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.

Abstracts

Background

Proteins arise from splicing of pre-mRNA 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.

Acknowledgements Supported by grants from NIAMS and the Alliance for Lupus Research (PLC)
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

A MIMETIC PEPTIDE OF SUPPRESSOR OF CYTOKINE +38, p < 0.05).

Materials and Methods

Previously, only rat anti-mouse monoclonal antibodies to CR2/C3d 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. 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 injection versus 101759 in PBS injected mice, p < 0.05) and proteinuria (mean 13115 mg albumin/dL/creatinine) in lymphadenopathy and splenomegaly were assessed by calliper readings and immune organ weighing at death. The capacity of SOCS1-KIR to mediate T lymphocyte effector function 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 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 mediate T lymphocyte effector function was accomplished through flow cytometric analysis of peripheral blood and immune organ analysis both directly ex-vivo and subsequent to culture.

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

Acknowledgements We thank Dr. Howard M. Johnson for a generous gift of SOCS1 and SOCS3 antibodies. We also thank Dr. Laurence Morel for critical review of this manuscript and Dr. Tenisha Wilson for technical support. The study was supported by a grant from the Lupus Research Institute, a BD Biosciences Research Grant, the NIH/NCATS Clinical and Translational Science Awards to the University of Florida TL1 TR000066 and UL1TR000064, a sub-award from NIH/NIAID/001AI101990, and the University of Florida.