chemokine in the serum (pg/mL) of SLE patients compared to HC, respectively, included: sCX3CL1 (478.6±46.19; 235.4±140.8) and MCP-1 (2657±466.2; 648.5±69.6) (p<0.00001), MIP1β (141.0±10.71; 79.31±10.69) (p<0.0003), and TNFα (59.07±6.64; 29.31±3.15) (p<0.0041). Additionally, the disease severity and endothelial dysfunction associated biomarker VEGF increased in SLE patients (445.6.3±48.1 pg/mL) compared to HC (231.8±47.03 pg/mL) (p<0.0069). Lastly, HC monocyte subsets were stimulated with TLR7 and 8 ligands and supernatants evaluated for the analytes described above, see table 2.

Abstract FRI0291 – Table 1

Monocyte Subset	Marker	Healthy Control (Mean ± SEM)		SLE Patients (Mean ± SEM)		P-Value	
		Percentage	MFI	Percentage	MFI	Percentage	MFI
CD14 ⁺	CCR1	71.18 ± 5.31	820.7 ± 55.8	71.81 ± 6.425	880.9 ± 110.4	0.799	0.775
CD16 ⁺	CCR1	19.41 ± 5.14	1768.2 ± 548.7	33.90 ± 7.86	1716.6 ± 473.9	0.108	0.329
CD14 ⁺ /CD16 ⁺	CCR1	34.87 ± 6.01	1993.3 ± 441.4	49.50 ± 7.13	1935.1 ± 396.4	0.124	0.420
CD14 ⁺	CD11b	97.58 ± 0.59	5289.5 ± 410.9	98.44 ± 0.29	6868.2 ± 626.6	0.985	0.007
CD16 ⁺	CD11b	55.22 ± 4.48	1289.6 ± 146.6	53.13 ± 4.34	2075.15 ± 324.4	0.819	0.021
CD14 ⁺ /CD16 ⁺	CD11b	93.82 ± 1.60	4580.8 ± 327.5	93.72 ± 2.32	5640.9 ± 579.0	0.861	0.102
CD14 ⁺	CD40	7.61 ± 2.72	612.2 ± 200.7	8.06 ± 3.08	806.7 ± 266.4	0.861	0.333
CD16 ⁺	CD40	7.37 ± 3.05	1640.6 ± 575.6	15.65 ± 6.09	1466.1 ± 473.6	0.200	0.511
CD14 ⁺ /CD16 ⁺	CD40	9.77 ± 2.92	1116.7 ± 225.2	12.33 ± 4.17	1389.1 ± 348.7	0.643	0.389
CD14 ⁺	CD163	65.77 ± 5.42	1499.4 ± 110.4	85.24 ± 3.08	2198.3 ± 171.0	0.006	0.001
CD16 ⁺	CD163	15.21 ± 3.06	563.88 ± 94.74	24.08 ± 3.34	757.6 ± 106.6	0.022	0.022
CD14 ⁺ /CD16 ⁺	CD163	61.00 ± 4.96	1358.0 ± 108.6	76.72 ± 5.36	2028.3 ± 198.0	0.014	0.014
CD14 ⁺	CX3CR1	42.82 ± 2.93	796.2 ± 70.58	63.0 ± 5.91	1062 ± 100.1	0.005	0.047
CD16 ⁺	CX3CR1	86.47 ± 2.53	2471.8 ± 223.5	85.50 ± 4.08	2947.3 ± 336.3	0.742	0.378
CD14 ⁺ /CD16 ⁺	CX3CR1	65.38 ± 4.66	2061.0 ± 164.6	81.23 ± 3.41	2578.1 ± 249.1	0.027	0.144

Abstract FRI0291 – Table 2

Cytokine / Stimulus	Stimulus	CD14 ⁺ Monocytes				CD14 ⁺ /CD16 ⁺ Monocytes				CD16 ⁺ Monocytes			
		pg/mL		Fold Change		pg/mL		Fold Change		pg/mL		Fold Change	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
CXCL1	Control	29.8	9.3	1.0	0.0	60.1	6.0	1.0	0.0	22.9	9.6	1.0	0.0
	ssRNA40 (TLR8)	44.2	0.4	2.4	0.8	62.7	5.0	1.0	0.0	41.4	8.4	2.0	0.5
	ssRNA40 (TLR7)	46.8	15.1	1.6	0.0	69.1	3.2	1.2	0.2	32.7	19.7	1.3	0.3
	ssRNA40 (TLR8)	65.5	2.4	2.5	0.9	74.3	0.2	1.3	0.1	49.3	13.9	2.3	0.4
MIP-1β	Control	80.9	26.9	1.0	0.0	936.7	199.6	1.0	0.0	181.3	87.3	1.0	0.0
	ssRNA40 (TLR7)	954.5	396.6	16.9	11.1	739.8	200.5	0.8	0.2	742.3	317.4	4.1	0.7
	ssRNA40 (TLR8)	317.1	215.0	3.1	1.3	1477.0	404.5	1.8	0.7	330.0	141.2	1.9	0.6
	ssRNA40 (TLR8)	1982.0	974.6	22.7	4.2	2529.0	1028.0	3.1	1.6	896.1	367.0	6.0	1.9
MIP-1α	Control	96.7	52.6	1.0	0.0	2368.0	609.8	1.0	0.0	213.6	80.0	1.0	0.0
	ssRNA40 (TLR7)	5464.0	1468.0	123.6	85.9	3411.0	984.3	1.8	0.7	2194.0	894.9	9.8	0.7
	ssRNA40 (TLR8)	3831.0	1368.0	90.4	18.6	3880.0	627.2	1.8	0.5	2330.0	1072.0	9.6	4.3
	ssRNA40 (TLR8)	1275.0	841.2	1.0	0.0	3182.0	1492.0	1.0	0.0	487.9	256.9	1.0	0.0
MCP-1	Control	4168.0	3028.0	9.9	7.6	1032.0	765.5	0.4	0.1	1265.0	614.7	3.1	0.5
	ssRNA40 (TLR7)	2979.0	3252.0	2.3	0.7	3415.0	1029.0	2.1	1.1	4894.0	4418.0	6.1	4.4
	ssRNA40 (TLR8)	12618.0	4296.0	12.0	2.8	5814.0	2057.0	4.5	3.1	11255.0	10153.0	28.9	9.0
	ssRNA40 (TLR8)	7.6	3.8	1.0	0.0	253.4	70.7	1.0	0.0	42.2	24.1	1.0	0.0
TNF	Control	1257.0	217.8	387.2	267.1	1331.0	252.4	6.3	2.2	1735.0	704.5	53.0	11.2
	ssRNA40 (TLR7)	26.0	18.3	2.9	0.7	392.1	75.9	2.1	1.1	99.9	45.8	2.6	0.6
	ssRNA40 (TLR8)	724.6	134.1	156.0	72.0	1379.0	233.9	7.3	3.7	592.7	142.4	22.3	7.2

Conclusions: Cell surface markers of activation and adhesion increased in SLE monocyte subsets compared to HC. In parallel, increased inflammatory cytokines and chemokines that attract monocytes to tissues were increased in the serum of these patients. Linking a possible source of this increase in serum analytes, HC monocyte subsets were stimulated with disease-relevant ligands and evaluated in culture. Along with increased monocyte expression of CX3CR1, preliminary data demonstrates an increase in CX3CL1 expression on endothelial progenitor cells (EPCs) and immature and mature circulating endothelial cells (iCECs and mCEC respectively) in active disease.

Disclosure of Interest: None declared

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FRI0292 DYSFUNCTION OF TFH, TREG AND TR1 CELLS IN APOE^{-/-} FASLG^Δ C57BL/6 MICE WITH LUPUS SYMPTOMS AND ATHEROSCLEROSIS

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Background: Cardiovascular disease due to atherosclerosis is currently recognised as one of the leading causes of death among patients with systemic lupus erythematosus (SLE). It is well established that dysfunction of lymphocytes contribute to the pathogenesis of SLE. Recent studies also showed infiltration of several subsets of lymphocytes in atherosclerotic lesions and their various contributions to atherosclerosis were uncovered in experimental models and patients. However, the predominant and specific subsets of lymphocytes that play critical role in the pathogenesis of SLE patients with cardiovascular complications remained to be elucidated.

Objectives: This study aims to define the dominant population of lymphocytes in mice with combination of lupus and atherosclerosis.

Methods: The mouse model of accelerated atherosclerosis in lupus (ApoE^{-/-} FasL^Δ B6 mice) was generated from apolipoprotein E-deficient (apoE^{-/-}) and FasL^Δ C57BL/6 mice. The lupus-like autoimmunity and atherosclerotic lesions was evaluated. The lymphocytes of spleen and peripheral blood were analysed by flow cytometry.

Results: The results of PCR and sequencing showed that the double-mutant ApoE^{-/-} FasL^Δ B6 mice were generated. Spleens from 5 month-old ApoE^{-/-} FasL^Δ B6 mice were significantly enlarged compared with wild type mice (WT mice). ApoE^{-/-} FasL^Δ B6 mice displayed a pattern of glomerulonephritis typically found in SLE and showed marked C3, IgG and IgM deposits in the glomeruli. Anti-dsDNA antibody and high levels of creatinine were detected in the serum of ApoE^{-/-} FasL^Δ B6 mice. These results indicated that the ApoE^{-/-} FasL^Δ B6 mice have typical characteristics of SLE. Oil red O staining revealed that there was significantly increased atherosclerotic lesion area at the proximal aorta in ApoE^{-/-} FasL^Δ B6 mice compared with WT mice (figure 1 a,b). The frozen section of myocardium stained by oil red O revealed that lipid deposited in myocardial cells of ApoE^{-/-} FasL^Δ B6 mice (figure 1 c,d). As expected, total cholesterol, LDL cholesterol and triglyceride were significantly increased, while HDL cholesterol decreased in the double-mutant mice. These results indicated that ApoE^{-/-} FasL^Δ B6 mice had accelerated atherosclerosis.

Conclusions: The ApoE^{-/-} FasL^Δ B6 mice simultaneously exhibit SLE and atherosclerosis characteristics. Our findings suggested that proinflammatory M1 macrophages and Th1 cells were increased, while the anti-inflammatory Treg and Tr1 cells were decreased and the imbalance of these cells and their releasing cytokines contributed to progression in atherosclerosis with SLE. Further studies may validate these cells as potential targets for treating SLE patients with atherosclerosis.

Disclosure of Interest: None declared

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FRI0293 IMBALANCE IN CIRCULATING SUBSETS OF INNATE LYMPHOID CELLS IS LINKED TO DISEASE ACTIVITY AND TYPE I INTERFERON SIGNATURE IN PRIMARY SJÖGREN'S SYNDROME

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Background: Recent studies indicate an important role for innate lymphoid cells (ILCs) in the pathophysiology of rheumatic diseases. In rheumatoid arthritis and spondyloarthritis elevated numbers of subsets of ILCs have been found at the site of inflammation producing cytokines including IFN-γ and IL-22 and in addition, group 3 ILC have been suggested to be involved in formation of ectopic lymphoid structures in rheumatic diseases, Shikhaigat Nat Rev Rheumatol 2017 Wenink A and R 2017). ILC3-like cells producing IL-22 have been found in the salivary glands of pSS patients. Ciccia ARD 2012 However, circulating ILC have not yet been studied in primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE). Furthermore, SLE and pSS are characterised by presence of a type I interferon (IFN) signature in a large proportion of the patients. Animal studies in HIV and asthma implicate type I IFN, produced by plasmacytoid dendritic cells (pDCs), to regulate the survival of group 2 and group 3 ILCs (ILC2 and

ILC3) via increase of Fas (CD95) expression, rendering the ILC more susceptible to apoptosis, Maazi JACI 2017, Zhang JCI 2015. Duerr Nat Immunol 2016

Objectives: In this study, we explored for the first time the frequency and phenotype of circulating ILCs in pSS and SLE and their relation to the IFN signature.

Methods: Frequencies and phenotypes of ILC subsets and pDCs were assessed by flow cytometry in peripheral blood of patients with pSS (n=20), SLE (n=20) and healthy controls (n=17). Patients were stratified by the presence or absence of an IFN signature as assessed by RT-qPCR on peripheral blood mononuclear cells as previously described. Brkic ARD 2013

Results: Results ILC1 numbers were increased in peripheral blood of patients with SLE as compared to healthy controls and in pSS patients ILC1 numbers correlated with disease activity (ESSDAI score), serum IgG levels and anti-SSB auto-antibodies (all p<0.05). Numbers of ILC1, ILC2 or ILC3 did not significantly differ between patients with SLE and pSS. However, patients with a high expression of the type I IFN signature had significantly decreased numbers of ILC2 and ILC3 (p=0.04 and p=0.02, respectively). The decrease of ILC2s and ILC3s was related to increased expression of Fas (CD95) on these cells in patients with a high type I IFN signature (both p=0.01).

Conclusions: Both in SLE and pSS, a type I IFN signature is related to reduced numbers of circulating ILC2s and ILC3s in association with increased Fas expression on these cells possibly rendering them more susceptible to Fas/FasL-dependent apoptosis at peripheral sites.

Disclosure of Interest: None declared

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FRI0294

INJURED PODOCYTES EXPRESS COMPLEMENT FACTOR H AND PROCESS IMMUNE COMPLEX DEPOSITION IN GLOMERULAR SUBENDOTHELIAL AREA

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Background: Glomerular Immune complex (IC) deposition causes glomerulonephritis in lupus nephritis. The reaction of intrinsic glomerular cells differs according to the area of IC deposition. Subepithelial IC deposits cause functional and structural changes on podocytes (glomerular visceral epithelial cells) through complement pathway activation as is well known in membranous glomerulonephritis. In this setting, podocytes can induce some complement factors including complement factor H (CFH) which serves not only to regulate the alternative pathway but also to process subepithelial IC deposition.¹ However, whether podocyte processing of IC deposition is limited to only the subendothelial area is obscure.

Objectives: To clarify the role of podocytes in IC deposition in the glomerular sub-endothelial area.

Methods: NEP25 mice genetically expressing human CD25 in podocytes were used. Intravenous immunotoxin for human CD25 (LMB2) provokes podocyte-specific injury (NEP25/LMB2). By shortening the period of LMB2 exposure to NEP25 mice, we mitigated the podocyte injury. We administered intraperitoneally IgG3-producing hybridoma clones, 2B11.3, which were previously established from an unmanipulated MRL/lpr mouse, to NEP25 mice (NEP25/hybridoma). Furthermore, we also injected short-term LMB2 to NEP25/hybridoma mice (NEP25/hybridoma/LMB2). We investigated IC deposits by immunofluorescence and electronic microscopic study. We measured complement regulatory factor mRNA expression including CFH, complement factor I (CFI), decay-accelerating factor (DAF), complement receptor 1-related gene/protein y (Cry), C3a receptor (C3aR) and C5a receptor (C5aR) of isolated glomeruli of each mouse by quantitative real time-PCR. In an *in vitro* study, we assessed CFH mRNA expression of immortalised mouse podocytes injured by puromycin.

Results: First, NEP25/LMB2 mice showed glomerular tuft collapse with epithelial cell hyperplasia, suggesting podocyte loss by light microscopy study as reported previously. mRNA expression of all complement regulatory factors but C5aR was decreased in NEP25/LMB2 mice as compared with NEP25 mice. This result suggests that podocytes produce these complement regulatory factors. On the other hand, when the podocyte injury was mitigated, CFH and C3aR mRNA expression increased, and CFI, DAF and Cry mRNA expression decreased as compared with NEP25 mice. Second, we detected IC deposition only in the glomerular sub-endothelial area without endocapillary proliferative lesion in NEP25/hybridoma mice. They showed increase of C3aR mRNA expression, and decrease of CFI and DAF mRNA expression as compared with controls. Notably, subendothelial IC deposition did not alter CFH mRNA expression. Third, NEP25/hybridoma/LMB2 (milder podocyte injury) showed decrease of IC deposits in glomeruli and increase of only CFH mRNA expression (1.7-fold) as compared to NEP25/hybridoma mice, while there was no significant change in mRNA expression of other

complement regulatory factors. Finally, puromycin-induced mild podocyte injury promoted CFH mRNA expression in cultured podocytes as compared to controls.

Conclusions: Mildly injured podocytes induce CFH expression, and serve to process IC deposition in the glomerular subendothelial area.

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FRI0295

INHIBITION OF CATHEPSIN S LEADS TO SUPPRESSION OF ANTIGEN SPECIFIC T CELLS FROM PATIENTS WITH PRIMARY SJÖGREN SYNDROME

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Background: Primary Sjögren syndrome (pSS) is an autoimmune disease characterised by an infiltration of T and B cells into exocrine gland tissue and its subsequent destruction¹. Antigen presenting cells, including B cells, foster T cell activation and anti-SS-A/SS-B producing plasma cells, eventually leading to disease progression and systemic complications².

Objectives: Cathepsin S (CatS) is crucially involved in MHCII processing in pSS mouse models³ and patients⁴. In this translational study we investigated the *ex vivo* effects of the CatS inhibitor RO5459072 in different bio-compartments, including specific T cells, of pSS patients and healthy controls.

Methods: *Ex vivo* CatS activity was assessed in different bio-compartments of 15 pSS patients and 13 healthy controls and in presence or absence of RO5459072 using commercial activity and quantification assays. In addition, antigen (5 µg/mL SS-A, 5 µg/mL SS-B, 5 µg/mL Influenza H₃N₂; 2 µg/mL Tetanus Toxoid and 100 ng/mL SEB) specific T cell responses were examined using 2 × 10⁵ PBMC/well IFN-γ/IL-17 Dual ELISPOT (48 hour incubation) and 5 × 10⁴ PBMC/well BrdU proliferation assays after (72 hour incubation) in presence or absence of RO5459072.

Results: pSS patients showed significantly higher CatS activity in tear fluid than healthy controls (two-tailed t-test p<0.01). RO5459072 significantly suppressed CatS activity in tears of pSS patients (two-tailed t-test p<0.01). CatS inhibition also exerted a strong and dose-dependent suppression of T cell responses towards SS-A and SS-B antigen in *ex vivo* derived pSS patient cells in Elispot and BrdU assays (table 1).

Table 1 Suppression of antigen specific T cell responses (Elispot) and suppression of cell proliferation (BrdU) in the absence or presence of RO5459072 (0–100 µM). One-tailed t-test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Conclusions: CatS activity in tear fluid seems to be a relevant biomarker for pSS disease activity. RO5459072 is a potent inhibitor of CatS and the pSS associated relevant antigen specific T cell responses.

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