AB0153

FUNCTIONAL CHARACTERIZATION OF THE SJÖGREN'S SYNDROME-ASSOCIATED LOCUS DDX6-CXCR5

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Background: Sjögren's syndrome (SS) is a chronic, heterogeneous disease with hallmark features of auto-inflammation and autoantibody production. We previously identified association between the *DDX6-CXCR5* locus and SS surpassing genome-wide significance.

Objectives: This study aims to determine the mechanism by which this association contributes to disease.

Methods: Fine mapping and imputation allowed enrichment of existing genetic datasets from a total of 1916 SS cases and 3684 controls with 971 testable variants in the *DDX6-CXCR5* interval. Candidate variants were prioritized using statistical and bioinformatics approaches. Electromobility shift assays (EMSAs) and pull-downs (PDs) followed by mass spectrometry (MS) were used to determine allelic-specific differences in binding using lysates from HSB-2 (T), Jurkat (T), Reh (B), Ramos (B), Daudi (B), THP-1 (monocyte), and HEK 293T (epithelial) cells.

Results: Bioinformatic analysis of the top associated variants after imputation (rs7125066 and rs7119038) in the *DDX6-CXCR5* region did not yield evidence of regional functionality. However, 46 other candidates that span the region of association were identified through imputation. Chromatin methylation pattern data from the Roadmap database showed several variants in this region were within transcription start sites or enhancer elements depending on the cell type and state. Using RegulomeDB, Haploreg, and other databases, rs4938572, rs12365699, rs57494551, and rs10892294 showed strong evidence affecting binding and/or expression of one or more target genes in the region and were selected for further study. Using EMSAs, statistically significant increase in binding for the risk allele as compared to the non-risk allele was found for rs4938572 ($p < 0.01$) for cell lysates from HSB-2, Reh, THP-1, Ramos, Jurkat, and Daudi but not HEK 293T cells. While decreased binding of risk allele for rs10892294 and rs12365699 were found for Ramos cells only, rs57494551 showed decreased binding for both Ramos and THP-1 cell lysate. The risk allele of rs57494551 also decreases binding using THP-1 cell lysate. Preliminary MS analysis showed several immune related transcription factors likely binding this region, including: IRF8, GTF1, RFX5, IKZF3, PAX5, and IKZF1. Cell type-specific expression of the genes in the region shows the expression of *DDX6* spans many immune cells subsets while the *CXCR5* expression is more restricted.

Conclusions: The genetic variants in the *DDX6-CXCR5* locus that were selected based on bioinformatics data resulted in a difference in the direction of binding, with the risk allele all residing on the same risk haplotype. The change in binding of protein to each associated variant likely would alter expression of both protein coding genes in a cell/context specific manner. Ongoing studies will assess the regulatory role of these sequences using luciferase assays and CRISPR/Cas9 based genetic modification of target SNPs in various cell types.

Disclosure of Interest: None declared

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AB0154

NOTCH3 IS UPREGULATED IN LUPUS NEPHRITIS AND ITS DEFICIENCY ACCELERATES LUPUS PROGRESSION

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Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that affects the kidneys in about 50% of patients. Lupus nephritis (LN) is a major risk factor for overall morbidity and mortality in SLE patients. Notch signaling constitutes an evolutionarily conserved pathway that determines major decisions in cell proliferation, survival, and differentiation.

Objectives: To address the role of Notch-3 signalling in the disease development of SLE and particularly LN.

Methods: We measured renal expression of Notch-3 in kidneys of both, LN patients and lupus-prone MRL.lpr mice. Notch-3^{-/-} and B6. Fas^{lpr/lpr} mice were

crossbred to generate a B6. Fas^{lpr/lpr} Notch-3^{-/-} genotype and we analyzed the spontaneous disease manifestations in 40 week-old mice. Furthermore, we administered antisense oligodeoxynucleotides (ODNs) targeting Notch-3 or scrambled controls in the more aggressive lupus-prone mice strain (MRL. Fas^{lpr/lpr}) mice starting at week 12 for 3 weeks and analyzed these mice at the age of 16 weeks.

Results: In 20-week old MRL.lpr mice, receptor Notch-3 and its target gene *Hes2* were significantly up-regulated in kidneys in comparison to 8 and 12 week-old littermates. *De novo* renal Notch-3 expression in lupus mice and SLE patients was especially pronounced in the glomerular compartment. B6. Fas^{lpr/lpr} Notch-3^{-/-} mice exhibited an aggravated lupus phenotype with earlier mortality, increased lymph nodes/spleen size, enhanced glomerular collagen IV deposition and increased immune cell infiltration in the kidneys. In line with this, application of anti-sense Notch-3 ODNs in MRL.lpr mice at the time of the transition from clinically inapparent to a manifested disease state (starting at week 12), resulted in accelerated disease progression as evidenced by enhanced lymphadenopathy, splenomegaly and an increase of inflammatory markers.

Conclusions: Overall, these results demonstrate that Notch-3 exerts protective effects in the course of SLE, and its deficiency or inhibition accelerates the development of SLE manifestations.

Disclosure of Interest: None declared

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AB0155

1,25 DIHYDROXYVITAMIN D SUPPLEMENTATION REDUCES HIND PAW EDEMA IN PRISTANE-INDUCED LUPUS MODEL

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Background: Systemic lupus erythematosus (SLE) is a multifactorial, autoimmune inflammatory disease with pleomorphic clinical manifestations involving different organs and tissues. Vitamin D (VITD) deficiency is highly prevalent and evidence is mounting that it contributes to the morbidity and mortality in SLE. VITD is an essential steroid hormone with well-established effects on mineral metabolism, skeletal health, and more recently established profound effects on immune system health.

Objectives: To evaluate the development of SLE after VITD supplementation in pristane-induced lupus model.

Methods: Female BALB/c mice divided into 3 groups: healthy animals (CO), SLE animals (PIL) and SLE animals + vitamin D supplementation (PIL+VITD). PIL +VITD group received a subcutaneous injection of Calcijex® [2 µg/kg] in PBS-Tween 20 buffer every second day during 180 days. Animals were monitored every 2 months for body weight, free exploratory locomotion, grip strength, endurance exercise performance and edema size. Interleukin 2 (IL-2), IL-4, IL-6, IFN-γ and TNF-α were measured by Luminex technology. The histological and immunofluorescence parameters (IgG, IgM and C3) of the kidney and the joints are under analysis. Data was analyzed with ANOVA Two-Way followed by Bonferroni and independent sample t-test. $p < 0.05$ was considered significant. All data are represented as Mean±SEM.

Results: When compared with CO group (5.12±1.23 g), both PIL (7.53±1.09 g) and PIL+VIT D (8.08±2.28 g) groups exhibited a greater body weight gain by day 180 of the experiment ($p < 0.05$). There was no statistically significant difference in free exploratory locomotion and grip strength between the groups, but both the PIL (29.39±6.75 min) and the PIL+VITD (25.75±12.48 min) groups showed increased fatigue compared to the CO group (42.08±3.47 min). Still, VITD supplementation reduced mean paw swelling to compared PIL group (0.207±0.29 vs 0.24±0.051, $p < 0.05$).

Conclusions: The supplementation of VITD improves the clinical severity of PIL-manifestations, such as the reduction of hind paw edema. Further analysis of the histology of the joints and the kidney will complement the evaluation of the vitamin D modulating effects on the immune system.

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AB0156

POLYSACCHARIDES FROM DENDROBIUM OFFICINALE AMELIORATE THE DEVELOPMENT OF MURINE SJÖGREN'S SYNDROME VIA INDUCING IL-10-PRODUCING B CELLS

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Background: Primary Sjögren's syndrome (SS) is a chronic autoimmune disease with salivary gland (SG) hypofunction and inflammation. Currently, effective

therapies for SS patients are still lacking. Polysaccharides derived from *Dendrobium officinale* (DOP) have been shown to possess immunomodulatory functions in various animal models. However, it remains unclear whether DOP can modulate immune responses and have potential therapeutic effects on SS.

Objectives: To investigate the immune modulatory and therapeutic effects of DOP on experimental SS (ESS) in mice.

Methods: Polysaccharides were extracted from *Dendrobium officinale* with removal of endotoxin. C57BL/6 mice were immunized with SG-derived proteins for ESS induction, followed by daily oral treatment of vehicle and different dosages of DOP. SG functions were assessed by monitoring saliva flow rates whereas lymphocytic infiltration and tissue destruction in SG were examined by histological analysis. Both T and B cell populations in peripheral lymphoid organs were analyzed by flow cytometry. The effects of DOP on IL-10-producing B cell (B10 cell) induction and Th17 cell differentiation were examined in culture. Moreover, TLR4 and TLR9 inhibitors were used to identify the function of TLR4/TLR9 in DOP-mediated effects.

Results: ESS mice with DOP treatment displayed significantly higher saliva flow rates than vehicle-treated ESS mice. DOP treatment ameliorated SG tissue destruction accompanied with profoundly reduced lymphocytic infiltration in ESS mice when compared with vehicle-treated counterparts. Moreover, DOP treatment markedly increased the numbers of B10 cells but decreased numbers of Th17 cells in peripheral lymphoid organs of ESS mice. Notably, DOP markedly increased frequencies and numbers of B10 cells but did not affect Th17 cells generation in culture. Moreover, TLR4 inhibitor, but not TLR9 inhibitor, significantly suppressed DOP-induced IL-10 production in B cells.

Conclusions: The results show that DOP treatment ameliorates ESS disease development by inducing regulatory B10 cells, suggesting that DOP might be a promising candidate for treating SS.

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AB0157 TARGETING T-CELL TRAFFICKING IN A MURINE MODEL OF SJÖGREN'S SYNDROME

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Background: Salivary glands of primary Sjögren's syndrome (pSS) are characterised by complex leukocyte infiltration organised into tertiary lymphoid structures (TLS). The mechanisms regulating leukocyte trafficking into inflamed salivary glands are poorly described, but dysregulated T-cell recruitment during inflammation is believed to contribute to disease onset and chronicity. We recently described a homeostatic pathway in which a B cell-derived peptide (PEPITEM), secreted in response to adiponectin, regulates T-cell trafficking during inflammation via sphingosine-1-phosphate activity on endothelial cells¹. Disruption of this pathway by downregulation of adiponectin receptors on circulating B cells has been demonstrated in type-1 diabetes and rheumatoid arthritis, suggesting a potential role for PEPITEM in the pathogenesis of autoimmune diseases, and indicating a role for adiponectin receptors as biomarkers in these conditions¹.

Objectives: We aimed to investigate the efficacy of PEPITEM as an inhibitor of T-cell trafficking in an inducible animal model of salivary gland inflammation that mimics the histological features of pSS, and to investigate the potential translatability of this pathway in patients with pSS.

Methods: Submandibular salivary glands of C57BL/6 mice were intra-ductally cannulated with luciferase-encoding replication-deficient adenovirus to induce TLS formation as previously described². Mice were administered daily either PBS or PEPITEM by intraperitoneal injection from day 0, and their salivary glands dissected at day 5 post cannulation. T-cell infiltration into salivary glands was assessed using a combination of flow cytometry, immunofluorescence, and qRT-PCR for inflammatory chemokines.

Results: B cells in sera from cannulated animals express lower levels of both adiponectin receptors 1 and 2 in comparison with non-inflamed control mice. In cannulated animals treated with PEPITEM, histological analysis of salivary glands revealed fewer, as well as less aggregated, infiltrating T cells. Both CD4⁺ and CD8⁺ numbers were significantly lower in the salivary glands of PEPITEM-treated animals. Furthermore, administration of PEPITEM also decreased mRNA transcripts for lymphotoxin beta, IL-7, lymphoid chemokines (CCL19 and CXCL13)

and T-cell chemokine receptor CCR7, cytokines and chemokines known to regulate ectopic lymphopoiesis in pSS. Samples from pSS patients are currently being assessed to validate the relevance of this pathway in pSS.

Conclusions: These results demonstrate that administration of exogenous PEPITEM can reduce T-cell influx into salivary glands. This may represent a rescue of the homeostatic regulation of leukocyte trafficking which is disrupted in inflammation. Our work suggests that PEPITEM should be considered to address therapeutic needs in chronic inflammatory conditions, and that the detection of decreased levels of adiponectin receptors could serve as a biomarker in pSS.

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AB0158 HISTOLOGICAL AND SEROLOGICAL CHARACTERISTICS CONTRIBUTE TO DIVERGENT OUTCOMES FOR INDIGENOUS PATIENTS WITH LUPUS NEPHRITIS

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Background: Renal outcomes for Indigenous Australians (IA) with Lupus Nephritis (LN) are worse than for other ethnic groups.

Objectives: To investigate whether differences in renal biopsy findings can explain the worse renal outcome in Indigenous patients

Methods: A single centre cohort study of 83 SLE patients undergoing a first renal biopsy at our institution for LN evaluation. Histological assessment included review of ISN classification, A/C/C sub classification, NIH tubulointerstitial activity and chronicity indices, tubulointerstitial index, semiquantitative IF scores for IgG, IgM, IgA, C3 and C1q deposits, localisation of electron dense deposits (EDD) and presence of thrombosis, vasculitis and tubuloreticular inclusions (TRI). Differences in histological and routine clinical findings including autoantibody profiles between IA patients (n=11) and the pooled data from Asian (n=29) and Caucasian (n=43) patients were analysed by non-parametric statistical methods.

Results: IA patients were younger at diagnosis (31 vs 38.5 years., p=0.08) and their biopsies contained fewer glomeruli (11 vs 21, p<0.01), more class I/II (28 vs 15%) and no class V lesions (0 vs 21%) (p=0.06 for overall comparison). IA patients were less likely to have cellular crescents (0 vs. 25%, p=0.03), more likely to have fibrous crescents and a low tubulointerstitial index (p=0.08). The overall AI (5.1 vs 4.9) and CI scores (1.1 vs. 1.3) or presence of full house IF deposits (67 vs 71%), renal thrombosis (0 vs 4%) or TRI (55 vs. 39%) was similar across groups (all p>0.3). IA patients had lower eGFR (43 vs 65, p=0.025), more often carried anti-SSA52kd Ab (73 vs 33%, p=0.02) and during a mean follow-up of almost nine years, had a higher proportion of patients developing ESRD (18 vs 3%, p=0.02).

Conclusions: IA patients with SLE who develop LN have fewer glomeruli, an increased frequency of mesangial abnormalities with absence of cellular crescents and membranous nephropathy and a high prevalence of anti-SSA 52KD Ab. Although based on small numbers, this suggests that lower nephron mass and immunological pathways involving IFN-inducible anti-SSA expression may contribute to LN development and worse renal outcome in Indigenous patients.

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AB0159 CYTOKINE PRODUCTION BY ACTIVATED PLASMACYTOID DENDRITIC CELLS AND NK CELLS IS SUPPRESSED BY AN IRAK4 INHIBITOR

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Background: In SLE, immune complexes containing self-derived DNA or RNA (RNA-IC) trigger the synthesis of several pro-inflammatory cytokines by immune cells. Treatment with anti-malarials, such as hydroxychloroquine (HCQ), which