**Conclusions:** Epigenetic cell counting is a promising novel tool to reproducibly and easily quantify immune cells in the (inflamed) labial salivary gland of sicca patients with relatively low amount of tissue needed (<1 $mm^3$). Considering the potential of this technique to include a huge number of (cell-specific) biomarkers we believe this opens up new standardized ways for salivary gland analysis with high relevance for patient classification, understanding of immunopathology and clinical trials.
**Disclosure of Interest:** None declared
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## THU0235 EXOSOME-DELIVERED MIR-146A REGULATES SENESCENCE OF BONE MARROW- MESENCHYMAL STEM CELLS FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS THROUGH TARGETING IRAK1 AND TRAF6

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**Background:** Exosomes are membrane nano-vesicles secreted by a multitude of cells that harbor biological constituents such as proteins, lipids, mRNA and microRNA. Recent study suggests that microRNAs can be transferred between cells and mediate target gene repression. Our research group revealed the senescence of bone marrow-mesenchymal stem cells from systemic lupus erythematosus patients,which participated in the development of SLE. However, the relationship between senescence of MSCs and miRNAs remains unclear.
**Objectives:** In this study, we investigated whether exosomes act as intercellular messengers delivering microRNA that modulate the senescence of BM-MSCs from SLE patients and its possible mechanism.
**Methods:** Tewelve female SLE patients and healthy subjects were enrolled in the study. All patients were females, and their age distribution was similar to that of the cases. Serum were collected from these persons. All BM-MSCs were Isolated by density gradient centrifugation. Serum-derived exosomes were extracted by Total Exosome Isolation reagent and confirmed by transmission electron microscope and western blot. The internalization of exosomes was detected by immunofluorescence. QRT-PCR was used to distinguish the difference of expression of miR-146a in exosomes between normal group and SLE group. Different exosomes stimulated normal BM-MSCs, then detecting expression of miR146a by qRT-PCR, detecting expression of IRAK1 and TRAF6 by WB, observing the activity of β-gal of cells, the changes of cytoskeletal structure by F-actin staining and the distribution of cell cycle by flow cytometry. We used miRNA mimics and miRNA inhibitor to interfere the expression of miR-146a.
**Results:** Serum-derived exosomes could be taken up by BM-MSCs through the plasma membrane due to treatment of BM-MSCs with exosomes. After stimulation of exosomes in normal MSCs, miR146a was decreased, but, IRAK1 and TRAF6 was activated. And, the cell volume and the number of SA-β-gal positive in SLE BM-MSCs was increased. The organization of cytoskeleton was neatly disordered. The rate of cell proliferation was decreased. The miR-146a mimics in SLE BM-MSCs can significantly reverse the senescence.
**Conclusions:** Exosomes-delivered miR-146a in the serum of SLE patients can promote the senescence of BM-MSCs through targeting IRAK1 and TRAF6. Exosomes play an important role in the pathogenesis of SLE.
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## THU0236 ANTIBODIES TOWARDS ATP-BINDING CASSETTE TRANSPORTER ABCA1: A NEW MECHANISM FOR ATHEROSCLEROSIS IN SLE?

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**Background:** Systemic Lupus Erythematosus (SLE) is considered an independent risk factor for cardiovascular disease and patients with SLE have an increased burden of atherosclerotic vascular disease[1]. High-density lipoproteins (HDL) are the plasma lipoproteins responsible for reverse cholesterol transport[2]. HDL protective effect on cardiovascular disease is attributed to the cholesterol efflux capacity as well as to its anti-oxidant and anti-inflammatory properties[3]. Dyslipidemia is frequent amongst patients with SLE, characteristically with low HDL levels.
ATP-binding cassette transporter ABCA1, also known as the cholesterol efflux regulator protein, is a ubiquitous cholesterol transporter that is highly expressed in macrophages. Its main function is to donate cholesterol to apolipoprotein A-I (ApoA-I) in lipid-poor HDL particles. As such, ABCA1 closely influences HDL levels and its role in atherosclerosis has been increasingly studied[4].
**Objectives:** This study was undertaken to determine if antibodies against ABCA1 can be detected in patients with SLE through enzyme-linked immunosorbent assay (ELISA).
**Methods:** Patients with SLE were divided in two groups: group A, with low damage (based on less than 4 SLICC criteria), and group B, with high damage (based on the presence of at least 4 SLICC criteria). Groups A and B were compared with a control group. 48 patients were enrolled (13 in group A and 35 in group B), and 18 age and gender-matched healthy controls were included in the control group. IgG anti-ABCA1 and anti-HDL antibodies were assessed by home-made ELISAs, using commercially available ABCA1 synthetic peptide and HDL from healthy donors.
**Results:** There were no differences between group A and the control group. Group B had higher titers of anti-ABCA1 antibodies when compared with group A (p=0.016) and the control group (p=0.022). For positivity we considered values superior to 3 standard deviations above the mean of healthy controls. Four patients showed positive anti-ABCA1 titers (11.4%).
**Conclusions:** This is the first time that naturally occurring antibodies against ABCA1 are detected by ELISA. These antibodies are increased in patients with SLE that have higher damage, measured by SLICC classification criteria. Future studies will determine their pathogenic role and the potential use of a standardized ELISA to detect anti-ABCA1 antibodies in clinical practice.
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**Disclosure of Interest:** None declared
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## THU0237 WHOLE TRANSCRIPTOME ANALYSIS OF APL TREATED HUVECS MAPS PROINFLAMMATORY AND PROCOAGULANT PATHWAYS

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**Background:** Antiphospholipid syndrome is an autoimmune thrombophillia characterized by recurrent thromboembolism and or pregrancy morbidity in the presence of antiphospholipid antibodies (aPL). β2GPI which is the major autoantigen in the syndrome forms complexes with anti-B2GPI autoantibodies that activate platelets, monocytes and endothelial cells. Previous studies have shown that anti-β2GPI-β2GPI complexes activate TLR4 and TLR6 on endothelial cells leading to NFκB, MAPK activation and Tissue Factor and proinflammatory cytokine expression.[1,2]
**Objectives:** To evaluate the whole transcriptome of endothelial cells that have been stimulated with aPL-B2GPI complexes.
**Methods:** Human umbilical Vein Endothelial cells (HUVECs) were isolated from 2 APS patients and 4 Healthy control women upon delivery. Healthy donor HUVEC were stimulated with IgG isolated from APS patients with high aPL titers and healthy individuals in the presence of B2GPI. Consequently total mRNA was isolated, cDNA libraries were created and whole transcriptome sequencing (RNASeq) was performed. Gene expression data were validated in protein levels with immunohistochemistry in placenta tissues from APS patients and healthy individuals.
**Results:** Whole transcriptome analysis of HUVECs stimulated with aPL-β2GPI complexes and IgG from healthy individuals revealed 680 differentially expressed genes, among which 377 were upregulated and 303 downregulated in the aPL stimulated endothelial cells. Characteristic examples of the upregulated genes are IL-6, IL-8, VCAM1, SELE and TGFB2 and TGFBR1. Bioinformatics analysis revealed that the upregulated genes belong mainly to the cytokine-cytokine receptor interaction (hsa053323), MAPK signaling pathway (hsa04010), TNF signaling pathway (hsa04668) and NOD-like receptor pathway (hsa04621). Characteristic examples of the downregulated genes include the CBX4, CBX8, BCOR and HDAC7 genes. Interestingly some of the proteins encoded by these genes play role in the epigenetic modification of DNA. Immunohistochemical staining on placenta biopsies from APS patients and healthy individuals for IL-6, IL-8, IL-18, NFkB, TF, TNF-a, E-SELECTIN, MAPK8, TGFB2 and TGFBR1 showed increased intensity in the signal of endothelial cells on APS specimens validating thus the RNASeq results in the tissues.
**Conclusions:** RNASeq of endothelial cells treated with aPL and B2GPI reveals a thoroughly analysed proinflamatory and procoagulant phenotype. Moreover differential expression of DNA modifying proteins suggests the possible epigenetic regulation of gene expression on endothelial cells in APS syndrome. Ongoing experiments aim to analyze histone acetylation and methylation status of the promoters of the selected genes that were shown to be differentially expressed.
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## THU0238 P-GLYCOPROTEIN MONOCLONAL ANTIBODY IMPROVES LUPUS-LIKE SYNDROME IN MRL/LPR MICE

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**Background:** Preventing steroid resistance and maintaining disease control are significant challenges to overcome in treating SLE patients[1]. P-glycoprotein (P-gp) of membrane transporters, a product of the multiple drug resistance (MDR)-1 gene, is known to play a pivotal role in the acquisition of drug resistance to chemotherapy in autoimmune diseases[2].Inhibition of P-gp could overcome such drug resistance[3,4]. So we observed the effect of P-gp monoclonal antibody on MRL/lpr lupus mice.

**Objectives:** To investigate the efficacy of P-glycoprotein monoclonal antibody in the treatment of the MRL/lpr mice.

**Methods:** Twenty four 14- week- old MRL/lpr female mice were divided into 3groups:group 1 (G1)were treated with P-glycoprotein monoclonal through caudal vein, group 2 (G2)were treated with P-glycoprotein monoclonal three times and group 0 (G0)were treated with 0.5ml normal saline as controls. Twenty-four hours proteinuria and body weight were assessed every two weeks.Enzyme linked immunosorbent assay (ELISA)was used to measure the levels of serum anti-dsDNA antibodies. The histopathology changes of the kidneys were observed.

**Results:** From the 22th week,the body weight of groups Gl and G2 increased significantly than that of the group G0 ($p<0.05$). At the 22th weeks,the 24 hours proteinuria in group Gl (1.9±1.1)mg and G2 (1.4±0.9)mg was decreased than that in group G0 (3.1+1.9)mg ($p<0.05$), and at the 26th weeks,that of groups G1 (2.4±1.4)mg and G2 (1.8±1.1)mg was also significantly decreased than in group G0 (5.3±2.2)mg ($p<0.01$). At week 26,serum creatinine decreased significantly in both groups G1 (7.0±2.9)umol/L and G2 (6.1±2.5)umol/L than in group G0 (12.7±1.3)umol/L ($p<0.05$). One week after treatment, the levels of anti-dsDNA antibodies in group Gl (43±19)x$10^2$ U/ml and G2 (45±32)x$10^2$ U/ml were both significantly decreased than those of the group G0 (87±39)x$10^2$ U/ml ($p<0.05$),and at the 26th weeks the difference between group G2 (35±11)xl$0^2$ U/ml and G0 (59±35)x$10^2$ U/ml was statistically significant. The nephron crescent formation in group G1 (0.11±0.05)and G2 (0.09±0.01)was significandy lower that of the group G0 (0.23±0.07) ($p<0.05$), and that of group G2 was significantly less that of group Gl ($p<0.05$).

**Conclusions:** P-glycoprotein monoclonal antibody is very effective in treating MRL/lpr mice.It is safe and free of rejection reactions.

Table 1. Effects of P-gp monoclonal antibody on the levels of anti-dsDNA antibodies, serum creatinine and the routine blood test of MRL/lpr mice ('X±s)

| Group | Number | ds-DNA ($\times10^2$U/ml) | Scr ($\mu$mol/ml) | WBC ($\times10^9$/L) | HB (g/L) | PLT ($\times10^9$/L) |
|---|---|---|---|---|---|---|
| G1 | 8 | 48±19 | 8.3±2.4* | 7.8±3.6* | 124±8 | 118±97* |
| G2 | 8 | 35±11# | 6.1±3.5# | 5.9±4.2# | 138±13 | 132±85# |
| G0 | 8 | 59±35 | 13.2±.8 | 3.1±2.9 | 110±11 | 76±61 |

Notes: *G1 vs G0 $p<0.05$, #G2 vs G0 $p<0.05$.

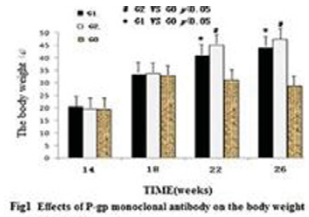


Fig1 Effects of P-gp monoclonal antibody on the body weight of MRL/lpr mice

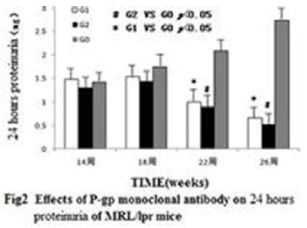


Fig2 Effects of P-gp monoclonal antibody on 24 hours proteinuria of MRL/lpr mice

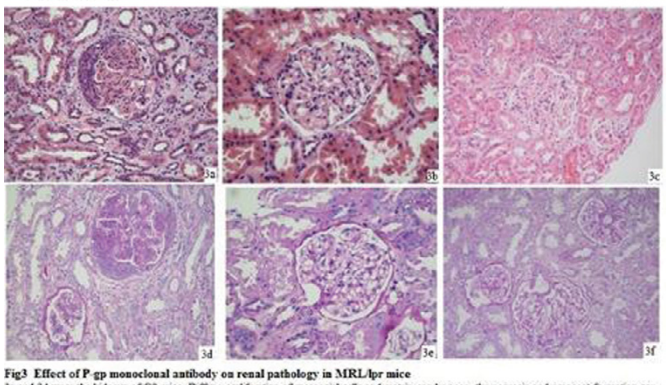

Fig3 Effect of P-gp monoclonal antibody on renal pathology in MRL/lpr mice
3a and 3d were the kidneys of G0 mice. Diffuse proliferation of mesangial cells and matrix can be seen, the necrosis and crescent formation can be seen in a few capillarie. Tubular lumen dilated and renal tubular epithelial cells shedding. Renal interstitial inflammatory cell infiltration, Arteriolar wall thickening and lumen stenosis. 3b and 3e were the kidneys of G1 mice. The mesangial cells and stroma were mildly proliferated, the basement membrane was thickened,but no crescent and no thickening of the epithelium. There was no inflammatory cell infiltration and fibrosis in the renal interstitium. 3c and 3f were the kidneys of G2 mice. There was no obvious inflammatory cell infiltration in the renal interstitium

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## THU0239 SERUM HMGB1 AND TLR4 LEVELS AS NOVEL BIOLOGICAL MARKERS FOR THE ACTIVITIES OF NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background:** Neuropsychiatric Systemic Lupus Erythematosus (NPSLE) is a severe complication of SLE, including a variety of neurological and psychiatric features. Previous studies have demonstrated the close relationship between NPSLE and inflammation. HMGB1-TLR4 signaling pathway is the up-stream pathway of NF-κB, which could upregulate the expression of various cytokines and other inflammatory mediators.

**Objectives:** The objective of the study was to explore the potential mechanism of HMGB1-TLR4 axis in SLE.

**Methods:** The study population consisted of 107 SLE patients and 43 age- and sex-matched healthy controls. 73 SLE patients had active disease. 36 of these had NPSLE. The serum anti-NR2A antibodies levels were measured by ELISA. Clinical and serological parameters were assessed according to routine procedures. HMGB1 and TLR4 levels were measured by ELISA.Statistical analyses were performed by using the chi-square test and the t-test.

**Results:** CNS manifestations accounted for 94% (34/36 patients), while involvement of the PNS was 6% (2/36patients). The majority of the manifestations were Seizure disorders (n=17; 47.2%),Headache (n=12; 33.3%), Cognitive dysfunction (n=10; 27.8%), Psychoses (n=8; 22.2%).Within the group of active patients those with NP manifestations had higher HMGB1 levels (0.451 (0.292 to 0.583)) compared to active patients with non-NP manifestations (0.356 (0.098 to 0.436)). In patients with NP (0.429 (0.313 to 0.526)) and non-NP (0.375 (0.196 to 0.478)) manifestations during active periods of the disease, TLR4 levels significant increased in comparison to the controls. TLR4 levels were significantly higher in active patients (0.401 (0.196 to 0.526)) compared to quiescent patients. There was a significant positive correlation between levels of HMGB1 and TLR4 in the total patients group ($P<0.0001$, r=0.939).We observed a correlation between HMGB1 levels and SLEDAI ($P<0.0001$, r=0.804). Also, TLR4 levels showed a significant correlation with SLEDAI ($P<0.0001$, r=0.809). HMGB1 levels correlated with anti-dsDNA levels ($P<0.0001$, r=0.558). Similarly, TLR4 showed a correlation with anti-dsDNA levels ($P<0.0001$, r=0.522).We observed a negative correlation in the total SLE group between C3, C4 and HMGB1 levels ($P<0.0001$, r=-0.545 and $P<0.0001$, r=-0.270 respective. Also, TLR4 showed a significant negative correlation with C3 and C4 ($P<0.0001$, r=-0.559 and $P<0.0001$, r=-0.285 respectively).

**Conclusions:** Our data suggest that HMGB1-TLR4 axis plays an important role in the pathogenesis of SLE as well as NPSLE.

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**Disclosure of Interest:** None declared

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## THU0240 DEFECTIVE REGULATION BY ATP-GATED IONOTROPIC P2X7 RECEPTOR DRIVES T FOLLICULAR HELPER CELL EXPANSION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background:** Systemic lupus erythematosus (SLE) is a chronic autoimmune