

Applicant's Name: Davinna Ligons, B.S.

Applicant's Division: Immunology

“Susceptibility to Autoimmune Myocarditis in Mice: Proliferation and Cell Death”

Davinna L. Ligons, Mehmet L. Guler and Noel R. Rose

Introduction: Autoimmune myocarditis and its sequela, dilated cardiomyopathy, is a chief cause of heart failure in young adults and is a major reason for heart transplantation. Our group established a mouse model of experimental autoimmune myocarditis (EAM) to study disease pathogenesis. In this model, immunization with cardiac myosin induces EAM in A.SW mice but not in B10.S mice.

Background: Investigating the genetic influence on disease, we discovered a susceptibility locus on chromosome 1 (*Eam1*). The *Eam1* locus overlaps with the diabetes susceptibility locus, *Idd5*, in NOD mice, which is known to be linked to resistance to cyclophosphamide (CP)-induced apoptosis in peripheral lymph node cells. Correlating with these findings, we also demonstrated that CD4⁺ T-cells of A.SW mice have significantly less CP-induced apoptosis compared to B10.S mice. Since then, we have found that CD4⁺ T-cells of A.SW mice have significantly less caspase 3, 8 and 9 activities than B10.S mice.

Hypothesis: We hypothesized that pathways regulating T-cell death including upstream pathways modulating activation and proliferation are defective in the susceptible A.SW mouse.

Methods: Splenocytes/lymphocytes were stimulated in culture with varying doses of anti-CD3 and anti-CD28 antibodies. *Activation:* cells were stained with anti-CD69 antibody and analyzed with flow cytometry. *Proliferation:* cells were stained with CFSE and analyzed with flow cytometry or alternatively, ³H thymidine and analyzed via a cell harvester/beta counter. *Cell death:* cells were stained with 7AAD/AnnexinV and analyzed with flow cytometry. Western blotting was used to detect the expression of FADD protein in stimulated splenocytes.

Results: Our data showed that CD4⁺ T-cells of the both B10.S and A.SW mice were activated to the same degree when stimulated *in vitro*. However, we found that, compared to B10.S mice, lymphocytes of A.SW mice proliferate significantly more, undergo significantly less cell death and express 32% more FADD when stimulated *in vitro*.

Conclusions: The increase in FADD expression and decrease in cell death in the A.SW mouse may account for the increase in proliferation. Defects in proliferation and cell death may in part explain why A.SW mice are susceptible to EAM and thus, will be the basis of future work investigating the genetic mechanisms of susceptibility to autoimmune myocarditis.