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, rapamycin (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 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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Background  Elevated levels of 9-O-acetylated sialyl gangliosides (9-OAG) are found on a number of cell types, but are most prominently expressed on B cells in systemic lupus erythematosus (SLE) as compared to controls. The 9-OAG on lupus B cells may be driving defective B cell survival and promoting autoimmunity. The specific mechanisms by which BCR-induced apoptosis is impaired by 9-OAG remain to be elucidated. Here we identify a novel cell-intrinsic program whereby 9-OAG acting via the BCR signalling program may be a potential therapeutic target for SLE.

Materials and methods  We generated a mouse model of lupus in which 9-OAG is depleted as a result of deficiency in CMP-Neu5Ac synthase (C57BL6.Cmpns−/−). C57BL6.Cmpns−/− mice were crossed with BXSB. Yaa mice and analyzed beginning at 5 wks of age to assess disease biomarkers. A schematic of the therapeutic approach is shown in Figure 1A. B cells from BXSB. Yaa, C57BL6.Cmpns−/− and BXSB. Yaa.Cmpns−/− mice were utilized for flow cytometric, histopathological, and cytotoxicity analyses. The results indicate that BCR induced apoptosis is significantly impaired in BXSB. Yaa.Cmpns−/− mice as compared to both BXSB. Yaa and C57BL6.Cmpns−/− controls. To assess if the increased 9-OAG levels on BXSB. Yaa.BCR−/− cells are responsible for the increased apoptosis resistance observed, we engineered BXSB. Yaa.BCR−/− mice to express an engineered BCR that lacks 9-OAG expression (BXSB. Yaa.BCR−/−.9OG−/−). BXSB. Yaa.BCR−/−.9OG−/− mice were analyzed beginning at 5 wks of age to assess disease biomarkers. The results indicate that BXSB. Yaa.BCR−/−.9OG−/− mice have improved survival and disease biomarkers as compared to BXSB. Yaa.BCR−/− controls.

Conclusions  The data presented provide compelling evidence that 9-OAG on B cells through the BCR signalling program may be a potential therapeutic target in lupus. The data also demonstrate that 9-OAG on B cells through the BCR signalling program may be a potential therapeutic target in lupus. The data also demonstrate that the increased 9-OAG levels on BXSB. Yaa.BCR−/− cells are responsible for the increased apoptosis resistance observed. Furthermore, the data indicate that BXSB. Yaa.BCR−/−.9OG−/− mice have improved survival and disease biomarkers as compared to BXSB. Yaa.BCR−/− controls.
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 sialic 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 this 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 prone B-1a cells. The CasD1 acetyltransferase may be a therapeutic target of relevance in lupus.

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