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-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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AI-08 B CELL PHENOTYPIC CHANGES IN ANTI-NUCLEAR ANTIBODY POSITIVE INDIVIDUALS PRIOR TO THE ONSET OF SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASE

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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 $(ANA)^+$ but lack clinical symptoms. Here we sought to determine whether ANA^+ 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^+ 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.

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.

AI-09 TREATMENT OF MRL/LPR MICE WITH A MAB BLOCKING BINDING OF C3D TO ITS RECEPTORS DECREASES ANTI-DNA AUTOANTIBODIES AND PROTEINURIA: SUPPORT FOR TARGETING THE CR2:C3D INTERACTION AS A THERAPEUTIC STRATEGY IN SLE

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Background The primary B cell receptors for antigen-bound complement C3 fragments are complement receptor 1 (CR1/CD35) and complement receptor 2 (CR2/CD21). In mice, as opposed to humans, CR1 and CR2 are derived through alternative splicing from a common gene designated *Cr2*. CR1 is the primary receptor for the C3b and C4b fragments of C3 and C4, respectively, while CR2 binds the TED domain within C3d and iC3b. The absence of CR1/CR2 in $Cr2^{-/-}$ mice impairs immunological responses to foreign antigens due to the lack of CR2/C3d costimulatory signals on B cells and impaired antigen retention on follicular dendritic cells. One might expect a similar humoral autoimmune enhancing role for CR1 and CR2 in systemic lupus erythematosus (SLE) through amplification of B cell responses to C3b/C3d-bound self-antigens. However, studies in murine models of SLE performed on a *Cr2*-/- background have demonstrated enhanced lupus-related autoimmunity. One potentially confounding factor with use of *Cr2*-/- mice is that CR1 is a high affinity receptor for C4b, which is itself necessary to maintain tolerance to lupus autoantigens in humans and mice.
Materials and methods Previously, only rat anti-mouse monoclonal antibodies (mAbs) to CR2/CR1 have been available, which are immunogenic in mice. We have developed novel non-immunogenic mouse anti-mouse mAbs targeted to CR2-specific ligand-receptor interaction. The first is a non-B cell depleting mAb that recognises and blocks CR2 interactions with C3d without directly affecting CR1 interactions with C4b or C3b. The second mAb recognises the C3d fragment and blocks its interaction with CR2 without directly affecting C3b or C4b interactions with CR1.
Results Using the MRL/lpr model of SLE, we have found that treatment with anti-CR2 mAb does not provide clinical benefit. Conversely, a single injection of anti-C3d mAb durably reduced anti-dsDNA antibody production (mean 383 R.U. in mAb 3d8b injected mice and 949 R.U. in control PBS injected mice, $p < 0.05$) and proteinuria (mean 13115 mg albumin/dL)/creatinine (g/dL) compared to 101759 in PBS injected mice, $p < 0.05$). The reduction correlated with reduced kidney damage and reduced BUN levels (46 ± 10 compared to 72 ± 38, $p < 0.05$). Notably, mice injected with anti-C3d mAb exhibited higher levels of CR1 and CR2 expression and trends toward normalisation of the splenic B cell compartment.
Conclusions Blocking C3d/TED domain interactions with its receptor(s) through ligand-directed interruption of binding represents a potential new therapeutic approach in patients with SLE. Whether interruption of C3d:CR2 interactions through targeting of CR2 itself will provide similar clinical benefit requires additional study.
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AI-10 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 pathlogies 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 cytometic 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 autoinflammatory 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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