AB0123 T-CELL SURFACE GLYCOSYLATION PATTERN ALTERATIONS IN SLE – A PUTATIVE LINK TO GALECTIN-1-MEDIATED IMMUNOREGULATORY DEFICIENCY

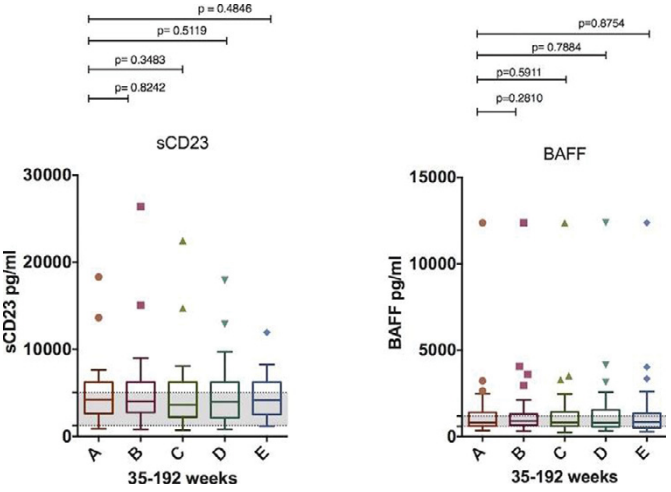
Á. Hornung[1], E. Szabó[2], Á. Czibula[2], É. Monostori[2], L. Kovács[1]. *[1]Department of Rheumatology and Immunology, University of Szeged; [2]Department of Genetics, Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary*

Background: We have previously found that activated T-cells from systemic lupus erythematosus (SLE) patients express lower amount of intracellular galectin-1 (icGal-1) than those of healthy controls and are resistant to the apoptotic effect of extracellular galectin-1 (ecGal-1), an endogenous immunoregulatory lectin. We also demonstrated that the *de novo* synthesized icGal-1 level affects apoptosis of T-cells induced by ecGal-1, since low icGal-1 expression resulted in reduced sentsitivity to ecGal-1 (Deák M *et al*). We have therefore proposed the defective icGal-1 production to be an explanation to the insufficient regulatory effects of ecGal-1 in SLE. However, altered binding of ecGal-1 to T-cells due to changes in surface glycosylation may also regulate the apoptotic activity of ecGal-1.
Objectives: We have herein hypothesized that the cell-surface glycosylation pattern, and consequently, lectin-binding ability in SLE T-cells is altered, and that an abnormal expression of glycosylation enzymes may account for these changes.
Methods: In order to analyse the glycosylation pattern of cell surface glycoproteins, lectin-binding assays were performed using 5 different plant lectins and human recombinant Gal-1 on resting and activated T-cells from patients with active SLE (n=8) with multi-colour flow-cytometry, and were compared with 15 healthy controls. mRNA levels of 13 glycosylation enzymes involved in the development of N-glycan structures on T-cells were measured with qPCR, and were correlated with the specific lectin binding data.
Results: As compared with the resting state, the increase in Gal-1 binding during activation was significantly lower in SLE T-cells than in controls, and the level of Gal-1 binding maximum was significantly reduced in SLE activated T-cells than in controls. Binding maximum of plant lectins that recognise high complexity N-glycans also increased less in SLE T-cells than in controls during activation. mRNA level of sialyltransferase ST3GAL6 was increased and neuraminidase Neu1 was decreased in active SLE patients as compared to controls. The ST6GAL1/NEU1 ratio in SLE patients positively correlated with the SLEDAI disease activity index.
Conclusions: SLE T-cells show decreased complexity of N-glycan structures. Increased ST3GAL6 and decreased Neu1 expression result in an increased density of terminal sialic acids, and this may explain the impaired Gal-1 binding. In addition to the previously described deficiency in icGal-1 expression upon activation, our present findings of an attenuated glycan complexity and a shift toward terminal sialylation provide a further mechanism of pathological T-cell activation and regulation of T-cell viability in SLE.
References:
[1] Deák M, Hornung Á, Novák J, Demydenko D, Szabó E, Czibula Á, Fajka-Boja R, Kriston-Pál É, Monostori É, Kovács L. Novel role for galectin-1 in T-cells under physiological and pathological conditions. Immunobiology. 2015 Apr;220(4):483–9.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5657

AB0124 SEROLOGICAL MEASURES OF B CELL FUNCTION IN PATIENTS WITH SLE; HOW ROBUST ARE THEY OVER TIME?

A. Hennessey[1,2], R. Marques[3], M. Leandro[1,2], D. Isenberg[1,2], G. Cambridge[2]. *[1]Division of Rheumatology, Department of Medicine, University College London Hospitals; [2]Centre for Rheumatology and Bloomsbury Rheumatology Unit, University College London, London, United Kingdom; [3]Serviço de Medicina Interna B, Centro Hospitalar Universitário de Coimbra, Coimbra, Portugal*

Background: In SLE the precise mechanisms whereby parent autoreactive B cells are generated and permitted to escape tolerance checkpoints, proliferate, persist and switch to pathogenic autoantibody production remains poorly understood. Hypotheses include defective negative central selection, defective peripheral selection with enhanced germinal center activity as well as positive selection of B cells via autoantigen presentation or T-independent mechanisms. Biomarkers to identify possible breaches in tolerance checkpoints would allow more effective intervention. We have therefore measured relative levels of soluble (s)CD23 (released during B cell differentiation from naive to memory B cell status) and the B cell activation factor, BAFF (survival factor and class-switch/differentiation promoter) in SLE sera to determine if relative levels of sCD23 and BAFF were of use as biomarkers to group patients based on B cell kinetics rather than clinical features. BAFF is often raised in SLE patients and can stimulate the aberrant differentiation of transitional B cells and of plasmablasts in vitro. Combining the 2 biomarkers could therefore indicate whether there was increased expansion of naïve B cells, and whether BAFF was a possible driver/consequence of autoimmunity in different patients.
Objectives: To determine whether relative levels of sCD23 and BAFF reflect disease activity or remain stable over time.
Methods: Stored SLE serum from patients who were Rituximab-naive, had >5 samples available in the biobank over at least 6 months (n=38). Samples were analysed for levels of sCD23 and BAFF via ELISA. Wilcoxon matched-pairs signed rank test were used to compare serial values. Clinical details including BILAG scores were also collected for the available time points. If positive dsDNA at any time during the time period this was deemed positive. The latest BILAG was utilised and reviewed, a patient was deemed not to have system involvement if they had an "E" for that system.
Results: Sera from 38 SLE patients (32 female, 6 male) with mean age of first sample 42 (range: 25–72). Minimum interval between 1st and last sample was 101 weeks (range: 35–192 weeks). Patients were then sorted into clinical groups according to levels of these serum markers. Normal ranges defined as: sCD23 (1235–5023pg/ml), BAFF (584–1186pg/ml). Group I (n=9): – High (H) sCD23, Normal (N) BAFF; Group II (n=11) – H sCD23, H BAFF; Group III (n=17) – N sCD23, N BAFF; Group IV (n=2) – N sCD23, H BAFF. Figure 1 shows the p values generated from the Wilcoxon matched-pairs signed rank test for each time period demonstrating nil significant change over time. Analysis of clinical data showed no differences in terms of organ involvement or anti-dsDNA -Ab status.



Conclusions: Within a cohort of SLE patients, soluble CD23 and BAFF is stable over time despite variation in disease activity. Grouping patients based on sCD23 and BAFF profile may be useful in identifying distinct B cell maturation pathways reflecting underlying autoimmune pathways which vary between patients.
Disclosure of Interest: A. Hennessey: None declared, R. Marques: None declared, M. Leandro Consultant for: Roche UK, Roche Basel and Genentech, D. Isenberg: None declared, G. Cambridge: None declared
DOI: 10.1136/annrheumdis-2017-eular.6780

AB0125 BRAIN IMMUNOPATHOLOGY OF LUPUS-PRONE FCΓRIIB-/-YAA MICE - IMPLICATION TO THE INNATE IMMUNE RELATED MECHANISM OF NEUROPSYCHIATRIC SLE

A. Nomura[1], G. Murayama[1,2], A. Chiba[1], S. Miyake[1]. *[1]Immunology; [2]Rheumatology, Juntendo University, Tokyo, Japan*

Background: Neuropsychiatric SLE (NPSLE) is a common manifestation of SLE and problems such as cognitive impairment or depression are elusive. The importance of innate immune related inflammation in the pathomechanism of neurodegenerative or psychiatric diseases has been recognized recently, and the importance of innate immunity in the pathogenesis of NPSLE has also been suggested[1)]. Therefore, we investigated innate immune mechanism of NPSLE by using lupus prone mice.
Objectives: This study is conducted to understand the brain immune pathology of lupus-prone FcγRIIB$^{-/-}$ *Yaa* mice in which innate immune stimulation is potentiated by the duplication of Toll-like receptor 7[2)].
Methods: Immune cell subsets and histopathology of brains were analyzed by flow cytometry and immunohistochemistry in FcγRIIB$^{-/-}$ *Yaa* mice compared with congenic mice at around 16 week-old when glomerulonephritis had developed. For flow cytometric analysis, microglia, myeloid lineage cells and lymphocytes were defined by staining with CD11b and CD45. Subsets of those cells and their activation status were analyzed. For histopathological analysis, microglia, brain macrophages, astrocytes and lymphocytes were immunostained and expression of MHC class I and class II were also analyzed.
Results: Flow cytometric analysis revealed increase in the number of microglial cells (CD11b^{+} CD45^{int}) and myeloid lineage cells (CD11b^{+} CD45^{high}) in the brains of FcγRIIB$^{-/-}$ *Yaa* mice compared with congenic FcγRIIB$^{+/+}$ mice. Mean fluorescence intensity of MHC class I was increased in microglia and myeloid lineage cells in FcγRIIB$^{-/-}$ *Yaa* mice. An increased percentage of CD3 positive cells compared to CD19 positive cells were observed and their expression of CD69, an activation marker, were increased in FcγRIIB$^{-/-}$ *Yaa* mice. In histopathology, number of macrophages and microglia identified by Iba1 (Ionized calcium binding adapter molecule 1) positive cells were increased in FcγRIIB$^{-/-}$ *Yaa* mice. In areas where MHC class I and class II were highly expressed on macrophages,

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.

References:

[1] Stock AD, Wen J, Doerner J, et al. Neuropsychiatric systemic lupus erythematosus persists despite attenuation of systemic disease in MRL/lpr mice. J Neuroinflammation.2015;12:205.

[2] Kawano S, Lin Q, Amano H, et al. Phenotype conversion from rheumatoid arthritis to systemic lupus erythematosus by introduction of Yaa mutation into FcγRIIB-deficient C57BL/6 mice. Eur J Immunol.2013;43:770–8.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2849

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%.

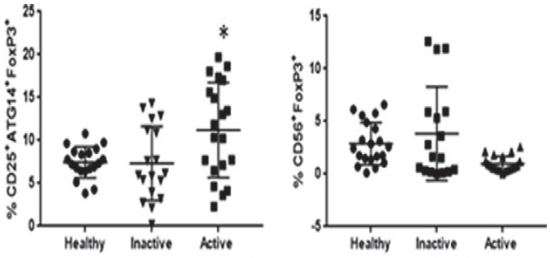


Figure 1: Expression in percentage of CD25$^+$ATG14$^+$FoxP3$^+$ (T reg) and CD56$^+$FoxP3$^+$ (NK cells) in SLE active and inactive patients and healthy controls. *p=0.04 Mann Whitney U test.

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

References:

[1] Lech M, Anders HJ. The pathogenesis of lupus nephritis. J Am Soc Nephrol. 2013, 24, 1357–1366.

[2] Chikte S, et. al. use of LysoTracker dyes: A flow cytometric study of autophagy. Cytometry. 2014, 85(2), 169–78.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6883

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

Acknowledgements: CTS-794 and ISCIII (FIS15/1333;RIER RD16/0012/0015)

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4735

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