

**Background** T cell activation depends upon a calcium signalling cascade that is regulated by voltage-gated potassium channels. Effector memory T cells ( $T_{EM}$ ), which are implicated in the immunopathogenesis of autoimmune diseases, express relatively high levels of the potassium channel Kv1.3. Dalazatide is a potent peptide inhibitor of the Kv1.3 channel that has recently shown safety and efficacy in a Phase 1 b plaque psoriasis trial. Evidence suggests that inflammatory cytokine producing  $T_{EM}$  cells might be involved in the immunopathology of lupus nephritis. The objective of this study is to provide proof-of-principle *ex vivo* data for therapeutically targeting chronic T cell activation in systemic lupus erythematosus (SLE).

**Materials and methods** Peripheral blood mononuclear cells from paediatric and adult SLE patients as well as healthy controls were studied. T lymphocyte subsets were assayed *ex vivo* for Kv1.3 expression by flow cytometry. The effect of dalazatide on inflammatory cytokine expression by  $T_{EM}$  cells activated by thapsigargin/phorbol myristate acetate (PMA) or ionomycin/PMA was evaluated by intracellular cytokine staining.

**Results** Kv1.3 expression by  $CD8^+ T_{EM}$  cells was significantly higher in patients with active lupus nephritis when compared to patients with inactive SLE or healthy controls. Dalazatide inhibited IFN- $\gamma$ , IL-17 and TNF- $\alpha$  production by both  $CD4^+$  and  $CD8^+ T_{EM}$  cells from SLE patients in a dose-dependent manner. Dalazatide-mediated inhibition was more significant in IFN- $\gamma$  and TNF- $\alpha$ -expressing  $CD4^+ T_{EM}$  cells from patients with active SLE compared to cells from patients with inactive disease.

**Conclusions** *Ex vivo* studies suggest that dalazatide inhibition of Kv1.3 on  $T_{EM}$  may be an effective strategy for treating SLE. In addition, Kv1.3 expression may be a useful biomarker of SLE disease activity.

AI-07

#### INCREASED "PROBLEMATIC" RNA SPLICING AND GREATER INTRON NUMBER IN SLE AUTOANTIGENS

<sup>1,2</sup>Brenda RS Temple, <sup>2</sup>Philip L Carl, <sup>3</sup>Philip L Cohen\*. <sup>1</sup>R.L. Juliano Structural Bioinformatics Core Facility; <sup>2</sup>Department of Pharmacology, University of North Carolina, Chapel Hill, NC, USA; <sup>3</sup>Section of Rheumatology, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

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**Background** Proteins arise from splicing of pre-mRNA using either the U2 spliceosome (most proteins); or the U12 spliceosome. The latter is believed to be less efficient, and it has been reported that autoantigens are much more likely than control proteins to use the U12 spliceosome. We set out to extend this work, and using new databases and a better contemporary understanding of splicing, to address the splicing mechanisms used for commonly encountered autoantigens.

**Materials and methods** We compared splicing characteristics of the UniProt autoantigen database with total genomic proteins, using the approach of Parada *et al.* (*Nucleic Acids Res.* 42:10564, 2014). Using this method, splice sites are given a "fit" score reflecting their fit to canonical scoring matrices for the two types of introns. We also determined the number of introns per gene and the average intron length.

**Results** We confirmed that autoantigens had more U12 spliceosome usage, although the difference was only twofold, much less than in the one previous study (Ng *et al.*, *J All Clin Immunol* 114:1463, 2004). Autoantigens had increased average number of introns per gene (24 vs 12) and an increase in noncanonical dinucleotides at the splice site. When they were scored for

"problematic" splices, autoantigens were three fold more likely to have problematic introns (39% vs 13%).

**Conclusions** Genes encoding autoantigens have more introns and more "problematic" introns than control proteins. This may result in greater numbers of splicing errors, giving rise to proteins toward which tolerance has not been established. This model predicts autoantigenic epitopes to be near splice sites and should encourage studies of more extensive databases of autoantigens to extend analysis.

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AI-08

#### B CELL PHENOTYPIC CHANGES IN ANTI-NUCLEAR ANTIBODY POSITIVE INDIVIDUALS PRIOR TO THE ONSET OF SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASE

<sup>1</sup>Joan Wither\*, <sup>1</sup>Nan Chang, <sup>1</sup>Babak Noamani, <sup>1</sup>Dennisse Bonilla, <sup>1,2</sup>Sindhu Johnson, <sup>3</sup>Larissa Lisnevskaia, <sup>4</sup>Earl Silverman, <sup>1</sup>Arthur Bookman, <sup>1</sup>Carolina Landolt-Marticorena. <sup>1</sup>University Health Network, Canada; <sup>2</sup>Mount Sinai Hospital, Canada; <sup>3</sup>Lakeridge Health Services, Canada; <sup>4</sup>Hospital for Sick Children, Canada

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**Background** Patients with systemic autoimmune rheumatic diseases (SARD) often have a prolonged pre-clinical phase during which they are anti-nuclear antibody (ANA)<sup>+</sup> but lack clinical symptoms. Here we sought to determine whether ANA<sup>+</sup> individuals who lack sufficient symptoms for a SARD diagnosis share the B cell phenotypic changes seen in SARD.

**Materials and methods** Healthy controls (HC) and ANA<sup>+</sup> individuals who: 1) lacked clinical symptoms of SARD (ANS); 2) had at least one clinical symptom of SARD (UCTD); or 3) had recently diagnosed steroid and immunosuppressive naïve SARD (SLE, SS, SSc, MCTD, DM) were recruited. PBMCs were stained with various combinations of fluorescently labelled antibodies and analysed by flow cytometry. Anti-nuclear antibodies were measured through the hospital laboratory. Whole blood IFN signature and BAFF RNA levels were measured by NanoString.

**Results** B cell phenotypes were examined for 32 HC, 38 ANS, 28 UCTD, and 59 early SARD patients. Patients with early SARD had a number of changes in their naïve and memory B cell subsets including: increased proportions of mature naïve (SSc) and T1T2 cells (SLE and SS), and decreased proportions of switched memory cells (all SARD). Similar decreases in the proportion of switched memory B cells were seen in ANS and UCTD patients, and as seen for the SARD patients, these cells were activated with elevated levels of CD86 as compared to HC. Significantly increased activation of the  $CD27^+IgD^-$  memory compartment was also seen in ANS, UCTD, SLE and SjD patients. Although significantly increased proportions of plasmablasts and/or  $CD138^+$  plasma cells were seen in early SARD patients, these were not seen in ANS and UCTD patients. Nevertheless, in pre-SARD individuals (ANS + UCTD) there was a significant positive correlation between the size of these cell subsets and ANA titer as well as the number of different anti-nuclear antibody specificities. As observed for early SARD patients, there was a trend to increased BAFF levels as compared to HC in pre-SARD individuals, which achieved statistical significance in UCTD patients. However, there was no association between the levels of BAFF and any of the B cell phenotypes, whereas the IFN signature was positively associated with the proportion of T1T2 cells.