AB0184

FLOW CYTOMETRIC IMMUNOPHENOTYPING OF SALIVARY GLANDS IN PRIMARY SJÖGREN'S SYNDROME

Paul Milne¹, Anastasia Resteu¹, Aleksandra Ivovic², Emmanuella Traianos³, David Storey³, Jessica Tarn³, Richard Siegel⁴, Peter Campbell², Wan Fai Ng³, Matthew Collin¹. ¹Newcastle University, Human Dendritic Cell Lab, Institute of Cellular Medicine, Newcastle upon Tyne, United Kingdom; ²Wellcome Sanger Institute, Cambridge, United Kingdom; ³Newcastle University, Musculoskeletal Research Group, Institute of Cellular Medicine, Newcastle upon Tyne, United Kingdom; ⁴National Institutes of Health, Autoimmunity Branch, Bethesda, United States of America

Background: Primary Sjögren's Syndrome (PSS) is a common autoimmune disease of unknown aetiology. It is characterised by inflammatory infiltration of exocrine glands, development of a sicca syndrome and a 20-fold increase in the risk of developing lymphoma. Standard pathological evaluation is based on a lymphocyte 'focus score' but little is known about the composition of the lymphoid infiltrate or its relationship to disease markers such as autoantibodies and the risk of lymphoma.

Objectives: The aim of the study was to use flow cytometry to characterise the lymphoid infiltrate in more detail.

Methods: Salivary glands were collected from 103 subjects attended the Newcastle Sjögren's clinic who had undergone minor salivary gland biopsy as part of the diagnostic investigations which also include testing for anti-SSA/SSB antibodies, Schirmer's tests and unstimulated oral salivary flow. 70 with confirmed PSS, 15 with potential or early-stage PSS and 18 with non-SS. Salivary glands were digested in collagenase for 3 hours and sort-analysed using a BD Biosciences FACSFusion flow cytometer. Sorted cells from 6 patients were Giemsa stained to observe cell morphology. All subjects have given their written informed consent according to the principles of Helsinki and the project has received local REC approval.

Results: Salivary glands contain multiple lymphoid populations including CD19+ B cells, CD19+CD38+ plasmablasts, CD19-CD38+ plasma cells, and predominantly central memory CD4+ and CD8+ T cells. The focus score is associated with an increase in the total number of lymphocytes of up to 10-fold. In particular there is a striking increase CD19-CD38+ cells with restricted kappa light chain expression associated with the most advanced cases. By morphology these cells have the appearance of plasma cells with frequent Russell bodies and occasional binucleated forms.

Conclusion: Flow cytometry of dispersed salivary gland demonstrates and association between lymphoid cells and the focus score in PSS patients. The dominant B cell population in PSS salivary gland is a CD19-negative tissue plasma cell with evidence of kappa light chain restriction in advanced disease.

Disclosure of Interests: Paul Milne: None declared, Anastasia Resteu: None declared, Aleksandra Ivovic: None declared, Emmanuella Traianos: None declared, David Storey: None declared, Jessica Tarn: None declared, Richard Siegel Employee of: Employee of Novartis since June 2018., Peter Campbell: None declared, Wan Fai Ng: None declared, Matthew Collin: None declared

DOI: 10.1136/annrheumdis-2019-eular.5510

AB0185

INTERFERON- γ AMPLIFIES IMMUNE RESPONSE MEDIATED BY TYPE I INTERFERONS IN PAEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS AND CORRELATES WITH DISEASE ACTIVITY

Gian Marco Moneta¹, Claudia Bracaglia¹, Ivan Caiello¹, Raffaele Pecoraro¹, Chiara Farroni², Fabio Basta¹, Luisa Bracci-Laudiero¹, Rita Carsetti², Fabrizio De Benedetti¹, Emiliano Marasco¹. ¹Bambino Gesù, Division of Rheumatology, Roma, Italy; ²Bambino Gesù, B Cell Physiopathology Unit, Roma, Italy

Background: Paediatric systemic lupus erythematosus (pSLE) is an autoimmune disorder of childhood characterized by the production of autoantibodies against nuclear antigens. In the last decade, several studies showed an up-regulation of genes induced by type I interferons (IFN α) in peripheral blood and tissues of pSLE patients². It has been reported that the expression of this group of genes, known as the type I IFN signature, correlates with disease activity². More recently, also the type II interferon (IFN γ) has been implicated in pSLE; however, its precise role has not been clarified yet³.

Objectives: To investigate the role of IFN γ in the pathogenesis of pSLE evaluating: 1) the expression levels of IFN γ -related genes in the peripheral blood of pSLE patients; 2) the possible cross-talk between IFN γ and type I IFNs.

Methods: Expression levels of IFN α -induced genes (IFI27, IFI44L, IFIT1, RSAD2, ISG15, SIGLEC1), IFN γ -induced genes (CXCL9, IDO1) and IFN γ itself were analysed by quantitative PCR (qPCR) in whole blood of pSLE patients and healthy donors (HDs). We developed a type II IFN score similarly to the type I IFN score described by Crow⁴. Peripheral blood mononuclear cells (PBMCs) from 6

HDs were stimulated *in vitro* with recombinant human IFN γ and IFN α 2b; gene expression was evaluated by qPCR. CXCL9 and CXCL10 in sera and supernatants from stimulated cells were measured by ELISA. Whole blood of pSLE patients and HD was incubated with an anti-IFN γ neutralizing antibody and iso-type control, and later type I and type II signatures were assessed. For each patient, SLEDAI was calculated.

Results: Expression levels of both IFN α -induced genes, IFN γ -induced genes and IFN γ were upregulated in pSLE patients with active disease (n = 21) compared to HDs and pSLE patients with inactive disease (n = 17). The type II IFN score correlated with the SLEDAI (r = 0.64, P < 0.01). As previously reported, also the type I IFN score correlated with SLEDAI (r = 0.67, P < 0.01). We also found increased serum levels of CXCL9 and CXCL10 in pSLE patients compared to HDs (P < 0.05).

In order to study a possible cross-talk between type I and type II IFNs, we stimulated *in vitro* HD PBMCs with recombinant IFN α 2b and IFN γ for 6 hours: we analysed the expression levels of type I and type II IFN signatures and assessed the production of CXCL9 and CXCL10 in the supernatants. IFN α 2b strongly up-regulated the expression of IFN γ , CXCL9 and IDO1. On the other hand, IFN γ induced the expression of the 6 IFN α -related genes. IFN γ , but not IFN α 2b, induced the release of CXCL9 in supernatants. Both IFN γ and IFN α 2b induced the production of CXCL10. IFN γ also up-regulated the expression levels of both TLR7 and TLR9, two potent inducer of IFN α . Finally, whole blood of pSLE was incubated with an anti-IFN γ neutralizing antibody for 24 hours: the type II signature was significantly downregulated (P < 0.05), whereas the type I signature was reduced without reaching statistical significance.

Conclusion: Our data suggest a potential role of IFN γ in the pathogenesis of pSLE. IFN γ -induced genes in whole blood and CXCL9 in serum are increased in pSLE patients. Type I and type II signatures are not strictly interferon-specific as IFN γ can induce the expression of type I genes. Moreover, blocking of IFN γ in the blood of pSLE patients reduces the type II signature confirming the presence of IFN γ in the blood of pSLE patients. IFN γ also induces the expression of TLR7 and TLR9, and IFN α induces the expression of IFN γ , thus establishing a positive crosstalk between IFN α and IFN γ that potentiate their reciprocal biological activity in pSLE.

REFERENCES

- [1] Petri M, et al. Lupus. 2009 Oct;18:980-9.
- [2] Munroe M, et al. Ann Rheum Dis 2016;75:2014-2021.
- [3] Rice GI, et al. Lancet Neurol 2013;12:1159-69.

Disclosure of Interests: Gian Marco Moneta: None declared, Claudia Bracaglia: None declared, Ivan Caiello: None declared, Raffaele Pecoraro: None declared, Chiara Farroni: None declared, Fabio Basta: None declared, Luisa Bracci-Laudiero: None declared, Rita Carsetti: None declared, Fabrizio De Benedetti Grant/research support from: Abbvie, SOBI, Novimmune, Roche, Novartis, Sanofi, Pfizer, Emiliano Marasco: None declared

DOI: 10.1136/annrheumdis-2019-eular.7613

AB0186

CORRELATIONS BETWEEN RENAL TRANSCRIPTOMIC PROFILES AND OUTCOMES IN A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

Pauline Montigny^{1,2}, Aurélie Degroof², Davide Brusa³, Frederic Houssiau⁴, Bernard Lauwerys^{2,4}. ¹Rheumatology Department – CHU UCL Namur, Yvoir, Belgium; ²Pôle de pathologies rhumatismales systémiques et inflammatoires – Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Bruxelles, Belgium; ³Flow Cytometry Platform, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Bruxelles, Belgium; ⁴Rheumatology Department, Cliniques Universitaires Saint Luc, Bruxelles, Belgium

Background: Factors contributing to poor renal outcomes in lupus nephritis (LN) are poorly understood. In our previous research, we found an association between the presence of activated immune effectors in the lupus kidney and disease severity. Here we investigated correlations between patterns of renal genes expression and biological parameters of renal disease activity in a mouse model of systemic lupus erythematosus (SLE).

Methods: We collected blood, urine, kidneys and spleens from 33 B6/Sle1. Sle2.Sle3 congenic mice, before disease onset at 3 months (n=8), and at different stages of disease progression, at 6 (n=7), 9-10 (n=12) and 17-18 (n=5) months. RNA was extracted from kidneys, and hybridized on Mouse Gene 2.0 ST exon arrays. Unsupervised hierarchical clustering studies and pathways analyses were performed on Genespring and DAVID softwares. Spleen and kidney CD8 T cell activation and differentiation markers were evaluated by flow cytometry (CD8, CD69, CD62L, CD44, CD122, CD25, CD44, CCR7, CD279, CD152, CD11a, CD103). Plasma urea and albuminuria were measured by immunoassay.

Results: 712 (out of 33,793) transcripts were overexpressed in kidneys from diseased mice (age > 6 months). These transcripts were significantly enriched in the following pathways: response to type I interferons, antigen processing and presentation, activation and regulation of B cells, complement activation, and regulation of cytotoxicity mediated by T cells.

Proportions of renal CD8 T cells with an effector phenotype were significantly increased in the kidneys from 6 months and older compared to younger mice, and compared to paired spleens. Proportions of renal effector CD8 T cells correlated significantly with transcripts involved in interferon signature, adaptive immune responses, cytotoxic T cells, chemotaxis, and correlated negatively with pathways associated with renal tubular cell functions.

Finally, we found a correlation between biological parameters of kidney function (plasma urea, albuminuria) and transcripts involved in pro-fibrotic pathways, chemokines and adaptive immune responses.

Conclusion: Our results confirm the link between the presence of activated immune effectors in the kidney and renal outcomes in a mouse model of SLE, similar to our previous observations in human LN, and warrant further functional studies on the role of kidney-infiltrating T and B cells in this model.

Acknowledgement: This research was funded in part by grants from Fonds de la Recherche Scientifique – FNRS, Fondation Roi Baudouin and Fondation Saint-Luc.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.5417

AB0187

CAN ARTIFICIAL INTELLIGENCE REPLACE MANUAL SEARCH FOR SYSTEMATIC LITERATURE REVIEW ON CUTANEOUS MANIFESTATIONS IN PRIMARY SJÖGREN'S SYNDROME?

Laure Orgeolet^{1,2}, Nathan Foulquier¹, Laurent Misery¹, Pascal Redou¹, Jacques-Olivier Pers¹, Valerie Devauchelle-Pensec¹, Alain Saraux¹. ¹CHU Brest, Brest, France; ²CHU Brest, Dermatology, Brest, France

Background: Manual systematic literature reviews are becoming increasingly challenging due to the sharp rise in publications. The task is particularly daunting when the study topic is complex.

Objectives: The primary objective of this literature review was to compare manual and in-house computer software retrieval of publications on the cutaneous manifestations of primary Sjogren's Syndrome (pSS). The secondary objective was to evaluate the prevalence of cutaneous manifestations in pSS.

Methods: We compared manual searching and searching with the in-house computer software BIBOT (1) designed for article retrieval and analysis. Both methods were used for a systematic literature review on a complex topic i.e., the cutaneous manifestations of pSS. Articles published in French or English between 1 January 1990 and 30 May 2018 were sought.

Results: The manual search retrieved 855 articles and BIBOT 1042 articles. In all, 202 articles were then selecting by applying exclusion criteria. Among them, 155 were retrieved by both methods, 33 by manual search only, and 14 by BIBOT only. Further selection was performed by reading the 202 articles, of which 54 were deemed relevant, including 23 providing data on the prevalence of one or more cutaneous signs in a cohort of patients with pSS. Cohort sizes and the nature and prevalence of cutaneous manifestations varied across publications. In all, 52 cutaneous manifestations were reported, of which the most common were cutaneous vasculitis (561 patients), xerosis (651 patients), and annular erythema (215 patients).

Conclusion: Agreement was good between the two methods. BIBOT was faster and automatically classified the articles in a chart. Combining the two methods retrieved the largest number of publications. The prevalence of cutaneous manifestations in patients with pSS varied considerably across studies. The advanced machine learning techniques used in artificial intelligence hold promise for literature reviews.

REFERENCES

- [1] Foulquier N, Redou P, Le Gal C, Rouvière B, Pers J-O, Saraux A. Pathogenesis-based treatments in primary Sjogren's syndrome using artificial intelligence and advanced machine learning techniques: a systematic literature review. *Hum Vaccin Immunother* 2018;14:2553-2558.

Disclosure of Interests: Laure Orgeolet: None declared, Nathan Foulquier: None declared, Laurent Misery: None declared, Pascal Redou: None declared, Jacques-Olivier Pers: None declared, Valerie Devauchelle-Pensec Grant/research support from: Roche-Chugai, Speakers bureau: MSD, BMS, UCB, Roche, Alain Saraux Consultant for: Roche SAS, Speakers bureau: Chugai Pharma France

DOI: 10.1136/annrheumdis-2019-eular.3981

AB0188

MOLECULAR NETWORKS IN MONOCYTES FROM SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS RELATED TO THEIR PHYSIOPATHOLOGY. MODULATORY EFFECTS OF ANTI-DSDNA ANTIBODIES AND MOLECULAR MECHANISMS UNDERLYING IN VIVOSTATIN TREATMENT

Alejandra M. Patiño-Trives¹, Pérez Sánchez Laura¹, María Galindo-Izquierdo², María Luque-Tévar¹, Alejandro Ibañez-Costa¹, Iván Arias de la Rosa¹, María del Carmen Abalos-Aguilera¹, Pedro Seguí Azpilcueta¹, Nuria Barbarroja Puerto¹, Eduardo Collantes Estevez¹, María A. Aguirre¹, Carlos Perez-Sanchez¹, Chary Lopez-Pedraza¹. ¹IMBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain; ²Hospital 12 de Octubre, Madrid, Spain

Objectives: 1. To characterize the mRNAs and microRNAs transcriptomes of monocytes from systemic lupus erythematosus (SLE) patients and their association with the pathophysiology of the disease. 2. To evaluate the role of anti-dsDNA antibodies in the regulation of these processes. 3. To investigate the molecular mechanisms involved in the efficacy of Fluvastatin in preventing the atherothrombotic risk.

Methods: Monocytes from peripheral blood of 81 SLE patients and 40 healthy donors (HD) were purified by negative immunomagnetic selection. Then, gene expression microarray (Agilent G4112F platform) and nCounter microRNA expression arrays (Nanostring) were performed. Functional categorization of altered genes and miRNAs was made using IPA software, and interaction networks were identified. Genes and miRNAs integrating the networks were validated in the whole cohorts by RT-PCR. Predicted miRNA-mRNA interactions were tested by microRNA over-expression or inhibition experiments. Serum and cellular inflammatory and oxidative profiles were evaluated by multiplex assay, PCR and specific commercial kits, respectively; phosphorylation status of intracellular proteins was analyzed by PathScan array. To evaluate the clinical significance of the parameters analyzed, correlation and association studies were performed. Mechanistic studies were developed to typify the specific effects of the anti-dsDNA antibodies on monocytes. Besides, the beneficial effects of ex vivo Fluvastatin treatment on the monocyte molecular profiles were assessed.

Results: Microarray identified 553 altered genes in SLE monocytes. Relevant biofunctions and disorders on which these genes were involved included inflammatory, immunological, cardiovascular, neurological, renal and reproductive disease. Analysis of miRNA profiles showed altered expression of 35 miRNAs in SLE monocytes. Sixty-one genes were inversely correlated and predicted as CVD-related target genes of 26 differentially expressed miRNAs. Transfection studies confirmed the relationship between specific miRNAs and their identified target genes.

Association of these genes and miRNAs with the anti-dsDNA positivity, early atherosclerosis and nephropathy, along with correlations with disease activity (SLE-DAI), activation of some intracellular signaling proteins, and levels of serum inflammatory and oxidative markers were demonstrated. *In vitro* studies demonstrated the specific modulation of several genes/miRNAs by anti-dsDNA, along with the increase of prothrombotic and proinflammatory mediators, the induction of apoptosis and the phosphorylation of intracellular proteins participating in renal and CVD-related signaling pathways. Besides, treatment of HD-monocytes with SLE patients' serum after Fluvastatin supplementation prevented the proinflammatory altered gene/miRNA profiles induced by serum from those patients before treatment.

Conclusion: 1. Gene and microRNA expression profiles allowed the identification of relevant genes and pathways altered in monocytes of SLE patients, associated with the pathogenesis of the disease, and modulated by anti-dsDNA. 2. Specific microRNA-miRNA regulatory networks control the biological processes and factors related to the CV pathology in SLE, which are prevented by Fluvastatin treatment.

Acknowledgement: Funded by ISCIII, PI15/01333, PI18/00837 and RIER RD16/0012/0015 co-funded with FEDER.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2019-eular.7142

AB0189

DECREASED URINE SEMAPHORIN 3A SECRETION PREDICTS THE EXTENT OF RENAL DAMAGE IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

Doron Rimar¹, Merav Lidar², Nasrin Eiza³, Elias Toubi³, Amal Silawy¹, Gleb Slobodin¹, Itzhak Rosner¹, Michael Rosenbaum¹, Abid Awisat¹, Nina Boulman¹, Lisa Kaly¹, Shira Ginsberg¹, Nizar Jiries¹, Zahava Vadasz³. ¹Bnai Zion Medical Center, Rheumatology, Haifa, Israel; ²Sheba Medical Center, Rheumatology, Ramat Gan, Israel; ³Bnai Zion Medical Center, Division of Allergy and Clinical Immunology, Haifa, Israel

Background: Semaphorins are a family of proteins, involved in axon-guidance, malignancy spread and angiogenesis. Semaphorin 3A (sema3A) is recognized also as "immune semaphorin", it is expressed on regulatory T cells and has been