by gene ontology (GO) and pathway enrichment analysis that were highly enriched in SLE T cells included mediators of adaptive responses and inflammation, and those regulating co-stimulation. By contrast, negative regulators of cell proliferation and function were found in the healthy control cluster, and diminished in SLE.

Conclusions Our data demonstrate altered transcriptional programs of lupus Tfh and Tcm cells, and therapeutic targets in disease. They also represent the first detailed transcriptional profiling, and single cell transcriptional profiling, of Tfh cells, the necessary and critical driver of humoral immunity in SLE.

AI-23   **MULTIPLEXED MECHANISTIC ASSAYS FOR CHARACTERISING SLE**

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Background SLE is a complex disease with very few approved therapeutic options. Unique opportunities exist to characterise blood cells, tissues such as kidney and skin, urine, serum and plasma as part of ongoing longitudinal cohort studies such as Accelerating Medicines Partnership (AMP) and Autoimmunity Centres of Excellence (ACE), and investigator initiated or company-sponsored clinical trials.

Materials and methods SLE blood and tissue samples are being studied using a variety of single cell measurements as well as studies of biofluids. Several of these technologies are becoming firmly established in the SLE field, including single cell RNA Seq of blood and dissociated kidney using recently-developed methods in several consortia related to SLE and cancer; low input RNA-Seq of bulk purified cells; Assay of Transposase Accessible Chromatin (ATAC Seq); CyTOF and EpiCyTOF developed through the ACE consortium; transcript profiling using many methodologies; meta analysis of existing transcript profiling datasets; autoantibody profiling using autoantigen microarrays, and arrays composed of secreted factors such as cytokines and chemokines; multiplexed ion beam imaging (MIBI); and unpublished imaging methods such as CODEX.

Results An overview of multiplexed methods will be presented and will focus on efforts by the Stanford ACE and collaborating investigators to develop methods specifically for the study of SLE. Historical methods will be compared, and ACE datasets on human SLE, and mouse models of SLE characterised as part of ALR studies, will be described that demonstrate unique roles for interferons and STAT signalling in lupus.

Conclusions Big data analyses and multiplexed assays of samples derived from SLE patients, as well as patients with related autoimmune diseases, have tremendous potential and should be included in all clinical trials, with a goal to better understand pathogenesis and to identify novel therapeutic targets.

AI-24   **CALCIUM/CALMODULIN KINASE CONTROLS T AND RENAL CELL FUNCTION**

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Background Molecular abnormalities in SLE T cells account for their aberrant function including cytokine production, cytotoxic responses and help to B cells.

Materials and methods Use of biochemical, molecular biology and engineered mice; study of kidney tissues and isolated kidney cells.

Results Calcium calmodulin kinase IV (CaMK4) is expressed at high levels in T cells from patients with SLE and accounts for the decreased production of IL-2 and the increased production of il-17. The mechanisms involved modification of transcription factors and epigenetic changes. CaMK4 drives proliferation of mesangial cells in lupus prone mice and the production of IL-6. In parallel CaMK4 suppresses the expression of nephrin in podocytes resulting in proteinuria and also advances the expression of CD86 enabling thus podocytes to provide costimulation to passer-by T cells. Targeted delivery of a CaMK4 inhibitor to CD4 T cells reverses autoimmunity in lupus-prone mice.

Conclusions CaMK4 accounts for the abnormal production of cytokines by SLE T cells, the proliferation of mesangial cells and the poor function of podocytes. Targeting CaMK4 and targeted delivery of CaMK4 inhibitors to T cells has proven promising in preclinical studies.

AI-25   **GLUCOSE OXIDATION IN LUPUS T CELLS**

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Background Autoreactive CD4$^+$ T cells are essential participants in the pathogenesis of Systemic Lupus Erythematosus (SLE). Immune substrate utilisation, including glucose metabolism, plays a central role in dictating the effector functions of CD4$^+$ T cells. We hypothesised that 1) SLE T cells have metabolic defects that enhance their pro-inflammatory functions, and 2) Inhibiting glycolytic metabolism in CD4$^+$ T cell may normalise CD4$^+$ T cell functions and reduce disease symptoms in SLE mice and in CD4$^+$ T cells from SLE patients.

Materials and methods We utilised four models of spontaneous lupus, B6.NZM2410.Sle1.Sle2.Sle3 Triple Congenic (TC), BWF1, BXSB.YAA and B6.lpr that differ in their genetic background as well as mechanisms of autoimmune activation. C57BL/6 (B6) served as a non-autoimmune control strain. CD4$^+$ T cells obtained from lupus-prone mice and controls, as well as from SLE patients and healthy controls (HC) were treated with metabolic inhibitors, including metformin, which inhibits mitochondrial complex I and activates AMPK, and the glycolytic inhibitor 2-Deoxy-D-Glucose (2-DG). Lupus-prone mice were treated with these drugs, either before or after disease onset. Glycolysis, oxygen consumption, activation and effector subset distribution were measured in CD4$^+$ T cells. Disease progression was assessed by measuring standard lupus biomarkers. Gene profiling was performed on CD4$^+$ T cells from SLE patients and HCs.

Results CD4 T cells from lupus mice and patients have a significantly higher metabolism as well as an enhanced mTOR activity

as compared to controls. A combination of inhibitors and gene expression studies showed that glucose oxidation rather than aerobic glycolysis is a major metabolic pathway of lupus T cells. *In vitro*, both metformin and 2-DG blocked IFNγ production and metformin increased IL-2 production. *In vivo*, a combined treatment with metformin and 2-DG normalised T cell metabolism and reversed disease phenotypes in all four lupus mouse models. Remarkably, the number of T_{FH} cells, which correlates with disease severity in patients, was normalised by the combination treatment in every model. Further, excessive IFNγ production by CD4 T cells from SLE patients was also normalised by metformin. Finally, $CD4^+$ T cells from lupus patients showed a metabolic signature with over-expression of genes promoting glucose utilisation, mitochondrial oxidative phosphorylation and sterol synthesis.

Conclusions The combination of a glucose inhibitor with metformin restores T cell function and reverses disease across diverse mouse models of SLE, and metformin treatment normalises the function of T cells from SLE patients. We propose that T cell metabolism may serve as a biomarker of disease activity and provides a novel target for immune intervention in SLE.

AI-26 | **INTESTINAL IGA AS PATHFINDERS TO IDENTIFY MICROBIOME PATHOBIONT CANDIDATES IN SLE**

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Background SLE is an archetypical systemic autoimmune disease, which has been attributed to interactions between genetic and environmental factors that are currently not well understood. Yet recent reports have begun to elucidate how intestinal bacteria influence the development of physiologic B-cell/T-cell responses, and can affect the pathogenesis of inflammatory and autoimmune conditions. Our studies are designed to shed light on the potential roles of the gut microbiome in SLE pathogenesis.

Methods We have assembled and characterised a cohort of 60 female SLE patients and 20 healthy controls. DNA from the unfractionated bacteria in faecal samples, and from the sorted endogenous IgA-coated and non-coated bacterial fractions, was then extracted. 16 S bacterial rRNA genes were then barcoded and amplified, and over 20,000 reads were determined per sample using illumina NGS technology. Faecal and serum total Ig and autoantibodies were measured by ELISA.

Results Our analysis showed less microbiome diversity in SLE than healthy controls ($p = 0.002$). This dysbiosis was treatment independent, with more severe intestinal dysbiosis and decreased bacterial diversity in patients with high disease activity, based on SLEDAI. In addition, SLE patients had increased representation of certain bacterial families, genus's and species, based on 16 S rRNA assignments of operational taxonomic units (OTUs). Patients with active disease displayed contractions of bacterial taxa with reported protective properties and reciprocal expansions of taxa with putative pathobiont properties. We also assessed IgA, which is the most prevalent antibody isotype made by the human body, and found evidence of exuberant levels in both intestinal and blood samples of SLE patients. While only a minority of bacterial taxa are specifically coated by endogenous intestinal IgA, IgA-coated bacteria in SLE patients had differential representation with recurrent taxa-specific expansion in SLE patients. Strikingly, *Prevotella copri*, which has recently been linked to new-onset RA, was significantly over-represented among the IgA-coated taxa only in SLE patients with high disease activity, and was not detected in healthy controls.

Conclusion Our studies provide the first evidence that SLE is associated with gut microbiome dysbiosis with expansions of specific bacterial taxa that may contribute to immune dysregulation. This imbalance was more significant in patients with high disease activity Characterisation of in vivo IgA-coated bacteria demonstrated that certain microbes taxa/species are preferentially recognised by the adaptive immune system of SLE patients, and these differ significantly from healthy adults. We are now studying these candidate pathobionts in longitudinal studies to address whether the microbiome predicts and/or tracks flares.

AI-27 | **BACTERIAL AMYLOIDS FROM BIOFILMS BREAK TOLERANCE IN LUPUS**

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Background Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease, in which infections are considered to play a pathogenic role, but the cellular and molecular culprits remain unknown. Bacterial biofilms are multicellular bacterial communities important in the establishment of chronic infection by pathogens. Bacteria produce amyloids, complex proteins with a conserved beta sheet structure, that strengthen the extracellular matrix of their biofilms. We have reported that bacterial and eukaryotic DNA is incorporated into curli fibres, functional bacterial amyloids present in *Salmonella* and *E. coli* biofilms. We found that curli/DNA complexes from biofilms activate conventional dendritic cells leading to production of pro-inflammatory cytokines, including Type I interferons, *in vitro* and *in vivo*, indicating that curli are a new class of danger signals. I.p. infections with curli-expressing *S.* Typhimurium or E. Coli Nissle, and also systemic administration of curli/DNA complexes purified from *Salmonella* biofilms accelerated onset in lupus-prone NZBxW/F1 mice and triggered autoantibodies production in non-predisposed mice, suggesting curli/DNA complexes as novel players in SLE pathogenesis.

Materials and methods In order to determine the cellular and molecular players in the acceleration of lupus by curli-expressing infections, bone marrow-derived cultures of conventional dendritic cells and macrophages were generated *in vitro* and B cells were isolated *ex vivo* from NZBxW/F1 lupus-prone mice, TLR-deficient mice and wild-type mice and stimulated with curli/DNA complexes purified from *Salmonella* biofilms. Moreover, lupus prone and control mice were infected i.p. and by oral gavage with curli-expressing or curli-deficient *S.* Typhimurium and the effects on dendritic cells, macrophages, and B cell activation, and production of autoantibodies were studied. A novel ELISA was developed to measure anti-curli antibodies in SLE patients and infected mice.

Results We found that Curli and DNA synergistically activate innate and adaptive immune cells, *in vitro* and *in vivo*, via TLR2 and TLR9, leading to a Type I interferon response. We found that curli/DNA complexes can directly stimulate proliferation and