FRI0277

METFORMIN REDUCES SALIVARY GLAND INFLAMMATION BY CONTROLLING B CELL DIFFERENTIATION AND REGULATING BALANCE OF TH17 AND TREG CELL IN NON-OBESE DIABETIC MICE

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Background: Sjögren's syndrome (SS) is a systemic autoimmune disease that affects exocrine glands and lymphoid organs. B cell hyperactivity and imbalance between T helper 17 (Th17) cells and regulatory T (Treg) cells are involved in pathogenesis of SS. Metformin, a commonly used anti-diabetic drug, is found to have immunomodulatory effect via AMP-activated protein kinase enhanced inhibition of mTOR-STAT3 signalling.

Objectives: We examined the therapeutic effect of metformin on SS by using animal model of SS, non-obese diabetic (NOD) mice.

Methods: Metformin (50 mg/kg) or vehicle (saline) was given per oral every day from 11 weeks after birth until 20 weeks. Salivary flow rate (SFR) was addressed on every 2 or 3 weeks between 11 weeks and 20 weeks. Histologic analyses of salivary gland and spleen were performed on week 20. Expression of inflammatory cytokine was determined by immunohistochemistry analysis and real-time PCR. Flow cytometry was performed with peripheral blood to examine Th17 and Treg cells and germinal centre (GC) B cell populations. Serum immunoglobulin level was measured by enzyme-linked immunosorbent assay. Splenic cells of NOD mice were treated with metformin or vehicle *in vitro* and cultured for 3 days.

Results: SFRs of metformin-treated mice recovered, whereas SFRs of those treated with vehicle declined. Histologic examination of salivary gland showed decreased infiltration of lymphocytes and reduced expression of IL-6 and TNF- α in metformin-treated mice. Relative expression of IL-6, TNF- α , and IL-17 mRNA in salivary gland and spleen also declined in metformin-treated mice. Flow cytometric analysis revealed decreased Th1 and Th17 cells and increased Treg cells in peripheral blood of mice treated with metformin. In addition, GC B cells and immunoglobulin levels were reduced in peripheral blood of mice treated with metformin. Decreased Tfh cells and increased Tfr cells were observed from *in vitro* cultures of splenic cells treated with metformin.

Conclusions: Metformin controls B cell differentiation and keeps balance between Th17 and Treg cells in NOD mice, in addition to reducing lymphocytic infiltration and inflammatory cytokine expression in salivary gland. Metformin has potential therapeutic effects on SS.

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FRI0278

ALTERATIONS IN MEMORY AND VISIO-SPATIAL LEARNING IN A PRISTANE-INDUCED LUPUS BALB/C MICE

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Background: Neuropsychiatric lupus is a condition that occurs in 30%–40% of systemic lupus erythematosus (SLE) patients and is associated with the presence of autoantibodies in central nervous system (CNS). The pristane-induced lupus in BALB/c mice is an experimental model that presents clinical and immunological similarities to SLE pathogenesis and it can be proposed as model to analyse affectations in visio-spatial learning and memory.

Objectives: To evaluate short and long term visio-spatial learning and memory in Balb/c pristane-induced lupus using Barnes maze test.

Methods: Fourteen female Balb/c mice were studied: 4 controls (single intraperitoneal injection of 0.5 mL NaCl 0.9%), 5 treated with pristane (single intraperitoneal injection of 0.5 mL pristane) and 5 treated with pristane plus lipopolysaccharide (LPS) 3 mg/kg 4 months post-administration of pristane. Short and long term visio-spatial learning and memory was evaluated in all groups at 7 weeks after the administration of LPS using Barnes maze test. The test consists of 3 phases: adaptation phase, acquisition phase and test phase. The short-term memory was evaluated during the acquisition phase (4 tests per day, D1–D4) and the long-term memory was evaluated 48 hour after the last test of the acquisition phase. Primary and total errors, primary and total latencies were analysed. The

results are shown as a mean $\pm$ SD. Kruskal-Wallis and Mann-Whitney U test was used for statistical analysis.

Results: All mice treated with pristane showed higher latencies to find the target hole in Barnes maze compared with controls during the short-term memory test (STP, D1–D4). In the long-term memory test (LTP) the highest latency in seconds (s) was observed in the pristane plus LPS group (64.6 $\pm$ 59.1 s) followed by the pristane group (62.4 $\pm$ 69.6 s) in comparison control group (7 $\pm$ 3.6 s).

Abstract FRI0278 – Figure 1. Total. Latency in Barnes maze test. Total latency in seconds between mice groups (pristane, pristane + LPS and control) in STP (A) and LTP tests (B) in Barnes's maze

Conclusions: The increased latencies in the groups pristane plus LPS and pristane with respect to controls suggests that the autoantibodies produced in this model could be involved in alterations of the CNS, modifying the learning and memory processes. These results also suggest that pristane may have the potential to alter the permeability of the blood-brain barrier by itself. However, more experimental studies are needed to confirm the results obtained in this study.

Disclosure of Interest: None declared

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FRI0279

DECREASED SPHINGOSIN-1-PHOSPHATE RECEPTOR 1 (S1P1) EXPRESSION IN ENDOTHELIAL PROGENITOR CELLS (EPCs) FROM SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) PATIENTS

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Background: SLE patients display an elevated cardiovascular risk when compared to the general population. Interferon- α (IFN- α) appears to drive atherogenesis in SLE at least in part, by promoting abnormal phenotype and function of EPCs. S1P1 is considered crucial for vasculogenesis and has been shown to attenuate IFN- α autoamplification by promoting IFN receptor 1 (IFNAR1) degradation.

Objectives: To characterise S1P1 expression in EPCs from SLE patients and its putative association to type I IFNs effects on vasculogenesis.

Methods: SLE subjects that fulfilled SLE ACR classification criteria were recruited. Healthy controls were selected based on age and gender. Disease activity was assessed by SLEDAI-2K index. EPCs were quantified by flow cytometry in PBMC subsets as those cells that coexpress CD133⁺/CD34⁺ in the CD3⁺/CD56⁺/CD79b⁺ gate. S1P1 expression was quantified in that subset. Control PBMCs were cultured in endothelial cell (EC)-enrichment medium for 2 weeks. Differentiated ECs were characterised by immunofluorescence (IF) as those cells that coexpress UEA-1 lectin and take up acetylated LDL. These cells were stimulated with recombinant IFN- α in the presence or absence of the S1P1 agonist CYM-5442 and the S1P1 antagonist W-146 to assess the effect of this pathway on EC differentiation and type I IFN induced gene expression.

Results: SLE and matched healthy controls (n=36/group) were compared. Demographic and clinical characteristics are depicted in table 1.

Abstract FRI0279 – Table 1. Demographic and Clinical features.

	Mean $\pm$ SEM
Age (years)	32.2 $\pm$ 1.35
Female (%)	34 (94.44)
Time since SLE diagnosis (months)	103.36 $\pm$ 13.46
Time since remission (months)	16.6 $\pm$ 3.07
SLEDAI-2K (points)	9.41 $\pm$ 1.15

SLE patients displayed decreased absolute EPC numbers when compared to controls (2463.43 $\pm$ 450.07 vs 39457.65 $\pm$ 9063.34 EPCs/ml, p<0.001). Decreased EPC numbers negatively correlated with SLEDAI-2K (r=−0.405, p=0.026). S1P1 expression was decreased in lupus EPCs when compared to controls (48.27 $\pm$ 1.55 vs 60.89 $\pm$ 1.66%,

$p < 0.001$) and this was independent of disease activity. Recombinant IFN- α decreased EPC differentiation in healthy control cells (32.44 ± 0.09 vs $79.69 \pm 0.27\%$, $p = 0.001$), and this was attenuated in the presence of CYM-5442 and exacerbated in the presence of W-146. Moreover, type I IFN regulated genes were modulated by these compounds.

Conclusions: The S1P1 pathway is involved in the regulation of EPC differentiation by type I IFNs. Defects in S1P1 signalling pathway in lupus EPCs may contribute to the development of endothelial dysfunction in SLE.

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FRI0280

A MOLECULAR NETWORK FOR FATIGUE IN PRIMARY SJÖGREN'S SYNDROME

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Background: Fatigue is a common phenomenon in primary Sjögren's syndrome (pSS) and other chronic inflammatory diseases, cancer, and neurodegeneration. The underlying mechanisms for fatigue are not completely understood, but increasing evidence point to a biological basis for the phenomenon.

Objectives: Following the sickness behaviour hypothesis for fatigue, where pro-inflammatory cytokines and particularly interleukin 1 β (IL-1 β) related signalling are essential, we wished to investigate how molecules that influence IL-1 β activity may influence fatigue through complex networks (IL-1 β , IL-1Ra, IL-1RII, IL-6 and S100B). We also hypothesised that the neuropeptide hypocretin-1 (Hcrt1), a regulator of sleep and wakefulness, could be an element in a network for fatigue.

Methods: In cerebrospinal fluid (CSF) from 49 patients with pSS, Hcrt1 was measured by RIA and the other proteins by ELISA. Fatigue was rated using the fatigue visual analogue scale (fVAS), and results analysed by univariate-, multiple regression, and principal component analysis (PCA).

Results: It was not possible to measure IL-1b due to very low concentrations in CSF. In simple univariate regression analysis with fatigue as a dependent variable a significant association was observed for depression ($R^2 = 0.20$, $p < 0.01$), and pain ($R^2 = 0.23$, $p < 0.01$) and the biochemical variable IL-1Ra ($R^2 = 0.09$, $p = 0.04$). In multiple regression with fVAS as dependent variable a model was obtained with depression, pain, and IL-1Ra as significant contributors ($R^2 = 0.37$; $p < 0.001$). In PCA two significant components were revealed (figure 1a). The first component (PC1) was dominated by variables related to IL-1 β activity. The second component (PC2) showed a negative correlation between IL-6 and Hcrt1. fVAS was then introduced as an additional variable. In this new model fVAS correlated with the IL-1 β related variables on PC1 and to a lesser degree with Hcrt1 on PC2 (figure 1b).

Abstract FRI0280 – Figure 1. a) PCA of biochemical variables only. b) PCA of biochemical variables including fatigue. Biplot illustrates scores of individuals and variables for PC1 and

PC2. Individual's scores are illustrated by dots. Arrows illustrate correlations of the variables to the components. Longer arrows mean higher correlation and arrows close to a component has higher contribution in generation of the component.

Conclusions: The main findings in this study indicate a functional network in which several IL-1 β related molecules in CSF influence fatigue in addition to the clinical factors depression and pain. The neuropeptide Hcrt1 seem to participate in fatigue signalling, but probably not through the IL-1 pathway.

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FRI0281

TRANSCRIPTOMIC PROFILING OF PDCs FROM PATIENTS WITH PSS IDENTIFIES CONSISTENTLY ALTERED GENE NETWORKS THAT INDICATE AN ACTIVATED PHENOTYPE AND ENHANCED ANTI-VIRAL STATE

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Background: Primary Sjögren's syndrome is an autoimmune disease characterised by lymphocytic infiltration of the exocrine glands and dryness of mouth and eyes. Type-I interferons (IFN) are thought to play an important role in pSS pathogenesis and plasmacytoid dendritic cells (pDCs) are capable of producing high levels of IFN. These cells infiltrate the salivary glands of pSS patients and their numbers correlate with local IFN-production.

Objectives: To understand the molecular mechanisms behind systemic dysregulation of pDCs, we performed RNA sequencing on pDCs isolated from peripheral blood of patients with pSS, non-Sjögren's sicca (nSS) and healthy controls.

Methods: We established two independent cohorts (each $n = 31$), of patients and controls. pSS patients ($n = 25$) were classified according to the 2002 AECG criteria. nSS patients ($n = 20$) presented with dryness complaints without a known cause, did not have any generalised autoimmune disease, and did not fulfil the classification criteria for pSS. Healthy donors ($n = 17$) were included as control group. Peripheral blood pDCs were isolated using MACS and RNA sequencing was performed for both cohorts. ± 20 million paired-end sequencing reads per sample were obtained using Illumina HiSeq 2500 platform.

Results: 8556 genes were differentially expressed (p -value < 0.05) between all three groups in the discovery cohort. Of these, 3144 genes were also differential in the replication cohort. We generated gene modules from both cohorts and found 5 gene clusters comprising 1259 genes that were consistently dysregulated in both analyses. Pathway analysis showed that the 5 modules contain genes associated with cellular activation, including a group of genes involved in IFN-signalling and viral sensing, as well as regulation of intracellular transport. Generally, pDCs from patients with nSS displayed an intermediate phenotype.

Conclusions: We mapped transcriptomic differences in circulating pDCs from patients with nSS and pSS and identified gene clusters that are robustly replicated in two independent cohorts. We found 5 gene clusters that are dysregulated in patients with pSS and indicate enhanced cellular activation, including IFN-signalling and viral sensing which are key pathways in pSS pathogenesis. nSS patients showed similar transcriptomic dysregulation at an intermediate level. These data can help us better understand the role of pDCs in pSS.

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FRI0282

MILK FAT GLOBULE EPIDERMAL GROWTH FACTOR 8 ON MONOCYTES IS A NOVEL BIOMARKER OF DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Milk fat globule epidermal growth factor 8 (MFG-E8) is an apoptosis-related secreted protein. It has been reported that MFG-E8 deficient or excess mice developed a systemic lupus erythematosus (SLE)-like autoimmune disease due to impaired clearance of apoptotic cells,¹ suggesting that abnormal expression of MFG-E8 is involved in the pathogenesis of SLE. Since elevated serum MFG-E8 level has been found in SLE patients,² it may possibly provide the