

activity is expected. The failure of F(ab')₂ fragments to bind is unexpected, pointing to a critical role for the Fc portion of the IgG molecule in stabilising antibody interaction. The Fc portion can contribute to anti-DNA by inducing a conformational change in the binding sites; contacting DNA; or forming Fc:Fc interactions to increase valency. This binding pattern can be called Fc-dependent monogamous bivalency. The findings suggest that agents that affect the Fc portion may be useful to therapeutically inhibit anti-DNA interactions.

AI-17 BAFF PROMOTES SYSTEMIC AUTOIMMUNITY VIA TACI-DEPENDENT ACTIVATION OF TRANSITIONAL B CELLS

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Background Although excess levels of B cell activating factor of the TNF family (BAFF, also known as BLyS) have been implicated in the pathogenesis of SLE, how excess BAFF promotes breaks in B cell tolerance is not completely understood. Transgenic mice (Tg) overexpressing BAFF develop an autoimmune disease resembling human SLE. BAFF binds to distinct receptors expressed on B cells, the BAFF receptor (BAFF-R) and transmembrane activator and CAML interactor (TACI). Since BAFF-R deletion results in loss of mature B cells, BAFF-R-dependent signals are presumed to explain BAFF-mediated autoimmunity. However, potential important roles for TACI in lupus pathogenesis have not been addressed.

Materials and methods After crossing BAFF-Tg and *Taci*^{-/-} mice, we used standard immunologic techniques to test the impact of TACI on BAFF-driven autoimmunity.

Results Despite prior evidence of a negative role for TACI in B cell activation, we discovered that TACI deletion resulted in a striking loss of class-switched serum autoantibodies. Loss of serum autoantibodies also correlated with protection from immune-complex glomerulonephritis in *Taci*^{-/-}.BAFF-Tg mice. Importantly, lack of autoimmunity was not explained by alterations in peripheral B cell development, since both BAFF-Tg and *Taci*^{-/-}.BAFF-Tg mice exhibited similar B cell hyperplasia, with equivalent expansion of the follicular (FM) and marginal zone (MZ) compartments. Rather, whereas surface TACI expression is usually limited to mature B cells, we discovered that excess BAFF integrates with dual B cell receptor (BCR)- and MyD88-dependent signals to promote TACI upregulation on transitional B cells. The novel TACI^{hi} subset of transitional B cells from BAFF-Tg mice are characterised by an activated, cycling phenotype and expressed activation-induced cytidine deaminase (AID) and T-bet. Single-cell cloning of B cell receptors from TACI^{hi} vs TACI^{lo} transitional B cells demonstrated that the TACI^{hi} cell subset is specifically enriched for autoreactivity and exhibits evidence of somatic hypermutation. Finally, consistent with a direct role in autoimmune pathogenesis, TACI^{hi} transitional B cells from BAFF-Tg mice spontaneously produce class-switched autoantibodies *ex vivo*.

Conclusion Our combined findings highlight a novel mechanism whereby BAFF promotes humoral autoimmunity via TACI-dependent activation of transitional B cells. In addition to SLE and other autoimmune disorders characterised by elevated BAFF, dysregulated transitional B cell activation is likely to be relevant a range of other clinical scenarios, including: autoimmune disease relapse after treatment with B cell-depletion therapies; *de novo*

humoral autoimmunity following stem cell transplantation; and, rapid IgM- and IgG-mediated antibody responses during pathogen challenges.

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AI-18 B CELL IFN- γ RECEPTOR SIGNALLING PROMOTES AUTOIMMUNE GERMINAL CENTRES VIA CELL-INTRINSIC INDUCTION OF BCL-6

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Background Dysregulated germinal center (GC) responses are implicated in the pathogenesis of human autoimmune diseases, including systemic lupus erythematosus (SLE). Although type 1 interferons (IFNs) are most frequently associated with lupus pathogenesis, type 2 interferon (IFN- γ) has also been shown to promote SLE. However, the respective impacts of these cytokines in promoting B cell activation during humoral autoimmunity have not been addressed.

Materials and methods We recently developed a chimeric murine lupus model in which Wiskott-Aldrich syndrome protein (WAS)-deficient B cells promote spontaneous humoral autoimmunity (Jackson, *et al. J Immunol* 2014). An important advantage of the WAS chimaera model is that dysregulated immune responses are limited to the B cell compartment, allowing genetic manipulation in a B cell-intrinsic fashion. In the current study, we contrast the impact B cell-intrinsic type 1 IFN vs. IFN- γ signals on autoimmune GC formation and the pathogenesis of SLE.

Results Although type 1 IFN prominently enhanced B cell responses *in vitro*, B cell-intrinsic IFNAR deletion exerted surprisingly minimal impacts on class-switched autoantibody titers and spontaneous GC formation *in vivo*. This finding suggested that other cytokines promote B cell activation in the WAS chimaera model. Notably, B cells directly initiated CD4⁺ T cell activation and T follicular helper cell formation via MHC Class II (MHC-II)-dependent antigen presentation. In addition, activated T cells exhibited prominent IFN- γ production that was lost following B cell-intrinsic MHC-II deletion, suggesting a direct role for IFN- γ in promoting autoimmune GC formation. Strikingly, B cell-intrinsic deletion of the IFN- γ receptor was sufficient to abrogate spontaneous GCs, class-switched autoantibodies and systemic autoimmunity. Mechanistically, although IFN- γ receptor signals increased B cell T-bet expression, B cell-intrinsic deletion of T-bet exerted an isolated impact on class-switch recombination to pathogenic IgG2c autoantibody subclasses without impacting GC development. Rather, in both murine and human B cells, IFN- γ synergized with BCR, TLR and/or CD40 activation signals to promote cell-intrinsic BCL-6 expression. Finally, IFN- γ driven BCL-6 expression in B cells was blocked using clinically-relevant Janus kinase inhibitors, ruxolitinib and tofacitinib.

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).

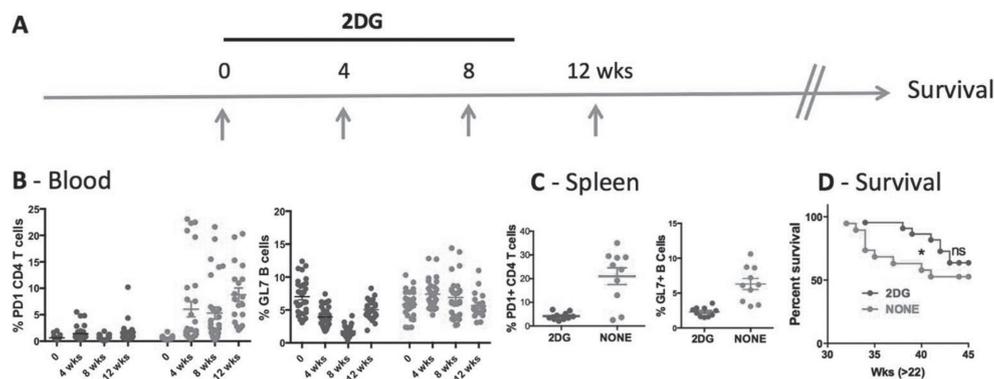
Conclusions Overall, the results highlight the potent and remarkable normalising effect of 2 DG in the prevention and treatment lupus-like autoimmune disease in mouse models with differing genetic and mechanistic etiologies. Given findings, we propose that that therapeutic inhibition of early steps in 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 \leq 0.05.