and extended the lifespan of many to beyond 80 wks of age (figure 1B and 1C). Safety assessments in C57BL6/J mice after 8 wks of treatment did not show any significant cardiac, behavioral, immunological and histopathological abnormalities. Alterations in serum chemistry analytes, including calcium and magnesium, but not markers of ketosis, were evident shortly after and persisted after 2DG exposure.

Conclusions The results highlight the potent and remarkable normalizing effect of intermittent 2DG therapy in the prevention and treatment lupus-like autoimmune disease with differing genetic and mechanistic etiologies and in its most precipitous forms. This mode of therapy did not incur untoward side effects. We thus propose that therapeutic inhibition of early steps in glycolysis by 2DG has broad potential for the treatment of lupus as well as related autoimmune disorders.

A. Treatments

B. Urine protein

C. Survival

Abstract AI-03 Figure 1 Intermittent 2DG treatment reverses ongoing autoimmune disease of aged BWF1 mice. Twenty-nine BWF1 female mice aged to 37 wks were randomized into two cohorts and monitored to >80 wks of age. (A) one cohort was treated with 2DG for 8 wks starting at 37 wks. All surviving mice were then treated at 53 wk for 8 wks with 2DG and again treated at 67–70 wks. (B) serial urine proteins. (C) overall survival.

anti-CD19 CAR T cell in eliminating B cells and disease development in a spontaneous mouse model of lupus.

Methods In these experiments, a modulatable CAR T cell system was used consisting of CAR T cells recognizing an antigenic site on a soluble anti-CD19 Fab ‘switch.’ Thus, activation and killing activity of CAR T cells was dependent on the presence of an independently injected anti-CD19 switch. To study CAR T cell efficacy, lupus-prone male BXSB mice at 3 months of age were initially conditioned with cyclophosphamide i.p., then 24 hour later given CAR T cells and either the anti-CD19 switch or PBS alone every other day (3 mice/group). Mice were followed for mortality, autoantibodies, proteinuria, and peripheral blood and spleen cells populations up to 9 weeks after CAR T cell transfer. Immunoglobulins and autoantibodies in sera were measured by ELISA. Flow cytometry was used to analyze immune cell populations and included a FITC-conjugated switch peptide to identify CAR-expressing T cells. Proteinuria was determined by dipstick and kidney sections were PAS-stained and scored for glomerulonephritis on a 0–4 scale.

Results An initial experiment documented that a single i.p. injection of 100 mg/kg of cyclophosphamide at 3 months of age did not reduce the development of lupus in BXSB male mice compared to PBS controls (4 and 3 mice/group). When mice treated with conditioning and CAR T cells were analyzed, the group given anti-CD19 switch but not PBS, had low levels of circulating B cells by one week after CART T cell transfer and for the duration of the experiment. Immunoglobulin and autoantibody levels were present in the PBS group, but undetectable in the anti-CD19 switch group at the end of the 9 week study. All PBS-, but none of the anti-CD19 switch-treated mice group developed severe glomerulonephritis (glomerulonephritis scores: 3.1 ± 0.07 vs 0.43 ± 0.23 , $p < 0.001$).

Conclusions In a small study, anti-CD19 CAR T cell treatment of lupus was highly effective in preventing the development of severe lupus glomerulonephritis. Strikingly, at 9 weeks after transfer, there was complete deficiency of circulating immunoglobulins suggesting that long-lived plasma cells either express sufficient levels of CD19 to be targeted by CAR T cells or less likely that the plasma cell population in lupus requires replenishment from newly generated B cells. These findings support the possibility of using the switchable CAR T cell approach to define the role of immune cell subsets in lupus and treatment of severe lupus.

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AI-04

IMMUNOTHERAPY FOR LUPUS IN A MOUSE MODEL TO DEFINE PATHOGENESIS AND THERAPEUTIC TARGETING

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Background Recent advances in immunotherapy using genetically modified chimeric antigen receptor (CAR) T cells have made it possible to selectively and completely eliminate cells expressing specific cell surface targets. Such a system could potentially be applied to lupus and other autoimmune diseases for identifying new targets in model systems and for therapy. To test the possible utility of such an approach, we therefore conducted a preliminary study to determine the efficacy of

AI-05

PLATELET RESPONSE TO IMMUNE COMPLEXES

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Background There is a growing appreciation for the contribution of platelets to immunity; however, our knowledge mostly relies on platelet functions associated with vascular injury and the prevention of bleeding. Circulating immune complexes (ICs) contribute to both acute and chronic inflammation in a multitude of clinical conditions through their interaction with

members of the Fcγ receptor (FcγR) family. FcγRIIA is the most abundantly expressed FcγR in human blood circulation. However, mice do not express FcγRIIA, and murine platelets are completely devoid of FcγRs.

Methods To model IC-mediated response more accurately, as they occur in humans, transgenic FcγRIIA (FcγRIIA^{TGN}) mice expressing FcγRIIA on platelets and certain leukocytes, were used in this study. As acute model, we intravenously injected ICs in FcγRIIA^{null} and FcγRIIA^{TGN} mice and monitored mouse reaction and platelet activation. To model platelet response to chronic exposure to ICs, we backcrossed NZB mice with FcγRIIA^{TGN} mice, generating NZB::FcγRIIA^{TGN} mice and crossed the mice with NZW mice, thus generating NZB::NZW::FcγRIIA^{TGN} and NZB::NZW::FcγRIIA^{null} mice. Platelet activation was monitored through time in these mice.

Results Platelet activation by acute exposure to ICs through a mechanism requiring expression of platelet FcγRIIA resulted in the induction of systemic shock. IC-driven shock was dependent on release of serotonin from platelet dense granules secondary to platelet outside-in signaling by αIIbβ3 and its ligand fibrinogen. On FcγRIIA activation, platelets underwent sequestration, but surprisingly they returned in the blood circulation with emptied granules after activation. Strikingly, reminiscent observations were made in lupus NZB::NZW::FcγRIIA^{TGN} mice. We found significant platelet activation and circulating degranulated platelets, uniquely in mice expressing FcγRIIA.

Conclusions Platelet activation in IC-mediated pathogenesis is well recognized. In lupus patients, platelets are found activated in blood circulation, however, to what extend ICs and FcγRIIA contribute to platelet activation was unknown. Here, we showed that the expression of FcγRIIA is critical to adequately examine platelet role in lupus.

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AI-06 LUPUS NEPHRITIS IS LINKED TO DYSBIOSIS, INCREASED GUT LEAKINESS AND IMMUNITY TO AN INTESTINAL COMMENSAL LACHNOSPIRACAEA SPECIES

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Background A transmissible agent has long been suspected in the pathogenesis of SLE, yet the potential contribution of the human intestinal microbiome has been little examined. We therefore characterized the gut microbiota of patients with SLE, with special interest in those with lupus nephritis (LN).

Methods Blood and fecal samples from SLE patients were obtained, with strict inclusion/exclusion of criteria. Fecal 16S rDNA sequencing, as well as cytokine and autoantibody assays were performed. In addition, sera from two independent lupus cohorts were studied for validation. Biomarkers of gut leakiness were assessed.

Results Compared to controls, the intestinal microbiome from SLE patients (n=61) showed decreased species richness diversity with reductions in taxonomic complexity most

pronounced in those with high disease activity. Notably, SLE patients had an overall 5-fold greater representation of a species in the Lachnospiraceae family of obligate anaerobic Gram-positive cocci, with reciprocal contractions of two other commensal species with putative protective properties. Abundance of the Lachnospiraceae species correlated with serum IgG to a cell wall component, postulated to represent a lipoglycan, from a strain of this same species (p=0.002, n=61, Spearman) but not with 7 other strains. There was also a significant direct correlation between SLEDAI scores and levels of these circulating anti-strain IgG antibodies (p=0.02, n=48). Levels of antibodies to strain-specific bacterial antigen, treated with RNase/DNase/proteinase K, were significantly higher in those with active nephritis at time of sampling compared to SLE without renal activity (Cohort 1 p=0.01 n=48; Cohort 2 p=0.006, n=28, Mann-Whitney). Levels of serum IgG anti-strain antibodies also significantly correlated with high-titer serum IgG to native DNA (p<0.0001, n=27), and inversely correlated with C3 and C4 levels. High titers of these anti-bacterial antibodies were associated with active Class III, IV and V (overlap) LN (Cohort 3).

Conclusions These findings suggest a novel paradigm for the pathogenesis of LN in which a common intestinal commensal bacteria may contribute to the immune-complex mediated disease process, with features akin to poststreptococcal GN but without outward signs and symptoms of clinical infection.

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AI-07 A NEW B CELL EFFECTOR PATHWAY WITH DEFECTIVE NEGATIVE REGULATION OF TLR7 SIGNALING IN HUMAN SLE

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Background B cell homeostasis is perturbed in SLE patients. In particular, many patients have a large expansion of IgD-CD27- B cells (DN). The DN population is heterogeneous for CXCR5 expression, and CXCR5- DN2 are the majority population in SLE patients but not in HCD (figure 1A). To further understand how these expanded cells differ from other B cell subsets and how they may be dysregulated in SLE, we phenotypically and functionally characterized DN2 in SLE patients and healthy control donors (HCD).

Methods B cells subsets were quantified by flow cytometry in HCD and two separate cohorts of lupus patients. Purified DN2 and other B cell subsets were flow sorted and transcriptionally analyzed using RNA sequencing. Toll-like-receptor 7 (TLR7) signaling after stimulation with R848 was measured by staining with anti-phospho-tyrosine specific anti-ERK. Antibody secreting cell differentiation was induced using *in vitro* stimulation of sorted B cell subsets with a combination of TLR7 and cytokines.

Results DN2 were only a minor B cell subset in HCD (less than 5%) but were elevated in 20% of cohort 1 patients and 60% of cohort 2 (figure 1B). In the patients with the largest