Conclusions Our study demonstrates that B cell-intrinsic IFN-γ receptor signals promote lupus pathogenesis via formation of spontaneous, autoimmune GCs. In addition, we have uncovered a novel cell-intrinsic program whereby IFN-γ, together with BCR-, TLR- and/or CD40 signals, orchestrates B cell expression of the GC master transcription regulator BCL-6. Our combined findings suggest that this IFN-γ signalling program may be a potential therapeutic target in SLE.

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AI-19 METABOLIC INHIBITION BY 2-DEOXYGLUCOSE PREVENTS AND REVERSES LUPUS IN MICE

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Background Glucose is a primary substrate for cellular respiration. Glucose utilisation increases in highly metabolic cells including activated, proliferating T cells and B cells as well as cancers. Lupus is a disorder in which autoreactive CD4+ T cell and B cells that deviate from normal homeostasis by their uncontrolled proliferation and differentiation to effector cells. Therapeutic limitation of glycolysis is therefore an attractive approach for attenuating the highly energetic, pathogenic processes inherent to lupus. Here we investigate the potential of several metabolic inhibitors that target early and downstream aspects of cellular respiration to identify inhibitors that show potential in the prevention and treatment of lupus.

Materials and methods Metabolic inhibitors included: 1) a classic glycolysis inhibitor, 2 deoxyglucose (2 DG); 2) a mitochondrial complex I inhibitor/AMPK activator metformin (MET); 3) an mTOR inhibitor, rapamicin (RAPA); and 3) a pyruvate dehydrogenase kinase inhibitor, dichloroacetate (DCA). The drugs were provided in drinking water or mouse chow for 4–8 wks. NZB X NZW F1 (BWF1) and BXSB.*Yaa* mouse models of lupus were evaluated in prevention studies and in treatment of mice documented to be undergoing autoimmune disease. Longitudinal and terminal immunophenotyping was performed using flow cytometric, serological, histopathological analyses.

Results 2 DG, MET, DCA and RAPA, and combinations thereof were applied prior to the onset of autoimmune disease to BWF1 and BXSB. *Yaa* mice. MET and DCA showed minimal effects and RAPA resulted in partial attenuation. In contrast, 2 DG acted potently to abrogate multiple disease biomarkers while not causing immunodeficiency. Given the strong immunologically normalising effects of 2 DG in disease prevention, we performed therapeutic interventions in which 2 DG was supplied for 8 weeks to already diseased BWF1 and BXSB.*Yaa* mice. Within 4 weeks of treatment, 2 DG normalised all cellular, serological and pathological features characteristic of the BWF1 and BXSB. *Yaa* lupus like syndromes. Furthermore, the lifespans of BXSB. *Yaa* mice were extended after withdrawal of treatment (Figure 1).

Conclusions Overall, the results highlight the potent and remarkable normalising effect of 2 DG in the prevention and treatment lupus-like autoimmune disease in mouse models with differing genetic and mechanistic etiologies. Given findings, we propose that that therapeutic inhibition of early steps in glycolysis, as exemplified by 2 DG, has broad potential for the treatment of multiple autoimmune disorders. Our current efforts are focused on: 1) the potential of 2 DG in treatment of other autoimmune severe diseases; and 2) evaluation of potential downsides of metabolic inhibition by 2 DG and other inhibitors of glycolysis.

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AI-20 DEFECTIVE BCR INDUCED APOPTOSIS LINKED TO ELEVATED LEVELS OF 9-*O*-ACETYLATED SIALYL GANGLIOSIDES ON B CELLS IN LUPUS PROVIDES A POTENTIAL THERAPEUTIC TARGET FOR LUPUS

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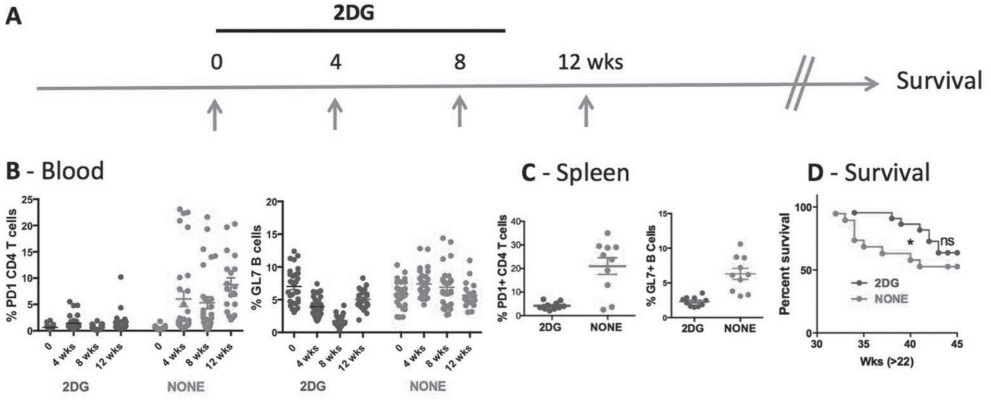

Abstract AI19 Figure 1 Exemplary data showing that treatment with 2DG reverses ongoing autoimmune disease of BXSB.*Yaa* mice. (**A**) Schematic of the therapeutic approach. (**B**) BXSB.*Yaa* mice were aged to 12 wks. FACS analysis of blood CD4+ T cells and B cells 0, 4, 8 of treatment and 12 wks (4 wks after treatment was withdrawn). (**C**) Analysis of CD4+ splenic CD4+ T cells and B cells 8 wks after treatment. (**D**) Survival of mice after withdrawal of treatment*. P ≤0.05.

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