

delivery, intrauterine growth restriction (IUGR) and preeclampsia. Conceiving in periods of low disease activity helps to reduce these complications.

Objectives: This project aims to describe the occurrence of pregnancy complications among women with immune-mediated rheumatic diseases and to study the associated clinical factors.

Methods: A retrospective longitudinal study was performed including consecutive pregnant women with immune-mediated rheumatic diseases seen in a multidisciplinary group for autoimmune diseases during pregnancy, in a tertiary hospital, between January 2010 and December 2015. Clinical and demographic data, as well as pregnancy outcomes, were collected through consultation of clinical files. The factors associated with pregnancy manifestations (premature delivery, flares during pregnancy, recurrent pregnancy loss and foetal growth restriction) were studied using Mann-Whitney, qui-square and fisher tests (SPSS 24.0). Significance level was set as <0.05.

Results: We included 151 gestations from a total of 140 women with a mean age of 32.5±4.4 years; 4 gestations were twin pregnancies. Within these 151 gestations, 54 (35.8%) women had SLE, 17 (11.3%) had Sjögren's syndrome, 17 (11.3%) had rheumatoid arthritis, 41 had APS (27.2%), 11 (7.3%) had Behçet's disease, 4 (2.6%) had systemic sclerosis, 8 (5.3%) had mixed connective tissue disease and 16 (10.6%) had other immune-mediated diseases. 35 (23.2%) had anti-SSA/La antibodies, 18 (11.9%) had anti-SSB antibodies, 6 (4.0%) had anti-URNP antibodies and 43 (28.5%) had anti-nuclear antibodies. Seven (4.6%) of the women developed gestational diabetes and 4 (2.6%) developed gestational hypertension. Furthermore, 54 (35.8%) women had had previous miscarriages. Prematurity occurred more frequently among neonates with IUGR (53.8% vs 46.1%; $p=0.04$), and was associated with gestational diabetes (21.4% vs 2.9%; $p=0.018$). It also occurred more frequently in multiple pregnancies (75% vs 16.4%; $p=0.001$), mothers taking glucocorticoids (28.6% vs 9.2%; $p=0.003$) and active rheumatic disease at conception (23% vs 6.8% $p=0.03$). No statistically significant differences were observed in the occurrence of different pregnancy complications among different diseases or in presence of different antibodies.

Conclusions: Our study proved a link between immune-mediated rheumatic diseases and specific pregnancy outcomes such as prematurity and IUGR. Outcomes were worse when taking glucocorticoids, when gestational diabetes were developed and when conception occurred in a period of active disease.

Disclosure of Interest: None declared

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AB0145 TLR4 SIGNALING PATHWAY MEDIATES THE SENESENCE OF BONE MARROW-MESENCHYMAL STEM CELLS FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Background: Previous studies of our research group revealed the senescence of bone marrow-mesenchymal stem cells from systemic lupus erythematosus patients, which participated in the development of SLE. "Inflammatory microenvironment" played a very important role in cellular senescence. In the preliminary experiments, we discovered the level of HMGB1 in serum and Peripheral blood mononuclear cells from SLE patients was higher than those of The healthy control group.

Objectives: The aim of this study was to investigate whether HMGB-1 can lead to senescence BM-MSCs from SLE patients and its possible mechanism.

Methods: Twelve 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. All BM-MSCs were isolated by density gradient centrifugation. Western Blotting and immunofluorescence were used to distinguish the difference of expression and localization of TLR4 signaling pathway between normal group and SLE group. Different concentrations (0.01, 0.1, 1, 10 μ g/ml) of HMGB-1 (the endogenous ligand of TLR4) stimulated normal BM-MSCs, then detecting expression of TLR4 signal 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 small interfering RNA (siRNA) to interfere the expression of TLR4.

Results: BM-MSCs from SLE patients showed prominent features of senescence, characterized by impaired capacities of proliferation, increased SA- β -gal activity, and disordered cytoskeleton distribution, and abnormal activation of TLR4 signaling transduction, high level of phosphorylated p65. I κ B α . After stimulation of HMGB1 in normal MSCs, TLR4 signaling 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 inhibitors of HMGB-1 and small interfering RNA (siRNA) of TLR4 can significantly reverse the senescence.

Conclusions: HMGB-1 binded to TLR4, and by activating MyD88/IRAK/TRAF pathway, promoted NF- κ B signal transduction, thereby affected the expression of cell cycle-related proteins, and then resulted in senescence of MSCs from SLE patients.

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AB0146 INVOLVEMENT OF PERIPHERAL CD8 T CELL SUBSETS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Although the mainstream of pathogenesis of systemic lupus erythematosus (SLE) is thought to be interactions between antigen-presenting cells like dendritic cells, helper T cells, B cells and cytokines, previous reports suggested CD8 T cells also involve in the pathogenesis of SLE^{1,2}. However the associations between subsets of CD8 T cells and clinical manifestations remains unclear.

Objectives: We conducted standard immunophenotyping analysis with peripheral blood from SLE patients and focused on CD8 T cell subsets to elucidate the association with clinical phenotype and serological markers.

Methods: Peripheral blood was obtained from inactive SLE patients and healthy subjects as controls and also from active SLE before and 3 months after treatment. CD8 T cell subsets were measured by flow cytometry with fresh whole blood samples.

Results: Thirty-four active SLE patients and 38 inactive patients and 22 healthy controls (HCs) whose age and sex were matched with those in SLE patients were enrolled. Mean SLE disease activity index (SLEDAI) was 14.2 and 1.8 in active and inactive patients, respectively. Among CD8 T cell subsets, the proportion of HLA-DR+ cells was significantly higher in SLE patients than HCs and positively correlated with SLEDAI ($p=0.016$, $\rho=0.283$), and was also higher in patients with nephritis than patients without nephritis ($p=0.074$), though it did not reach statistical significance. The proportion of naive CD8 T cells positively correlated with the titer of anti-dsDNA antibody ($p=0.011$, $\rho=0.30$) and C1q immune complex levels ($p=0.043$, $\rho=0.25$), and negatively correlated with serum complement levels ($p=0.019$, $\rho=-0.34$). The proportion of central memory CD8 T cells (Tcm) negatively correlated with SLEDAI ($p<0.001$, $\rho=-0.43$), the titer of dsDNA antibody ($p<0.001$, $\rho=-0.51$) and C1q immune complex levels ($p<0.001$, $\rho=-0.44$), and positively correlated with serum complement levels ($p<0.001$, $\rho=0.49$), and was lower in the patients with nephritis ($p=0.041$), skin rash ($p<0.001$), and fever ($p=0.002$) than the patients without them. The proportion of effector memory CD8 T cells (Tem) was lower in SLE and negatively correlated with the titer of anti-dsDNA antibody ($p=0.028$, $\rho=-0.26$). Sixteen active patients were treated with prednisolone (PSL) 50mg (mean) with concomitant immunosuppressant; eight were treated with cyclophosphamide. Mean SLEDAI decreased from 15.9 to 3.9 at 3 months after the treatments. The proportion of naive CD8 T cells significantly decreased ($p=0.003$), and the proportions of HLA-DR+ cells and Tem increased at 3 months ($p=0.011$ and $p=0.007$, respectively). The proportion of Tem increased and negatively correlated with SLEDAI only with those who were treated with cyclophosphamide.

Conclusions: Pathological state of SLE positively correlated with the proportion of naive CD8 T cells and negatively correlated with the proportion of Tem and these subsets were affected by treatment with PSL and immunosuppressant particularly cyclophosphamide. These results indicate that CD8 T cells are involved in pathophysiology and could potentially become a biomarker or a treatment target in SLE patients.

References:

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AB0147 THE EFFECT OF TREATMENT WITH HYDROXYCHLOROQUINE ON SOLUBLE TISSUE FACTOR LEVELS IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES AND ANTIPHOSPHOLIPID SYNDROME WITH AND WITHOUT UNDERLYING SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Antiphospholipid syndrome (APS) is characterized by venous, microvascular and/or arterial and/or obstetric morbidity (obstetric APS) in patients who are persistently positive for antiphospholipid antibodies (aPL)[1]. The mainstay of treatment is based on anticoagulation therapy; however, increasing interest is currently received by the antimalarial hydroxychloroquine (HCQ). The use of HCQ has been associated with a reduced risk of thrombosis but HCQ's antithrombotic mechanism of action is unclear particularly in patients with aPL and APS.

Objectives: The aim of our study was to assess soluble tissue factor (TF) levels in HCQ naïve-patients with persistent aPL or APS at baseline and 12 weeks after commencing HCQ. We hypothesise that HCQ lowers levels of soluble TF.

Methods: Twenty-two individuals with APS with or without other associated autoimmune disease (20 females, two males, median age 55 (range 18–70) years) had blood samples taken before and 12 weeks after starting HCQ 200mg. Plasma was stored at -80°C and thawed to measure TF using Imubind TF kit (Invitech Ltd, Cambridgeshire, UK). The assay was performed according to the manufacturer's instructions. Patient characteristics are outlined in table 1. Statistical analysis was performed using SPSS Version 22. For continuous normally distributed data a two-tailed student's paired t-test was performed. A value of $p=0.05$ was considered as significant. There are no previous data in this area, so we were unable to do a power calculation to work out study size. Our study is therefore a pilot study.

Results: Soluble TF levels were above our normal range (40–300 pg/ml) prior to the commencement of HCQ and were significantly reduced (pre level mean (SD) 401.8 (152.8) pg/ml versus post 300.9 (108) pg/ml ($p=0.010$).

Table 1: Demographic data	
Variables	APS (N=22)
Age: median (range)	47 (18-69)
Sex (female:male)	20:02
Ethnicity (white:asian:black)	(20:1:1)
aPL subtype	
LA* positive	19
IgG or IgM aCL positive (>99th centile)	5
IgG or IgM anti-Beta2GPI antibody positive (>99th centile)	0
aCL and anti-Beta2GPI antibody positive (>99th centile)	0
aCL antibody and LA* positive (>99th centile)	3
Anti-Beta2GPI antibody and LA* positive (>99th centile)	0
aPL complication	
Thrombotic APS	
Previous arterial thrombosis	10
Previous venous thrombosis	7
Previous arterial and venous thromboses	4
Obstetric APS	
Previous recurrent 1st trimester pregnancy loss	2
Previous pre-eclampsia	1
Previous intrauterine growth restriction	1
Previous stillbirth	3
Treatment	
Warfarin	14
Heparin	0
Aspirin	3
Autoimmune profile	
Antinuclear antibodies (ANA)	10
Extractable nuclear antibodies (ENA)	2
Double stranded DNA antibodies (dsDNA)	1
Key: aCL - anticardiolipin; anti-Beta2GPI - anti-Beta2glycoprotein1; LA - lupus anticoagulant. *LA detected by either DRVVT, dilute aPTT or TSVT	

Conclusions: There was a significant reduction in soluble TF levels in this patient cohort of patients with aPL and APS after commencing HCQ. Our previous work has shown that HCQ has not affected complement turnover, VEGF levels, thromboelastometry findings or CRP levels. Our findings of a reduction of soluble TF levels in aPL positive patients after the commencement of HCQ maybe a key mechanism by which HCQ reduces thrombotic risk. Further studies of a larger patient cohort are required to confirm our observation.

References:

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AB0148 INDUCTION OF HO-1 EXPRESSION IN MONOCYTES MIGHT PREVENT KIDNEY DAMAGE IN LUPUS NEPHRITIS (LN)

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Background: Systemic lupus erythematosus (SLE), is an autoimmune disease characterized by autoantibody synthesis and inflammation. During disease course, up to 70% of SLE patients will develop LN. Emerging evidence has demonstrated that infiltrating monocytes and macrophages are associated with LN pathogenesis. We have previously demonstrated that HO-1, a haem-degrading enzyme with anti-inflammatory properties is decreased in peripheral monocytes of SLE patients. Therefore, we decided to explore the contribution of HO-1 expression to LN pathogenesis.

Objectives: To explore the role of HO-1 in modulating innate immunity through a cytoprotective effect in monocytes of LN nephritis patients. Accordingly, we examined the expression of HO-1 in circulating monocytes, and the effect of HO-1 induction in reactive oxygen species (ROS) production and the phagocytic activity of monocytes from peripheral blood of LN patients and healthy controls (HC).

Methods: SLE patients with proliferative LN confirmed by renal biopsy (Class III, IV or V ISN/RPS) were recruited at Hospital Clínico de PUC. All individuals signed an informed consent form. Monocytes were purified from peripheral blood mononuclear cells (PBMCs) of LN patients and HC using pan-monocytes MACS kit. Subpopulations of monocytes and HO-1 expression were measured by FACS. ROS was determined using CellRox Kit. The phagocytic ability of monocytes was assessed by FACS and the total phagocytosis was calculated as the percentage of cells with engulfed beads.

Results: We found that monocytes from LN patients show significant differences when compared to HC in all the parameters analyzed. The percentage of CD16+ inflammatory monocytes was higher in LN patients ($6.72\pm 0.98\%$) compared to HC ($4.07\pm 0.48\%$) ($p<0.05$). HO-1 protein expression is decreased in circulating LN monocytes (4789 ± 911 vs 1572 ± 481 , $p=0.005$). Baseline levels of ROS are elevated in LN monocytes with similar values that the ones found in monocytes from HC treated with a ROS inducer (HC: 3509 ± 584 ; HC+TBHP: 8436 ± 1909 ; LN: 8355 ± 1714). Furthermore, phagocytic activity is increased in LN monocytes ($77.97\pm 3.31\%$) compared to HC ($39.63\pm 2.75\%$). Moreover, our preliminary data indicate that HO-1 induction, using cobalt protoporphyrin (CoPP), leads to downregulation of ROS production in LN (~60%) and HC (~40%) leaving both in similar levels of ROS production. In addition, phagocytic activity is also decreased in LN and HC monocytes in the presence of CoPP (~30%).

Conclusions: Decreased HO-1 expression in circulating monocytes of LN patients leads to higher ROS production and phagocytic activity. ROS level and phagocytosis are reduced when we induce HO-1 expression with CoPP. We propose that HO-1 induction might exert a cytoprotective role in LN by regulating innate immunity. FONDECYT N° 1150173.

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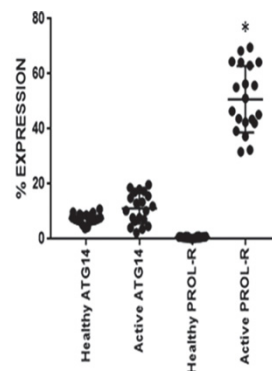
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AB0149 PROLACTIN AND AUTOPHAGY IN SYSTEMIC LUPUS ERYTHEMATOSUS: CLINICAL SIGNIFICANCE OF CORRELATION BETWEEN PRL-R+ (RECEPTOR), CD19+, ATG14+, AND CD25+ EXPRESSION ON B AND T REGULATORY CELLS

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Background: Systemic Lupus Erythematosus (SLE) is a prototype of autoimmune diseases with excessive anti-nuclear autoantibodies production. B cells activation with immune complex formation is the main characteristic of SLE with abnormalities in immune cells, dysregulation of apoptosis, and defects in the clearance of apoptotic materials. Autophagy, a highly conserved protein degradation pathway, is essential for removing protein aggregates and misfolded proteins in cells and its defects contributes to SLE pathogenesis. On the other hand, multiple evidences in humans and experimental models suggest that prolactin (PRL) is associated with active SLE and participates in the immune dysregulation, and one of the mechanisms of PRL action is the inhibition of apoptosis.

Objectives: Analyze the relationship between PRL receptors (PRL-R) on B cells and markers of autophagy on T regulatory cells and the association, if any, with clinical characteristics of SLE.



Expression of PRL-R and ATG14 in active SLE patients and healthy controls. * $p<0.026$ Dunn's multiple comparisons test.