reaction of astrocytes and patchy increase of lymphocytes were also observed. Furthermore, MHC class I and class II were also highly expressed in the vascular endothelium in FcγRIIB$^{-/-}$ *Yaa* mice.

Conclusions: Activation of myeloid lineage cells and reactive changes of glial cells and endothelial cells were observed in the central nervous system of lupus-prone FcγRIIB$^{-/-}$ *Yaa* mice. These results imply the role of innate immune mechanisms in the pathology of NPSLE.

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AB0126 AUTOPHAGY AND SYSTEMIC LUPUS ERYTHEMATOSUS: CLINICAL SIGNIFICANCE OF ATG14+, FOXP3+, AND CD25+ EXPRESSION ON T REGULATORY CELLS AND NK CELLS

A. Rodríguez[1], E.J. Zurita[1], A.R. Durán[2], R. Bustamante[3], A. Sánchez[3], M.Á. Saavedra[3], C. Arroyo[4], G. Medina[5], L.J. Jara[3]. *[1]Immunology Department, Escuela Nacional de Ciencias Biologicas; [2]Immunology Department, Escuela Nacional de Ciencias Biológicas; [3]Rheumatology Department; [4]Banco de Sangre, Hospital de Especialidades "Dr. Antonio Fraga Mouret", Centro Médico la Raza, IMSS; [5]Rheumatology Department, Hospital de Especialidades "Dr. Antonio Fraga Mouret", CENTRO, Mexico City, Mexico*

Background: Autophagy is a highly conserved protein degradation pathway, essential for removing protein aggregates and misfolded proteins in healthy cells. Autophagy and autophagy-molecules expression have been implicated in autoimmune diseases. Systemic Lupus Erythematosus (SLE) is a prototype of autoimmune disease whose main characteristic is the loss of immune tolerance. Recent evidences suggest that autophagy, and autophagy-related proteins participate in SLE immune regulation. However, little is known about the SLE clinical significance of autophagy-related proteins, T regulatory, and NK cells.

Objectives: To evaluate the expression of ATG14$^+$ (autophagy-related key regulator protein), FOXP3$^+$, CD25$^+$T regulators, CD56$^+$NK cells in active and inactive SLE patients.

Methods: The expression of ATG14$^+$, FOXP3$^+$, CD25$^+$, and CD56$^+$ were measured by flow cytometry, and expressed in percentages in T and NK cells of SLE patients (1997, ACR criteria), and healthy controls. Active SLE was considered by SLEDAI ($\geq$4). The organs affected and treatments were evaluated.

Results: A total of 40 SLE patients and 20 healthy controls were included. The mean expression of autophagy in 20 active SLE patients was 11.19% in comparison with inactive SLE patients, 7.13%, (p=0.04), and in healthy donors, 7.445% (p=0.0281). The FOXP3$^+$ expression in NK cells in active SLE was lower in comparison with inactive patients (0.98% vs 3.82% respectively). In healthy donors was 2.89%.

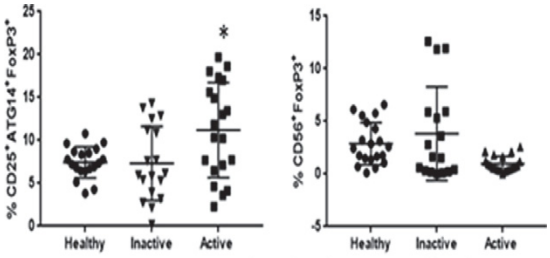

Figure 1: Expression in percentage of CD25$^+$ATG14$^+$FoxP3$^+$ (T reg) and CD56$^+$FoxP3$^+$ (NK cells) in SLE active and inactive patients and healthy controls. *p=0.04 Mann Whitney U test.

Conclusions: We found that in active patients autophagy is higher than in inactive patients. In inactive patients FOXP3 expression in NK cells is normal. These results can be due to the effect of the different treatments given according to clinical manifestations. Autophagy-related key regulator protein may be a new target of SLE treatment

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AB0127 ANTI-DS-DNA ANTIBODIES REGULATE ATHEROTHROMBOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS THROUGH THE INDUCTION OF NETOSIS, INFLAMMATION AND ENDOTHELIAL ACTIVATION

C. Perez-Sanchez[1], M.A. Aguirre[1], P. Ruiz-Limon[1], M.C. Abalos-Aguilera[1], I. Arias-de la Rosa[1], N. Barbarroja[1], Y. Jimenez-Gomez[1], P. Segui[1], E. Collantes-Estevez[1], J.A. Gonzalez-Reyes[2], J.M. Villalba[2], M.J. Cuadrado[3], C. Lopez-Pedrera[1]. *[1]IMIBIC/Reina Sofia Hospital/University of Cordoba; [2]Department of Cell Biology, Physiology and Immunology, ceiA3, University of Cordoba, Cordoba, Spain; [3]Lupus Research Unit and St. Thomas Hospital, London, United Kingdom*

Background: The role of anti-dsDNA in the pathogenesis of the systemic lupus erythematosus (SLE) has been clearly established. However, the influence of these autoantibodies in the atherothrombotic status of SLE patients has not yet been evaluated

Objectives: 1. To analyse in vivo the involvement of anti-dsDNA antibodies in the development of CVD in SLE patients. 2. To evaluate in vitro the mechanisms underlying the effects of anti-dsDNA antibodies in these processes

Methods: The study was conducted in 50 SLE patients and 38 healthy donors. Endothelial function was assessed by measuring the post-occlusive hyperaemia using Laser-Doppler. Various markers of oxidative stress, inflammatory cytokines, prothrombotic mediators and NETosis, were quantified in purified leukocytes and plasma from SLE patients and controls. Activation of intracellular pathways was analyzed in monocytes using pathscan intracellular signaling array. In vitro, purified neutrophils, monocytes and lymphocytes from healthy donors and endothelial cells (ECs) were treated separately and in a trans-well co-culture system with anti-dsDNA antibodies isolated from the serum of SLE patients. Then, markers of inflammation, thrombosis, oxidative stress and NETosis were evaluated by flow cytometry (protein), RT-PCR (mRNA) and electron microscopy

Results: SLE patients showed impaired micro-vascular endothelial function (reduction of hyperaemia post occlusion area) and altered expression levels of pro-inflammatory proteins (IL6, IL8, MCP-1 and PCR), prothrombotic molecules (TF), oxidative stress markers (peroxides and mitochondrial membrane potential) and netosis-related molecules (NE, MPO and cell free-DNA). Monocytes from anti-dsDNA-positive SLE patients showed activation of various pathways related to inflammation, thrombosis and apoptosis (ErK, STAT-3, p38, JNK, GSK, Bad and Caspase-3). Association studies demonstrated that molecules related to inflammation and thrombosis, endothelial dysfunction, oxidative status and netosis were linked to the occurrence of thrombotic events, as well as to the presence of anti-dsDNA antibodies. In vitro treatment of purified leukocytes with anti-dsDNA antibodies promoted an increase in the production of NETosis, levels of peroxides and percentage of cells with altered mitochondrial membrane potential, as well as enlarged expression of a number of proinflammatory and prothrombotic molecules. In vitro treatment of HUVEC with anti-dsDNA antibodies promoted an increase in endothelial activation molecules (ICAM-1, VCAM-1 and E-selectin).

Conclusions: 1. Positivity for anti-dsDNA antibodies is linked to an increased pro-atherothrombotic status in SLE patients. 2. Anti-dsDNA antibodies, in vitro, promote NETosis on neutrophils, apoptosis on monocytes, modulate the expression of molecules related to inflammation and thrombosis, and induce endothelial activation. Together, that data suggest the involvement of such autoantibodies on atherothrombosis development in SLE

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AB0128 ALTERATIONS OF THE SPLICING MACHINERY COMPONENTS IN LEUKOCYTES FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS INFLUENCES ITS DEVELOPMENT AND ATHEROTHROMBOTIC PROFILE AND DRIVES THE THERAPEUTIC RESPONSE

C. Perez-Sanchez[1], M.A. Aguirre[1], S. Pedraza-Arévalo[1], M. del Río-Moreno[1], P. Ruiz-Limón[1], N. Barbarroja[1], Y. Jimenez-Gomez[1], I. Arias-de la Rosa[1], M.C. Abalos-Aguilera[1], P. Segui[1], E. Collantes[1], J. Castaño[1], R.M. Luque[1], M.J. Cuadrado[2], C. Lopez-Pedrera[1]. *[1]IMIBIC/Reina Sofia University Hospital/University of Cordoba, Cordoba, Spain; [2]Lupus Research Unit, St Thomas Hospital, London, United Kingdom*

Background: Recent studies emphasize the relevance of alternative splicing in the development of genetic and autoimmune diseases and suggest therapeutic possibilities based on the modulation of this process.

Objectives: To identify alterations in the leukocyte splicing machinery of patients with systemic lupus erythematosus (SLE) and to evaluate its influence on the development and activity of the disease, its atherothrombotic profile, and the response to specific therapies.

Methods: An array of selected components of the major-(n=12) and minor-spliceosome (n=4) and associated splicing factors (n=28) was developed, and their expression levels were evaluated using a Fluidigm methodology, in purified leukocytes from 36 SLE patients and 29 healthy donors (HD). In parallel, an extensive clinical/serological evaluation was performed. Carotid intimate media thickness (CIMT) was used as atherosclerosis marker. Endothelial activity was

monitored by laser-doppler, and pro-inflammatory and oxidative stress markers were quantified by flow cytometry and RT-PCR. In a parallel cohort of SLE patients (n=12), the effects of in vivo treatment with ubiquinol on spliceosome components was evaluated.

Results: As a general feature, a significant reduction in splicing factors and spliceosome components was found in all the leukocytes of SLE patients. Interestingly, we found a specific altered profile of splicing factors and spliceosome components when compared monocytes (U2AF1, FBP11, SRSF9), lymphocytes (RBM22, PRP8, SRSF5) and neutrophils (RNU4, CA150). The reduced levels of some components of spliceosome in both monocytes and neutrophils were linked to the occurrence of thrombotic events, foetal loss and arterial hypertension. In lymphocytes, those reduced levels were strongly related to the positivity for anti-dsDNA antibodies in SLE patients, thus suggesting that reduced spliceosome machinery would contribute to increase in altered autoantigen assembly, inducing increased autoantibody production. Correlation studies demonstrated an inverse relationship among reduced levels of spliceosome components/splicing factors and high activity of the disease (measured as SLEDAI), endothelial dysfunction, and increased expression levels of peroxides and peroxynitrites, as well as of altered mitochondrial membrane potential in monocytes and neutrophils. In vitro treatment of leukocytes from HDs with anti-dsDNA promoted a reduction in spliceosome components associated with the expression of proinflammatory and oxidative mediators. Finally, in vivo treatment with ubiquinol reversed reduced expression in SLE of spliceosome components related to their proaterothrombotic profile.

Conclusions: These results reveal the existence of SLE- associated spliceosome alterations -promoted by anti-dsDNA antibodies-which could be related to the development and activity of this autoimmune condition and have influence on the induction of mechanisms that drive atherothrombosis as well as the therapeutic response.

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AB0129 HMGB1+ MICROPARTICLES IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITH LUPUS NEPHRITIS

C. Burbano [1], J.A. Gómez-Puerta [1], M. Rojas [1], G. Vasquez [1], J. Orejuela [1], C.H. Muñoz [2], A. Vanegas-García [2], D. Castaño [1]. *[1]Universidad de Antioquia; [2]Hospital Universitario de San Vicente Fundación, Medellín, Colombia*

Background: High mobility group box protein 1 (HMGB1) is a nuclear DNA-binding protein that can function as an alarmin when is released from activated and dying cells. In association with nucleosomes, HMGB1 may contribute to the pathogenesis of systemic lupus erythematosus (SLE). Some previous reports have associated HMGB1 with the pathogenesis of cutaneous lupus and lupus nephritis (LN). HMGB1 may also be contained in microparticles (MPs). These vesicles have a wide spectrum of biological activities in intercellular communication, and they compete with apoptotic cells to bind mononuclear phagocytes.

Objectives: To evaluate the association of MP-HMGB1+ circulating with LN and to correlate them with LN activity.

Methods: Blood samples from 60 SLE patients were used to isolated MPs from platelet-poor plasma by centrifugation and their count, cell source and phenotype were characterized by flow cytometry. Renal pathology was reported using the standardized International Society of Nephrology/Renal Pathological Society classification. Inactive lupus nephritis (LN) was defined by the presence of one or more of the following criteria: 24 hrs proteinuria 500 mg/dl or inactive urine sediments (<5 red cells/HPF) and no red cell casts and no leucocyturia (<5 white cells/HPF) and stable serum creatinine.

Results: Mean age of SLE patients was 31.9±10.8 years, and mean disease duration was 7.8±6.2 years. 73% patients had LN and 89% were female. Patients with LN had significantly higher frequency of MP-HMGB1+, no significant differences were found among patients with active versus inactive LN or among patients with proliferative vs non-proliferative LN; MP-HMGB1+ had a moderate positive correlation with disease activity (SLEDAI, r=0.367, p=0.020), anti-C1q antibodies titers (r=0.42, p=001) and 24 hours proteinuria (r=0,33, p=,032), but no correlation was found with activity or chronicity indexes on renal biopsies. A ROC curve for MP-HMGB1+ and renal involvement showed a good discriminative ability (AUC 0.706). A cutoff of 15.7% of MP-HMGB1+ showed the best discrimination threshold with a sensitivity of 63.3% and specificity of 83.3%.

Conclusions: In our cohort of patients with SLE, MP-HMGB1+ was significantly higher in patients with LN and in patients with active disease. Given the multiple implication of HMGB1 in SLE, including the active kidney recruitment of mononuclear phagocytes, we consider that MP-HMGB1+ could be considered as a potential biomarker for LN in SLE patients.

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AB0130 MESENCHYMAL STEM CELLS ALLEVIATE SLE THROUGH PROMOTING TREG CELLS BY HLA-G

C. Chen, J. Liang, G. Yao, Z. Zhang, S. Huang, L. Sun. *The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China*

Background: Soluble human leukocyte antigen-G (sHLA-G) is a non-classical HLA class I molecule, exhibiting strong immunosuppressive properties by inducing the differentiation of T regulatory cells (Treg). Mesenchymal stem cells (MSCs) transplantation alleviates disease progression in systemic lupus erythematosus (SLE) patients. However, the underlying mechanisms are largely unknown.

Objectives: The aim of the present study is to explore whether sHLA-G is involved in upregulating Treg cells by MSCs, which contributes to therapeutic effects of MSCs transplantation in SLE.

Methods: The serum sHLA-G levels of SLE patients and healthy controls were detected by ELISA. The percentages of peripheral blood CD4 + ILT2 +, CD8 + ILT2 +, CD19 + ILT2 + cells and Treg cells were determined by flow cytometry. Ten patients with active SLE, refractory to conventional therapies, were infused with MSCs and serum sHLA-G was measured 24 h after infusion. Peripheral blood mononuclear cells (PBMCs) were isolated from SLE patients and co-cultured with UC-MSCs for 3 days at different ratios (50:1, 10:1, and 2:1) with or without anti-HLA-G antibodies, and the frequencies of CD4 + CD25 + Foxp3 + T cells were then determined by flow cytometry.

Results: The concentrations of serum sHLA-G were comparable between SLE patients and healthy controls. However, there was a negative correlation between sHLA-G levels and SLE disease activity index (SLEDAI) scores in active SLE patients (SLEDAI >4). We found that serum sHLA-G levels were negatively correlated with blood urea nitrogen, serum creatinine and 24-hour urine protein in SLE patients. The sHLA-G levels were significantly lower in SLE patients with renal involvement than those without renal involvement. The expression of ILT2 on CD4 + T cells from SLE patients decreased significantly compared to that of healthy controls. A positive correlation between the frequencies of Treg and CD4 + ILT2 + T cells was found in SLE patients. The levels of sHLA-G increased 24 h after UC-MSC transplantation. The frequencies of Treg cells and the expressions of ILT2 on CD4 + Tcells were significantly increased 24 h after transplantations. *In vitro* studies showed that MSCs increased the frequency of Treg cells in SLE patients in a dose-dependent manner, which was partly abrogated by the anti-HLA-G antibody.

Conclusions: Our results suggested that MSCs might alleviate SLE through upregulating Treg cells, which was partly dependent on sHLA-G.

Disclosure of Interest: None declared

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AB0131 PLATELET MICROPARTICLES (PMPS) ARE HIGHER IN PATIENTS WITH SLE COMPARED TO HEALTHY CONTROLS

C.-L.M. Murphy, V. Ripoll-Nunez, C. Periculeous, T. McDonnell, S. Croca, I. Giles, A. Rahman. *Rheumatology, University College London Hospital, London, United Kingdom*

Background: Patients with SLE have an increased risk of cardiovascular disease (CVD) and reasons for this are unknown. Microparticles (MPs) are membrane-bound particles released by many cells, 70–90% are derived from platelets. MPs are a rich source of autoantigens, including DNA and have many functions including thrombosis and inflammation. PMPs can induce foam cell formation and promote adherence of platelets to endothelial lesions. MPs are potentially important in the pathogenesis of autoimmune rheumatic disease and in cardiovascular disease.

Objectives: To determine if EMPs and PMPs are increased in patients with SLE. Our secondary aim was to determine if there was a difference between those with/without subclinical cardiovascular disease and healthy controls.

Methods: Atherosclerotic plaque was previously determined in patients with SLE who had no known history of CVD by carotid and femoral ultrasound scans. Plaque and thickened intima media thickness (IMT) >0.1cm were defined according to the Mannheim Carotid Consensus. Data regarding plaque area, IMT and echolucency were collected. Plasma was stored at -80° at the time of scan. Plasma from n=57; plaque (n=16), no plaque (n=23) and healthy controls (HC) (n=18) were analysed for presence of endothelial and platelet microparticles (EMPs and PMPs), as per protocol. Samples were stained with Annexin V and platelet and endothelial antibodies, CD42a, CD31, CD105 and CD144. MPs were measured using flow cytometry. Statistical analysis was carried out using PRISM.

Results: A total of 57 plasma samples were tested. The average age of those with SLE was 45±12 years. 96% were female. 56% were Caucasian, 18% Asian, 21% Afrocaribbean, 5% other ethnicity. 18% of the SLE patients were smokers. Of the 18 HC, the average age was 37±7 years, 83% were female. 88% were Caucasian and 12% other ethnicity. 11% of the HCs were smokers. PMPs were significantly higher in patients with SLE compared to healthy controls (p=0.025). Patients with SLE without plaque had more PMPs compared to healthy controls