SLE, Sjögren's and APS – etiology, pathogenesis and animal models

AB0148

EXTRACELLULAR VESICLE-MEDIATED DELIVERY OF EBV SMALL RNA (EBER1) ACTIVATES LUPUS NEPHRITIS RELATED ANTIVIRAL IMMUNITY IN TUBULAR EPITHELIAL CELLS VIA TLR3

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Background: In lupus nephritis (LN), genetic and environmental factors drive the chronic activation of antiviral defenses leading to immune complex-mediated glomerular and tubular damage. Increasing evidence suggests the involvement of extracellular vesicles (EVs) in autoimmune disease. Currently a role for EVs in the pathogenesis of lupus nephritis has not been proposed.

Objectives: To investigate the role of EVs in the pathogenesis of LN.

Methods: To determine the presence of EVs in kidneys, biopsies from LN patients and IgA- nephropathy and Focal Segmental Glomerulosclerosis control patients were used. Serum samples from SLE patients and from RA patients as controls were used to determine the presence of circulating EVs. Primary renal tubular epithelial cells (TEC) were cultured, and Kidney injury molecule-1 (KIM1) expression was assessed by FACS. Exosomes were analyzed by electron microscopy and western blot. mRNA analysis was performed by qPCR. TLR3 inhibition was performed with TLR3/dsRNA complex inhibitor and with hydroxychloroquine.

Results: We show that EVs deliver virus-derived small RNA and activate TEC via toll-like receptor 3 (TLR3). Highly specific stem-loop RT-PCRs revealed Epstein Barr Virus (EBV)-encoded small RNAs in LN biopsies while quantitative EBV-DNA PCR, sensitive to a single copy was negative. In situ hybridization failed to detect nuclear EBV-EBER1 (i.e. EBV-infected cells) in LN biopsies. However, we observed atypical EBER signal in the cytoplasm of TECs in LN but not in disease control biopsies, suggestive of uptake of extra-renal EBER. Consistent with this, we detected EBER1 in circulating EVs of SLE sera. The LN tissues express strongly elevated levels of TLR3, Interferon induced transmembrane-1 and -3, and TNF α . Primary TEC cultured in vitro endocytose EBER1-EVs secreted by EBV-infected B cells via phosphatidylserine receptors such as KIM-1. Importantly, EV-EBER1 uptake by TEC triggers antiviral immunity and pro-inflammatory cytokine secretion in a Toll-like receptor 3 (TLR3)-dependent manner. Treatment with hydroxychloroquine (HCQ) or a small molecule inhibitor that blocks TLR3-RNA interactions strongly reduced the pro-inflammatory effects of EBER1.

Conclusions: We propose that small RNA-loaded EVs exacerbate pre-existing autoimmunity in SLE patients by engaging tubular epithelial TLR3, supporting the rationale for TLR3-blockade as therapeutic strategy in the treatment of lupus nephritis.

Disclosure of Interest: None declared

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AB0149

DECREASED MICRORNA-130A EXPRESSION DRIVES ACTIVATION OF CLASSICAL DENDRITIC CELLS FROM PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Background: Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and dryness of mouth and eyes. Classical dendritic cells are very potent antigen presenting cells known to induce strong T-cell proliferation and cytokine production.

Objectives: Considering the critical role of microRNAs (miRNAs) in regulation of gene expression, we investigated miRNA expression in circulating CD1c⁺ dendritic cells (cDCs) of patients with pSS.

Methods: Two independent cohorts consisting of pSS patients and healthy controls were established: a discovery cohort (15 pSS, 6 HC) was used to screen the

expression of a large panel of 758 miRNAs. An independent validation cohort (14 pSS, 11 HC) was used to test the reproducibility of the results. cDCs were isolated from peripheral blood using MACS and miRNA profiling of 758 targets was performed using the OpenArray platform in the discovery cohort. A selection of 16 differentially expressed miRNAs was measured in the validation cohort using a custom-made array. Isolated cDCs from HC were stimulated with a panel of Toll-like receptor (TLR) ligands and the expression of miR-130a and miR-708 was measured by qPCR. The effect of transfection with miR-130a on protein synthesis was analysed by using the pulsed stable isotope labelling by amino acids in cell culture (pSILAC) method (quantitative mass spectrometry-based technique) in a HEK-293T cell-line.

Results: A total of 24 miRNAs was downregulated in pSS patients versus HC in the discovery cohort ($p < 0.05$, with a difference between the groups of $> \log_2$). Of the 16 miRNAs that were selected for replication, the decreased expression of miR-130a and miR-708 in pSS was validated. Activation of cDCs via TLR3 and TLR7/8 induced downregulation of both miRNA-130a and miRNA-708. Transfection with a miR-130a mimic resulted in downregulation of several proteins with a seed match for the miRNA. These proteins are known to be involved in membrane trafficking and cell activation through CREB/NF- κ B signalling.

Conclusions: miR-130a and miR-708 are significantly downregulated in cDCs of patients with pSS. We show that the expression of these miRNAs is decreased upon cDC activation and that upregulation of miR-130a decreases the expression of proteins involved in the CREB/NF- κ B pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of circulating cDCs from pSS patients.

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AB0150

DIMINISHED EXPRESSION OF PD-L1 BY ACTIVATED B CELLS IS CHARACTERISTIC OF SLE

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Background: Programmed cell death 1 (PD-1) and its ligands PD-L1 and PD-L2 are known to play an important role in immune response regulation via a co-inhibitory signal during T cell activation. Genome-wide association studies in humans and the fact that PD-1 knock-out mice develop a lupus-like pathology point toward the involvement of the PD-1 pathway in the pathogenesis of systemic lupus erythematosus (SLE).^{1,2} However, little is known regarding the role of this pathway in B cells from patients with SLE.

Objectives: This study addressed the kinetics of PD-1, PD-L1 and PD-L2 expression on B cells from patients with SLE.

Methods: Blood samples were obtained from healthy donors (HD) and SLE patients. PBMCs from HD and SLE patients were stimulated with IL2/IL10, aBCR (IgG/IgM/IgA), CpG oligodeoxynucleotides (CpG) and CD40L alone or in combination. The membrane expression of PD-1, PD-L1 and PD-L2 on CD19⁺CD20⁺B cells was measured by FACS at baseline and after 48 hour stimulation.

Results: At baseline, the expression of PD-1, PD-L1 and PD-L2 on B cells between HD and SLE patients was similar. Notably, 48 hour stimulation of B cells with IL2/IL10, aBCR, CpG and CD40L gave rise to twofold reduced expression of PD-L1 by SLE compared to HD B cells. Interestingly, PD-L1 expression by SLE B cells was increased only in presence of CD40L, whereas in HD the mix of CpG, aBCR, and IL2/IL10 led also to a significant increase in PD-L1 expression.

Abstract AB0150 – Figure 1. Diminished PD-L1 expression in activated B cells may explain increased activation of T cells in SLE. (A) CD40L activated HD B cells upregulate PD-L1 that suppress activated T cells via PD-1. (B) CD40L activated SLE B cells fail to upregulate PD-L1 leaving activated T cells unchecked

Conclusions: The data suggest that activation of B cells from SLE patients require CD40/CD40L interaction to increase PD-L1 expression, which is, however, reduced compared to HD. Diminished PD-L1 expression upon B cell stimulation is consistent with impaired inhibition of T cells in SLE (figure 1).

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AB0151

CHARACTERIZATION OF THE PLASMATIC GLYCOPROTEIN AND LIPOPROTEIN PROFILES OF SYSTEMIC LUPUS ERYTHEMATOSUS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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Background: Systemic Lupus Erythematosus (SLE) is an autoimmune disease associated with systemic inflammatory processes. Chronic inflammation alters the number and type of non-cellular elements, including lipoproteins and glycoproteins.

Objectives: Here we investigate the plasmatic lipoprotein and glycoprotein profiles associated with SLE.

Methods: Plasma samples were obtained from 50 healthy control individuals and 50 SLE patients. All patients fulfilled the ACR criteria for classification of SLE. ¹H-NMR was used to determine the lipoprotein and glycoprotein profiles from cases and controls. For the lipoprotein profiles, VLDL, LDL, IDL and HDL concentration, lipid composition and particle properties were quantified. The glycoprotein profile was decomposed in four reproducible peak regions LMW, Glyc-B, Glyc-A, Glyc-Lipid and baseline, and quantified for concentration, position and aggregation state. Association analysis to disease was performed using logistic regression fitting for age, gender and body mass index. Classifiers were built using recursive-partition algorithm and classification accuracy was evaluated using receiver operating curves.

Results: We found a significant perturbation of the lipoprotein profile in SLE patients compared to controls. A statistically significant ($P<0.05$) reduction of cholesterol-LDL and HDL lipoproteins as well as an increase in triglyceride VLDL was found in SLE. Particle number for LDLs, were found to be reduced compared to controls ($P<0.05$), and mostly due to lower quantities of large LDL particles ($P<0.005$). The glycoprotein profile was also significantly altered for several NMR measures. The most significant changes were related to the reduction of the width and area of the Glyc-Lipid peak in SLE patients ($P<0.005$ and $P<0.05$,

respectively). Using the plasma molecular patterns to predict the presence of SLE, we found a significant classifier for the lipoprotein pattern (ROC AUC(95% CI)=0.85(0.78–0.93)), and an optimal classifier for the glycoprotein pattern (ROC AUC(95%CI)=0.90(0.84–0.97))

Conclusions: SLE is characterized by perturbations of the plasmatic lipoprotein and glycoprotein profiles. The increase of triglyceride VLDL in patients compared to controls, could explain the increased risk for cardiovascular disease observed in SLE. Our results also show that SLE patients have a significantly different glycoprotein profile compared to controls. In both cases, plasmatic ¹H-NMR profiling could be a valuable source of informative biomarkers for SLE.

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AB0152

MICROARRAYS GENE EXPRESSION PROFILING OF MONOCYTES FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS: NOVEL GENES AND PATHWAYS INVOLVED IN THE DISEASE

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Objectives: 1. To identify altered genes and pathways in monocytes from Systemic Lupus Erythematosus (SLE) patients involved in the pathogenesis of the disease. 2. To evaluate the contribution of anti-dsDNA antibodies to the regulation of these processes.

Methods: Eighty three subjects, including 53 SLE patients and 30 healthy donors (HDs), were recruited. Total RNA was extracted from monocytes isolated of 6 subjects, analyzed as exploratory cohort- and microarray studies were performed in an Agilent G4112F platform (Whole Human Genome Microarray 44k). By using the Ingenuity Pathway Analysis Software (IPA) the altered gene, pathways, and network profiles were identified and functionally categorised. The top differentially expressed genes were validated by RT-PCR in monocytes purified from all the subjects enrolled. The inflammatory profile was evaluated in serum by multiplex assay. The activation of intracellular proteins was analysed by PathScan intracellular signalling protein array. Correlation and association studies were performed with clinical and analytical variables. The effect of anti-dsDNA antibodies in the altered gene expression signature was evaluated by *in vitro* studies on monocytes from HD

Results: Microarray gene expression profiling identified 553 significantly altered genes in monocytes from SLE patients in relation to HDs ($p<0.05$ and fold change >2). IPA analysis showed that the altered genes were mainly related to inflammatory and immunological disease (32.4%), as well as cardiovascular (22.5%), neurological (21.9%), musculoskeletal (10%), renal (6.5%), dermatological (4.5%) and reproductive system disorders (2.2%). The top altered canonical pathway identified was the interferon signalling, and the main altered genes, validated by RT-PCR, were PGC1- α (master regulator of mitochondrial biogenesis and oxidative stress), IFI27, IFI44, IFI44L, IFIT1, IFI6 and RSAD2 (interferon signalling), EDNRB (endothelin receptor involved in the vascular system homeostasis), SERPINB10 (serpine related to haematopoiesis and apoptosis), CDKN1B and CCND2 (proliferation regulators), and IL22RA1 and CMKLR1 (inflammatory mediators). The altered gene signature was associated with the presence of anti-dsDNA antibodies, and clinical features of SLE (early atherosclerosis and nephropathy), and correlated with the disease activity and the levels of inflammatory serum markers (CRP, ESR, C3, C4, IL8, MCP1, IFN γ , IP10, PDGFBB). The activation of numerous intracellular signalling proteins was also noticed. *In vitro* studies demonstrated the modulation of several genes by effect of anti-dsDNA.

Conclusions: Gene expression profile allowed the identification of relevant genes and pathways altered in monocytes of SLE patients, which were associated with the pathogenesis of the disease and modulated, at least partially, by anti-dsDNA. The identification of relevant genes whose products regulate specific pathological processes might contribute to the development of targeted therapies in SLE

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