AB0184 FLOW CYTOMETRIC IMMUNOPHENOTYPING OF SALIVARY GLANDS IN PRIMARY SJÖGREN'S SYNDROME

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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.

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AB0185 INTERFERON- γ AMPLIFIES IMMUNE RESPONSE MEDIATED BY TYPE I INTERFERONS IN PAEDIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS AND CORRELATES WITH DISEASE ACTIVITY

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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.

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AB0186 CORRELATIONS BETWEEN RENAL TRANSCRIPTOMIC PROFILES AND OUTCOMES IN A MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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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.