and phenotypic characterization was performed. All experiments were conducted by flow cytometry.

Results: KLRG1 was expressed in a statistically significant lower amount on total NK cells from SLE patients compared to HS ($p=0.0007$). Analysis of CD56^{dim} and CD56^{bright} populations showed less amount of KLRG1 in SLE patients compared to HS ($p=0.0051$ and $p=0.0068$) (figure 1). The low levels of KLRG1 on NK cells inversely correlated with the SLEDAI-2K ($p=0.029$) and were inversely associated with the presence of arthritis ($p=0.029$), arthralgias ($p=0.0024$) and hematological disorders ($p=0.025$). A direct association between KLRG1 expression on NK cells and the use of HCQ was found ($p=0.001$). Analyzing the two main subpopulations of NK cells, we observed an inverse association between KLRG1 on CD56^{dim} cells and arthritis ($p=0.0089$), arthralgias ($p=0.0001$), hematological disorders ($p=0.0329$) and SLEDAI-2K ($p=0.0105$). KLRG1 on CD56^{dim} was directly associated with the use of HCQ ($p=0.0010$). KLRG1 on CD56^{bright} cells was also directly associated with the use of HCQ and MMF ($p=0.03$ and $p=0.014$).

Fig.1

Abstract AB0162 – Figure 1

Conclusions: This study shows for the first time a reduced expression of KLRG1 in SLE patients. The inverse associations between the levels of this receptor on NK cells and the SLEDAI-2K and clinical features, together with the direct association with HCQ and MMF, suggest a possible role of KLRG1 in the pathogenesis of this disease. KLRG1 could be a possible therapeutic target in SLE.

REFERENCES:

- Spada R, Rojas JM, Barber DF. Recent findings on the role of natural killer cells in the pathogenesis of systemic lupus erythematosus. *J Leukoc Biol*. 2015 Oct;98(4):479–87.
- Henson SM, Akbar AN. KLRG1—more than a marker for T cell senescence. *Age (Dordr)*. 2009 Dec;31(4):285–91.
- Armstrong DL, Reiff A, Myones BL, et al. Identification of new SLE-associated genes with a two-step Bayesian study design. *Genes Immun*. 2009 Jul;10(5):446–56.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6444

AB0163

INCREASED ERYPTOSIS LEVELS IN ERYTHROCYTES TREATED WITH ANTIBODIES FROM APS PATIENTS: ERYPTOSIS AS NEW ACTOR IN APS PATHOGENESIS

M. Vomero¹, F. Ceccarelli¹, A. Finucci¹, C. Barbati¹, T. Colasanti¹, C. Pensa¹, F. R. Spinelli¹, C. Perricone¹, L. Novelli¹, L. Massaro¹, F. R. Mauro², R. Foà², F. Conti¹, G. Valesini¹, C. Alessandri¹. ¹Lupus Clinic; ²Department of Cellular Biotechnologies and Hematology, Sapienza University of Rome, Rome, Italy

Background: Erythrocytes (RBCs) are highly sensitive cells constantly exposed to several stress stimuli including inflammatory mediators. Despite the absence of nuclei and the lack of crucial elements in the machinery of apoptosis, they have developed a rapid self-destruction process called eryptosis. During this process the externalization of phosphatidylserine (PS) activates the correct elimination of erythrocytes by phagocytes preventing inflammation and intravascular hemolysis. It has been recently demonstrated that PS-exposing erythrocytes are able to adhere to endothelial cells causing an impairment of circulation,¹ suggesting a possible involvement of eryptosis in the increased risk of thrombotic episodes typical of antiphospholipid syndrome (APS).

Objectives: Enhanced eryptosis is known to contribute to several pathological conditions but the role of this process in APS has not been investigated yet. For this reason, we evaluated the effect of antibodies (Abs) purified from APS patients and healthy subjects positive for antiphospholipid antibodies without clinical manifestations (aPL carriers) on eryptosis activation. Moreover, spontaneous eryptosis levels in APS, aPL carriers, autoimmune haemolytic anaemia (AIHA) and healthy donors (HD) were analyzed.

Methods: 30 patients with primary APS (M/F 7/23, mean age 50.5±8.2 years), 17 aPL carriers (M/F 4/13, mean age 48.6±8.3 years) were recruited after written informed consent. Moreover 13 AIHA patients and 17 HD were also enrolled as positive and negative control group respectively. Ammonium sulfate precipitation is used to purify Abs from sera of APS and aPL carriers subjects. RBCs, isolated from whole blood by centrifugation, were incubated with APS and aPL carriers Abs at concentration of 20µg/mL and after 4 hours the percentage of annexin V-positive cells (PS-exposing cells) was analyzed by flow cytometry. The same technique was used to estimate spontaneous eryptosis levels in all cohorts studied.

Results: *In vitro* Abs from APS induced eryptosis in RBCs isolated from HD after 4 hours of culture compared to untreated and RBCs stimulated with Intravenous Immunoglobulins (IVIg), both $p=0.02$. On the contrary, Abs from aPL carriers had no effect on the percentage of PS-exposing RBCs (figure 1). *Ex vivo*, APS patients showed higher levels of spontaneous eryptosis compared to HD ($p=0.001$). As expected, eryptosis was upregulated in AIHA patients compared to all populations studied ($p<0.0001$). Interestingly, the percentage of annexin V-positive RBCs was lower in aPL carriers respect to APS patients ($p=0.001$). No significant correlation between eryptosis and clinical parameters was found.

Abstract AB0163 – Figure 1

Conclusions: In this study we demonstrated a new aspect of APS pathogenesis based on the capacity of Abs isolated from APS patients, and not those from aPL carriers, to stimulate eryptosis suggesting a possible contribution of this process in APS clinical manifestations.

REFERENCE:

- Borst O, Abed M, Alesutan I, et al. Dynamic adhesion of eryptotic erythrocytes to endothelial cells via CXCL16/SR-PSOX. *Am J Physiol Cell Physiol* 2012;302:C644–51.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6331

AB0164

NUMBERS OF B-LYMPHOCYTES INCREASE WHEN FORMATION OF LYMPHOEPITHELIAL LESIONS PROGRESSES IN SALIVARY GLANDS OF PRIMARY SJÖGREN'S SYNDROME PATIENTS

M.S. Van Ginkel¹, E.A. Haacke^{1,2}, H. Bootsma¹, S. Arends¹, J.F. van Nimwegen¹, G.M. Verstappen¹, F.K. Spijkervet³, A. Vissink³, B. van der Vegt², F.G. Kroese¹.

¹Department of Rheumatology and Clinical Immunology; ²Department of Pathology and Medical Biology; ³Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, Netherlands

Background: The major histopathological finding in salivary glands of primary Sjögren's syndrome (pSS) patients are periductal lymphocytic infiltrates (foci). In association with these infiltrates, lymphoepithelial lesions (LELs) may be formed. LELs are defined by infiltration of lymphocytes within the epithelium with hyperplasia of ductal cells. Although it has been suggested that B-lymphocytes predominate in LELs, the relative role of B- and T-lymphocytes in LEL formation is not known. Since LELs are a characteristic finding in salivary glands of pSS patients, quantification of B- and T-lymphocytes in the different stages of LEL development will give more insight in their formation as well as in the pathogenesis of pSS.

Objectives: To quantify the numbers of B- and T-lymphocytes in different stages of LEL development in parotid and labial gland biopsies of pSS patients.

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

DOI: 10.1136/annrheumdis-2018-eular.6637

AB0166

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

S. Nayar¹, B.A. Fisher¹, J. Campos¹, D.H. Gardner¹, A. Dumusc², V. Iannizzotto¹, C. Smith¹, C.D. Buckley¹, S.J. Bowman³, C.G. Mueller⁴, F. Barone¹.

¹Rheumatology Research Group, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK; ²rheumatology department, Departement de l'appareil locomoteur, Lausanne University Hospital (CHUV), Lausanne, Switzerland; ³University Hospitals Birmingham National Health Service Foundation Trust, Birmingham, UK; ⁴Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

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

DOI: 10.1136/annrheumdis-2018-eular.6563

AB0166

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

S. Soliman^{1,2}, K. Vanarsa², A. Swilling², J. Merrill³, C. Mohan². ¹Rheumatology and Rehabilitation, Faculty of Medicine, Minia University hospital, Minya, Egypt; ²biomedical engineering, university of Houston, Houston; ³Oklahoma Medical research foundation, Oklahoma, United States

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

DOI: 10.1136/annrheumdis-2018-eular.1133

AB0167

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

S. Kansurkar, M.K. Rai, D.P. Misra, V. Agarwal. Clinical Immunology and Rheumatology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGI), Lucknow, Uttar Pradesh, India, Lucknow, India

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