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Acknowledgement: Center for Genes, Environment and Health Ms. Kendra Walton

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.707

AB0179 THE TRANSCRIPTION FACTORS IKZF1 AND IKZF3 CONTROL B CELL ACTIVATION AND DIFFERENTIATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: The transcription factors *IKZF1* (Ikaro) and *IKZF3* (Aiolos), essential for the maturation, differentiation and survival of B cells, have been linked to Systemic Lupus Erythematosus (SLE). The cereblon modulator Ibrdomide, which induces degradation of *IKZF1* and *IKZF3*, is undergoing clinical trials in SLE. However, the role of *IKZF1* and *IKZF3* in aberrant plasmablast development and pathogenesis of SLE has not been fully elucidated.

Objectives: To assess the mechanism of *IKZF1* and *IKZF3* control of gene expression underlying activation and differentiation of B cells in SLE patients.

Methods: CD19⁺ B cells were isolated from the peripheral blood of patients with SLE recruited at Barts Health NHS Trust (n=25). B cells were cultured for 5 days and stimulated with IL-2, IL-10, IL-15, CD40L and TLR-7 ligand Resiquimod to induce plasmablast differentiation together with ibrdomide (10 nM) or control at either day 0 or 18h prior to harvest at day 4. At day 5, cells were harvested for fluorescence-activated cell sorting (FACS) and measurement of IgG and IgM in supernatants by ELISA. RNA extraction and RNA-sequencing were performed on FACS-sorted CD27⁺ IgD⁺ naïve B cells and CD20^{low}CD27⁺CD38⁺ plasmablasts and matched baseline B cells.

Results: Ibrdomide from day 0 (n=9) significantly reduced the number of CD20^{low}CD27⁺CD38⁺ plasmablasts (number of sorted CD20^{low}CD27⁺CD38⁺ plasmablasts mean±SD 48727±86122 in untreated, 3248±8335 in ibrdomide, p=0.03). Accordingly, ibrdomide significantly inhibited the production of IgM and IgG from SLE B cells (p=0.017 and 0.050, respectively). Ibrdomide given at day 4 did not affect the numbers of plasmablasts or the production of IgM and IgG (n=16). However, ibrdomide induced a significant modulation of several genes both in naïve B cells and plasmablasts, as assessed by RNA-seq on sorted cells (400 and 461 differentially modulated genes in naïve B cells and plasmablasts, p adjusted <0.05). Pathway analysis showed that ibrdomide treatment resulted in downregulation of JAK-STAT signalling downstream of IL12 (FDR=7.92E-04), IL12 signalling (FDR0.0014), and p53 signalling regulation of cell death (FDR=0.0043) and showed a trend to upregulation of RUNX1 signalling and Rho GTPase cycle.

Conclusion: Our work confirms the importance of *IKZF1* and *IKZF3* in modulating gene expression required for B cell differentiation in SLE, as shown by the ability of ibrdomide to inhibit the differentiation of SLE B cells into plasmablasts. Inhibition of *IKZF1* and *IKZF3* modulated the expression of several transcriptional programmes in naïve B cells and plasmablasts. While larger scale analysis will be needed to confirm the functional consequences of *IKZF1* and *IKZF3* modulation in naïve B cells and plasmablasts, our results confirm their relevance in SLE as important regulators of B cell activation and differentiation.

Acknowledgement: This study was supported by research funding from Celgene Corporation.

Disclosure of Interests: Sotiria Manou-Stathopoulou: None declared, Felice Rivellesse: None declared, Daniele Mauro: None declared, Katriona Goldmann: None declared, Debasish Pyne: None declared, Peter Schafer Shareholder of: Celgene corporation, Employee of: Celgene Corporation, Michele Bombardieri Grant/research support from: Celgene, Consultant for: Medimmune, Costantino Pitzalis Grant/research support from: Celgene, Myles Lewis Grant/research support from: Celgene

DOI: 10.1136/annrheumdis-2019-eular.6343

AB0180

VALIDATION OF COMPLEX IMMUNOPHENOTYPING STRATIFICATION OF PATIENTS WITH LUPUS AND SJÖGREN'S SYNDROME WITH THERAPEUTIC POTENTIAL

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Background: There is a current paucity in effective treatments for patients with primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE). Their overlapping symptoms mean that they are often treated with similar biologic therapeutics, despite being distinct diagnoses. Our pilot research sought to stratify patients by novel, complex immune signatures, to potentially facilitate a more personalised approach to prescribing medication, irrespective of clinical diagnosis. From a cohort of 40 patient patients with SLE, pSS or combined SS/SLE, and 33 healthy controls; five distinct clusters were originally identified.

Objectives: Here we aimed to validate the original patient endotypes from our pilot data with a new patient cohort, and refine the identified clusters to be of more realistic clinical benefit.

Methods: A new cohort of patients with SLE (n=32), pSS (n=31) and SS/SLE (n=15) donated peripheral blood samples while attending appointments at UCLH clinics. None had received B-cell depletion therapy in the preceding 48 months. Complex phenotyping of peripheral T and B-cell subsets was performed by flow cytometry. Unsupervised clustering, statistical comparison to healthies and Receiver Operator Characteristic (ROC) analysis, as per our previous work, were used to stratify patients using immune signatures into distinct endotypes considering only those markers where sensitivity and specificity were above 80% (AUC>0.80). Characteristics of new clusters were compared with previous findings to refine the number of markers and unique groups.

Results: Five distinct endotypes across the three disease groups were identified after unsupervised clustering of CD19+ B-cell, CD4+ and CD8+ T-cell subsets. Two of these clusters serve to validate groupings from our original data- one with a distinct B cell fingerprint (Bm1, IbM5 and Total CD19+) with AUC=0.87, AUC=0.81 and AUC=0.83 respectively, and another with a T cell fingerprint (Total CD4+, Total CD8+, CD8+ naïve, CD8+ effector memory T cell subsets) with AUC>0.80 for all of them. Clinical diagnoses and symptoms treatment did not differ significantly between clusters.

Conclusion: Our results demonstrate a novel method to re-classify patients using clustered immune phenotype abnormalities. Two distinct groups demonstrated either B or T-cell immunophenotypes, likely reflective of their underlying immunopathogenesis. Stratification by patient endotype has the potential to facilitate a better-informed selection of biologic therapeutics for individual patients; and highlights pathways for further mechanistic research.

Acknowledgement: British Sjögren's syndrome Association and Versus Arthritis

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.5696

AB0181

ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) OF THE BAFF GENE AND FATIGUE IN PRIMARY SJÖGREN'S SYNDROME

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Background: Primary Sjögren's Syndrome (SS) is characterized by B lymphocyte hyperactivity with B cell activating factor (BAFF) acting as an important regulator. Single Nucleotide Polymorphisms (SNPs) of the *BAFF* gene have been implicated in the pathogenesis of several autoimmune diseases including lupus and SS both of which are characterized by heightened fatigue levels, often compromising quality of life.

Objectives: To explore potential associations between several *BAFF* gene SNPs and fatigue status experienced by primary SS patients.

Methods: Fatigue status was assessed by Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT-F) scale in 178 primary SS patients. Five SNPs (rs9514827, rs1041569, rs9514828, rs1224141, rs12583006) of the *BAFF* gene were tested in DNA extracted from peripheral blood of all patients enrolled in the study using the RFLP-PCR method. A cut-off value of <30 was used, which indicates severe fatigue. Analysis of *BAFF* SNPs in association with fatigue levels was performed by the online platform SNPStats.

Results: The frequency of T/T genotype of both rs9514828 and rs1224141 was reduced in primary SS patients with severe fatigue compared to those without

(17.9% vs 36.7%, $p=0.0094$ and 51.8 vs 69.2%, $p=0.027$, respectively. The corresponding ORs [95%CI] in the dominant model were 0.38 [0.17-0.82] and 0.48 [0.25-0.92], respectively. No statistically significant associations between fatigue status and other SNPs tested were detected.

Conclusion: We report novel associations between genetic makeup and primary SS-associated fatigue with the rs9514828 and rs1224141 *BAFF* T/T genotypes decreasing the likelihood of fatigue development among these patients. These findings need to be validated in multi-center studies.

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Acknowledgement: This research is co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

Disclosure of Interests: Christina Maria Flessa: None declared, Adrianos Nezos: None declared, Evangelia Zampeli Speakers bureau: Roshe, Astrazeneca, Haralampos M. Moutsopoulos: None declared, Clio Mavragani: None declared

DOI: 10.1136/annrheumdis-2019-eular.7024

AB0182 RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KAPPA B LIGAND (RANKL)/RANK AND OSTEOPROTEGERIN (OPG) PATHWAY ACTIVATION IN SJOGREN'S SYNDROME

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Background: Primary Sjogren's syndrome (SS) is an autoimmune exocrinopathy characterized by chronic salivary and lacrimal glands dysfunction. Recent studies suggest that activation of the receptor activator of nuclear factor-kappa B ligand (RANKL)/RANK and osteoprotegerin (OPG) pathway is implicated in the development of autoimmunity.

Objectives: The aim of this study is to investigate the contribution of RANK/RANKL/OPG pathway in primary SS pathogenesis.

Methods: Sixty-six primary SS patients fulfilling the American/European criteria and 13 subjects with oral and/or ocular dryness without satisfying the aforementioned criteria (sicca controls, SC) and 11 healthy controls (HC) have been enrolled. RANKL and OPG serum levels were determined by ELISA in 29 SS patients and 11 HC. Total RNA was extracted from minor salivary glands biopsies (MSG) from 43 SS patients and 13 SC and RANKL mRNA expression was assessed by qRT-PCR. Protein expression of RANKL was determined by Western Blot and immunohistochemistry (IHC) in total protein extract and paraffin embedded tissue respectively, derived from MSG biopsies of 6 primary SS patients and 5 SC. Statistical analysis was performed with SPSS 24.0 package.

Results: Increased serum levels (pg/ml) of RANKL (mean±SD=4.197±897.6vs mean±SD=144.8±290.2, $p=0.013$) and OPG (mean±SD=173.4±57.52, mean±SD=48.42±21.89, $p=0.015$) were detected in SS patients compared to HC. Higher RANKL at both mRNA and protein level by IHC were detected, in MSG derived from primary SS patients compared to SC. RANKL protein expression was significantly increased in MSG biopsies from primary SS patients compared to SC by Western Blot (mean±SD=2.468±1.896 vs mean±SD=0.5138±0.823, $p=0.0335$).

Conclusion: Increased serum levels, mRNA and protein expression of RANKL in association with increased OPG titers in SS patients compared to controls, suggests that the activation of RANK/RANKL/OPG pathway contributes to SS pathogenesis.

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Acknowledgement: Research Grant from the Greek Rheumatology Society and Professional Association of Rheumatologists

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.7099

AB0183 THE ROLE OF THE PHOSPHOLIPASE LP-PLA2 ACTIVITY IN SJOGREN'S SYNDROME RELATED LYMPHOMAGENESIS: A NEW SERUM BIOMARKER?

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Background: One of the major complications of primary Sjogren's syndrome (SS) is the development of B-cell non-Hodgkin's lymphoma (B-NHL). The contribution of tissue macrophages in the pathogenesis of B-NHL, based on previous histopathological studies, appears to be significant. Extracellular lipoprotein-associated phospholipase A2 (Lp-PLA2) is a product of tissue macrophages which is found in the circulation associated with lipoproteins and has been involved in both cardiovascular and malignant diseases, including B-NHL lymphoma.

Objectives: The purpose of the current study was to investigate the role of serum Lp-PLA2 activity as a potential biomarker for the development of B-NHL in the setting of primary SS.

Methods: Extracellular Lp-PLA2 activity was determined by measuring [³H]PAF degradation products in liquid scintillation counter in serum samples obtained from 48 primary SS patients, 9 primary SS patients complicated by lymphoma (SS-lymphoma) and 40 healthy controls. Similar results were obtained in an independent cohort of 25 primary SS patients, 17 primary SS-lymphoma and 10 healthy controls, when a commercially available ELISA kit was implemented. Statistical analysis was performed with the Graph Pad software.

Results: The activity of Lp-PLA2 was statistically significant increased in patients with primary SS-lymphoma compared to primary SS patients without lymphoma [mean±SD (nmol/min/ml): 62.0±13.4 vs 47.7±14.7, $p=0.003$ and 19.7±4.7 vs 15.2±3.2, $p=0.005$, respectively], as well as healthy controls [mean±SD (nmol/min/ml): 62.0±13.4 vs 52.0±16.3, $p=0.03$ and 19.7±4.7 vs 15.7±3.1, $p=0.06$, respectively]. No statistical significant differences in Lp-PLA2 activity were detected between primary SS patients without lymphoma development and healthy controls.

Conclusion: Evaluation of extracellular Lp-PLA2 activity in primary SS patients could be a useful serological biomarker for B-NHL development in the context of primary SS.

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Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.5758