FSCV in ex vivo slice preparations as well as in anesthetized mice in vivo. DA transients were elicited in the dorsolateral striatum (DLS), dorsomedial striatum (DMS); and nucelus accumbens core (NAcc). In some experiments mice were crossed with DAT-Cre animals and channelrhodopsin 2 (ChR2) was virally expressed in DA neurons to allow optical stimulation of DA transients. RESULTS/ANTICIPATED RESULTS: As previously reported, SAPAP3 KO mice showed excessive grooming compared to control littermates at the age assessed (4-5 months). DA transients evoked by a single electrical pulse in slices from SAPAP3 KO mice were not significantly different from those observed in slices from control littermates in any of the regions tested including the DLS, DMS and NAcc. However, when four electrical pulses were applied at a frequency of 10Hz to mimic DA neuron bursting, the magnitude of DA transients observed in the DMS and NAcc of SAPAP3 mice were greater than those evoked in control littermates.Interestingly, phasic stimulation produced similar DA transients in the DLS of both genotypes suggesting that phasic DA signaling was not globally altered. To confirm this finding we crossed SAPAP3 KO mice with DAT-Cre mice and injected ChR2 containing virus into the midbrain to selectively express ChR2 in DA neurons. Transients were then optically evoked resulting in selective activation of DA neurons. Optical stimulation produced a pronounced enhancement of DA release in SAPAP3 KO mice specifically in the DMS and only following phasic-like stimulation. DISCUSSION/SIGNIFICANCE OF IMPACT: These exciting findings suggest that DA signaling in SAPAP3KO mice is dysregulated in a very precise manner that is sub-region specific as well as dependent on the pattern of stimulation. These results suggest that targeted therapies that can modulate these specific modes of dopaminergic signaling in these distinct striatal subregions could provide improved efficacy in OCD patients that are resistant to SSRI treatment.

3439

Role of the Airway Microbiome in Viral Bronchiolitis Associated Respiratory Failure

Emily Wasserman[1], Stefan Worgall, M.D., Ph.D.[1] and Anurag Sharma, Ph.D.[1]
[1]Weill Cornell

OBJECTIVES/SPECIFIC AIMS: This study aims to determine if a bronchiolitis specific microbiome exists and how it evolves through disease course. Objective 1. Determine the microbiome profile of the airway in virus induced bronchiolitis-associated respiratory failure. Objective 2. Identify changes in the airway microbiome profile through the course of virus induced bronchiolitis associated respiratory failure, and the relationship between microbiome composition and clinical respiratory status. Objective 3. Determine the impact of rhinovirus infection on the lung and stool microbiome in a murine asthma model. METHODS/STUDY POPULATION: Objectives 1 &2: We are conducting a single-center prospective case-control study of patients admitted to the Komansky - Weill Cornell Pediatric Critical Care Unit. Infants less than two years of age with a diagnosis of bronchiolitis requiring intubation and mechanical ventilation are enrolled as subjects. Infants less than two years of age intubated and requiring mechanical ventilation without primary lung pathology are enrolled as controls. To evaluate our primary objective, tracheal aspirates will be collected from both subjects and controls on the day of intubation. We will perform 16s RNA sequencing on the tracheal aspirate samples and compare the resulting microbiomes. To evaluate secondary objective, we will collect tracheal aspirates of our study population on a daily basis and map the microbiome in parallel with objective measures of respiratory status including oxygen index and successful extubation. Both subjects and controls are being enrolled as a convenience sample. Objective 3: Mice, heterozygous for the sptlc2 gene (Spltc2 +/−) demonstrate reduced de-novo sphingolipids and increased airway hyperresponsiveness with methacholine challenge. Airway hyperresponsiveness is a cardinal feature of asthma. This airway hyperresponsiveness is exacerbated in the setting of rhinovirus (Figure 1). Using 16s sequencing, we will examine the lung microbiome of Sptlc2 +/− ad Sptlc2 +/+ at 1- and 7-days following rhinovirus infection. RESULTS/ANTICIPATED RESULTS: This clinical study is currently IRB approved and enrollment is ongoing. We have enrolled 12 subjects and 5 controls. Sample analysis will begin following the 2018-2019 respiratory season, with an anticipated cohort of 20 subjects and 20 controls. With regards to the murine studies, we have demonstrated that the lung microbiome of Sptlc2 +/− and Sptlc2 +/+ mice is similar at baseline (Figure 2) and remains similar following 1-day infection with rhinovirus. We do however, see a distinct change in the microbiome profile of the stool of Sptlc2 +/− mice following rhinovirus infection (Figure 3). Lung analysis at day 7 post infection is pending. DISCUSSION/SIGNIFICANCE OF IMPACT: These studies will lay the groundwork for detailing the functional role of the airway microbiome in bronchiolitis, with the objective of developing new modalities for disease treatment and prevention. In addition our murine studies allow us to view the microbiome in the context of sphingolipid deficiency, providing a potential mechanistic link to rhinovirus and ORMDL3 associated asthma.

3582

Scavenger Receptor Expression is Differentially Affected by DNAzyme-Gold Nanoparticle Conjugates

Cory Sylber[1], Jessica Petree[1], Nusaiba Baker[1], Khalid Salaita[1] and Cherry Wongtrakool[1]
[1]Emory University

OBJECTIVES/SPECIFIC AIMS: Scavenger receptor (SR) surface proteins are highly conserved motifs and are implicated in the uptake of nanotherapies. Gold nanoparticles functionalized with DNAzymes (DzNP) represent a promising novel nanotherapy for lung diseases such as asthma, particularly because they can be delivered directly to the lung. Our lab has been studying the therapeutic potential of a DzNP targeting GATA-3, a master transcription factor regulating Th2 inflammation. Although nanoparticle uptake through scavenger receptors has been described in macrophages in other models, the role of SRs in DzNP uptake in the lung is poorly understood. We hypothesize that scavenger receptors mediate DzNP uptake in alveolar macrophages. To begin examining this hypothesis, we examined whether DzNP exposure and uptake regulates gene expression of MARCO and MSR1, two class A scavenger receptors. METHODS/STUDY POPULATION: Using a silver stain, we measured dose dependent DzNP uptake in murine alveolar macrophages (MH-S). Using qRT-PCR, we measured gene expression of scavenger receptors MSR1 and MARCO in murine alveolar macrophages (MH-S) and after 24 hour exposure to 2251 DzNP, a DzNP targeting GATA-3, and dextran sulfate sodium (DSS), a known SR-A blocker. RESULTS/ANTICIPATED RESULTS: 2251 DzNP uptake in alveolar macrophages is dose dependent. MARCO gene expression levels significantly increase in murine alveolar macrophages when cultured with increasing concentrations of 2251 DzNP (10 pM-2 nM) or DSS 25-200 ug/ml) for 24 hours. However, MSR1 gene expression

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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 thought MSR1.

3486

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

Jonathas Fernandes Queiroz Almeida[1], Aline Souza[1], Hong Ji[1] and Kathryn Sandberg[1]
[1]Georgetown - 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 sRF 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.

3502

Stimulating iNKT Cell-Mediated Neuroblastoma Cytotoxicity in a Mouse Model

Kevin Owen McNerney[1], Hamid Bassiri[2], Spyridon Karageorgos[2] and Priya Khurana[2]
[1]University of Pennsylvania School of Medicine and [2]Children'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 intraperitonealy 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 cytomtetry 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.

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