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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)x10^2 U/ml and G2 (45±32)x10^2 U/ml were both significantly decreased than those of the group G0 (87±39)x10^2 U/ml ($p<0.05$),and at the 26th weeks the difference between group G2 (35±11)xl0^2 U/ml and G0 (59±35)x10^2 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 (μmol/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 $p<0.05$.

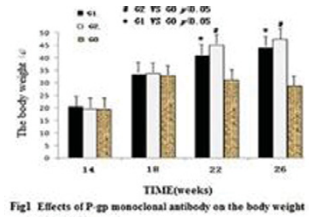

Fig1 Effects of P-gp monoclonal antibody on the body weight of MRL/lpr mice

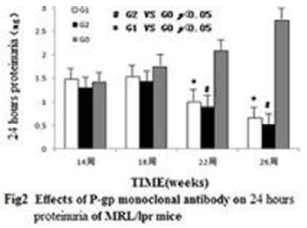

Fig2 Effects of P-gp monoclonal antibody on 24 hours proteinuria of MRL/lpr mice

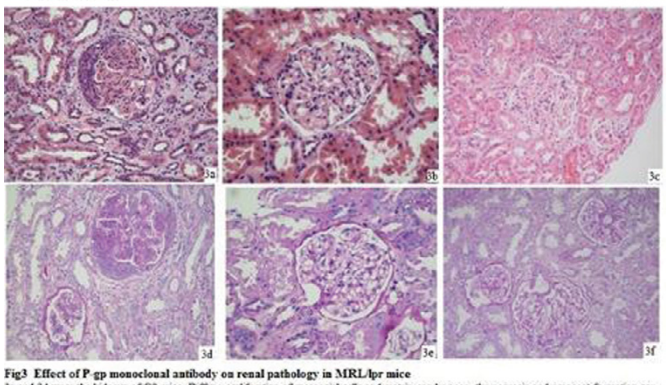

Fig3 Effect of P-gp monoclonal antibody on renal pathology in MRL/lpr mice
3a and 3d were the kidneys of G0 mice. Diffuse proliferation of mesangial cells and matrix can be seen, the necrosis and crescent formation can be seen in a few capillarie. Tubular lumen dilated and renal tubular epithelial cells 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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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

disease of unknown aetiology. The deregulated activation of T follicular helper (Tfh) cells in secondary lymphoid organs may play a pivotal role in the activation of B cells and production of autoantibodies. Both murine and human Tfh cells were shown to be sensitive to extracellular ATP via purinergic P2X7 receptor. P2X7 is a non-selective ionic channel that in the presence of high concentrations of ATP or prolonged stimulation opens to a pore permeable to molecules up to 900 Da and causes cell death. Mice deleted for P2rx7 show a significantly worsened outcome of pristane-induced SLE. Expansion of circulating Tfh (cTfh) cells has been correlated with increased levels of autoantibodies and more severe clinical manifestations in SLE.

Objectives: Our aim was to investigate the possible role of P2X7 receptor activity in driving cTfh expansion in a cohort of SLE patients. Patients with primary antiphospholipid syndrome (PAPS) and healthy donors (HD) served as normal controls.

Methods: Forty-two adult patients with SLE (SLEDAI >4), 14 patients with primary antiphospholipid syndrome (PAPS) and 34 sex- and age-matched healthy donors (HD) were included. Circulating Tfh cells were isolated as a CCR7loPD1+ cells. In 32 patients with SLE, we investigated permeability of Tfh cells to Yo-Pro-1 staining over time at FACS upon stimulation with the P2X7 selective agonist BzATP and the presence of a correlation (Spearman's rho) between Tfh cells expansion and Yo-Pro uptake. We analysed *in vitro* differentiation of CXCR5+PD1+ Tfh cells and sensitivity of this pathway to BzATP in CD4 naïve cells isolated from 4 SLE and 4 healthy donors in the presence of a mixture of Activin A and IL-12, with or without BzATP.

Results: As previously reported, SLE patients had a significant expansion of the CCR7loPD-1+ cTfh cells [SLE (n=42): 38.3±12.8% versus HD (n=34]): 21.6±4.5%, ****$p<0.0001$]. There was no significant difference in the representation of cTfh cells between PAPS patients ([n=14] 22.6±6.6%) and HD (p=0.6714). The analysis of BzATP induced Yo-Pro-1 permeability revealed a significantly increased resistance to P2X7-mediated cell death in SLE patients as compared to HD and PAPS patients (fold increase of Yo-Pro-1-positive cells at 450 sec, HD [n=28]: 5±2.3%; SLE [n=32]: 3±1.7%, ****p=0.003; PAPS [n=14]: 5±2.9%, **p=0.0042). Furthermore, Spearman's rho showed a significant correlation between the percentage of cTfh cells and the degree of Yo-Pro uptake (r -0.37). *In vitro* generation of Tfh cells from HD naïve cells revealed a significant response to inhibition by BzATP, which was not present when SLE naïve cells were used.

Conclusions: The CCR7loPD-1+ cTfh cells are significantly expanded in SLE but not PAPS patients compared to HD. The degree of expansion correlates with diminished sensitivity to P2X7-mediated cell death. This defective regulation is present also within *in vitro* differentiation of Tfh from naïve cells isolated from SLE patients. Our data suggest a selective defect of P2X7-mediated control of Tfh cell generation and expansion in SLE.

Disclosure of Interest: None declared

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THU0241 DECREASED CIRCULATING CXCR3+CCR9+ TH CELLS ARE ASSOCIATED WITH ELEVATED LEVELS OF THEIR LIGANDS CXCL10 AND CCL25 IN THE SALIVARY GLAND OF PATIENTS WITH SJÖGREN'S SYNDROME TO POTENTIALLY FACILITATE CONCERTED MIGRATION

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Background: Primary Sjögren's syndrome (pSS) is characterized by dryness and lymphocytic infiltration in the salivary glands. Both CXCR5+ T follicular helper (Tfh) cells and CCR9+ Tfh-like cells and their specific chemotactic ligands CXCL13 and CCL25 are present at increased levels in the salivary glands of pSS patients. Recently, we and others found that CCR9+ Th cells are elevated in pSS peripheral blood and co-express CXCR3 and other chemokine receptors, known to be differentially expressed by Th cell subsets. CCR9+ Th cells play an important role in mucosal immunity and have been shown to produce high levels of IFN-γ, like CXCR3+ Th1 cells. Since ligands of CXCR3 (CXCL9/10/11) are abundantly expressed in the salivary glands of pSS patients the potential role of this receptor in conjunction with CCL25 was studied in comparison with other chemokine receptors.

Objectives: To study potential chemokine interactions causing enhanced migration of CCR9+ T cells into the salivary glands in pSS.

Methods: CXCL10, CCL25, CXCL13, CCL17 and CCL20 mRNA and protein expression in the salivary gland of pSS and non-Sjögren's sicca (nSS) patients was assessed (mRNA: n=9 vs n=9 and protein: n=26 vs n=34, respectively). Frequencies of CXCR3, CCR9, CXCR5, CCR4 and CCR6 expressing Th cells in blood of pSS patients and healthy controls were assessed by flow cytometry (n=11 vs n=11). Chemotaxis assays (n=3 HC, n=5 pSS) were performed to study migration induced by CXCL10 and CCL25.

Results: CCL25, CXCL10 and CXCL13 expression were increased in pSS compared to nSS patients, both at mRNA and protein level (all $p\leq0.02$). CCL17 and CCL20 expression were low and detectable in only few patients. Protein levels of CXCL10 and CXCL13 correlated with lymphocytic focus scores and all 3 chemokines correlated with serum IgG levels in pSS (all $p<0.05$). CCL25 protein levels strongly correlated with CXCL10 (r=0.545, p=0.004) but not with CXCL13. A relative decrease of CXCR3+ cells was found in the CCR9+ Th subset in the peripheral blood of pSS patients (p=0.04), which was most pronounced in the effector and effector memory subsets (64% vs 26%, p=0.03 and 51% vs 27% p=0.01, respectively). CCR4 or CCR6-expressing CCR9+ Th cells and CXCR3 or CCR6-expressing CXCR5+ Th cells were not decreased. To test the hypothesis that CXCR3 ligands and CCL25 facilitate migration, co-migration of lymphocytes in response to CXCL10 and CCL25 was studied. CXCL10 and CCL25 induced synergistic Th cell chemotaxis in vitro (p=0.02 and $p<0.01$ as compared to CCL25 or CXCL10 only, respectively).

Conclusions: The decreased frequency of CXCR3+CCR9+ Th cells in blood of pSS patients may be due to a concerted action of overexpressed ligands at the site of inflammation. Elevated expression of ligands CXCL10 and CCL25 in the salivary gland and the synergistic effect on chemotaxis in vitro indicate a potential role for these chemokines in formation of lymphocytic infiltrates in exocrine glands of pSS patients.

Disclosure of Interest: None declared

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THU0242 BIOLOGICAL PATHWAY ANALYSIS IN PRIMARY SJÖGREN'S SYNDROME ASSOCIATED LYMPHOMA

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Background: Lymphoma development is a serious complication of Primary Sjögren's syndrome (pSS). To date, the biological processes that may be involved in pSS-associated lymphoma are not fully understood.

Objectives: The aim of our study is to use microarray gene expression data from a well-defined cohort of pSS patients to identify biological processes that may be relevant to pSS-associated lymphoma.

Methods: pSS patients and healthy controls from the UK primary Sjögren's syndrome registry (UKPSSR) were used in this study. All patients fulfilled the AECG criteria. Whole genome gene expression data from whole blood RNA samples (n=144) stratified into five clinical subsets (pSS=61, pSS with lymphoma=16, pSS with other cancers=21, pSS with paraproteinemia=23 and healthy controls=23) were used for the pathway analysis. A list of 68 differentially expressed genes in pSS-associated lymphoma compared with pSS (non lymphoma) were uploaded into the Ingenuity Pathway Analysis (IPA) analytic tool. Similar approach was also used for comparison between the lymphoma and other pSS subject groups. Finally, we also examined the regulators and pathways involved in the genes from the gene expression signature in pSS-associated lymphoma we have previously described (*BMS1*, *NUDT14* and *MGST3*) [1].

Results: In pSS-associated lymphoma the top canonical pathway was "Aryl Hydrocarbon Receptor (AHR) signaling," which includes *MGST3*. Several other canonical pathways also included the genes of the 3-gene biosignature of pSS-associated lymphoma. The downstream effects and gene-gene interactions were explored through molecular networks analysis. Furthermore, important upstream regulators of the 3 biosignature genes include *NFE2L2*, *PPARA* and *TOCF1* were identified.

The pathway analyses of the other pSS subgroups showed 67 common canonical pathways showed among all the pSS subgroups. Focusing on pSS-associated lymphoma versus healthy controls, 94.9% of the canonical pathways in this comparison were in common with the canonical pathways identified when comparing pSS-associated lymphoma with pSS. The "Interferon Signaling pathway" was the top pathway for all pSS subgroups comparing with healthy controls. In addition, all the non-lymphoma pSS subgroups showed similar patterns in the downstream analysis which differ from the pSS-associated lymphoma group.

Conclusions: The pathway analysis revealed different possible pathways that might be involved in lymphoma development in pSS and indicates a unique gene expression signature exist in pSS-associated lymphoma. These results might provide a deeper understanding and a direction for future studies to investigate lymphoma development in pSS patients.

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Disclosure of Interest: None declared

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