

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 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 auto-inflammatory 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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