model is our ability to turn disease on and then off, simply by providing, or not providing, DOX. In mice on DOX for 4 wks and then off DOX for 2 wks, autoantibody titers markedly decrease and skin lesions resolve with minimal if any residual scarring. Subsequent DOX re-administration, without the transfer of additional T cells or any additional irradiation, leads to the rapid recurrence of autoantibody production and skin disease, thereby recapitulating lupus flares.

Conclusions We have now leveraged the hyperactivity of TLR9-deficient mice to develop a novel T cell dependent model of cutaneous inflammation that is strikingly similar to human CLE. This model provides a means for characterising both T and B cell memory responses elicited by autoantigens, and determining to what extent the primary vs secondary responses can be limited by TLR antagonists.

AI-15 DECREASED INTRACELLULAR CALCIUM FLUX IN FOLLICULAR HELPER T CELLS AFTER T CELL RECEPTOR STIMULATION

[1]**Abhinav Seth***, [2]Edward I Herman, [1]Jason S Weinstein, [1]Jin-Young Choi, [1,2]Joseph E Craft. *[1]Departments of Internal Medicine (Rheumatology); [2]Immunobiology, Yale University School of Medicine, New Haven, CT 06520, USA*

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Background Follicular helper T (Tfh) cells are a specialised subset of CD4+ helper T cells that are required for B cell maturation in germinal centres and subsequent antibody formation following infection or immunisation with thymus dependent antigens. Tfh cells have also been implicated in mediating pathogenic autoantibody production in lupus and modulation of their function has been shown to ameliorate end organ disease in murine models of lupus. Understanding the molecular determinants of Tfh cell function may allow for the development of specifically targeted immunomodulating therapies for lupus and other autoantibody mediated diseases. In this study, we have systematically characterised the ability of Tfh cells to flux calcium in response to T cell receptor stimulation.

Methods B6 mice were immunised in bilateral foot pads with a mixture of papain and 4-Hydroxy-3-nitrophenylacetyl conjugated to ovalbumin (NP-OVA). After 5 days, inguinal and popliteal lymph nodes were harvested and lymphocytes were labelled with fluorophore-conjugated antibodies to allow identification of different T cell subtypes by flow cytometry. Cells were loaded with the calcium sensitive dyes Fluo4 and FuraRed to allow ratiometric imaging of intracellular calcium. Cells were stimulated with anti-CD3 antibodies to initiate T cell receptor (TCR) signalling and the intracellular calcium concentration was monitored in naïve T cells, Tfh cells and other effector T cells subtypes. Similar experiments were conducted using T cells obtained from the spleens of 1) B6 mice infected with the helminth *Nippostrongylus brasiliensis*, 2) B6 mice acutely infected with lymphocytic choriomeningitis virus or 3) 6 month old lupus-prone, B6.*Sle1*.Yaa, mice.

Results Tfh cells, relative to naïve T cells or to their Th1 or Th2 counterparts, exhibit significantly reduced calcium flux upon TCR stimulation in the context of NP-OVA immunisation (2.4-fold reduction, $p < 0.0001$), helminth infection (3.7-fold reduction, $p < 0.0001$), viral infection (2.3-fold reduction, $p < 0.0001$) or autoimmune activation in lupus-prone mice (3.3-fold reduction, $p < 0.0001$). These findings are not due to generalised defects in signalling as Tfh cells retain the ability to activate MAP kinases following TCR stimulation, suggesting a specific alteration in the ability of Tfh cells to handle calcium.

Conclusion Our results demonstrate that Tfh cells have a selective defect in calcium mobilisation upon TCR stimulation. The altered calcium handling profile of Tfh cells likely contributes to the unique molecular program of these specialised cells. These results have important implications for designing therapeutic strategies to selectively target Tfh cells in autoimmune disease.

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AI-16 THE ROLE OF FC IN THE BINDING OF ANTI-DNA ANTIBODIES TO DNA

David S Pisetsky*, Nancy A Stearns. *Division of Rheumatology and Immunology, Department of Medicine, Duke University Medical Centre Medical Research Service, VA Medical Centre*

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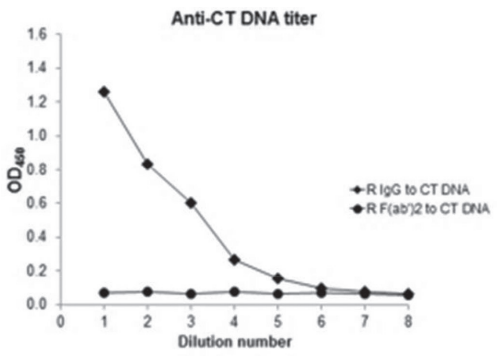
Background Antibodies to DNA (anti-DNA) are the serological hallmark of systemic lupus erythematosus (SLE) and mediate pathogenesis via the formation of immune complexes. While the avidity of these antibodies is high, it depends on monogamous bivalency, a mode of antibody binding in which both IgG combining sites interact with an extended piece of DNA. In the current study, we investigated this interaction further by assessing the activity of Fab and F(ab')2 preparations of IgG from plasmas of SLE patients.

Materials and methods Using purified IgG, Fab fragments were generated by papain digestion while F(ab')2 fragments were prepared with pepsin. The binding to native calf thymus (CT) DNA was assessed by ELISA using an anti-human IgG (Fab specific) peroxidase reagent. In these experiments, the concentrations of IgG and fragments were determined on the basis of an equivalent number of binding sites. Control antigens were tetanus and an EBV antigen preparation. IgG and fragments from normal human subjects were used as controls for binding to foreign antigens.

Results For each of the SLE IgG preparations studied, Fab and F(ab')2 fragments failed to bind significantly to DNA in the ELISA (Figure 1). In contrast, the Fab and F(ab')2 fragments were active against the tetanus and EBV antigens. The binding of the fragments from SLE patients to the foreign antigens was similar to that of normal human subjects.

Conclusions These results define a new pattern of anti-DNA binding. Since a Fab fragment can bind monovalently, a lack of



Abstract AI-16 Figure 1 The binding of intact IgG and F(ab')2 fragments to DNA was determined by ELISA using calf thymus DNA as antigen.

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

[1]Holly M Jacobs, [1]Christopher D Thouvenel, [1]Sarah Leach, [1]Tanvi Arkatkar, [1,2,3,4]**David J Rawlings***, [1,3,4]Shaun W Jackson. *[1]Seattle Children's Research Institute; [2]Departments of Immunology; [3]Departments of Immunology and Pediatrics; [4]University of Washington School of Medicine Seattle, WA, USA*

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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 CENTRESVIA CELL-INTRINSIC INDUCTION OF BCL-6

[1,2]**Shaun W Jackson***, [1]Holly M Jacobs, [1]Tanvi Arkatkar, [3]Elizabeth M Dam, [1]Nicole E Scharping, [1,4]Nikita S Kolhatkar, [5]Baidong Hou, [3]Jane H Buckner, [1,2,4]David J Rawlings. *[1]Seattle Children's Research Institute, Seattle, WA, USA; [2]University of Washington School of Medicine; [3]Departments of Immunology and Pediatrics; [4]Benaroya Research Institute, Seattle, WA, USA; [5]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China*

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Background Dysregulated germinal centrecenter (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.