, anti-dsDNA either became non-significant or considerably less significant than anti-histone/nucleosome antibodies. When excluding anti-histone or anti-nucleosome positive patients, the associations between interferon-inducible factors MCP-1/IP-10 and anti-dsDNA were lost. In contrary, when excluding anti-dsDNA positive patients, associations with anti-histone and anti-nucleosome remained significant. SA100A12 associations with anti-dsDNA antibodies remained significant after exclusion of anti-histone positive patients, but were lost when excluding anti-nucleosome positive patients.

Conclusion: Using uni- and multi-variate analyses as well as patient stratification, levels of mainly IFN-induced inflammatory biomarkers correlate strongly with anti-histone and anti-nucleosome antibodies compared to other ANA specificities including anti-dsDNA. Our results, from two lupus cohorts with different ethnicities, suggest that autoantibodies against DNA-complexes or DNA-associated proteins rather than anti-dsDNA antibodies per se may drive the induction of the interferon signature in SLE.

REFERENCES: NIL.

Acknowledgements: NIL.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.4423
diseases, chronic infections, pregnancy, cancer, abdominal surgery or gastrointestinal bleeding were excluded. Demographic, clinical data, as well as Anti-nuclear antibodies and complement were obtained from records. No participant received antibiotics, probiotics or synbiotics 3 months prior to the study. DNA was extracted with the DNAeasy PowerLyzer PowerSoil DNAeasy kit, Qiagen (Hilden, Germany) according to the manufacturer’s specifications. E. gallinarum was detected by endpoint polymerase chain reaction assay.

Results: Sixty patients were included, where most subjects were women (51, 85%). Mean age was 41.79 ± 16.8 and time of diagnosis 107.03 months (± 95.46). E. gallinarum and Enterococcus spp were found in 7 (11.8%) cases. The most frequent MEX-SLEDAI parameter was arthritis in 34 (56.6%) cases, followed by acute cutaneous lupus in 23 (38.3%). Clinical manifestations and paraclinical findings are shown in Table 1. We found a significant difference in E. gallinarum positive patients in creatinine levels (0.98 vs 1.14 ± 0.72, p < 0.001). When analyzing ESR by age-adjusted upper limit, significance was lost (11.14 vs 19.49, p > 0.05).

Conclusion: Prevalence of E. gallinarum in SLE stool samples was 11.7%. Serositis, higher mean creatinine and lower mean ESR was more common in E. gallinarum positive subjects. Further research is needed to better understand E. gallinarum dysbiosis in SLE.

Table 1. Sociodemographic, clinical and paraclinical features.

<table>
<thead>
<tr>
<th>Sociodemographic, clinical, and paraclinical features</th>
<th>E. gallinarum Positive</th>
<th>E. gallinarum Negative</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, Kg/m²</td>
<td>n=7</td>
<td>n=59</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>37.14 ± 20.6</td>
<td>42.3 ± 13.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Months since diagnosis</td>
<td>75.95 ± 59.3</td>
<td>112.72 ± 99.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>58.89 ± 9.16</td>
<td>68.19 ± 15.96</td>
<td>0.06</td>
</tr>
<tr>
<td>Size, m</td>
<td>1.61 ± 0.08</td>
<td>1.59 ± 0.06</td>
<td>0.20</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>0 (0.0)</td>
<td>5 (9.43)</td>
<td>0.42</td>
</tr>
<tr>
<td>Alopecia</td>
<td>2 (28.57)</td>
<td>11 (20.75)</td>
<td>0.51</td>
</tr>
<tr>
<td>ANRs</td>
<td>3 (42.85)</td>
<td>31 (58.49)</td>
<td>0.61</td>
</tr>
<tr>
<td>Serositis</td>
<td>4 (57.14)</td>
<td>4 (7.54)</td>
<td>0.001</td>
</tr>
<tr>
<td>Nephritis</td>
<td>1 (14.28)</td>
<td>8 (15.09)</td>
<td>0.95</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>0 (0.0)</td>
<td>6 (11.32)</td>
<td>0.37</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>0 (0.0)</td>
<td>2 (3.77)</td>
<td>0.72</td>
</tr>
<tr>
<td>MEX-SLEDAI, mean ± SD</td>
<td>1.71 ± 0.29</td>
<td>1.83 ± 2.12</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Acknowledgements: No Acknowledgements to declare.

Disclosure of Interests: None Declared.

DOI: 10.1136/annrheumdis-2023-eular.4955

Background: Activated T cells make a significant contribution to inflammation in systemic lupus erythematosus (SLE). Their ability to secrete proinflammatory cytokines and express activating NK receptors allows them to mediate inflammation. We know that cellular metabolism regulates the activation of T cells. A phase II study has reported on efficacy of DMF in cutaneous lupus [1]. Evidence from patients with multiple sclerosis indicates that dimethyl fumarate (DMF), an electrophile, targets cellular metabolism to modulate T cell activation and function [2]. However, the potential of DMF to modulate T cell metabolism and activation in SLE is not known.

Methods: All experiments were performed using isolated T cells from freshly drawn whole blood samples from patients with SLE. T cells were isolated using negative selection using Stem cell or Miltenyi magnetic bead separation kit. Isolated T cells were activated with anti-CD3 and IL-2 and incubated with either DMF at 25µM concentration or DMSO alone for 48 to 72 hours. Analysis of cytokines in supernatants was performed using cytometric bead arrays. FlowJo software was used to analyse flow cytometry files. Graph Pad Prism software was used to perform statistical analysis.

Results: In Seahorse experiments, after 7 days of incubation dimethyl fumarate (DMF) inhibited the oxygen consumption rate (OCR) and extra cellular acidification rate (ECAR) in isolated T cells when compared with samples incubated with vehicle, dimethyl sulfoxide (DMSO). Our results revealed that DMF significantly inhibited: 1) aerobic glycolysis and oxidative phosphorylation in activated CD4+ T cells from patients with SLE (n=4), in vitro; 2) T cell activation and proliferation as assessed by a reduction in the frequency of CD69 (n=4) and Ki67 (n=2) positivity, respectively. Collectively, these results suggest that DMF inhibits T cell activation and proliferation in patients with SLE. After 7 days of incubation, DMF significantly inhibited the expression of activating NK receptors CD58 and NKGD2 on CD4+ T cells whereas DMF seemed to have a trend towards enhancing the expression of inhibitory NK receptors NK2A and CD158b (n=6). After 7 days of incubation, DMF significantly reduced CD4+ T cell intracellular expression of IFN-γ, TNF-α, IL-17 and secretion of pro-inflammatory cytokines IFN-γ and TNF-α in supernatants (n=6).

Conclusion: Our data indicated that DMF modulates metabolic programming, both glycolysis and oxidative phosphorylation, to inhibit activation, proliferation, and secretion of proinflammatory cytokines from CD4+ T cells from patients with SLE. These results provide strong mechanistic rationale for considering dimethyl fumarate as a novel therapeutic agent to treat systemic lupus erythematosus.

References:

Acknowledgements: The study received full funding support from the biomedic research centre, University College Hospital. Dr. Reddy’s work was supported by MRC-CARP fellowship award.

Disclosure of Interests: Loren Kell: None declared, Samuel Taylor: None declared, Kavina Shah: None declared, Roel De Maeyer: None declared, David Isenberg Consultant of: no competing interest with submitted work, Grant/research support from: Roche Glycart, no competing interest with submitted work.

Funding: This work was supported by the MRC-CARP fellowship award.

ABO2125 SPECIFIC AUTOANTIBODY CONTENT OF CIRCULATING IMMUNE COMPLEXES IN SLE – PHENOTYPIC CHARACTERIZATION AND CLINICAL ASSOCIATIONS

Keywords: Biomarkers, Autoantibodies, Systemic lupus erythematosus

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Background: Systemic Lupus Erythematosus (SLE) is characterised by autoantibody production and immune complex (IC) formation. The relative abundance of different autoantibodies within circulating ICs as compared to serum in SLE is hitherto unclear; moreover, the clinical relevance of the IC-carried fraction of SLE-specific and associated autoantibodies is mostly unknown.

Methods: Circulating immune complexes (CICs) isolated from sera and plasma of SLE patients with or without nephritis were immunoprecipitated with the corresponding autoantibody. After final wash, the autoantibody content of the immunoprecipitates were measured by ELISA. The IC antibody content was compared between SLE patients with or without nephritis and the association with clinical and laboratory parameters were evaluated.

Results: A total of 90 samples, including sera (n=65) or plasma (n=25) with or without nephritis were included. The clinical parameters assessed were: disease activity, renal involvement and clinical manifestations. The IC autoantibody content was increased in SLE patients with nephritis vs. those without nephritis (p<0.001).

Conclusion: The results indicate that circulating immune complexes in SLE contain high antibody content of specific autoantibodies. Further studies are needed to evaluate the clinical relevance of these findings.