

Parallel Session 18: Autoimmunity and the environment

35 A DIET HIGH IN FIBRE DIET CAN MODERATE INFLAMMATION AND KIDNEY PATHOLOGY IN A MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background and Aims Systemic Lupus Erythematosus (SLE) is a complex, multifactorial autoimmune disease mediated by the deposition of immune complexes in tissues such as the kidney, skin and brain, with the ensuing inflammatory cascade driving progressive tissue damage and dysfunction. Mice lacking Lyn tyrosine kinase (*Lyn*^{-/-} mice) develop an autoimmune disease similar to SLE, driven by dysregulation of the immune system, immune complex deposition in tissue and systemic inflammation culminating in progressive glomerulonephritis. The gut microbiome has been shown to have an immunoregulatory effect on the development of autoimmune and inflammatory diseases, in large part due to the production of short chain fatty acids from the fermentation of dietary fibre.

Methods To determine whether dietary fibre could moderate systemic autoimmune and inflammatory pathology, *Lyn*^{-/-} mice and control C57BL6/J mice were fed a high fibre diet (HFD) or a standard control diet from weaning until 42 weeks old.

Results On the control diet, *Lyn*^{-/-} mice developed dysbiosis, lymphopenia, splenomegaly from enhanced splenic myelopoiesis, hyperactivation of immune cells, and pathogenic IgG anti-dsDNA autoantibodies that deposited in the kidney glomeruli leading to glomerulonephritis. These hallmarks of inflammation and autoimmune disease were significantly reduced in *Lyn*^{-/-} mice fed a HFD, indicating that dietary intervention is effective at dampening chronic systemic inflammation and glomerular pathology.

Conclusions These findings highlights the contribution of diet and the gut microbiome in regulating systemic immune responses and controlling autoimmunity, inflammation, and preventing the progression of immunopathology and suggests that fibre supplementation may improve outcomes for those living with SLE or other chronic systemic inflammatory diseases.

36 IDENTIFICATION OF DISEASE-ASSOCIATED GUT MICROBIOTA IN LUPUS-PRONE BAFF-TG MICE

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Background and Aims Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with environmental and genetic contributing factors. The gut microbiota (GM) interacts with the immune system to maintain homeostasis. However, microbiome dysbiosis has been shown to lead to the development of autoimmune diseases. We aimed to investigate the role of GM in SLE-prone BAFF-Tg mice and study the possible benefit of GM-targeted treatments.

Methods We used 16S metagenomics to compare the GM composition, before or after disease onset, and before or after treatment of established disease with several different fibre-enriched diets or antibiotics. Gut bacteria composition was identified by sequencing V3-V4 regions on an Illumina MiSeq platform in a 96-plex library configuration, and bioinformatics analysis was performed using QIIME software. Matching data on mouse disease levels was obtained by flow cytometry, auto-antibody ELISA, and kidney histology.

Results BAFF-Tg mice exhibited distinct GM compositions compared to WT, both before and after disease onset, with certain families of bacteria expanded or replaced prior to disease progression. GM-targeted therapy by high-fibre dietary modulation or antibiotics reduced anti-dsDNA autoantibodies to undetectable levels.

Conclusions GM dysbiosis, of some particular bacterial species we identified, can be linked to the level of disease development in this lupus-prone mouse model. Therapeutic strategies targeting GM, including easily implementable dietary modulations and antibiotics, could be investigated further as novel avenues for treating and managing SLE.

Free Communications 1 – Biomarker discovery

37 TOWARDS PATIENT STRATIFICATION IN SYSTEMIC LUPUS ERYTHEMATOSUS USING BASELINE AUTOANTIBODY SIGNATURES

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Background and aims Patients with systemic lupus erythematosus (SLE) have a wide spectrum of clinical manifestations and disease activity. This has made it extremely difficult to demonstrate superiority of novel treatments in clinical trials. Our goal is to establish an autoantibody classification system of SLE subgroups of which one represents a more homogeneous SLE population with active disease.

Methods We first established the global SLE autoantibody reactivity profile by comparing SLE serum samples with healthy controls. High-content profiling revealed an extended autoantibody repertoire with reactivity to cytokines, interferon (IFN) and IFN pathway proteins. Based on screens with >700 SLE samples, we designed the multiplex NavigAID SLE array consisting of 86 diagnostic and novel antigens.

Results Starting with 86 NavigAID SLE antigens we stratified SLE into five subgroups with reactivity towards distinct subsets of antigens. For example, patients with nephritis could be subclassified into two subsets according to the presence of anti-dsDNA or anti-neutrophil cytoplasmic antibodies (ANCA) revealing the heterogeneity of SLE. Reactivity to anti-IFN pathway proteins was associated with high disease activity, whereas patients with low disease activity had only few autoantibodies. We also found SLE patients who were tested positive for anti-nuclear autoantibodies (ANA), but did not exhibit the typical SLE reactivity profile. These patients may have been misclassified based on their positive ANA test result and maybe considered as potential outliers in clinical trials.

Conclusions Autoantibody profiling using 86 antigens provides an opportunity for identifying subgroups of patients with distinct marker profiles for designing clinical trials and evaluating clinical response in defined patient subgroups.

38

DISCOVERY AND EVALUATION OF A MULTIPLEXED MASS SPECTROMETRY PANEL FOR MEASURING CANDIDATE PEPTIDE BIOMARKERS IN URINE FROM PATIENTS WITH LUPUS NEPHRITIS

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Background and aims Lupus nephritis (LN) is a clinical manifestation of systemic lupus erythematosus (SLE) associated with significant morbidity and mortality. Although proteinuria is highly correlated with disease progression in LN, the composition of the LN urinary proteome remains poorly characterised. To address this issue, complementary mass spectrometry (MS)-based approaches were used to identify candidate urinary biomarkers and a targeted proteomics panel was developed to further assess levels in LN samples.

Methods LN urine samples were profiled using three MS-based methods: 2D SDS-PAGE, chemical labelling using isobaric mass tags, and data-independent acquisition (DIA). A quantitative, multiple reaction monitoring method was developed to further evaluate levels of these candidate peptide biomarkers in a larger cohort.

Results Using these discovery proteomic approaches >2600 proteins were identified, 290 of which are up-regulated in LN samples. While chemical labelling enabled identification of more total proteins, DIA outperformed chemical labelling in identification of proteins significantly up-regulated in LN samples. Further evaluation of a selected panel revealed increases in the majority of candidate peptide biomarkers in LN samples compared to healthy controls, including peptides from proteins involved in inflammation and adaptive immunity.

Conclusions These results indicate that peptides from proteins involved in inflammation and adaptive immunity can be quantified in urine of LN patients using a multiplexed MS-based method. Results from this study will be used to inform longitudinal and interventional studies focused on understanding the biological implications of these candidate biomarkers and to direct development of novel tools to evaluate disease progression and treatment efficacy of current and future LN therapeutics.

39

PERIPHERAL IMMUNOPHENOTYPING IDENTIFIES THREE SUBGROUPS BASED ON T CELL HETEROGENEITY IN LUPUS PATIENTS

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Background and aims To elucidate the diversity of systemic lupus erythematosus (SLE), we stratified SLE patients based on immunophenotyping.

Methods Peripheral blood mononuclear cells were obtained from 80 active SLE patients (with one or more BILAG category A, or two or more BILAG category B). Circulating B, T and dendritic cells were defined based on flow cytometric analysis for human immune system termed "the Human Immunology Project". Based on these results, the immunophenotype was visualised by principal component analysis and SLE patients classified into subgroups by cluster analysis.

Results Principal component analysis indicated that the immunophenotype of active SLE patients was consistent with T and B cell axes. Among these correlations, Th17 and Treg cells were statistically close, and showed positive correlation ($p < 0.001$). Furthermore, Tfh and Th1 cells were also statistically close, and showed positive correlation ($p = 0.04$). The same pattern was also noted between Tfh and plasmablasts ($p = 0.02$). Cluster analysis showed that SLE patients were divided into three subgroups (with high proportions of plasmablasts in all groups): patients did not show any characteristic features other than increased plasmablasts (T cell-independent group), patients with high percentage of Tfh cells (Tfh-dominant group), and patients with high proportions of activated Treg and memory Treg and low proportion of naïve Treg (Treg-dominant group).

Conclusions Our study indicates that SLE patients can be divided into three subgroups based on T cell heterogeneity. This heterogeneity should be taken into consideration not only in basic research but also in patient selection in clinical trials for development of new drugs.

40

ELUCIDATING GENETIC PATHWAYS IN SLE AND STRATIFYING PATIENTS VIA WHOLE GENOME SEQUENCING

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Background and aims Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease. Twin studies indicate a strong genetic contribution to lupus, yet often the pathogenic variant remains unknown. A better understanding of the individual genetic causes of SLE will enable personalised therapies. Using next generation sequencing technologies (WES/WGS) it is now possible to identify rare/novel gene variants that cause disease.

Methods We have used WES/WGS to identify rare genetic variants with strong effects that contribute to SLE and complex autoimmunity. The effect of variants on protein function were evaluated using *in vitro* biochemical and over-expression assays. Immunophenotyping of patient PBMCs and the use of bespoke mouse models engineered by CRISPR/Cas9 to harbour patient-specific variants were used to dissect disease mechanisms.

Results We identified a genetic variant in TREX1 as a cause of cerebral SLE, providing proof of principle that rare genetic variants do contribute to complex autoimmunity. The patient was revealed to be a prime candidate for tailored therapies