through endosomal TLR inhibition effectively blocks IFN- α , is standard of care. However, few patients experience complete remission.

Objectives: We asked how an IL-1 receptor associated kinase 4 (IRAK-4) inhibitor I92 (ND-2158, Nimbus Discovery),¹ acting downstream of TLR 7/9, affects RNA-IC-induced cytokine production compared to hydroxychloroquine (HCQ).

Methods: Plasmacytoid dendritic cells (pDCs) and natural killer (NK) cells were isolated from peripheral blood mononuclear cells (PBMCs) of healthy individuals. PBMCs from 15 SLE patients were depleted of monocytes. Cells were stimulated with RNA-IC, consisting of IgG from SLE patient sera and U1snRNP particles, in the presence or absence of I92 or HCQ. Cytokines were measured by immunoassays or flow cytometry. RNA-sequencing was performed on RNA-IC stimulated pDCs from four healthy individuals and the effect of I92 and HCQ was assessed.

Results: RNA-IC induced IFN- α , TNF- α , IL-6, IL-8, IFN- γ , MIP1- α and MIP1- β production in pDC and NK cell co-cultures. I92 reduced the pDC and NK cell derived cytokine production by 74.95%. HCQ interfered with cytokine production in pDCs, but not in NK cells. In monocyte-depleted SLE PBMCs I92 blocked TNF- α , IFN- γ , MIP1- α and MIP1- β production more efficiently than HCQ. IL-8 production was high in monocyte depleted PBMC from SLE patients, and not blocked by neither drug, despite significant inhibition of IL-8 in pDC-NK co-cultures from healthy individuals. Following RNA-IC activation of pDCs, 975 differentially expressed genes were observed (FDR<0.05), many connected to cytokine pathways, cell regulation and apoptosis. The IRAK4 inhibitor significantly changed more RNA-IC induced genes than HCQ (492 vs. 65 genes). Several top upregulated genes were reversed by both I92 and HCQ, including *IFNA2*, *IFIT2-3*, *OASL*, *CXCL10*, *CD274*, *TNFSF10*, *APOL6*. Genes such as *DKK4*, *LAD1* and *EAF2* were significantly more downregulated by I92 than by HCQ.

Conclusions: Whereas both HCQ and the IRAK4 inhibitor block important pro-inflammatory cytokines, the IRAK4i I92 exhibits a broader inhibitory effect than HCQ on pathways triggered by RNA-IC, which suggests that IRAK4 inhibition could be a future therapeutic option in SLE and possibly other systemic autoimmune diseases characterized by the presence of ICs containing nucleic acid.

REFERENCE:

- [1] Kelly PN, Romero DL, Yang Y, et al. Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy. *J Exp Med* 2015;212:2189–201. doi:10.1084/jem.20151074

Disclosure of Interest: K. Hjorton: None declared, N. Hagberg: None declared, E. Israelsson Employee of: AstraZeneca, O. Berggren: None declared, J. Sandlyng: None declared, K. Thorn Employee of: AstraZeneca, J. Mo Employee of: AstraZeneca, M.-L. Eloranta: None declared, L. Ronnblom: None declared
DOI: 10.1136/annrheumdis-2018-eular.6369

AB0160 LOSS OF LET-7 MICRORNA UPREGULATES IL-6 IN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS TRIGGERING TREG/TH17 IMBALANCE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

L. Geng, X. Xu, J. Liang, C. Zhao, L. Sun. *Department of Rheumatology and Immunology, The Drum Tower Clinical Medical School of Nanjing University, Nanjing, China*

Background: Systemic lupus erythematosus (SLE) patients exist an imbalance between CD4⁺CD25⁺FoxP3⁺ T regulatory (Treg) and IL-17-producing cells (Th17). Correction of this Treg/Th17 imbalance may have therapeutic impact for SLE patients. Our previous study demonstrated that bone marrow derived mesenchymal stem cells (BMSCs) from SLE patients are defective in immune modulation, which might be involved in Treg/Th17 imbalance and disease onset, but the mechanisms are not clear yet. The expression imbalance of microRNAs (miRNAs) can cause cell dysfunction, which participates in a variety of diseases development. As an important computer predicted target of let-7, interleukin-6 (IL-6) is an important negative immune regulatory factors secreted by BMSCs. Our previous experiments found that the expressions of let-7 are markedly down-regulated in SLE BMSCs by microRNA array analysis, and the synthesis of IL-6 is significantly higher in SLE BMSCs compared to normal controls.

Objectives: To investigate whether loss of let-7 could play its role in SLE BMSCs immune modulation defects by directly up-regulating IL-6 mRNA, which might contribute to Treg/Th17 imbalance in PBMCs from SLE patients, to further understand the pathogenesis of SLE.

Methods: BMSCs were isolated, cultured and expanded from iliac crest bone marrow of four healthy donors and four SLE patients. Real-time PCR was used to further determine the let-7 expression and IL-6 mRNA level in BMSCs from healthy controls and SLE patients. The important computer predicted target of let-7, IL-6 was determined using the luciferase reporter assay. Let-7-mimics, let-7-inhibitor, and let-7-negative control were transfected to BMSCs with using Lipofectamine 2000. BMSCs from healthy controls and SLE patients were co-cultured

with PBMCs from SLE patients for 72 hours to detect their effect on the ratio of Treg/Th17.

Results: Compared to normal controls, the expression of let-7 was markedly down-regulated in BMSCs from SLE patients by RT-PCR, and the synthesis of IL-6 mRNA and protein levels were significantly higher in BMSCs from SLE patients. Compared to let-7-negative controls, transfection of BMSCs with let-7-mimics markedly lower synthesis of TGF- β 1 mRNA, and let-7-inhibitor led to an opposite effect. The mean value of Treg/Th17 was significantly decreased in PBMCs from SLE patients compared to normal controls. Transfection of normal BMSCs with let-7-inhibitor caused significant downregulation of Treg/Th17 compared to let-7-negative controls, while transfection with let-7-mimics led to an opposite effect.

Conclusions: SLE patients exist an imbalance between Treg and Th17 cells, which might be associated with up-regulation of IL-6 secretion of BMSCs by loss of let-7.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.6937

AB0161 INTERLEUKIN-37: A THERAPEUTIC FOR LUPUS NEPHRITIS IN MRL-FASLPR MICE

L. Ding¹, X. Hong¹, Z. Huang², D. Liu¹. ¹Department of Rheumatology and Immunology, Shenzhen People's Hospital, the Second Clinical Medical College of Jinan University; ²Department of Immunology and Microbiology, Biological Therapy Institute, Shenzhen University School of Medicine, Shenzhen, China

Background: Systemic lupus erythematosus (SLE) is a serious and noticed autoimmune disease, and lupus nephritis (LN) as one of its major complications without effective treatment. IL-37 has been identified as a natural inhibitor of innate immunity. Although increasing evidence shown that serum IL-37 correlated with SLEDAI and nephritis activity, However, it is still unclear that the therapeutic effect of IL-37 on lupus mice *in vivo*.

Objectives: This study aims to determine the inhibitory effect of IL-37 in lupus mice and lupus nephritis *in vivo*, and to expose the IL-37 related mechanisms of cell and molecules that inhibits the inflammation in lupus nephritis.

Methods: MRL-Fas^{lpr} mice with mild and advanced disease were treated with lentiviral vector pLVX-IL-37b. The protein levels of IL-37b in serum and tissue, IL-17, IL-6, IL-10 and TGF β -3 produced by cell culture from spleen and kidney of mice were measured by ELISA, the relative mRNA expression of these cytokines in PBMCs was detected by RT-PCR, and the frequency of Th17 and Treg cells in splenocyte were determined by FACS.

Results: Our results show that IL-37 was highly effective in prolonging survival, alleviating the pathologic characteristics of lupus nephritis (LN) in MRL-Fas^{lpr} mice by reducing the autoimmune complex deposition such as IgM, IgG and C3, decreasing the production of inflammatory cytokines such as IL-17, IL-6 and promoting the anti-inflammatory cytokines IL-10 and TGF β -3. We also found that IL-37 protect lupus mice from LN associated with increasing the frequency of Treg cells and reducing the frequency of Th17 cells.

Conclusions: These results indicate the protective action of IL-37 in LN, and strengthen the support for IL-37 as a new target for therapy of SLE.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2018-eular.4378

AB0162 KLRG1 EXPRESSION IS REDUCED ON NK CELLS FROM SLE PATIENTS AND INVERSELY CORRELATES WITH DISEASE ACTIVITY AND CLINICAL FEATURES

L. Novelli, C. Barbati, F. Ceccarelli, M. Vomero, C. Perricone, F. Morello, C. Garufi, F.R. Spinelli, C. Alessandri, G. Valesini, F. Conti. *Sapienza University of Rome, Rome, Italy*

Background: Systemic Lupus Erythematosus (SLE) is a multifactorial autoimmune disease, with different immunological alterations and clinical phenotypes. Natural killer (NK) cells participate in the regulation of several immune responses but their function in SLE is not well understood.¹ KLRG1 (killer cell lectin like receptor G1) is a trans-membrane protein expressed in humans especially on NK cells, working as an inhibitory receptor of their cytotoxic activity. Thus, KLRG1-mediated signal might have a role in preventing autoimmunity, increasing the activation threshold of NK cells.² KLRG1 gene also, emerged as a disease susceptibility gene for SLE in four different ethnic groups.³

Objectives: The aim of this study was to characterize KLRG1 expression on NK cells (both CD56^{dim} and CD56^{bright}) from SLE patients compared to healthy subjects (HS) and to investigate its possible correlations with clinical features, including SLE disease activity.

Methods: We enrolled 14 patients (14F, mean age $\pm$ SD 37 $\pm$ 10.7 years, mean disease duration $\pm$ SD 10.5 $\pm$ 7.9 years) affected by SLE according to the 1997 ACR criteria, and 7 HS (7F, mean age $\pm$ SD 33 $\pm$ 5.5 years). Disease activity was measured by SLEDAI-2K. Peripheral blood mononuclear cells (PBMCs) were purified

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.