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.

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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.

References:

[1] Al-Ali S, et al. Whole blood transcriptomic signature of primary Sjögren's syndrome associated lymphoma. Biomarkers and Targeted Therapeutics in Sjögren's (BATTS) Conference, 2016.

Disclosure of Interest: None declared

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