

Adaptive Immunity

AI-01 KV1.3 EXPRESSION ON SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) URINARY LEUKOCYTES

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Background Effector memory T lymphocytes (T_{EM}) implicated in the immunopathogenesis of SLE have been detected in the urine of patients with lupus nephritis. As T_{EM} depend on the voltage-gated potassium channel Kv1.3 for activation-induced calcium signalling, activated T_{EM} represents a potential target for the novel Kv1.3 blockade therapy dalazatide. In active SLE nephritis, Kv1.3 is upregulated on peripheral blood CD8 + T_{EM} and cytokine production was inhibited in SLE T lymphocytes. We therefore hypothesized that Kv1.3 is expressed on urinary leukocytes from patients with lupus nephritis.

Methods Urinary cells were isolated in 33 samples from patients with SLE ages 15–41 years, (mean 19, IQR 16.2–19.3). Immunofluorescence was performed to quantify and characterize cells expressing Kv1.3. Urinary leukocytes were defined as non-epithelioid cells by morphology. Leukocyte subsets were defined as CD3+ T lymphocytes, CD20+ B lymphocytes, or CD14+ macrophage.

Results In the urine, leukocytes expressing Kv1.3 were found in samples from every subject studied. Overall, Kv1.3 expression was detected on a mean of 3.5% of leukocytes (range 0.1%–13.4%; IQR 0.8%–5.1%) with higher levels in patients with active disease (4.8%, IQR 1%–6.7%) compared to patients with inactive disease (2.1%, IQR 0.8%–2.7%, *p*=0.04). CD3+ lymphocytes expressing Kv1.3 were found in all subjects (mean 52% of CD3+ cells, IQR 33%–100%). In 90% of subjects, Kv1.3 was detected on CD20+ B lymphocytes (mean 68%, IQR 46%–100%), and in 90% of subjects CD14+ macrophages (mean 69%, IQR 46%–100%). Patients with class III or IV nephritis had increased frequencies of leukocytes expressing Kv1.3 (6%) compared to patients with Class V nephritis (2%, *p*=0.01) or no nephritis (1.8%, *p*=0.01).

Conclusions Kv1.3 is detectable on SLE urinary B lymphocytes, T lymphocytes, and macrophage, implying that inflammatory cells in the kidney may be targeted by this channel. Peripheral blood cell expression and functional data suggest that SLE activated T_{EM} lymphocytes may be susceptible to inhibition by dalazatide.

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AI-02 DISSECTING IMMUNE PHENOTYPES IN SLE

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Background There are many possible explanations for the high percentage of failed clinical trials in systemic lupus erythematosus (SLE). Our preferred explanation relates to the heterogeneity of immune phenotypes in SLE patients. We have, therefore, put

considerable effort into dissecting immune phenotypes among patients.

Methods First, we hypothesize that in each SLE patient, either myeloid cells or B cells drive the disease. This hypothesis is supported by the transcriptional profiles of SLE patients and both serologic analyses and transcriptional profiles of unaffected sisters of SLE patients. This paradigm, if confirmed, will influence the appropriate therapy for maintenance of remission. Second, we believe we can identify patients in whom IgG autoantibodies are made by plasma cells maturing through an extrafollicular pathway and patients in whom IgG autoantibodies are made by germinal center derived plasma cells.

Results Patients might be stratified by plasma cell differentiation pathway so that therapies could be designed to target only one subset of plasma cells and therefore might be less immunosuppressive.

Conclusions These approaches to patient stratification need further exploration in clinical trials for distinct therapeutic interventions.

AI-03 EFFICACY AND SAFETY OF INTERMITTENT 2-DEOXYGLUCOSE THERAPY IN MOUSE MODELS OF LUPUS

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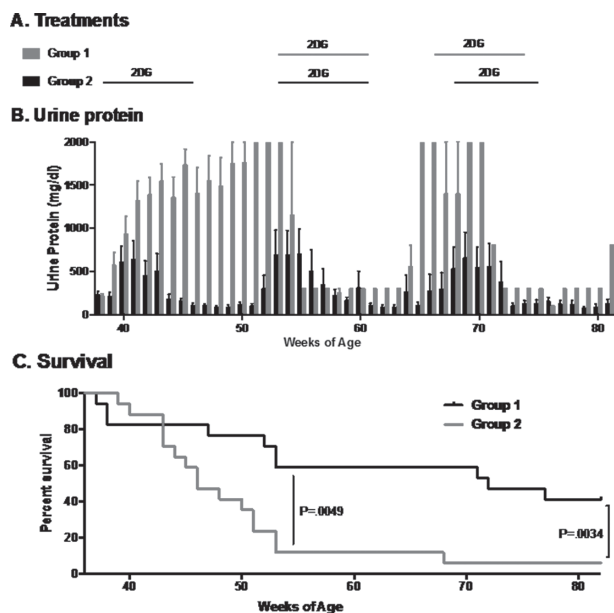
Background Glucose is a primary substrate for cellular respiration. Glucose utilization increases in highly metabolic cells including activated, proliferating T cells and B cells as well as cancers. Lupus is a disorder in which autoreactive CD4 +T cell and B cells deviate from normal homeostasis by their uncontrolled proliferation and differentiation. Therapeutic limitation of glycolysis is therefore an attractive approach for attenuating such highly energetic, pathogenic processes inherent to lupus. Here we investigate the potential of the classic glycolysis inhibitor, 2-deoxyglucose (2DG), to prevent and reverse severe forms of autoimmune lupus-like disease in mice. As untoward side effects of 2DG therapy have also been reported, we further evaluate potential complications and biomarkers of 2DG therapy.

Methods For autoimmune disease studies, the following mouse models for spontaneous lupus-like autoimmune disease were utilized: NZB × NZW F1 (BWF1) females (adult onset) and BXS_B.*Yaa Cd8-/- Il15-/-* males (acute adolescent onset). 2DG was provided *ad libitum* in acidified drinking water (2DG 2–6 g/L) for intervals of 8 wks. Mice were monitored longitudinally for weight loss, proteinuria, and survival. To assess potential effects of 2DG in non-diseased mice, C57BL6/J mice treated with 2DG and controls were analyzed for a series of metabolic, serum chemistry, cardiac, behavioral, immunological and histopathological phenotypes after short and long term exposure.

Results An 8 wk 2DG treatment of young BXS_B.*Yaa Cd8-/- Il15-/-* mice prevented their development of prototypic cellular abnormalities and greatly extended lifespans. Given the strong normalizing effects of 2DG in disease prevention, we performed therapeutic interventions in which 2DG was intermittently supplied to 37 wk old BWF1 mice (figure 1A). This treatment abrogated proteinuria even in heavily diseased mice

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.



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