

levels have minimal change when exposed to low concentrations of 2251 DzNP and DSS. At higher concentrations of 2251 DzNP and DSS, MSR1 expression levels are decreased. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Alveolar macrophages exhibit a dose dependent increase in MARCO gene expression levels with increasing concentrations of 2251 DzNP and DSS, but MSR1 gene expression is not affected in a similar fashion. 2251 DzNP-induced increases in MARCO gene expression suggests that 2251 DzNP may facilitate its own uptake through MARCO. 2251 DzNP exposure negatively regulates MSR1 expression at higher doses and suggests that 2251 DzNP may inhibit its own uptake through MSR1.

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Sex Differences in the Effects of Severe Food Restriction on Electrolyte Balance

Jonathas Fernandes Queiroz Almeida¹, Aline Souza¹, Hong Ji¹ and Kathryn Sandberg¹

¹Georgetown - Howard Universities

OBJECTIVES/SPECIFIC AIMS: The goal of this study was to determine if there are any sex differences in the pathophysiological effects of sFR. **METHODS/STUDY POPULATION:** Male Fischer rats (4-month-old) were maintained on a control (CT) (ad libitum regular chow; n=8) or sFR (60% reduction of daily food intake, n=8) diet for 2 weeks. On days 1, 2, 3 and 14, the rats were placed in metabolic cages for food and water intake and 24-hour urine collection. Body weight (BW) is measured daily. After 2 weeks, the animals are given free access to normal chow for 3 months. Short-term and long-term effects of sFR on blood pressure and heart rate will be measured. **RESULTS/ANTICIPATED RESULTS:** After 2 weeks, the male CT group gained 7% BW (p <0.05), while BW in the sFR males was reduced by 12% (p<0.05 vs. CT). In contrast, female controls did not gain BW while the sFR females lost 18% of their BW. Water intake was reduced by 35%, which was similar to the reduction in females (p=0.18). The hematocrit of sFR male rats was higher (51.1%) than the CT group (45.2%, p<0.05), which was most likely due to the 6% reduction in plasma volume. A similar effect on hematocrit was observed in sFR females. Similarly, also to female rats, sFR had no effect on Na⁺ and K⁺ plasma or urine concentrations by day 14 in the male rats. **DISCUSSION/SIGNIFICANCE OF IMPACT:** sFR has similar effects on electrolyte balance in males and females. Ongoing studies will determine if there is any sex difference in the effects of sFR on blood pressure, heart rate and susceptibility to hypertension and cardiac injury.

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Stimulating iNKT Cell-Mediated Neuroblastoma Cytotoxicity in a Mouse Model

Kevin Owen McNerney¹, Hamid Bassiri², Spyridon Karageorgos² and Priya Khurana²

¹University of Pennsylvania School of Medicine and ²Children's Hospital of Philadelphia

OBJECTIVES/SPECIFIC AIMS: Overall Research Aim: To develop an iNKT-cell engaging reagent ("CAB") to induce neuroblastoma-directed cytotoxicity in vitro and in a mouse model of neuroblastoma. **Objective 1:** Explore the contribution of different GD2 affinities to the cytotoxicity against neuroblastoma cells in vitro. **Objective 2:** Determine whether use of different stimulatory glycolipids (alpha-GalCer

vs. C34) alter the activation and cytotoxicity of iNKT cells against neuroblastoma in vitro. **Objective 3:** To analyze survival of an immunocompetent mouse model of neuroblastoma treated with C34-loaded vs alpha-GalCer-loaded CAB molecule, and to analyze the tumor microenvironment in each treatment condition. **METHODS/STUDY POPULATION:** CAB molecule will be generated by fusing a CD1d protein to an scFv domain for GD2 using cloning techniques. Previous work by our group has used a streptavidin-biotin system to link CD1d to an antibody against GD2, which is large and immunogenic. Protein expression of this novel fusion protein will occur in HEK293 cells. This new CAB molecule will then be loaded with alpha-GalCer or C34 for use in cytotoxicity and in vivo experiments. **Cytotoxicity Assessment:** Chromium assays will be used to assess the specific cytotoxicity generated by iNKT cells against neuroblastoma cells in vitro. iNKT cells will be activated by "CAB's" with relatively high and low affinity for GD2, and also with Alpha-GalCer and C34 glycolipid antigen. flow cytometry will be used to assess for CD107a and Interferon Gamma. **Mouse Model of Neuroblastoma:** TH-MYCN +/+ mice will be used as an immunocompetent model of neuroblastoma. These mice have the MYCN gene under the control of a tyrosine hydroxylase promoter, and spontaneously develop neuroblastomas by 2 weeks of life which are uniformly fatal by 8 weeks of life. In vivo survival studies will be conducted by injecting CAB of relatively high and low affinity, loaded with glycolipid antigen intraperitoneally into TH-MYCN+/+ mice starting at 2 weeks of age, twice weekly. There will also be a matched negative control. **Treatment groups are listed below:** 1. alpha-GalCer loaded high-affinity Cab 2. alpha-GalCer loaded low-affinity Cab 3. C34-loaded high-affinity Cab 4. C34-loaded low-affinity Cab 5. Unloaded high-affinity Cab 6. Unloaded low-affinity Cab **Enrollment** will be 6 mice per group for the survival curves. **Tumor Microenvironment analysis:** 2 additional mice will be included in each group listed above to be sacrificed 2 weeks into treatment for tumor assessment with flow cytometry for iNKT cell, NK cell, T-Lymphocyte frequencies as well as interferon-Gamma expression. **RESULTS/ANTICIPATED RESULTS:** **Objective 1:** We expect to find that the highest affinity scFv domains for GD2 result in the greatest amount of cytotoxicity against neuroblastoma cells via iNKT cells. **Objective 2:** We expect that the C34 molecule will induce the greatest amounts of iNKT cell activation against neuroblastoma cells and higher cytotoxicity against neuroblastoma, which has not been shown previously. **Objective 3:** We expect to see prolonged survival of mice treated with the high affinity GD2 CAB loaded with C34 or alpha GalCer compared with the low affinity CAB loaded with C34 or alpha GalCer. We also expect that the C34 loaded CAB in both groups will have prolonged survival when compared with the alpha-GalCer loaded CABS of either affinity. **DISCUSSION/SIGNIFICANCE OF IMPACT:** iNKT cells have been shown previously to confer an improved prognosis in neuroblastoma and other malignancies. Furthermore, high risk neuroblastomas tend to downregulate expression of a chemokine that attracts iNKT's to the site of the neuroblastoma. Directing iNKT to the site of neuroblastoma holds promise as an effective immunotherapy option. Our preliminary data demonstrate that CABS directed against GD2 are capable of exerting cytotoxicity of neuroblastoma in vitro. Furthermore a trend towards prolonged survival has been shown in TH-MYCN mice in early experiments. The development of a novel antibody that has reduced immunogenicity, incorporates a glycolipid antigen that does not induce iNKT cell anergy, and is specific for the GD2 tumor specific antigen has potential to result in increased iNKT-mediate neuroblastoma cytotoxicity and prolonged survival in TH-MYCN+/+ mice.