Results: IgG production by CD19⁺ B cells *in vitro* upon multiple stimulation was significantly increased in pSS patients as compared to HC (p=0.015). Notably, the IgG production was further enhanced by the addition of rnsBAFF to the culture, and that enhancement was significantly higher in pSS patients than HC (p=0.011). Moreover, the proportion of CD38^{high}IgD⁺ B cells among CD19⁺ B cells was significantly and positively correlated with not only serum IgG level (p<0.0001), but also the amount of IgG produced *in vitro* by the multiple stimulated CD19⁺ B cells (p=0.042). The mean fluorescence intensity of BR3 expression in CD38^{high}IgD⁺ B cells was significantly higher in pSS patients than HC (p=0.029), whereas no significant difference was observed between pSS and HC for total CD19⁺ B cells.

Conclusions: Our results suggest that CD38^{high}IgD⁺ B cells, which are known as activated B cells, highly react to BAFF in pSS patients as compared to HC. Our findings may shed light on the mechanism of B cell activation triggered by BAFF during the pathogenesis of pSS.

Disclosure of Interest: None declared

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FRI0275 ALTERED PROTEIN TYROSINE PHOSPHATASES ACTIVITY AND DISEASE SPECIFIC EXPRESSION PATTERN OF CD45 AND CD148 ON VARIOUS LYMPHOCYTE SUBSETS IN AUTOIMMUNE DISEASES

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Background: Previous studies demonstrated impaired B cell receptor (BCR) response in patients with systemic lupus erythematosus (SLE). Besides diminished cytokine production upon TLR9 stimulation¹ it was found that phosphorylation of Syk² and downstream Ca²⁺ flux was reduced upon BCR stimulation. This led to the hypothesis of a "post activation" state of peripheral B cells (BCs) in autoimmunity which might be related to altered protein tyrosine kinase (PTK) versus phosphatase (PTP) activities. Prior studies in SLE showed enhanced PTP but not ser/thr phosphatase (PSP) activities in BCs.³ Receptor PTPs (RPTPs) CD45/CD148 are described as regulators of initial steps of BCR signalling by regulating Lyn which phosphorylates CD79 ITAMs and thus activates Syk.⁴

Objectives: This study aimed at testing the hypothesis that altered phosphatase activity (PA) is a hall mark of autoimmunity. So, expression of RPTPs CD45/CD148 were compared on lymphocyte subsets in patients with rheumatoid arthritis (RA), primary Sjögren's Syndrome (pSS), SLE and healthy donors (HD). To check whether increased global PA is restricted to BCs of patients with SLE, the study extended analysis to T cells (TCs) and studied patients by including cohorts of RA and pSS.

Methods: Peripheral blood samples were analysed for expression of CD45 and CD148 in patients and HDs using flow cytometry (n(HD/RA/pSS/SLE)=13/8/18/22). PTP and PSP activities were analysed in isolated BCs and TCs lysates from patients and HD using a protein activity assay (n(HD/RA/pSS/SLE)=21/6/13/10).

Results: Compared to HDs CD45 expression is significantly enhanced on peripheral monocytes and CD3⁺/CD4⁺ as well as CD3⁺/CD4⁺ TCs from patients with RA. Further, CD3⁺/CD4⁺ TCs of patients with RA express more CD148 (Tb1 1). In patients with SLE, CD148 expression is decreased on CD148⁺ memory B cells. In addition, this study confirmed that enhanced PTP activity is characteristic of SLE BCs since TCs of any of the cohorts and BCs of patients with RA and pSS showed PTP and PSP activities comparable to HD.

Abstract FRI0275 – Table 1. Significant differences found in RA and SLE compared to HD.

		CD45	CD148		PTP activity	PSP activity
RA	monocytes	↑0.0354	ns	TCs	ns	ns
	CD3 ⁺ /CD4 ⁺	↑0.0325	↑0.0451			
	CD3 ⁺ /CD4 ⁺	↑0.0277	ns			
	naïve BCs	ns	ns			
SLE	memory BCs	ns	ns	TCs	ns	ns
	monocytes	ns	ns			
	CD3 ⁺ /CD4 ⁺	ns	ns			
	CD3 ⁺ /CD4 ⁺	ns	ns			
	naïve BCs	ns	ns	BCs	↑ P _{PT} = 0.0122	ns
	memory BCs	ns	↓0.0064		↑ P _{PSP} = 0.0055	

↑: significantly increased; ↓: significantly decreased; ns: not significant; PP1/PP2: phosphopeptide 1/2

Conclusions: This study identified characteristic differences of PTP activities in BCs from patients with SLE compared to HD which were not identifiable by an enhanced CD45 or CD148 expression on PBMCs and did not correlate with disease activity. This suggests intrinsic abnormality in SLE BCs. While there is evidence that abnormal PTP is present in SLE BCs, further studies are needed to identify underlying individual PTPs to further analyse their pathogenic and potential therapeutic relevance. Among the tested autoimmune conditions increased

CD45 expression on TCs and monocytes was specific to RA, which might indicate disease dependent patterns of PTP/PSP expression.

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FRI0276 ABERRANT DISTRIBUTION OF CD3+CD56+ NKT-LIKE CELLS IN PATIENTS WITH SJÖGREN'S SYNDROME

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Background: CD3⁺CD56⁺ NKT-like cells are a subset of T cells co-expressing NK cell markers, and bridge innate and adaptive immunity. However, the phenotype and the potential role of NKT-like cells in the pathogenesis of Sjögren's Syndrome (SS) remains elusive.

Objectives: To elucidate the distribution of CD3⁺CD56⁺ NKT-like cells in peripheral blood and labial salivary gland (LSG) of SS patients, as well as the immune function of NKT-like cells in SS patients.

Methods: SS patients (n=110) and healthy controls (HC, n=94) were enrolled. Peripheral CD3⁺CD56⁺ NKT-like population, proliferation and apoptosis potential, surface chemokine receptors and proinflammatory cytokines secretion were quantified by flow cytometry. The infiltration of NKT-like cells in LSG was examined by immunofluorescence. Serum and tissue levels of CX3CL1 were detected by ELISA and immunohistochemistry, respectively. *In vitro* transwell migration assay was used to examine the chemotaxis of NKT-like cells induced by chemokines. NKT-like cells were stimulated with CX3CL1 and were quantified the TNF-α and IFN-γ secretion by flow cytometry.

Results: Peripheral CD3⁺CD56⁺ NKT-like cells from SS patients were significantly lower than those from HC (3.09%±2.35% vs 5.37±4.06%, p<0.001), which was negatively correlated with ESSDAI (r=-0.523, p=0.009) and serum RF level (r=-0.586, p=0.015). The proliferation rate (35.1%±10.69% vs 36.4±10.75%, p=0.812) or apoptosis rate (13.01%±12.15% vs 12.48±5.28%, p=0.338) of NKT-like cells from SS and HC were comparable. However, NKT-like cells infiltrations into LSG were observed in SS patients but were rarely presented in HC. Serum level of CX3CL1 (936.8±99.82 vs 887.8±82.77 pg/ml, p=0.015) and epithelial CX3CL1 of LSG were higher in SS patients than those in HC. CX3CR1 were expressed on NKT-like cells from both SS and HC (68.25%±22.38% vs 70.06±17.34%, p=0.777). Furthermore, migration assay showed that CX3CR1 promoted NKT-like cells chemotaxis with a dose-dependent manner. Functionally, NKT-like cells from SS patients expressed higher level of CD69 (10.97%±6.75% vs 2.69±1.82%, p<0.0001). Peripheral NKT-like cells from both SS patients and HC secreted high level of TNF-α (70.29%±22.74% vs 81.33±10.76%, p=0.137) and IFN-γ (62.42%±16.66% vs 64.34±14.50%, p=0.759), and low level of IL-4 (4.39%±3.42% vs 2.64±1.90%, p=0.131) and IL-17 (0.30±0.28 vs 0.31±0.30, p=0.967). Importantly, CX3CL1 promoted TNF-α and IFN-γ production by NKT-like cells *in vitro*.

Conclusions: NKT-like cells were aberrantly depleted in peripheral blood and accumulated in LSG in patients with SS, which was driven by CX3CL1- CX3CR1 axis. NKT-like cells from SS were activated and secreted high level of TNF-α and IFN-γ, which were further promoted by CX3CL1. Thus, NKT-like cells were implicated in the pathogenesis of SS, which might be a potential therapeutic target for SS.

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