Background Genetic and epigenetic mechanisms that may contribute to lupus susceptibility in humans and mouse models are of interest especially if they provide enzymes that could function as potential therapeutic targets. We have discovered an enzymatically mediated *O*-acetylation event found prominently in B cells in humans with lupus and in MRL/+ mice. Detailed studies in MRL/+ mice indicate that this modification may allow for a break in B cell tolerance and may be a major component of lupus susceptibility in these mice as well as possibly in humans as well.
Materials and methods We have used a catalytically dead Influenza C hemagglutinin esterase Ig fusion protein and a bovine coronavirus hemagglutinin esterase – Ig fusion protein as tools to respectively identify and remove 9-*O*-acetylated silaic acid on B cells in humans and in MRL/+ mice. Enzymatic approaches were used to identify the type of glycoconjugate that exhibits enhanced 9-*O*-acetylation of sialic acid moieties. We created a CasD1 knockout mouse to examine the role of this enzyme in *O*-acetylating sialic acid moieties in vivo. Genetics, whole genome sequencing and RNA-seq approaches are being used to identify the mechanism underlying ths lateration and its link to lupus susceptibility.
Results Increased 9-*O*-acetylation of sialic acid on naive B cells is observed on approximately two-thirds of subjects with active SLE. Markedly increased levels of 9-*O*-acetyl sialic acid are also observed in the earliest B lineage cells in lupus prone MRL/+ mice and this high level is maintained throughout B cell development and well before these mice exhibit any features of disease. This increased 9-*O*-acetylation of sialic acid was not observed on glycoproteins or mucins on MRL/+ B cells but was restricted to gangliosides. Acetylated gangliosides protected these B cells from BCR-dependent apoptosis. Deacetylation of sialic acid on MRL/+ B cells restored anti-IgM mediated apoptosis to wild type levels. We used a *CasD1* knockout mouse to establish that this enzyme is required for the 9-*O*-acetylation of sialic acid in vivo. Increased 9-*O*-acetylation of sialic acid in MRL/+ mice is dominantly inherited and the molecular basis of this striking change is being investigated using genetics and whole genome sequencing.
Conclusions Enhanced 9-*O*-acetylation of sialic acid on B cells in lupus prone mice and in humans may represent a potential mechanism by which B cell tolerance is abrogated in lupus subjects and in lupus-prone mice. The CasD1 acetyltransferase may be a therapeutic target of relevance in lupus.
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AI-21 DEFECTIVE IKAPPABNS LEADS TO LOSS OF PERITONEAL B1 CELLS AND A REDUCTION IN INDUCED BUT NOT SPONTANEOUS AUTOIMMUNE HEMOLYTIC ANAEMIA

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Background The role of the B1a subset in cells in autoimmunity remains controversial. Here we identify a spontaneous mutation in IkappaBNS associated with severe reduction in the peritoneal B1a, but not other B cell subsets and use this mutation to study its contribution to autoimmunity.
Materials and methods NZB, NZB.NZW-*Lbw2*, B6-*Nfkbid*^{bumble} (IkappaBNS-deficient mice), and crosses were bred and maintained at TSRI and experiments approved by Scripps IACUC. Flow cytometry, mapping, sequencing, Ig and autoantibody ELISA, and direct Coomb's test were by standard procedures. For in vivo poly(I:C) stimulation, 200 ug were given i.p. to 6–10 wk-old mice 2x/wk for 8 wks. B cells were stimulated in vitro with 30 ug/ml goat F(ab')$_2$ anti-mouse IgM in RPMI 10% FCS.
Results A chromosome 4 NZB subcongenic line, NZB.NZW-*Lbw2SE*, was discovered to exhibit very low B-1a cells in the peritoneal cavity and reduced serum IgM, but with no detectable effect on B cells in other lymphoid compartments including the MZ subset in the spleen. Mapping and a complementation study with *Nfkbid* (IkappaBNS gene)-deficient mice, identified a spontaneous hypomorphic K100N mutation of IkappaBNS, a member of the nuclear IkappaB family that serves as modulators of NF-kB function. Notably, in contrast to complete deletion of IkappaBNS, which affects multiple immune cell types, the phenotype of the NZB-SE mutation, named *lowb1*, was limited to peritoneal B-1a cells. The absence of low B-1a cells did not reduce susceptibility to spontaneous autoimmune hemolytic anaemia. However, *lowb1* mice were resistant to poly(I:C)-induced autoimmune hemolytic anaemia indicating that B-1a cells could play a role in modulating environmental factors.
Conclusions These studies suggest a limited role for B1a cells in autoimmune hemolytic anaemia and identify the nuclear Ikappa family as a modulator of autoimmunity.

AI-22 SINGLE CELL PROTEIN AND TRANSCRIPTIONAL PROFILING OF CD4+ FOLLICULAR B HELPER T (TFH) AND CENTRAL MEMORY (TCM) CELLS IN SLE: PHYSIOLOGICAL AND PATHOLOGICAL PHENOTYPES

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Background Follicular B helper T (Tfh) cells expand in the circulation of patients with systemic lupus erythematosus and are correlated with pathological outcomes (Choi, *et al., Arthritis Rheum.* 2015). These cells are both necessary and sufficient to drive pathogenic humoral autoimmunity in murine lupus, and likely in SLE.
Materials and methods We performed multidimensional single cell secretome profiling on Tfh cells from patients with SLE and controls, in parallel with transcriptome (RNA-seq) analysis (including single cell) of Tfh and central memory (Tcm) cells.
Results We find that single Tfh and Tcm cells in SLE are polyfunctional cytokine producers, for example, co-secreting both B-helper IL-21 and inflammation-inducing IFN-γ in excess of that seen in controls. Given their pathogenic potential in lymphoid organs and in disease-affected end organs, such as the lupus kidney, and to dissect the mechanisms underlying the aberrant secretome phenotypes, we performed transcriptome (RNA-seq) analysis (including single cell) of Tfh and central memory (Tcm) cells from SLE patients and healthy donors. Principal component analysis (PCA) of single-cell transcriptomes was done using the top 2000 differentially expressed genes based on ANOVA-testing, revealing four distinct clusters of Tfh and Tcm cells, separating cells from healthy donors and SLE patients. Pathways determined

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.

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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.

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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