THU0233

ANTIBODIES AGAINST COMMENSAL STREPTOCOCCAL SPECIFIC PROTEIN (SSP) AND MITOCHONDRIAL IMMUNO- DOMINANT PROTEIN(MIP) IN DIAGNOSIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND OTHER SYSTEMIC AUTOIMMUNE DISEASES

C. H. Hsu¹, C. J. Jung², Y. M. Kuo^{1,3}. ¹College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China; ²School of Medicine, College of Medicine, Taipei Medical University, Department of Microbiology and Immunology, Taipei, Taiwan, Republic of China; ³College of Medicine, National Taiwan University, Graduate Institute of Clinical Medicine and Dept of Internal Medicine, Taipei, Taiwan, Republic of China

Background: Streptococcal infection has well known to cause rheumatic fever with various presentations similar to SLE. Besides, both streptococcal-blood stream infection and SLE had aggravated neutrophil extracellular traps (NETs) formation. Previously, we did report a streptococcal induced endocarditis rat model, and identified layers of neutrophil extracellular traps (NETs). According to our findings, specific immunoglobulin G (IgG) could bridge bacteria to host and these IgGs are required to induce the formation of NETs. Whether oral commensal bacteria could induce pathogenic antibodies which promote NET formation remained unknown.

Objectives: So we aimed to search for novel autoantibodies in SLE through antibody repertoire screening which recognize whole proteins derived from *Streptococcus mutans*, and investigated to find cross-reactive antibodies presented in the serum of lupus patients and do the correlation between serum MPO(myeloperoxidase)-DNA, a marker of NETosis.

Methods: The streptococcal specific protein (SSP) was identified through LC-MS and by a proteomics survey. We then purified the target protein in streptococci with expression vector, and antibody level will be detected quantitatively. We recruited patients with SLE, other systemic autoimmune diseases (AIDs) patients to elucidate the performance of this biomarker. Besides, we pursued the Basic Local Alignment Search Tool (BLAST) and searched for cross-reactive autoantigens.

Results: 79 lupus patients and 95 patients with other systemic autoimmune disease were enrolled. By using cut-off value 1.06(set according to area under the receiver operating characteristic curve) of anti-SSP o.d. value (174 samples), 27 of 79 (34.2%) SLE patients have positive results while only 7/95 (7.4%) was detected in control group. The specificity and sensitivity of the anti-SSP for diagnosis of SLE was 92.6% and 34.2% respectively. According to BLAST and B cell-epitope prediction algorithms, The P32-55 epitopes were identified and we synthesized a highly immunogenic and surface-accessible epitope related protein. We named the protein to be MIP (mitochondrial immuno-dominant protein.) Most of the sera from SLE patients could recognize MIP and anti-MIP correlated with serum MPO-DNA($r=0.41$, $p=0.039$).

Conclusion: The novel anti-SSP antibody against commensal streptococcal specific protein can differentiate SLE and other AIDs. Further investigation to determinate whether the anti-SSP antibody or the associated immune-complex could induce or aggravate NETs formation is warranted.

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THU0234

INFLUENCE OF THE SPLICING MACHINERY DYSREGULATION ON THE PATHOGENESIS OF LUPUS AND THE KIDNEY INVOLVEMENT

C. Lopez-Pedrer¹, M. Luque-Tévar¹, A. Ibañez-Costa², A. M. Patiño-Trives¹, M. A. Aguirre¹, P. S. Laura¹, M. D. C. Abalos-Aguilera¹, I. Arias de la Rosa¹, P. Seguí Azpilcueta¹, M. Espinosa¹, N. Barbarroja Puerto¹, E. Collantes-Estévez¹, J. P. Castaño Fuentes², R. M. Luque Huertas², C. Perez-Sanchez¹.

¹IMIBIC/Reina Sofia Hospital/University of Cordoba, Córdoba, Spain; ²Department of Cell Biology, Physiology and Immunology, University of Cordoba, Córdoba, Spain

Background:

Objectives: The aim of this study was to evaluate whether alterations in the splicing machinery of immune cells from Systemic Lupus Erythematosus (SLE) patients could influence the development and activity of the disease and the kidney involvement.

Methods: The study was conducted in 41 SLE patients and 34 healthy donors (HD). Monocytes, lymphocytes and neutrophils were purified by immunomagnetic selection. Then, selected elements of the splicing machinery and a set of genes related to inflammation, renal and cardiovascular disease (including interleukins, adipocytokines, chemokines, and oxidative stress markers, among others) were evaluated using a microfluidic qPCR array (Fluidigm). Besides, the inflammatory profile in plasma, including the analysis of 27 proteins, was evaluated by using the Bioplex assay. In parallel, an extensive clinical/serological evaluation was performed; comprising disease activity and cardiovascular and renal involvement along with autoantibodies, complement factors, and acute phase reactants. Correlation and association studies and logistic models among those clinical and analytical parameters were developed. Mechanistic *in vitro* studies were carried out by incubation of HD-leukocytes with anti-dsDNA-IgG purified from SLE patients and changes promoted in both, splicing machinery and leukocyte inflammatory profile, were assessed.

Results: A significantly altered expression of spliceosome components was found in all the leukocyte subsets: 27, 12 and 11 components were differentially expressed in monocytes, lymphocytes and neutrophils, respectively, in SLE patient's vs HD. In parallel, a number of genes codifying for proteins involved in inflammation, fatty acid metabolism, oxidative stress and migration were found altered and correlations with spliceosome components were further identified. Besides, those statistical analyses demonstrated multiple links among altered spliceosome components and the clinical profile of these patients, such as the activity of the disease (SLEDAI), the occurrence of obstetric complications and the presence of arterial hypertension. Logistic regression and ROC curve analyses identified a signature composed in each leukocyte subset by 3 altered spliceosome components that could differentiate between SLE and HDs. Remarkably, the levels of a high number of those altered components were associated to the presence of lupus nephritis (LN). Moreover, ROC curve analyses allowed to identify several cell-specific spliceosome components as potential biomarkers of renal disease. In patients with LN we could also identify a distinctive inflammatory profile in plasma in relation to patients without renal involvement, which further correlated with the altered expression of a number of spliceosome components in each leukocyte subset. Lastly, the *in vitro* treatment of HD leukocytes with anti-dsDNA promoted the alteration of several spliceosome components found also altered *in vivo* in SLE lymphocytes, monocytes and neutrophils.

Conclusion: 1)The splicing machinery is greatly altered in leukocytes from SLE patients, regulated, at least partially, by anti-dsDNA antibodies and closely related to the activity of the disease, including obstetrical complications, hypertension and lupus nephritis. 2)Specific components of the spliceosome in SLE leukocytes subsets might be used as potential biomarkers to typify the disease, particularly kidney involvement.

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THU0235

VITAMIN D AND INTERFERON SIGNATURE GENE EXPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS

R. Magro¹, C. Saliba², L. Camilleri², C. Scerri², A. Borg¹. ¹Mater Dei Hospital, Rheumatology, Msida, Malta; ²University of Malta, Msida, Malta

Background: Vitamin D deficiency is more prevalent in patients with systemic lupus erythematosus (SLE) as a result of sun avoidance.¹ The potential negative impact of vitamin D deficiency on SLE disease activity has been shown in a number of studies.² The expression of the interferon signature genes in SLE correlates positively with disease activity, and these genes are thought to mediate the clinical manifestations of the disease.³

Objectives: The aim of this study was to establish whether a relationship exists between serum 25-hydroxyvitamin D level and the interferon signature gene expression in whole blood of SLE patients.

Methods: Informed consent was obtained from 92 SLE patients who were over the age of 18 and who fulfilled the SLICC classification criteria for SLE. The patients were interviewed and blood samples were taken. SLE disease activity was measured by SLE disease activity index-2K (SLEDAI-2K). RNA extraction was performed from whole blood. QuantiGene Plex technology

was used to measure the expression of 12 interferon signature genes in the extracted RNA. The study was approved by the University Research Ethics Committee.

Results: 92.4% of the cohort studied were female. 58.7% were receiving vitamin D3 supplementation at a mean dose of 1031IU daily. 27.2% had vitamin D insufficiency (25-hydroxyvitamin D 21-29ng/ml) and 15.2% were vitamin D deficient (25-hydroxyvitamin D <20ng/ml). Mean serum 25-hydroxyvitamin D was 30.75ng/ml (standard deviation 9.53ng/ml). Median SLEDAI-2K was 4 (range 0-12). Serum 25-hydroxyvitamin D had a significant negative correlation with body mass index (BMI) ($R=-0.258$, $p=0.006$) but there was no significant negative correlation with SLEDAI-2K or with the expression of the interferon signature genes. The expression of most interferon signatures genes measured (IFI35, OAS1, MX1, IFITM1, STAT2, IFIT3, IFIT1, STAT1, SOCS1) had a significant positive correlation with SLEDAI-2K.

Conclusion: This study did not show a significant relationship between serum vitamin D level and disease activity. In keeping with this, there was no significant negative correlation between serum 25-hydroxyvitamin D and interferon signature gene expression. Further prospective studies and randomised controlled trials are required to study this relationship in greater depth.

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THU0236

EFFICACY AND SAFETY OF NON-MITOGENIC ANTICD3 ANTIBODY ADMINISTRATION IN THE TREATMENT OF LUPUS-PRONE MICE

M. Morita¹, S. Masuyama¹, M. Mizui¹, Y. Isaka¹. ¹Osaka University, 2-2, Suita, Japan

Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by the production of autoantibody and systemic tissue damages including glomerulonephritis. Immune responses mediated by autoreactive T-cells, as well as by autoantibody, is involved in the development and progression of end-organ damages¹. Biologic agents which manipulate T-cell function such as CTLA4-Ig and anti-CD40L have been revisited and tried to treat human SLE, however, both of them failed to demonstrate efficacy.

A mouse specific anti-CD3ε mAb, clone 145-2C11 (2C11) is known to be immunosuppressive by down-modulation of TCR and depletion of T-cells². Administration of Fc-deleted 145-2C11 F(ab')₂ to lupus-prone mice was reported to reduce lymphadenopathy and prolong survival, but had no significant effect on anti-DNA antibody titer³. The mechanisms by which 2C11 ameliorates lupus are still unclear.

In this study, we used non-mitogenic Fc-modified silent 145-2c11 (2C11S), which disables interaction between target cells and Fc receptor-bearing cells, abolishes antibody directed cytotoxicity, and has longer half-life than F(ab')₂. 2C11S is expected to exert its effect in safe and stable as compared with functional parent-2C11 (2C11P) antibody.

Objectives: The purpose of our study is to clarify the difference between 2C11S and 2C11P and to examine their therapeutic effects against murine lupus-prone (NZB/W F1) mice.

Methods: 20 µg of 2C11P (absolute antibody), 2C11S (absolute antibody), or isotype control immunoglobulin G1 κ (IC)(BioLegend) were administered intraperitoneally to C57BL6 mice. The difference of their action on T-cells were evaluated in a time series from peripheral blood. Plasma cytokine levels were measured within 24 hours after antibody administration.

In NZB/W F1 mice from weeks 10 or 20, 2C11P, 2C11S, and IC were administered (100 µg / week, 4 times, intraperitoneally). Plasma anti-dsDNA antibody titer, spleen and kidney blood cell subpopulation, and histology of renal tissue were evaluated before and/or after treatment.

Results: Duration of reduced TCR expression in 2C11S group was approximately twice as long as that in 2C11P group, and the levels of plasma TNF-α was not increased in 2C11S group while significant increase was observed in 2C11P group (IC; mean 48.3 ± SD 16.7 pg/ml, 2C11S; 57.9 ± 6.12, 2C11P; 168 ± 50.6, IC VS 2C11S; $p>0.99$, IC VS 2C11P; $p=0.03$, ANOVA).

In NZB/W F1 mice, the number of follicular helper T (Tfh) cells in spleen significantly decreased in 2C11S group (IC; median 9.0×10^4 [interquartile range 8.5×10^4], 2C11S; 1.8×10^4 [1.0×10^4], 2C11P; 1.0×10^5 [9.4×10^4], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p>0.99$, Kruskal-Wallis). The number of germinal center B (GCB) cells in spleen also decreased in 2C11S group (IC; 1.2×10^5 [1.7×10^5], 2C11S; 9.0×10^3 [2.3×10^4], 2C11P; 8.0×10^4 [2.3×10^5], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p>0.99$). The number of infiltrating CD4⁺T-cells in kidney significantly reduced in 2C11S group (IC; 3.4×10^3 [1.0×10^4], 2C11S; 6.4×10^2 [8.8×10^2], 2C11P; 1.2×10^3 [4.4×10^3], IC VS 2C11S; $p=0.048$, IC VS 2C11P; $p=0.23$). In addition, the rate of increase in anti-dsDNA IgG titers significantly decreased in 2C11S group (IC; 2.3 [1.3], 2C11S; 0.9 [1.0], 2C11P; 1.3 [1.4], IC VS 2C11S; $p=0.03$, IC VS 2C11P; $p=0.24$). Finally, glomerular hypercellularity was markedly alleviated only in 2C11S group (IC; 4.4×10 [8.4], 2C11S; 3.8×10 [1.1], 2C11P; 3.9×10 [8.2], IC VS 2C11S; $p=0.02$, IC VS 2C11P; $p=0.57$).

Conclusion: 2C11S did not induce cytokine release with maintaining longer effect on TCR down-modulation. 2C11S reduced autoantibody production by suppressing GCB differentiation, possibly through down-regulation of Tfh cell number. Consequently, 2C11S ameliorated lupus nephritis. On the other hand, 2C11P did not show therapeutic effect.

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THU0237

UNCOVERING DIFFERENCES BETWEEN THE SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND HEALTHY IMMUNOMES USING MULTI-PARAMETRIC INTERROGATION

K. Nay Yang^{1,2}, J. G. Yeo^{1,3}, M. Wasser¹, P. Kumar¹, S. P. Tang⁴, S. K. Ng⁴, S. L. Poh⁵, T. Arkachaisri^{1,2,6}, S. Albani^{2,3,5}. ¹Translational Immunology Institute, Singhealth/Duke-NUS Academic Medical Institute, Singapore, Singapore; ²Duke-NUS Medical School, Singapore, Singapore; ³KK Women's and Children's Hospital, Singapore, Singapore; ⁴Selayang Hospital, Paediatric Rheumatology Unit, Department of Paediatrics, Kuala Lumpur, Malaysia; ⁵Translational Immunology Institute, Singhealth/Duke-NUS Academic Medical Institute, Singapore, Singapore; ⁶KK Women's and Children's Hospital, Rheumatology and Immunology, Singapore, Singapore

Background: Systemic lupus erythematosus (SLE) is a complex, systemic autoimmune disease that interferes with the balance between regulation and immunity, resulting in immune system dysfunction. Disease course is unpredictable due to alternating remissions and flares¹.

Disease activity and treatment response are measured with composite scores such as the SLE Disease Activity Index (SLEDAI)². However, disease heterogeneity can impede reliable patient assessments. Mechanistic insights into SLE are required for better assessment.

Current studies are mainly descriptive, and a complex disease like SLE is best interrogated with multi-parametric, holistic approaches such as mass cytometry (CyTOF).

Objectives: (1)To characterise immune signatures of newly diagnosed SLE patients and in the process:

a.Study the roles of B and T cells in SLE

b.Gain a holistic understanding of the adaptive immune response

(2)To compare immunological profiles of newly diagnosed SLE patients with age-matched healthy controls

We hypothesise that significant differences exist between immunomes of newly diagnosed SLE and healthy subjects.

Methods: Peripheral blood mononuclear cells (PBMCs) of 5 SLE subjects (median age 125 months) were tested with CyTOF. Data was uploaded to an online analytical platform, the Extended Polydimensional Immunome Characterization (EPIC) discovery tool, for comparison with 51 age-matched controls in its database.

Subsequently, normalization and FlowSOM (Flow cytometry analysis by Self-Organising Maps) clustering to 50 nodes were performed with 37 functionally and phenotypically important immune markers. The Mann-Whitney U test identified significantly different cluster frequencies.

Results: Correspondence analysis comparing global differences in cluster frequencies showed segregation of SLE subjects away from healthy controls. Multiple significant differences were identified ($p < 0.05$). Notably, a memory CD4⁺CD152⁺PD1⁺ T cell subset (CD4⁺CD152⁺PD1⁺CD45RO⁺CD25⁺FoxP3⁺) was enriched in SLE (median: 2.17%, interquartile range: 1.66 to 7.74% of CD45⁺ PBMCs) versus control (1.34%, 1.06 - 1.58%; $p = 0.00267$). Expression