T cell subpopulations, and total and KLH-specific immunoglobulin (Ig) levels were monitored. Additionally, gene expression in blood was analysed using the NanoString nCounter platform.

Results: Atacept was well-tolerated at all dose levels tested. All animals in the treatment arms had quantifiable levels of atacept in serum throughout the study. Reduction of serum IgM and IgG was detected 7 days after treatment, with a continuous reduction in the mean serum IgM and IgG levels observed until Day 29 (table 1). In animals treated with 3 and 30 mg/kg atacept, a significant decrease in serum anti-KLH IgG levels was observed versus vehicle-treated controls, beginning at Day 11 (table 2). A minor reduction in absolute CD3-CD20+, IgD + B cell numbers was seen in response to treatment, but no changes in T cell subsets were detected. Changes in gene expression following atacept treatment were predominantly observed in B cell-related and Ig genes.

Abstract FRI0272 – Table 1. Mean% reduction of total IgM and IgG relative to baseline

Mean reduction	Day 8			Day 29		
	0.3 mg/kg	3 mg/kg	30 mg/kg	0.3 mg/kg	3 mg/kg	30 mg/kg
IgG	7.1	14.2	16.3	10.6	27.2	46.3
IgM	9.1	17.1	22.3	21.5	52.3	64.1

Table 2 Mean% reduction of KLH-specific IgM and IgG relative to vehicle group

Mean reduction	Day 11			Day 29		
	0.3 mg/kg	3 mg/kg	30 mg/kg	0.3 mg/kg	3 mg/kg	30 mg/kg
KLH-specific IgG	17.3	55.2	49.7	39.4	78.8	71.5
KLH-specific IgM	15.1	33.4	40.1	40.4	67.8	66.6

Conclusions: This study showed that atacept modulates B cell responses, IgM and IgG levels, and Ig isotype switching in a KLH-antigen-challenged cynomolgus monkey model, thus supporting its use in the treatment of antibody-mediated diseases. Additionally, gene expression PD markers identified in this study will be used in subsequent clinical trials as exploratory PD readouts.

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FRI0273 RNA SEQUENCING DETECTION OF GENE DYSREGULATION IN EPITHELIAL SORTED CELLS FROM SALIVARY GLAND TISSUE REVEALS INTERESTING PATHWAYS INVOLVED IN SJÖGREN'S SYNDROME PATHOPHYSIOLOGY

E. Rivière¹, N. Tchitchek¹, G. Nocturne^{1,2}, S. Boudaoud¹, J. Pascaud¹, A. Virone¹, A. Thai³, N. Allaire³, B. Jagla⁴, M. Mingueneau³, X. Mariette^{1,2}, ¹Immunology of viral infections and autoimmune diseases, IDMIT, CEA – Université Paris Sud – INSERM U1184, Le Kremlin Bicêtre and Fontenay aux Roses; ²Rheumatology, Université Paris Sud, Le Kremlin Bicêtre, France; ³Immunology Research, Biogen, Cambridge, USA; ⁴Biomarker Discovery Platform UtechS CB and Hub de Bioinformatique et Biostatistiques C3BI, Institut Pasteur, Paris, France

Background: Primary Sjögren's syndrome (pSS) is a chronic autoimmune disorder characterised by lymphocytic infiltrates and destruction of the salivary glands. Several lines of evidence support the hypothesis that salivary gland epithelial cells (SGECs) are not only the target of autoimmunity in pSS patients but may also play a role for its initiation and maintenance.

Objectives: To establish high-resolution molecular maps of SGEC from pSS patients compared to controls using RNASeq analysis.

Methods: Patients had pSS according to 2016 EULAR/ACR criteria and controls had sicca symptoms without any antibodies and with normal lip biopsy. SGEC, B, T CD4 and CD8 lymphocytes were sorted from salivary gland biopsies from 9 pSS patients and 4 controls, using a FACS ARIA cell sorter. Total RNASeq profiling was performed using MiSeq (Illumina). For SGEC subset, 4 samples were excluded due to a contamination by B lymphocytes, thus analysis was performed on 5 pSS and 4 controls using R software, to identify transcriptional differences

between pSS and control SGEC. Functional Enrichment analysis was performed using Ingenuity Pathway Analysis software.

Results: In SGEC, 495 genes were differentially expressed between pSS and controls. 280 genes were up-regulated, and 215 genes were down-regulated. Enrichment analysis (table 1) highlighted IL-7 signalling pathways (including IL-7, STAT5A, STAT1 genes) and interferon signalling (including OAS1, IFIT3, IFI6, TAP1 genes). Other genes potentially involved in immune responses and interactions between SGEC and lymphocytes were significantly up-regulated, including bone marrow stromal cell antigen 2, HLA-DRA, BAFF-R and IL-23 A (table 2). These results need to be confirmed by RT qPCR. However, consistent results have already been obtained in our laboratory, showing that IL-7 serum level is increased in pSS patients compared to controls and that SGECs produce IL-7 after interferon stimulation. The analysis of the non-coding RNA part and the other sorted cells subtypes is ongoing.

Abstract FRI0273 – Table 1. Pathways identified as statistically over-represented with Ingenuity Pathways Analysis in epithelial cells from pSS patients compared to controls

Pathway	-log p-value
Primary Immunodeficiency Signalling	4,08
Interferon Signalling	3,52
B Cell Development	2,89
Role of JAK2 in Hormone-like Cytokine Signalling	2,73
IL-7 Signalling Pathway	2,51

Abstract FRI0273 – Table 2. Selection of genes differentially expressed between pSS and controls in SGEC

Gene Symbol	log2 fold-change	p-value
IL-7	2.56	0.002
BST2	4.08	0.0002
HLA-DRA	1.84	0.0372
IL-23 A	3.59	0.0155
BAFF-R	4.94	0.0097

Conclusions: Immune interactions between SGEC and B or T lymphocytes could represent a key in the understanding of the initiation and/or maintenance of autoimmunity in pSS. Our study highlights the key role of epithelial cells in activation of immune cells. *In vitro* experiments are needed to confirm these results and elucidate the molecular mechanisms.

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FRI0274 ELEVATED REACTIVITY OF CD38^{HIGH} B CELLS AGAINST BAFF CONTRIBUTES TO IGG OVERPRODUCTION IN PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

E. Ishioka-Takeji, K. Yoshimoto, K. Suzuki, H. Yasuoka, K. Yamaoka, T. Takeuchi. Keio University School of Medicine, Tokyo, Japan

Background: Primary Sjögren's syndrome (pSS) is often accompanied by hypergammaglobulinemia and production of autoantibodies, such as anti-Ro/SSA and anti-La/SSB antibodies. These serological aberrations suggest that abnormally activated B cells play a key role in the pathogenesis of pSS. We have previously reported that the proportion of peripheral CD38^{high}IgD⁺ B cells among CD19⁺ B cells is significantly elevated in pSS patients and positively correlated with serum anti-Ro/SSA, anti-La/SSB titer, total IgG, and the European League against Rheumatism (EULAR) Primary Sjögren's syndrome disease activity index (ESSDAI). B cell activating factor belonging to TNF family (BAFF) is a well known cytokine which promotes differentiation, proliferation and survival of B cells. It has been reported that serum BAFF level is increased in pSS patients compared to HC and that BAFF is also highly expressed in salivary glands. Based on these background, it is conceivable that BAFF plays a pivotal role in the pathogenesis of pSS.

Objectives: To elucidate the involvement of BAFF in IgG overproduction in pSS.

Methods: Peripheral CD19⁺ B cells were prepared from pSS patients (n=16) and gender-matched HC (n=10) by using CD19-microbeads. The cells were stimulated *in vitro* with an anti-IgM antibody, recombinant human CD40 ligand and recombinant human IL-4 ('multiple stimulation') with or without recombinant human soluble BAFF (rhBAFF) for 96 hours. The amount of IgG produced by the cells in the culture supernatants was measured by ELISA. The proportion of B cell subsets, characterised by anti-CD19, anti-IgD and anti-CD38 antibodies, and the expression level of a BAFF receptor (BR3) in the subsets were analysed by FACS. Disease activities of the pSS patients were quantified based on the ESSDAI scores. The serological data of the patients were collected by clinical records.

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 rbsBAFF 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

F. Szelinski^{1,2}, S. Fleischer², S. Weissenberg^{1,2}, K. Reiter¹, T. Dörner^{1,2}.

¹Department of Rheumatology and Clinical Immunology, Charité; ²German Rheumatism Research Center Berlin (DRFZ), Berlin, Germany

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

H. Chen, L. Zhao, W. Xu, Z. Chen, J. Sun, F. Zhang. Rheumatology, Peking Union Medical College Hospital, Beijing, China

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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