Chugai, Eisai, Taisho Toyama, UCB, Janssen, Eli Lilly, and NIPPON KAYAKU., Speakers bureau: Astellas, BMS, Daiichi-Sankyo, Mitsubishi Tanabe, Pfizer, Ayumi, Takeda, Chugai, Eisai, Taisho Toyama, UCB, Janssen, Eli Lilly, and NIPPON KAYAKU.
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THU0221 DRY EYE IN SJÖGREN'S SYNDROME: CHEMOKINE AND CYTOKINE TEAR SPECTRUM

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Background: Previous studies have linked the participation of multiple chemokines and cytokines in the physiopathology of primary Sjögren's syndrome (PSS), however data regarding their presence in tears is scarce

Objectives: To evaluate a panel of chemokines/cytokines in the tears of patients with PSS and correlate them with ocular symptoms as well as objective ocular tests.

Methods: We included 21 patients with PSS (EULAR/ACR criteria). A single expert ophthalmologist in dry eye evaluated the patients and assessed the tear film break-up time, Schirmer-I test, tear meniscus height, the Van Bijsterveld staining score and the SICCA Ocular Staining Score (OSS). We classified lacrimal dysfunction severity in two categories (1=mild, mild/moderate or moderate, and 2= moderate/severe and severe). We scored the ESSPRI, and ocular dryness VAS as well as the Ocular Surface Disease Index (OSDI), a 12-item scale for the assessment of symptoms related to dry eye disease and their effect on vision. Tear samples were collected using sterile tear flow strips, that were immediately frozen at -86°C until assayed. Once defrosted, the tears were extracted from the strips using a buffer containing 0.5 M NaCl and 0.5% Tween-20. We tested IFN- γ , IL-10, IL-12, IL-17A, IL-1 β , IL-2, IL-21, IL-23, IL-5, IL-6, IL-8, TNF- α , BAFF, CXCL10 and CCL2 by Luminometry. We also included 21 healthy controls without dry eye, to test chemokines/cytokines that after our initial screening were meaningful.

Results: Most patients were females (90.4%), mean age 59.3 $\pm$ 13 years and median disease duration 7.9 years (0.5-27). All of them had ocular and oral symptoms. The median tear film break-up time was 6 seconds (2-9), median Schirmer-I test 6 mm (1-25), median lacrimal meniscus height 1.5 mm (0.5-2), median Van Bijsterveld staining score 10 points (2-18), median OSS 7 points (2-11), median ESSPRI score 6.7 points (2-9.2) and median ocular dryness EVA score 9 points (1-10).

We did not detect most of the evaluated chemokines/cytokines with the exception of IL-8, CXCL10, and CCL2. The former was similar in both, patients and controls. PSS patients had lower levels of CXCL10 (472.8 pg/ μ L vs 1652 pg/ μ L, $p=0.01$) and CCL2 (1.08 pg/ μ L vs 9 pg/ μ L) than controls. Indeed, patients with worst lacrimal dysfunction severity had the lowest levels of CXCL10 (239.3 pg/ μ L vs. 646.2 pg/ μ L, $p=0.02$). We found correlations among CXCL10 and CCL2 ($T=0.30$, $p=0.02$) and lacrimal meniscus height ($T=0.55$, $p=0.005$), as well as with CCL2 and lacrimal meniscus height ($T=0.57$, $p=0.01$). None of the other variables were correlated.

Conclusion: We identified CXCL10 and CCL2 as the main chemokines in tears of patients with PSS. CXCL10 seems to participate in the normal eye homeostasis.

Disclosure of Interests: None declared

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THU0222 PLASMACYTOID DCS FROM PATIENTS WITH SJÖGREN'S SYNDROME ARE TRANSCRIPTIONALLY PRIMED FOR ENHANCED PRO-INFLAMMATORY CYTOKINE PRODUCTION

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Background: Type-I IFN activity is associated with pathogenesis and increased disease activity in primary Sjögren's syndrome (pSS). In addition, deficiency for the type-I IFN receptor in mice prevents experimental-Sjögren's syndrome. Plasmacytoid dendritic cells (pDC) are the premier type-I IFN producing immune cells and aberrances in their functional

properties may underlie pSS immunopathology. Assessing the molecular basis of this may provide a better understanding of pSS pathogenesis and new opportunities for therapeutic intervention.

Objectives: To delineate the dysregulation of pSS pDCs using RNA-sequencing and compare their transcriptional profile to pDCs obtained from patients with non-Sjögren's sicca (nSS) and healthy controls (HC).

Methods: All pSS patients met the classification criteria. nSS patients presented with dryness complaints without a known cause, did not have any generalized autoimmune disease including pSS as evaluated by an experienced rheumatologist, and did not fulfil the classification criteria. pSS (n=25), nSS (n=20), and HC (n=17) donors were included in two independent cohorts (n=31 each). Circulating BDCA-4 expressing pDCs were isolated and RNA-sequencing was performed, after which data-driven networks and modular analysis were used to identify signatures of consistently differentially-expressed genes. pSS and HC pDCs were cultured in the presence of endosomal TLR ligands, after which gene expression and secreted cytokine levels were measured.

Results: We identified signatures of consistently co-expressed and differentially expressed genes that indicated transcriptional activation in patient pDCs, which was remarkably reproducible in two independent cohorts. These included a type-I IFN-associated signature, a ribosomal protein signature, and a transcriptional machinery signature. Corroborating the transcriptomic profile, stimulated pSS pDCs produced higher levels of type-I interferon upon *in vitro* stimulation. nSS patients formed an intermediate group in which some patients were molecularly similar to pSS patients. Finally, we developed a discriminative classifier on the basis of the identified transcriptional profiles that discriminated pSS patients from HC with 100% sensitivity and 80% specificity, and identified a group of pSS-like patients within the nSS group.

Conclusion: Circulating pSS pDCs exhibit a transcriptional signature similar to activated pDCs and are primed for enhanced production of pro-inflammatory cytokines, including type-I IFN. Our data provide in-depth characterization of the aberrant regulation of pSS pDCs and substantiate their perceived role in the immunopathology of pSS and other type-I interferon-associated autoimmune diseases.

Disclosure of Interests: None declared

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THU0223 CHRONIC ADRENERGIC STIMULATION OF MINOR SALIVARY GLANDS OF PATIENTS WITH PRIMARY SJÖGREN'S DRIVES ER STRESS AND ACTIVATION OF THE UNFOLDED PROTEIN RESPONSE

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Background: Sjögren's syndrome (SS) is a common autoimmune disease in which the main targets of immune injury are specific secretory epithelia, such as salivary and lacrimal glands. Stress appears to play a significant role in the initiation of this entity.

Objectives: The aim of the present study was to investigate whether chronic stress plays a role in triggering endoplasmic reticulum (ER) stress in salivary gland epithelial cells from SS patients.

Methods: Minor salivary gland biopsy specimens were obtained from six SS patients and six control patients with sicca symptoms not fulfilling AECG criteria [1]. The expression and cellular localization of β 1-, β 2- and α 1-adrenoceptors and the levels of cAMP were measured by immunofluorescence. The morphology of the ER was evaluated *in situ* by Transmission Electron Microscopy (TEM). Primary salivary gland epithelial cell lines (SGEC) derived from minor salivary gland biopsies, were established by the explant out-growth technique [2] and were treated with epinephrine and norepinephrine. The protein levels of the ER stress markers GRP78/Bip and C/EBP homologous protein (CHOP) were determined by immunoblot analysis.

Results: *In situ* immunofluorescence staining revealed increased expression of β 1-, β 2- and α 1 adrenoceptors as well as cAMP levels in tissues derived from SS patients compared to controls. TEM evaluation of salivary tissues from SS subjects revealed extensive dilation of the ER lumen compared to controls. Treatment of SGEC with epinephrine and norepinephrine did not influence cell survival (cell viability assay). To mimic chronic stress *in vitro*, epinephrine was applied for 10 days on SGEC. It was found that 20 μ M Epinephrine induced severe ER stress on SGEC, as attested by increased expression of GRP78/Bip and CHOP, after 3 and 6 days of treatment. The expression of ER stress markers returned to basal levels after 10 days of treatment.

Conclusion: Our data revealed that adrenergic receptors are differentially expressed by salivary epithelium of SS patients compared to control tissues. This finding was further substantiated by the increased levels of cAMP, indicating a profound sympathetic stimulation of this secretory tissue. The dilated ER lumen of the salivary gland epithelial cells from SS patients confirmed the occurrence of ER stress. Moreover, the finding that catecholamines induce severe ER stress *in vitro* in SGEc under conditions resembling chronic stress suggests a causal relationship between sympathetic tone and ER stress. Deciphering the role of chronic stress in ER machinery will provide important insights that may lead to novel therapeutic targets for SS.

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Disclosure of Interests: None declared

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THU0224 THE HEPATITIS VIRUS PRE S1 PROTEIN RETARDS THE ONSET OF LUPUS-LIKE GLOMERULONEPHRITIS IN NZB/W F1 MICE

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Background: Infection can trigger autoimmunity but the nature of the pathogen could enhance or inhibit the onset of the disease. In this study we tested the tolerogenic effect of the surface Hepatitis B Virus capsid protein preS1 in a lupus mouse model.

Objectives: To evaluate the effect of administration of Hepatitis B virus preS1 subunit to NZB/WF1 mice

Methods: Mice (10 each group) were injected with a total volume of 200µl of 32.5 µg PreS1 in 200µl PBS (group 1), 200µl PBS (group 2), 3 times at 11th, 14th and 17th weeks of age. Urine samples were collected weekly to evaluate proteinuria by reactive strips. Blood samples were collected before every injection, at 22th and 28th weeks, and at death to evaluate levels of anti-C1q and anti-dsDNA by home-made ELISA tests. Tissues were harvested for histological analyses. Proteinuria free and survival rates were analyzed by Kaplan-Meier method using Mantel-Cox test for comparisons. Neurocognitive functions were evaluated with Staircase, Y-Maze and Forced Swim Test.

Abstract THU0224 – Figure 1

Results: Anti-dsDNA and Anti-C1q levels (OD±SD) were significantly higher in PBS than in preS1 treated mice (Figure 1). Mean proteinuria levels (mg/dl±SD) were higher in PBS than in preS1 treated mice. Proteinuria free survival rate (<300 mg/dl) was lower in group 2 than group 1 (p=0.016; Figure 2). Also survival rate was significantly lower in PBS than in preS1 injected mice (p=0.033). Memory deficits (p<0.01) and anxiety-like behavior was pronounced in PBS treated mice. Severe segmental and focal glomerulonephritis in both groups with large proliferation of mesangial and endothelial cells and Russell bodies were found by histological analysis. Mice treated with preS1 had more chronic inflammation in the kidneys and extramedullary hematopoiesis in the spleen and liver. Notably, in salivary glands, severe acute inflammation was observed in group 1 and chronic inflammation in group 2.

Conclusion: HBV capsid protein preS1 causes a delay in the onset of lupus-like glomerulonephritis and ameliorates behavioral disorders in NZB/BF1 mice. This peptide seems to induce a tolerizing rather than a pathogenic effect in these autoimmune prone mice models.

Abstract THU0224 – Figure 2

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THU0225 THE MOLECULAR PROFILING OF MONOCYTES FROM PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME IDENTIFIES SEVERAL NETWORKS RELATED TO THEIR ATHEROTHROMBOTIC STATUS. ROLE OF ANTIPHOSPHOLIPID ANTIBODIES ON MONOCYTE MIRNA SECRETION

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Objectives: 1. To characterize the mRNAs and microRNAs transcriptomes of monocytes, key immune cells in the atherothrombotic pathology of Antiphospholipid Syndrome patients (APS). 2. To evaluate the role of antiphospholipid antibodies (aPL) in the regulation of these processes. 3. To identify and characterize circulating microRNAs secreted by activated monocytes.

Methods: Monocytes from peripheral blood of 40 APS patients and 40 healthy donors were purified by negative immunomagnetic selection. Gene expression microarray (Agilent G4112F platform) and nCounter microRNA expression array (Nanostring) were performed on purified RNA. Functional categorization of altered genes and miRNAs was made using IPA software, and miRNA-mRNA interaction networks showing inverse correlation expression were identified. Genes and miRNAs integrating the networks were validated in the whole APS cohort by RT-PCR, as well as on a set of thrombotic non-autoimmune patients. Predicted miRNA-mRNA interactions were tested by microRNA transfection experiments.

In parallel, a miRNA array in the plasma of those patients was performed, and common deregulated miRNAs between monocytes and plasma were identified. Monocytes isolated from HD were treated *in vitro* with purified aPLs and the altered gene/miRNA expression induced in monocytes was evaluated. Moreover, the effects of aPLs on the secretion of common deregulated microRNAs in the supernatant of cultured cells were assessed.

Results: Microarray identified 518 altered genes in APS monocytes. Relevant biofunctions on which these genes were involved included hematological and cardiovascular system development and function, inflammatory response, and embryonic development, among others. Gene alterations were validated in the whole cohort, demonstrated to be stable along the time, divergent of the gene profile in monocytes from non-autoimmune thrombotic patients, and associated to thrombotic recurrences and early atherosclerosis. Analysis of miRNA profiles showed altered expression of 22 miRNAs in APS monocytes. Fifty-four genes were inversely correlated and predicted as CVD-related target genes of 19 differentially expressed miRNAs in monocytes. Association of these genes and miRNAs with the