Methods: The study population consisted of 15 pSS patients, who fulfilled the ACR-EULAR criteria and underwent salivary gland biopsies of both the parotid and labial gland. Patients were not treated with biologicals and 13 patients were not using other immunosuppressive therapy. Presence and severity of LELs were evaluated on HE stained sections. Severity of lesions was scored from stage 0 to stage 3 (stage 0: lymphocytic ductal infiltration without hyperplasia of the epithelium; stage 1: lymphocytic ductal infiltration and <50% hyperplastic epithelium; stage 2: lymphocytic ductal infiltration and ≥50% hyperplastic epithelium; stage 3: lymphocytic ductal infiltration and fully circumferentially hyperplastic epithelium with occluded lumen). Numbers of B- and T-lymphocytes within LELs were counted (10 ducts per biopsy) by using Image J cell counter on serial CD20 and CD3 stained sections. High molecular weight cytokeratin staining was used to identify ductal borders. Generalized estimating equations (GEE) were used to analyse the numbers of B- and T-lymphocytes and B/T ratio's over the different stages of severity of LELs.

Results: B- and T-lymphocytes can both infiltrate within the ductal epithelium forming LELs in salivary glands of pSS patients. T-lymphocytes were present in all LELs, scattered through the whole ductal epithelium. Whereas B-lymphocytes were found in clusters, mostly located in the hyperplastic area of the ductal epithelium. With higher severity of LELs, the numbers of B- and T-lymphocytes increased significantly, in both the parotid and labial gland. The numbers of B-lymphocytes increased relatively more with higher severity of LELs than T-lymphocytes. This has led to an increased intraepithelial B/T ratio in more pronounced LELs. This increased B/T ratio was even more pronounced in parotid than in labial glands. In both glands, there was a predominance of T-lymphocytes in lower LEL stages. In more severe LEL stages, B-lymphocytes outnumbered the T-lymphocytes.

Conclusions: Given the relative increase in the number of B-lymphocytes with higher severity of LELs as well as their close association with proliferating ductal epithelial cells, we conclude that B-lymphocytes play a major role in the hyperplasia of the ductal epithelium.

Disclosure of Interest: None declared

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AB0166

RANK/RANK-LIGAND INTERACTION REGULATES PATHOGENIC T CELL RECRUITMENT IN SJÖGREN'S SYNDROME

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Background: The RANKL (ligand)-RANK-OPG triad, members of the TNF(R) superfamily, is implicated in lymphoid organ development and bone homeostasis. It has recently been demonstrated that RANK-activated astrocytes release CCL20 and attract T cells to the central nervous system in a model of Multiple Sclerosis and that transgenic RANK expression in the skin promotes aberrant epithelial cell proliferation and is sufficient to induce ectopic formation of tertiary lymphoid structures (TLS). Ductal epithelial cells (SGEs) have been implicated in Sjögren's Syndrome (SS) pathogenesis where they mediate immune recruitment by expression of pro-inflammatory chemokines and support the formation of pre-malignant myoepithelial lesions.

Objectives: A combination of human and mouse studies were used to address the role of RANK-RANKL interaction in primary (p) SS.

Methods: Salivary glands (SGs) and saliva samples from patients recruited in the OASIS cohort (University of Birmingham) were studied to evaluate this pathway in human disease. Consecutive stimulated saliva samples (n=69) were analysed using Proseek Multiplex INF⁹⁶ × ⁹⁶, covering 92 unique inflammation-related protein biomarkers. Taking advantage of a viral induced model of pSS we studied the effect of this pathway with a RANKL blocking antibody and by inducing gain of function with direct cannulation in the salivary glands of recombinant RANKL. Murine SGs were studied by immunofluorescence, flow cytometry and qPCR on total tissue and sorted cells.

Results: Fourteen proteins in saliva were significantly separated between pSS and sicca controls, and elevated levels of just two proteins, RANKL and TNFβ, could classify pSS or sicca with 75% accuracy. Levels of salivary RANKL and CCL20 were strongly correlated (r=0.6; p<0.01). We demonstrated that both human and murine inflamed SGEs upregulate both RANK and CCL20, a chemokine known to recruit pathogenic T cells. Upregulation of RANKL was found in human Th2 cells, classically associated with humoral responses and germinal centre (GC) formation. SGs from mice treated with anti-RANKL antibody showed decreased epithelial proliferation, reduced T cell infiltration and defective TLS

establishment. On the contrary, viral infected SGs treated with recombinant RANKL showed increased T cell infiltration, CCL20 expression and enhanced differentiation of GC B cells.

Conclusions: *In vivo* RANK/RANKL interaction mediates recruitment of activated T cells that are skewed toward a Th2 phenotype. These, in turn, will favour the establishment of TLS in the SG. Those data were confirmed in human pSS, where expression of RANK is found in inflamed epithelium and RANKL detection in saliva is able to differentiate patients with pSS from sicca controls, thus candidates this pathway both for drug targeting and patient stratification.

Disclosure of Interest: None declared

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AB0166

TRACKING OF MUCOCUTANEOUS AND MUSCULOSKELETAL FLARES IN SLE USING SERUM FAS, FERRITIN, IGFBP2 AND STNFRIL

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Background: SLE is a multisystemic autoimmune disease characterized by unpredictable disease course with periods of flares and remission. The lack of reliable methods which can predict a disease flare hampers the exploration of effective and preventive strategies for disease relapses.

Objectives: To study the performance of serum FAS, sTNFRIL, Igfbp2 and Ferritin as biomarkers for tracking lupus flares in non-renal SLE patients.

Methods: Twenty-nine patients, who met the requirements of American College of Rheumatology (ACR) for classification of SLE, were recruited from Oklahoma Medical Research Foundation (OMRF) for serological testing of all four serum protein markers. None of the patients had lupus nephritis. Serum samples were obtained over 4 consecutive visits. Serum FAS, sTNFRIL, Igfbp2 and Ferritin molecules were measured in all patients. Lupus disease activity was assessed by both SLEDAI and BILAG activity indices.

Results: In our study, all 4 tested biomarkers (FAS, Ferritin, Igfbp2 and sTNFRIL) showed significant correlations with SLEDAI and BILAG. FAS (r= 0.36, p<0.0001 for SLEDAI & r= 0.29, p= 0.0002 for BILAG), Ferritin (r= 0.13, p=0.0494 for SLEDAI & r= 0.22, p= 0.0035 for BILAG), Igfbp2 (r= 0.24, p= 0.0013 for SLEDAI & r= 0.18, p= 0.0106 for BILAG) and sTNFRIL (r= 0.30, p<0.0001 for SLEDAI & r= 0.19, p= 0.0112 for BILAG). When serial disease activity changes were examined, different serum markers performed better in different SLE patients, tracking with mucocutaneous or musculoskeletal disease flares. In studying a total of 72 disease intervals, FAS and Ferritin exhibited the highest concordance with concurrent disease activity (58–62%), followed by Igfbp2 (50%) and sTNFRIL (46%), all of which were superior to the performance of complement C3, C4 and anti-DNA. Furthermore, adding FAS to other tested molecules increased its ability to track concordant disease activity changes (81% for FAS±Ferritin; 76% for FAS±Igfbp2 and FAS±sTNFRIL).

Conclusions: Serum FAS and Ferritin emerge as potential serum markers for tracking mucocutaneous or musculoskeletal disease flares in SLE patients.

Disclosure of Interest: None declared

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AB0167

METFORMIN HAS ANTI-INFLAMMATORY POTENTIAL BY REDUCING P-GP EXPRESSION ON PBMCs OF PATIENTS WITH LUPUS

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Background: Metformin causes immunomodulation by activation of AMP-kinase and thereby inhibition of mTOR pathway. Metformin reduces disease activity in lupus mouse models.¹ A study has shown that its use in lupus patients decreased corticosteroid dose and prevented flares.² P-glycoprotein expression is linked to drug resistance and increased cytokine production. Metformin inhibits expression of P-glycoprotein (P-gp) in cancer cells.³ There is scarce data on effect of metformin on P-gp expression and cytokine secretion on immune cells in autoimmune diseases like lupus.

Objectives: To study the effect of metformin on P-gp expression in PBMCs of lupus patients and its effect on inflammatory cytokines secretion. To determine optimum concentration required for such an effect.

Methods: PBMCs of nine lupus patients (Mean age 30 yrs, all females) were cultured using RPMI medium and then stimulated with PMA/ ionomycin, with or without increasing dose of metformin (0.01, 0.1, 1, 10 mM/L) for 24 h. Cytokines IL-1b, IL-6, IFN-γ and IL-10 were analyzed by ELISA in culture supernatant. Cell