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 1b 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-γ, IL-17 and TNF-α 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-γ and TNF-α-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**

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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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Conclusions B cell phenotypic abnormalities precede the onset of clinical disease in ANA+ individuals and have a pattern suggesting ongoing activation through T-B collaboration.

TREATMENT OF MRL/lpr MICE WITH A MAB BLOCKING +10 compared to 72 +38, p < 0.05).

A MIMETIC PEPTIDE OF SUPPRESSOR OF CYTOKINE SIGNALLING-1 AMELIORATES LUPUS PATHOLOGY IN A SPONTANEOUS MOUSE MODEL

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Background Although aberrant antibody production is a lupus disease hallmark, abundant evidence implicates a dysregulated peripheral T lymphocyte repertoire in the onset and progression of lupus. Notably, the intracellular protein suppressor of cytokine signalling-1 (SOCS1) has been shown to regulate T lymphocyte effector functions and modulate lupus-like pathologies in rodent models. Significantly, it has been previously shown that a peptide (SOCS1-KIR), capable of mimicking SOCS1, was effective in mitigating T lymphocyte effector functions associated with lupus disease progression. The peptide has been shown to function through the inhibition of the janus kinases Jak2 and Tyk2. We first test the hypothesis that administration of SOCS1-KIR would partially restore SOCS1 function in animals genetically deficient in SOCS1. We next test the hypothesis that SOCS1-KIR administration will have efficacy in modulating lupus disease pathologies in the MRL lpr/lpr spontaneous model of lupus, whose disease is mediated by a genetic defect in Fas mediated apoptosis of (auto) immune cells. We also assess peptide mediated changes in T lymphocyte effector functions.

Materials and methods SOCS1 heterozygous mating pairs were obtained from St. Jude and used to generate SOCS1-/- mice. SOCS1-KIR (10 micrograms/gram) was administered daily at birth. 3–4 month old female MRL lpr/lpr mice were purchased from Jackson labs and received 10 micrograms/gram of peptide 3 x week. SOCS1-KIR mediated changes in the survival SOCS1-/- mice and onset of skin pathologies in MRL lpr/lpr mice were assessed by Kaplan Meier curves. SOCS1-KIR mediated changes in lymphadenopathy and splenomegaly were assessed by calliper readings and immune organ weighing at death. The capacity of SOCS1-KIR to modulate T lymphocyte effector functions was accomplished through flow cytometric analysis of peripheral blood and immune organ analysis both directly ex-vivo and subsequent to culture.

Results SOCS1 deficient mice (SOCS1-/-) die of a systemic autoimmune disease within 21 days after birth. The administration of SOCS1-KIR significantly prolonged the survival of SOCS1-/- mice. The enhanced survival of SOCS1-/- mice was correlated to enhanced peripheral Foxp3+ cells. The administration of SOCS1-KIR to MRL lpr/lpr mice significantly inhibited spontaneous skin lesion formation and lymphadenopathy. The amelioration of lupus pathology in MRL lpr/lpr mice was correlated to decreased frequencies of interferon gamma producing memory T lymphocytes and increased levels of PD1, which promotes apoptosis.

Conclusions Together these results suggest that a peptide mimic of SOCS1 may have value as a therapeutic for lupus.

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