Proceedings

and

Abstracts

MU College of Veterinary Medicine

presents

The 37th Annual

Phi Zeta Research Day

May 9, 2014
Phi Zeta Research Day
Friday
May 9, 2014

**Poster Presentations:**
9:00 a.m – 12:00 p.m.
1:00 – 4:00 p.m.

All Interns, Residents, Grad Students, Postdocs
Veterinary Professional Students

**Oral Presentations:**
1:30 – 3:30 p.m.

2nd & 3rd year Residents & Grad. Students

**Oral Presentations:**
1:30 – 2:30 p.m.
2:30 – 3:30 p.m.

*W235 Vet. Med. Bldg. (Classroom)*
Veterinary Professional Students
Advanced Grad Students & Postdocs

**Oral Presentations:**
1:30 – 3:00 p.m.

*W233 Vet. Med. Bldg. (Classroom)*
Interns, 1st year Residents & Grad. Students

**Keynote Address:** 12:00 p.m., E-126 Vet. Med. Bldg., Auditorium

*“Animal Models for Inflammatory Bowel Disease”*

Al Jergens, DVM, PhD, DACVIM
Professor and Associate Chair for Research and Graduate Studies
Department of Veterinary Clinical Sciences
College of Veterinary Medicine
Iowa State University

**Social Hour:** 5:00 p.m., Adams Conference Center Atrium

**Initiation/Awards Banquet:** 6:00 p.m., H. Richard Adams Conference Center

Sponsors: College of Veterinary Medicine Dean’s Office and Office of Research,
Nestle Purina, Inc., Zoetis, and the Niemeyer Lecture Series
CVM/Phi Zeta Research Day  
May 9, 2014

**POSTER CATEGORIES**

_H. Richard Adams Conference Center_

(9:00 a.m. – 12:00 p.m., Interns, Residents, Graduate Students, Postdocs)
(1:00 p.m. – 4:00 p.m., Veterinary Professional Students)

<table>
<thead>
<tr>
<th>#</th>
<th>Presenter</th>
<th>Department</th>
<th>Advisor</th>
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<tbody>
<tr>
<td>1</td>
<td>Anna Anandan</td>
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<td>Lisa Anderson</td>
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<td>Pamela Zgoda</td>
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## Interns, 1\textsuperscript{st} Yr Residents & Graduate Students

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<tr>
<td>34</td>
<td>Delia Bouhan</td>
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## 2\textsuperscript{nd} & 3\textsuperscript{rd} Yr Residents & Graduate Students

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## Advanced Graduate Students & Postdoctoral Fellows

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<tr>
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<td>Lydia Cook</td>
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<td>Rachel Olson</td>
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### PRESENTATION CATEGORIES

#### KEYNOTE ADDRESS (Vet Med Auditorium, E126 Vet Med): 12:00 – 1:00 p.m.

#### 2nd/3rd Yr Residents & Grad Students (1:30 p.m. – 3:30 p.m.) – E126, Auditorium

**Moderator: Brenda Beerntsen**

<table>
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<tr>
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<td>Pamela Fry</td>
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<td>Julie Trzil</td>
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<td>Carol Reinero</td>
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#### Veterinary Professional Students (1:30 – 2:30 p.m.), W235 Vet. Med. Classroom

**Moderator: Jeff Bryan**

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<tr>
<td>1</td>
<td>Kari Chesney</td>
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#### Interns, 1st Yr Residents & Graduate Students (1:30 – 3:00 p.m.), W233 Vet. Med. Classroom

**Moderator: John Middleton**

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<td>Christa Bernhard</td>
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#### Advanced Grad Students and Postdoctoral Fellows (2:30 – 3:30 p.m.), W235 Vet. Med. Classroom

**Moderator: Jeff Bryan**

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<tr>
<td>1</td>
<td>Eric Coate</td>
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<td>Sheila Grant</td>
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# JUDGES:

2\textsuperscript{nd} & 3\textsuperscript{rd} Residents, Grad Students - Posters (9:00am-12:00pm, Adams)

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<th>Name</th>
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<th>Department</th>
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<tr>
<td>Sandra Bechtel</td>
<td>Assistant Professor</td>
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<tr>
<td>Charlie Brown</td>
<td>Professor</td>
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Interns, 1\textsuperscript{st} Year Residents, Grad Students - Posters (9:00am-12:00pm, Adams)

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<tr>
<td>Craig Franklin</td>
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Veterinary Professional Students-Posters (1:00pm-4:00pm, Adams)

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<tr>
<td>Tim Evans</td>
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<td>Joan Coates</td>
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Advanced Graduate Students & Postdoctoral Fellows- Posters (9:00am-12:00pm, Adams) & Presentations (2:30pm-3:30pm, W235)

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<td>Tom Reilly</td>
<td>Clinical Associate Professor</td>
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<td>Mick Calcutt</td>
<td>Associate Professor</td>
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Veterinary Professional Students- Presentations (1:30pm-2:30pm, W235 Vet Med)

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<tbody>
<tr>
<td>Al Jergens</td>
<td>Professor</td>
<td>Iowa State U.</td>
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<tr>
<td>James Amos-Landgraf</td>
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Interns, 1\textsuperscript{st} Year Residents, Grad Students - Presentations (1:30pm-3:00pm, W233)

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<tr>
<td>Susan Schommer</td>
<td>Clinical Assistant Professor</td>
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<td>Mike Lewis</td>
<td>Professor</td>
<td>Vet Med and Surg</td>
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2\textsuperscript{nd} & 3\textsuperscript{rd} Residents, Grad Students – Presentations (1:30pm-3:30pm, Auditorium)

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<tr>
<td>Craig Emter</td>
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<td>Chris Baines</td>
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Moderators for Presentations:
Jeff Bryan: Professional Students, Advanced Grad Students & Postdocs (1:30pm, W235 Vet Med)
Brenda Beerens: 2\textsuperscript{nd} & 3\textsuperscript{rd} Residents, Grad Students Presentations (1:30pm, Auditorium)
John Middleton: Interns, 1\textsuperscript{st} Year Residents, Grad Students (1:30pm, W233 Vet Med)
1. EFFECTS OF VINCLOZOLIN ON NRF2 TESTICULAR EXPRESSION IN SEXUALLY MATURE BOARS. Anna Anandan, Tim Evans (sponsor), Tom Reilly and Susan Schommer. Veterinary Medical Diagnostic Laboratory.

Many chemicals can interfere with normal endocrine function. Adverse reproductive effects have been associated with exposures to these endocrine disrupting chemicals (EDCs), especially in experimental rodent models. Swine are more similar to humans than rodents, so the porcine model might be more accurate, than traditional rodent models, in predicting reproductive risks associated with EDC exposures in humans. Vinclozolin (VCZ), a dicarboximide fungicide, has been associated with antiandrogenic effects in animal models. Preliminary data suggest that adult boars are more susceptible than adult male rodents to the adverse reproductive effects of VCZ. Oxidative stress is one mechanism by which EDCs damage testes, and VCZ and its metabolites can induce oxidative damage in various cells. Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates endogenous antioxidant responses, and Nrf2 localization and expression within rodent testes change in response to oxidative stress. The objective of this research was to refine a porcine model for human risk assessment, by determining whether Nrf2-related changes in oxidatively stressed testes, reported in rodents, also occur in boars exposed to EDCs. Paraffin blocks and flash-frozen samples of testicular tissue, collected from sexually mature boars orally exposed to 0, 0.25, 5, or 100 mg of VCZ per kg of body weight for two weeks, were used to characterize Nrf2 distribution and to evaluate Nrf2 as a biomarker for VCZ-induced testicular insult. It was hypothesized that the distribution and expression of Nrf2 within porcine testes will change in a VCZ dose-dependent manner. Immunohistochemical staining, RT-PCR, and Western blots, were used to evaluate VCZ-related effects on testicular Nrf2 distribution and expression in boars. Although further study is needed, this research suggests that VCZ alters the distribution and expression of Nrf2 within porcine testes. It is hoped that, in the future, this research will show how Nrf2 can be used in a porcine model for human risk assessment, as a biomarker for EDC-related oxidative damage.

2. FREELY-BEHAVING VIDEOFLUOROSCOPIC CHARACTERIZATION OF DYSPHAGIA IN CANINE DEGENERATIVE MYELOPATHY. Lisa Anderson, Sarah Weiss, Joan Coates, Teresa Lever (sponsor) Departments of Veterinary Medicine and Surgery and Biomedical Sciences

Canine degenerative myelopathy (DM) has recently been proposed as a naturally occurring disease model for human amyotrophic lateral sclerosis (ALS). Dysphagia (swallowing impairment) is identified in almost all patients with ALS at some point in the disease process. Clinical signs of dysphagia have also been reported in DM-affected dogs with end-stage disease. We hypothesize that functional biomarkers for dysphagia can characterize canine DM and are distinct from other diseases that result in swallowing impairments. We are currently investigating multiple quantitative and qualitative measures of swallowing using contrast videofluoroscopy with various food and liquid consistencies, which include inter-swallow interval, time to maximal pharyngeal constriction, and time to upper esophageal sphincter closure. The study protocol allows dogs to consume foods and liquids containing either barium or Omnipaque (iohexol) contrast agents while standing unrestrained in a custom-designed polycarbonate, radiolucent kennel. The kennel setting eliminates need for physical restraints and force-feeding techniques in food-motivated dogs. The procedure is digitally recorded for subsequent frame-by-frame analysis for measurements. The signalment for each dog is recorded for correlation with disease onset and progression. To date, 7 Pembroke Welsh Corgis have been tested, and the videos are currently being analyzed in a blinded manner. Three dogs were DM-affected and have been tested a second time within 3 months. Understanding of dysphagia in canine DM may lead to subclinical detection of this phenotype and support canine DM as a disease model for ALS.
FUNCTIONAL ANALYSIS OF THE COXIELLA BURNETII PROTEIN COM1. Kendall Annetti, M. Pennella and Guoquan Zhang (sponsor), Department of Veterinary Pathobiology

*Coxiella burnetii* is an obligate intracellular, Gram-negative bacterium and the causative agent of Q fever. Although it has been reported that the *C. burnetii* Com1 protein is an immunoreactive outer membrane protein and has been applied as a reference protein for many assays, the native function of the *C. burnetii* protein Com1 remains unclear. The amino acid sequence of Com1 shares a conserved catalytic site with the well-studied disulfide oxidoreductase enzyme, DsbA, in *Escherichia coli*. In several bacterial pathogens DsbA-like enzymes are involved in the disulfide bond formation of secreted virulence factors, such as toxins, adherence factors, and motility mechanisms. Since there is no established oxidative protein folding pathway in *C. burnetii*, we investigated the role of Com1 as a putative disulfide oxidoreductase. The *com1* gene was amplified by PCR from *C. burnetii* genomic DNA and cloned into the pET28a expression vector. The construct sequence was verified by sequencing and transformed into *E. coli* expression strain. Com1 protein was purified by immobilized metal affinity chromatography. Putative oxidoreductase activity of Com1 was measured as the rate of insulin B chain precipitation from solution in the presence of DTT. The insulin assay indicated that Com1 acts as a disulfide oxidoreductase. Complementation of Com1 in DsbA knock-out *E. coli* was carried out to elucidate its role in the oxidative protein folding pathways. Further *com1* gene knock-out in *C. burnetii* is required to understand the function of the protein in live cells. Stipend support was provided by the Department of Veterinary Pathobiology, University of Missouri and project supplies were provided by NIH/NIAID 1RO1AI083364-01 to Guoquan Zhang.

EVIDENCE OF LUNG REMODELING AS ASSESSED BY COMPUTED TOMOGRAPHY IN EXPERIMENTAL AND SPONTANEOUS FELINE ASTHMA. Alina Banuelos, Isabelle Masseau and Carol Reinero (sponsor), Department of Veterinary Medicine and Surgery.

In allergic asthma, airway remodeling (permanent architectural changes in the lung) is a prominent feature. Computed tomography (CT) has appeal as a minimally-invasive diagnostic. The purpose of this study was to compare indices of airway remodeling between cats with experimentally-induced and spontaneous asthma and healthy unaffected cats using CT. We hypothesized experimental and spontaneous feline asthma would have similar remodeling changes noted on CT and would be significantly different from healthy cats. Scans were performed using a multidetector CT scanner with a restraining device that avoids the need for anesthesia in experimentally asthmatic research cats (n=5), spontaneously asthmatic pet cats (n=5) and healthy research cats (n=5). Mean±standard deviation of lung attenuation normalized to tracheal lumen attenuation for each cat was determined using 3D Slicer software. Data analysis was performed using one-way ANOVA with significance of P<0.10. Experimentally asthmatic and spontaneously asthmatic cats had significantly (P=0.038 and P=0.095, respectively) increased lung attenuation (more positive lung density) than healthy cats. In addition, there was no significant difference in degree of heterogenicity of attenuation between the experimentally and spontaneously asthmatic cats (P=0.380). Although the normalized lung volume was smaller in pet cats compared to research cats, lung volume was similar between groups (P = 0.221). In conclusion, this small pilot study showed CT-derived measures of airway remodeling were similar in experimental and spontaneous asthma and were significantly different from healthy cats. CT may be useful in experimentally and spontaneous feline asthma “models” to investigate new therapies targeting remodeling.
EFFECTS OF GM-CSF ON THE FUNCTION OF PMNS FROM HEALTHY DOGS AND DOGS WITH NEOPLASIA. Celia Friedman Cowan, Yan Zhang, Sandra Axiak-Bechtel, Juliana Amorim, Kaoru Tsuruta and Amy DeClue (sponsor), Department of Veterinary Medicine and Surgery.

Cancer is the leading cause of death in adult dogs, and while chemotherapy is a common treatment, most dogs eventually relapse and die. Chemotherapy is a harsh treatment that suppresses the number and function of polymorphonuclear cells (PMNs) in addition to immunosuppression present from the cancer. PMNs are key in the innate immune system, especially in the recognition of lipopolysaccharide (LPS) of gram-negative bacteria via toll-like receptor 4 (TLR4) and exhibit phagocytic, cytotoxic, and anti-tumor functions. Therefore, dogs with cancer, especially those undergoing chemotherapy, have greater risk of infection (and reduced anti-tumor cytotoxicity), limiting the amount of chemotherapy treatment that can be used. By increasing function of PMNs, it is possible that more chemotherapy could be tolerated, leading to higher rates of remission and longer survival times. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine involved in proliferation and differentiation of blood cells. It enhances cytotoxic and phagocytic capabilities of PMNs and increases TLR4 expression in people. The use of GM-CSF in dogs with cancer could help reduce infection rates and increase anti-tumor cytotoxicity. Preliminary data in our laboratory shows that dogs with cancer have impaired immune function. We plan to evaluate if GM-CSF can restore PMN function in dogs with cancer. We plan to stimulate cells with varying concentrations of GM-CSF or a control solution to evaluate PMN function including changes in cytokine (IL-6, IL-10, TNF) production using bead-based multiplex cytokine assays and TLR4 expression using flow cytometry. So far, we have found that PMNs exposed to GM-CSF expressed increased levels of TLR4. The ultimate goal of the study would be to apply our results in a clinical trial in tumor-bearing dogs with the hope of improving outcomes in dogs with cancer.

RELATIONSHIP BETWEEN SERUM TOTAL PROTEIN AND IMMUNOGLOBULIN G LEVELS IN CALVES FED MATERNAL COLOSTRUM OR A COMMERCIAL COLOSTRUM REPLACEMENT PRODUCT. Lauren Geiger and Patrick Pithua (sponsor). Department of Veterinary Medicine and Surgery.

The physiology of the bovine placenta prevents the transfer of immunoglobulins from mother to offspring. As a result, the immature immune system of the calf is completely dependent upon the colostrum it receives to prevent disease. Colostrum intake, therefore, is one of the most important factors in calf management. Failure of passive transfer can predispose calves to disease and even increase neonatal mortality risk. Currently, producers measure serum total protein to ascertain if acceptable passive transfer has occurred. Consensus in literature appears to point to a cut off of greater than or equal to 5.0 or 5.2 g/dl serum total protein as a marker of acceptable passive transfer of immunity; however, few studies have been conducted to determine whether serum total protein is an accurate predictor of serum immunoglobulins in vivo. For this project, 23 calves were fed either maternal colostrum, lacteal-derived colostrum, or plasma-derived colostrum. Serum samples were taken before colostrum ingestion and subsequently at 36 hours and weekly for up to 8 weeks after colostrum. Each sample was examined for serum total protein levels using a refractometer and immunoglobulin G levels using radial immunodiffusion assay kits. After analysis of data, it was determined that the thresholds determined by this study are comparable to currently utilized ranges. This demonstrates that it is not necessary for producers to employ different cutoffs of serum total protein levels used to determine passive transfer status when using various colostrum replacement products. Research Support: Patrick Pithua Student Support: University of Missouri College of Veterinary Medicine
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EFFECT OF DOG TRAINING ON OFFENDER SELF ESTEEM AND LOCUS OF CONTROL.
Nicole Haarmann, Rebecca Johnson (sponsor), George Lombardi, MU Research Center for Human Animal Interaction

Introduction: Puppies for Parole is a program that pairs offenders with shelter dogs to train the dog to the Canine Good Citizen level. The program teaches offenders necessary skills to encourage successful rehabilitation and societal reentry and gives the participants the opportunity to repay public debts caused by their crimes. Anecdotal reports have shown improvement in offender behavior, better interaction with the staff, and increasing prison safety and security, however, no formal research has been conducted to scientifically validate the effects on offenders.

Specific Aim: We will identify to what extent participation in the Puppies for Parole program is associated with improved self-esteem, more internal locus of control, and better self-perceived health in the inmates. It also aims to learn the offenders' perceptions of participation in the shelter dog program.

Methods: The study uses a repeated measures, non-random, two group experimental design with a treatment group of offenders training shelter dogs (TG) and a control group of offenders (CG) with their usual activities at the facility. Data are collected at baseline, 8 weeks, and 16 weeks after enrollment in the study. Data collected at baseline includes demographic information and dog ownership history.

Self-perceived health, self-esteem, locus of control, requests for medical services, conduct violations, and grievances filed are collected at all three intervals. Data on personal factors such as requests for medical services, mental health services, conduct code violations, and grievances filed were collected for both groups. The treatment group also responded to questions about their perception of the program.

Results and Conclusions: Three hundred eleven offenders were enrolled in the program (n=137 in the TG and n=174 in the CG.) The average participant was 34 years old (TG=34.4, CG=34.7), Caucasian (TG=70%, CG=59%), never married (TG=42%, CG=44%), and had a high school education equivalent (TG=74%, CG=80%). Data is currently being analyzed and will be presented. The study will provide empirical outcome measures to a widely used program.

AN INVESTIGATION FOR THE USE OF BETA ADRENERGIC RECEPTOR AGONISTS TO TRANSFORM WHITE ADIPOSE TISSUE INTO A BROWN-LIKE ADIPOSE TISSUE.

Nicholas Harrison, Harold Laughlin (sponsor) and Nathan Jenkins. Department of Biomedical Sciences.

Obesity has become an epidemic in both the human population, as well in the pet population, and is causing a growing concern of the health consequences associated including type 2 diabetes, metabolic diseases, coronary heart disease, and stroke, just to list a few. Recent research has pointed to systemic inflammation as a result of the enlarged white adipose cells that result in these health consequences. Brown and white adipose tissue both have different properties, brown being more metabolically active, and does not contribute to the inflammatory response as much as does white adipose. Beta adrenergic stimulation is known to increase the metabolism of adipose tissue. The experiments summarized here were designed to test the hypothesis that chronic treatment with a beta 2 adrenergic receptor agonist would result in the conversion of white adipose into a more brown like state, creating a healthier tissue. Total RNA was extracted from adipose tissues of mice treated with either isoproterenol or saline via subcutaneously implanted osmotic mini-pumps. RNA was converted to cDNA, and real time polymerase chain reaction was used to determine the relative quantities of each gene in the tissue. By looking at the up or down regulation of genes such CP1, UCP, PCG, and TNF, we determined whether Beta 2 agonist altered phenotype of adipose tissue as proposed. There was indeed an upregulation of genes seen in brown adipose tissue, and a decrease in inflammatory genes, leading us to the conclusion that there was in fact browning of the adipose tissue.

Research support:

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ACUTE HYPOXIA (AH) ACTIVATES NNOS AND ASTROCYTE ASSOCIATED NNOS CONTAINING CELLS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN).

Amanda Hoffman, K. Max Coldren, and Cheryl M. Heesch (sponsor), Dalton Cardiovascular Research Center.

Patients (obstructive sleep apnea) and animals exposed to chronic hypoxia develop hypertension, but acute hypoxia (AH, 2 hr) results in hypotension. The PVN integrates multiple cardiorespiratory signals and is activated during AH. Previous studies found that neuronal nitric oxide synthase (nNOS) containing cells in the PVN, which are neither spinally projecting (pre-sympathetic) nor vasopressinergic, are activated during AH. Since astrocytes closely associated with neurons modulate synaptic transmission (tripartite synapse), we evaluated association of astrocytes with both nNOS and pre-sympathetic PVN cells. Pre-sympathetic PVN cells were retrogradely labeled prior to exposure to 10% (AH) or 21% (normoxia, N) oxygen. Immunohistochemistry identified activated (FOS-IR) cells, nNOS, and astrocytes (GFAP). AH increased overall activation of PVN cells (N, 50±5; AH, 170±26) and specifically activated nNOS cells (N, 17±3; AH, 55±9). A substantial percentage of spinally projecting (N = 36±7; AH = 41±6%) and nNOS cells (N = 38±7; AH = 37±7%) were associated with astrocytes. Of nNOS cells activated by AH, 35±5% were associated with astrocytes, while 65±5% of activated nNOS cells were not associated with astrocytes. Spinally projecting cells were not activated by AH. We propose that astrocytic glutamate transporters remove glutamate from the synapse and blunt excitability of both nNOS and presympathetic neurons. Nitric oxide (NO) from activated nNOS cells is highly diffusible and increases presynaptic GABA release. Thus, increased NO may limit further cell excitation and contribute to inhibition of spinally projecting cells, preventing increased sympathetic activation via the PVN. (P<0.05) [Student support: Zoetis Animal Health; Research support: NIH HL98602, CMH]

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EPIGENETIC ANALYSIS OF THE SLC6A4 SEROTONIN TRANSPORTER IN BOTTLENOSE DOLPHINS (TURSIOPS TRUNCATES).

Casandra M. Jacobs, CE Hagan, JM Amos-Landgraf (sponsor), Brookfield Zoo/Chicago Zoological Society, Department of Veterinary Pathobiology.

There are many theories as to why dolphins swim into shallow waters and beach themselves. In our study, we believe that this atypical stranding behavior could be a stress-related response. Studies have shown that early life stress causes epigenetic modifications to stress-related genes, resulting in abnormal behavior in rodents and humans. We hypothesize that methylation patterns of stress genes will differ in stranded dolphins compared with healthy animals. Our first goal is to sequence and quantify methylation of a known stress gene, the serotonin transporter (SLC6A4). In humans, methylation of the SLC6A4 promoter is linked with stress exposure. We will analyze the promoter region of the serotonin transporter, SLC6A4, in dolphins using sequencing and quantitative methylation analysis through bisulfite pyrosequencing. This is a technique that determines methylation status at CpG dinucleotides. Treatment of DNA with bisulfite converts cytosine residues to thymine only if unmethylated; methylated cytosine residues remain unconverted. This will yield single nucleotide resolution information about the methylation status of the SLC6A4 promoter. This is the first known behavioral epigenetic analysis in dolphins. The results of our sequence and methylation studies will next be used to make comparisons between stranded and non-stranded dolphins. We expect our ongoing studies to illuminate aspects of stress biology that may inform how these animals are treated and cared for in captivity.
IN VITRO EFFECTS OF OXIDIZED LOW DENSITY LIPOPROTEIN ON CANINE JOINT TISSUES.
Christopher R. Kennedy, Keiichi Kuroki, Aaron Stoker, James L. Cook (sponsor), Comparative Orthopaedic Laboratory

Patients with primary osteoarthritis (OA) commonly have cardiovascular disease (CVD) and it has been reported that cardiovascular mortality is directly proportional to the extent of OA in affected individuals. Although the high incidence of concurrent OA and CVD may be merely an independent feature of advanced age and/or obesity, major risk factors for both, one can speculate that there is a direct link between the two. Altered lipid metabolism may be the underlying cause and could help link OA and CVD. It has been hypothesized that oxidized low density lipoprotein (oxLDL), a causative material of atherosclerosis, is a key molecule that connects these diseases. The aim of this project was to test the hypothesis that oxLDL would induce histological and biochemical changes compatible with OA in a co-culture joint model. All procedures were approved by the institution’s animal care and use committee.

Six mm cartilage and four mm synovium explants were obtained from 6 dogs that were euthanized for reasons unrelated to the project. Cartilage and synovial explants were co-cultured in medium with oxLDL at concentrations of 0, 10 or 100 ug/ml and samples of liquid media (n=6 of each group) were collected on days 3, 6, 9 and 12 of culture for biochemical evaluation. Explants (n=6) were collected on days 6 and 12 for evaluation of cell viability, GAG, and histopathology. There was a significant decrease (P<0.05) in viable cell density as well as ADAMTS4 and MMP enzyme activity in samples cultured for 12 days with oxLDL compared to controls. The study findings suggested that oxLDL has an adverse effect on chondrocyte health.

COMPARISON OF BACILLUS SPORE DISPLAY SYSTEMS. Nathaniel Kollias¹, Hsin-Yeh Hsieh², Che-Min Su², George Stewart², 1. College of Veterinary Medicine 2. Department of Veterinary Pathobiology and Bond Life Sciences Center and 3. Department of Biochemistry and Center for Agroforestry

Tethering of enzymes to a solid support has been shown to enhance their activity and stability in a variety of industrial and biological applications. Recently, bacterial endospores have been used as particle display systems for covalent attachment of proteins in an environmentally accessible format. Spores are dormant forms of certain soil bacteria, which have the ability to resist environmental degradation for long periods of time do to their unique protein coat. Thus by attaching exogenous proteins to such a stable biological platform in theory should allow various enzymes to remain stable for long periods of time. The best studied spore forming bacterium is that of Bacillus subtilis (nonpathogenic bacterium), but also consists of Bacillus anthracis and Bacillus thuringiensis (nonpathogenic). Genetic engineering of the B. subtilis bacterium has shown fusion of foreign proteins to certain spore coat proteins results in surface display of the foreign protein, enzyme activity, and immunogenicity of the attached proteins. The spore coat proteins used in these studies are CotB, CotC, CotG, and CotX. However, incorporation of the fusion protein into the spore coat is thought to be relatively inefficient, thus limiting the amount of foreign protein that can be displayed on the spore surface. Conversely, B. thuringiensis and B. anthracis have an additional structural layer, the exposporium, which is external to the spore coat. The
architecture of the exosporium is composed of a hairlike nap layer consisting of the BclA glycoprotein. Our laboratory has identified the protein sequence, which targets BclA to the spore surface, which allows us to express high levels of foreign proteins on the *B. thuringiensis* exosporium surface. This study was initiated to compare and contrast the relative expression levels of foreign proteins on the surface of *B. thuringiensis* versus *B. subtilis* spores. Enhanced green fluorescent protein was incorporated into spores of *B. thuringiensis* using the BclA system or spores of *B. subtilis* using the CotB, CotC, or CotG systems. Fluorescence levels relative to spore titer were determined.

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TESTIS SPECIFIC Y-LIKE GENE IN CANINE PROSTATE CARCINOMA: GENE EXPRESSION ANALYSIS AND ITS POTENTIAL AS A BIOMARKER. Kristin E. Koth, Jeffrey N. Bryan, Senthil R. Kumar (sponsor), Department of Veterinary Surgery.

Introduction: Testis specific Y-like protein (TSPYL-5) is a member of TSPY-L family of gene, whose functions are currently unknown. This gene is one of the frequent targets of epigenetic silencing in human cancers and reported to possess tumor suppressor function. It is not known whether this gene is present in any of the canine cancers. We hypothesize that this gene could be expressed in canine prostate cancer cells and could function as a biomarker for this disease.

Materials and Methods: A canine prostate carcinoma cell line (ACE-1) was grown in DMEM-F12 media with 10% FBS at 37°C. Total RNA was extracted from the cells using RNA extraction kit (Qiagen, USA). cDNA was synthesized from the total RNA using a iScript Reverse Transcription Supermix (BioRad, USA). The resultant cDNA served as a template for PCR amplification in the presence of specific forward and reverse primers. The amplified PCR products were analyzed by 1% agarose gel electrophoresis. In order to analyze the variation in gene expression due to hypermethylation, the PCR was performed using cDNA from ACE-1 cells treated with two demethylating agents, 6-thioguanine (6-TG) and 5-aza-2-deoxy cytidine (5-10 μM), respectively.

Results: Our preliminary investigation indicated that the TSPYL-5 like gene is indeed expressed in canine prostate carcinoma cells. Treatment of ACE-1 cells with 6-TG or 5-aza-2-deoxy cytidine, respectively partially induced the TSPYL-5 expression in ACE-1 cells. These results suggest that the methylation of TSPYL-5 like gene in canine prostate carcinoma cells could be low in frequency.

Conclusions: For the first time we report the presence of TSPYL-5 like gene in canine prostate carcinoma cells. It is not clear whether its expression in canine prostate carcinoma cells could play a part in tumor suppressor function. Alternately, the presence of this gene may play a role in cell resistance and growth. Further, inherent expression of TSPYL-5 like gene could implicate a role as a biomarker in canine prostate carcinoma.

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IMPROVING HIV-1 NEUTRALIZING ANTIBODY KD-247 AGAINST MULTIPLE HIV-1 CLADES. Benjamin Langsten¹, Dandan Lui², Yee Tseuy Ong², Dallas Pineda², George Smith³ and Stefan Sarafianos¹,⁴ (sponsor), ¹ College of Veterinary Medicine, ² Christopher Bond Life Sciences Center, Department of Molecular Microbiology & Immunology, ³ Department of Biological Sciences and ⁴Department of Biochemistry

HIV-1, which has many different clades, has claimed more than 25 million lives in the last thirty years. Clade B is most common in the United States and Europe, while non-clade B is more common in other parts of the world, especially sub-Saharan Africa. KD-247 is a humanized antibody that targets the third hypervariable(V3) loop of HIV-1 gp120. The tip region of V3 loop has a conserved sequence of GPGR among clade B HIV-1 and a sequence of GPGQ among non-clade B HIV-1. KD-247 is effective against the V3 loop in clade B, but not non-clade B. We have generated a library of KD-247 variants in the heavy or light chain of the antibody using the phage display technology to improve binding and neutralizing efficiency of KD-247 to both clade B and non-clade B HIV-1. The binding efficiency is measured by ELISA. On-going studies involve testing variants with modifications in the CDR3 of the heavy chain, which has been show by other HIV-1 neutralizing antibodies to have interaction with their targets. This study will further provide insights into the interaction of neutralizing antibody with GPGR and GPGQ V3 loop.
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PRESERVATION OF UNAFFECTED CARTILAGE DURING OPEN SURGICAL PROCEDURES.
Leanne M. Mathew, AM Stoker (sponsor), J. Farr and JL Cook, Comparative Orthopaedic Laboratory,

Introduction: Surgical treatment of focal cartilage defects often requires open arthrotomy with exposure of unaffected cartilage to non-physiologic environmental conditions. The objective of this study was to determine an optimal method for preserving cartilage health during surgical exposure.

Methods: With IACUC approval, femoral condyles (FC) and tibial plateaus (TP) were removed from dogs (n=6) euthanatized for reasons unrelated to this study. Each FC or TP was assigned to one treatment group: Control (no treatment), Saline drip, Saline sponge, Media drip, Media sponge, or hyaluronic acid (HA). Tissues were exposed to operating room conditions for 2 hours. For drip treatments, tissues were lavaged every 15 minutes with saline or media, while sponge-treated tissues were covered in gauze saturated with saline or media during the 2-hour exposure. HA was applied liberally at time 0 to cover the entire cartilage surface for this group. Cartilage was collected at time 0 and 2 hours to determine water content and percent cell viability. Differences between time points and among treatments were analyzed using t-Test and ANOVA with significance set at p<0.05.

Results: Cell viability was significantly higher in cartilage treated with HA, Saline sponge, and Saline drip compared to Controls (p<0.05), and all treatment groups had significantly higher water content than Controls (p< 0.05) after the 2-hour exposure. The Controls also had significantly (p< 0.05) lower cell viability and water content after the 2-hour exposure when compared to time 0 samples. Discussion: These data indicate that unaffected cartilage can be detrimentally altered by exposure during relatively short operations and that saline or HA can prevent potential damage to cartilage health during surgery.

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NANOPARTICLE-MEDIATED GENE TRANSFER MODULATING EQUINE CORNEAL WOUND REPAIR, Valentina Moshnikova and Rajiv Mohan, Department of Veterinary Medicine and Surgery

Infections and/or injury to the cornea are known to cause haze (opacity) and/or neovascularization (growth of new blood vessels) and consequently vision impairment in ~1.5 million Americans every year and are also among the leading causes of blindness worldwide. Nanomedicine may provide new ways to treat and prevent these conditions. Recently developed nanoparticles are proving to be excellent agents for delivery of therapeutic genes and peptides to specific tissues or cells without significant toxicity, with potential for developing treatments for corneal diseases that do not require repeated applications and provide long-term relief. Dr. Mohan has already demonstrated that polymeric PEI-DNA nanoparticles are effective for delivery of genes into corneal fibroblasts and have low levels of cytotoxicity. Moreover, it has been shown that delivery of soluble TGF-beta type II receptor using PEI-DNA nanoparticles can inhibit transformation to myofibroblasts and corneal fibrosis. Now we transfected corneal fibroblasts with Smad7 gene, which is expected to inhibit TGF-beta signaling. We used PCR to detect Smad7 mRNA to test for efficient delivery of the gene with PEI-DNA nanoparticles. We evaluated the effect of the transfection on fibroblast transformation following incubation in presence of TGF-beta. We used immunohistochemistry, PCR and Western blot to measure the levels of smooth muscle actin (SMA), a typical marker of fibrosis. Equine corneal fibroblasts transfected with Smad7 gene had decreased SMA expression, which reflects decreased transformation to equine corneal fibroblasts. This result shows that gene therapy based on anti-fibrotic genes such as Smad7 has potential to inhibit or prevent corneal fibrosis.
USE OF ORAL FLUIDS TO DETECT PATHOGEN PRESENCE IN SWINE. Justin M. Nash, C.E. Snider and S. Schommer (sponsor)

Blood, urine, fecal and tissue samples are often used for the detection of pathogen presence in an animal. However, these sample collection methods are often difficult to perform or even impossible to obtain on live animals. Studies have shown that Porcine Circovirus (PCV), Porcine Reproductive and Respiratory Syndrome Virus (PRRS) and Swine Influenza Virus (SIV) can all be detected in oral fluids. The goal of this study is to develop and optimize real-time PCR assays for detection of Hepatitis E (HepE) and PCV using oral fluid samples obtained from pigs. Samples were obtained from two different swine populations using pieces of cotton rope hung in the pigs' enclosures. Approximately 5 mL of oral fluid suspension was collected and saved, while the rest of the fluid was centrifuged to separate supernatant and pelleted fractions. All three fluid fractions were extracted using established Veterinary Medical Diagnostic Laboratory DNA and RNA extraction procedures. The samples were then screened for the presence of multiple pathogens, including PCV, general Mycoplasma species, Mycoplasma hyopneumoniae, and porcine parvovirus using a conventional PCR assay. Presence of general Mycoplasma species, with the exception of M. hyopneumoniae, were detected, while the samples were negative for other pathogens. However, detection of Mycoplasma species in oral fluids has not been previously described. Next, samples previously determined as either positive or negative for PCV on the conventional PCR assay will be used to create and validate a real-time PCR assay for PCV detection. A real-time PCR assay will then be validated for detection of HepE, and used to assess its ability to detect pathogen presence in oral fluids.

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ANALYSIS OF PERIOPERATIVE BLOOD TRANSFUSION NECESSITY AMONG VARIOUS TYPES OF SURGERY IN DOGS. Courtney Nelson and F. A. Mann. Department of Veterinary Medicine and Surgery

As challenging major surgeries are increasingly performed by veterinarians, the necessity for perioperative blood transfusions can be expected. Based on clinical impression, some surgeries may be more likely than others to hemorrhage significantly enough to require whole blood or packed red blood cell transfusion. A blood transfusion is generally warranted when the dog's packed cell volume (PCV) falls below 20%, because at this point cardiac and pulmonary function is impaired. Within the abdominal cavity, surgeries at particular risk for hemorrhage include gastrectomy, liver lobectomy, and splenectomy, among others. Because of the vascular anatomy within the thoracic cavity, intra thoracic surgery might be expected to be at risk for life-threatening hemorrhage. Additionally, there are some extra-abdominal/extra-thoracic surgeries known for hemorrhagic tendencies, such as neoplastic thyroidectomy, rhinotomy, and, in some instances, perineal hernia. We are comparing the three categories of surgery (intra-abdominal, intra-thoracic, and extra-abdominal/extra-thoracic) based on PCV and total protein throughout the surgery to see whether one of the groups has a higher prevalence of requiring transfusion of whole blood or packed red blood cells in dogs. Also, we are exploring the need for transfusion among specifically selected surgeries. We hypothesize that among the three categories of surgery, there will be no difference in transfusion requirement. We also hypothesize that there will be no difference in transfusion requirement among gastrectomy, liver lobectomy, splenectomy, neoplastic thyroidectomy, rhinotomy, and perineal hernia.
OBJECTIVE DETECTION AND QUANTIFICATION OF COMPENSATORY LAMENESS IN HORSES WITH INDUCED FOOT PAIN. Shanna Nelson and Kevin Keegan (sponsor), Department of Veterinary Medicine and Surgery.

Background: To minimize pain associated with lameness, horses reduce the load on the lame limb by changing their gait to move the center of body mass (CBM) away from the limb with primary (or true) lameness. With primary front limb (FL) lameness, the CBM is shifted caudally leading to compensatory (or false) hind limb (HL) lameness. With primary HL lameness, the CBM is shifted cranially leading to compensatory (or false) FL lameness. Compensatory lameness (CL) can be confusing and compromise the identification of the limb with primary lameness (PL). Aim: to objectively assess CL in horses with induced unilateral FL or HL lameness.

Hypotheses: 1- CL is always present with severe primary lameness but inconsistently present with mild to moderate primary lameness 2- CL is always less severe (relative to threshold) than primary lameness; 3- CL is more common with primary HL lameness than with primary FL lameness.

Methods: Fifteen horses were alternately subjected to experimental FL and HL lameness induced with a shoe with an adjustable screw to apply increasing pressure on the toe. Horses were then trotted in hand in a straight line on a hard surface until veterinarians were able to identify the limb with induced PL. Asymmetries in vertical motion of the head and pelvis were measured with an inertial sensor-based system (ISBS) and lame limbs were identified based on criteria recommended by the manufacturer. Values of MINDIFF and MAXDIFF for each evaluation were adjusted by subtracting baseline values. Lameness severity was calculated with the following formulae: for FL= √(MINDIFF² + MAXDIFF²)/threshold; for HL, when primary FL lameness was induced, =(MINDIFF - MAXDIFF)/threshold; when primary HL lameness was induced, =(MINDIFF + MAXDIFF)/threshold. For each horse, a trial with CL and the least severe PL was selected.

Results: Compensatory lameness was not always present when severe PL (>4 x threshold) was induced, but the incidence of CL was higher (38/83 trials, 45.8%) with severe PL than with mild PL (≤4 x threshold) (1/38 trials, 2.6%) (p<0.001); CL was less severe than PL regardless of the limb with induced PL (induced FL, p=0.006; induced HL, p=0.040); CL was observed more often with primary FL lameness (42%) than with primary HL lameness (25%) (p=0.049).

Conclusions: Objective assessment allows characterization of CL which can help clinicians identify the limb with PL. The relatively small number of horses and the fact that all horses had pre-existing lameness were the main limitations of this study.

AN AQUEOUS EXTRACT OF BITTER MELON INHIBITS BREAST CANCER CELL GROWTH BY TARGETING mTOR PATHWAY. Alyssa Scagnelli, Mohamed Alalem, Alpana Ray and Bimal K. Ray (sponsor), Department of Veterinary Pathobiology.

While breast cancer is considered to be a more easily treatable carcinoma, there are still some forms of the disease that have a poor prognosis due to a high metastatic rate. Successful treatment of breast cancer can be achieved through research on identifying new molecular targets. Bitter melon (BM), a widely used vegetable in Asia, South America and Africa, has recently been reported to be effective in reducing cancer growth. But the exact mechanism of the beneficial effect of BM is not fully understood. Here, we report that an aqueous extract of BM has more profound growth inhibitory effect on breast cancer cells as compared to normal breast epithelial cells. A dose-dependent effect of BM extract on growth of the cells was monitored using wound repair and quantitated by MTT cell proliferation assay. Since BM has been shown to activate adenosine monophosphate-activated protein kinase (AMPK) in cancer cells and activation of AMPK results in mTOR inhibition, we have investigated whether BM could exert its anti-proliferative effect by targeting mTOR function. mTOR is a central regulator of various intracellular and extracellular stimuli. It promotes cell growth and survival through stimulation of protein synthesis. Being a serine/threonine kinase, mTOR may also have a crucial role in the regulation of gene transcription via modulating the activity of some transcription factors, particularly, those involved in cell proliferation and differentiation such as Kruppel-like factor 4 (KLF-4). We have assessed the effect of BM in the regulation of mTOR function in breast cancer cells.
SUBCUTANEOUS AND INTRAPERITONEAL TAMOXIFEN INJECTIONS IN AN OUTBRED RAT STOCK. Rebecca J. Schehr, Carin E. Ahner, Marina R. McCoy, Aaron C. Ericsson, Elizabeth C. Bryda (sponsor), Department of Veterinary Pathobiology.

Tamoxifen has been used frequently for inducing Cre recombinase-based gene knock out in mice but less is known about its effects in rats because few genetically engineered rat models carrying tamoxifen-inducible Cre recombinase genes exist. An attempt to use a standard mouse protocol to induce Cre recombinase expression in a genetically engineered rat strain resulted in 100% neonate mortality 5 to 7 days after injection, prompting a study to determine the best dose and route of tamoxifen administration in rats. The hypothesis was that neonatal rats tolerate lower tamoxifen doses than neonatal mice and that tamoxifen injected subcutaneously (SQ) will be absorbed more slowly than tamoxifen injected intraperitoneally (IP), leading to reduced mortality. Sprague-Dawley (SD) rats aged 8-12 weeks were given two injections, 24 hours apart of tamoxifen in sunflower oil at 100, 40 or 3.72 mg tamoxifen/kg body weight. Neonatal SD rats were given two injections, one at birth and one 24 hours later, at the same doses used in the adults. In adults, no mortality was noted at any dose tested; however, rats injected at 100 mg/kg showed less percent weight gain as compared to control groups. There was 100% mortality in 3 of 4 groups of neonates injected at 100 mg/kg. Necropsy, performed on at least one animal from each adult and neonatal group, showed that both adult and neonatal rats receiving SQ injections formed a capsule around the sunflower oil, reducing the oil’s dispersion from the injection site. Histology, performed on adult animals only, showed that the oil pocket capsules were comprised primarily of macrophages and fibrous material. Rats that received IP injections showed multiple white foci on the mesentery of the abdominal organ. Overall, the results showed that neonatal rats cannot tolerate the 100 mg/kg tamoxifen dose routinely used in mice; however, route of administration does not appear to matter.

EFFECT OF LITHIUM CHLORIDE ON INFLAMMATORY PROCESSES IN THE ADULT HORSE: NEUTROPHIL PHAGOCYTOSIS AND OXIDATIVE BURST CAPACITY ASSESSED USING FLOW CYTOMETRY. Elizabeth Schroepfer, Jonathan Tomkovitch, Mindy Wolfe, Charles Wiedmeyer, Alex Bukoski, Amy DeClue, Tim Evans, & Philip Johnson (sponsor), Veterinary Medical Diagnostic Laboratory and Department of Veterinary Medicine and Surgery.

Laminitis is a devastating disease of the equine hoof for which effective preventive and therapeutic strategies are currently lacking. Laminitis occurs following activation of the innate immune system and the degradation of the hoof’s basal epithelial cell attachments to both adjacent cells and to the underlying basement membrane. Recently, it has been shown that reduced expression of β-catenin and integrin-β4 in hoof lamellar basal epithelial cells is a component of laminitis resulting from activated innate inflammation. This finding may explain diminished cell-to-cell and cell-to-basement membrane attachment, and is possibly a consequence of suppressed canonical Wnt signaling pathways. Lithium chloride (LiCl) both supports Wnt signaling and inhibits innate inflammatory responses. Therefore, LiCl administration might prevent laminitis through support of canonical Wnt signaling pathways and inhibition of innate immune responses. As a first step toward employing LiCl for prevention of laminitis, we investigated the effect of systemically-administered LiCl on neutrophil function (a proxy for innate immune responsiveness). Blood was obtained from 8 healthy, adult horses before (time 0), during (+2 h), and at the conclusion of a 24-hour treatment period with LiCl. A titrated dose intended to maintain a steady state plasma Li concentration in the 0.8-1.2 mM therapeutic range was used. In order to ensure that the circulating Li concentration remained in the therapeutic range throughout the 24-hour treatment period, plasma Li concentration was measured every 4 h as a basis for adjustment of LiCl dose. Neutrophils (phagocytic capacity and oxidative burst capacity) were assessed via flow cytometry.
ROLE OF MITOCHONDRIAL GSK3β IN MITOCHONDRIAL PERMEABILITY TRANSITION AND CELL DEATH. Christina Scudder and Christopher Baines (sponsor), Dalton Cardiovascular Research Center.

Glycogen synthase kinase 3β (GSK3β) is thought to be involved in pathways that lead to opening of the mitochondrial pore, making it a promoter of Mitochondrial Permeability Transition (MPT) and thus a mediator in the process of cell death. While GSK3β is located throughout the cell, there appeared to be a specific form that localizes to the mitochondria. Co-staining for HA-tag and ATP synthase in cells over-expressing this form confirmed that it is indeed a mitochondrially localizing form. Further examination of this mitochondrial GSK3β will include an evaluation of ROS production, calcium retention capacity, and oxidative cell death in cells over-expressing active, inactive, and normal forms of the protein. It is hypothesized that ROS production will be increased, calcium retention capacity will be reduced, and oxidative cell death will be increased in cells over-expressing active forms of mitochondrial GSK3β.

EFFECT OF INTERLEUKIN 17 RECEPTOR DEFICIENCY ON THE DEVELOPMENT OF EXPERIMENTAL LYME BORRELIOSIS. Darcie R. Sidelinger, Carrie E. Lasky, and Charles R. Brown (sponsor), Department of Veterinary Pathobiology

Lyme disease is caused by infection with the spirochete, *Borrelia burgdorferi*, and is the most common vector-borne disease in the United States. Individuals who are not treated with antibiotics near the time of infection will typically develop secondary disease manifestations such as arthritis and carditis. Despite much effort, the immunological mechanisms driving the development of disease are not clearly defined. Interleukin 17 (IL-17) is a cytokine that is a significant contributor to the inflammatory response through recruitment of macrophages and neutrophils to the site of infection. It has been found in the synovial fluid of Lyme disease patients and in the serum of *Borrelia*-infected mice, and has been suggested to play an important role in the development of Lyme arthritis. To directly test the requirement for IL-17 activity in Lyme arthritis, we infected arthritis-susceptible C3H mice deficient in the common A chain of the IL-17 receptor and followed the development of disease. Severity of arthritis and carditis will be determined by histology on days 21 and 35 post-infection and levels of spirochete DNA in tissues will be measured using real-time PCR. Production of *Borrelia*-specific IgM and IgG antibodies will be measured from serum and cytokines will be measured directly from tissue homogenates. These studies will definitively assess the requirement for IL-17 signaling for the development of disease in the murine model of Lyme borreliosis.
HYPOXIA ALTERS GLIAL CELL mRNA AND PROTEIN EXPRESSION IN THE nTS. Tessa K. Smith and David D. Kline (sponsor), Dalton Cardiovascular Research Center.

The brainstem nucleus tractus solitarius (NTS) receives and integrates visceral afferent input to influence cardiorespiratory function, including the increase in breathing and blood pressure during low oxygen (hypoxia). The glutamatergic sensory afferent-NTS neuron synapse is closely associated with glia which actively contribute to synaptic signaling. However, little is known the extent or the mechanism by which glia in the NTS “tripartite synapse” (post-synaptic neuron, presynaptic terminal and glial cell), influences synaptic transmission during hypoxia to augment cardiorespiratory function. We hypothesize that after undergoing sustained hypoxia (10% O2, 24 hr) a down-regulation occurs of both glial cell proteins and mRNA that result in enhanced synaptic transmission. We examined mRNA (RT-PCR) and protein expression (immunohistochemistry) of the glial glutamate re-uptake transporters EAAT1 & 2 which remove glutamate from the synaptic cleft. Data shows EAAT's are located in identified astrocytes and surround synaptic vesicles. The amount of EAAT2 protein and mRNA in NTS is greater relative to EAAT1 during normoxia. Following sustained hypoxia, mRNA of both glutamate transporters is down-regulated, but there is no change in protein. This indicates that 24 hours of hypoxia is an insufficient amount of time to see changes at the level of protein synthesis. With more time spent in hypoxic conditions, we would expect to see fewer glutamate transporters present, leaving more glutamate in the synaptic cleft and, as a result, more synaptic and neuronal activity occurs to increase cardiorespiratory function.

A WHOLE-BLOOD FUNCTIONAL ASSAY FOR IDENTIFYING GLUCOCORTICOID RESISTANCE IN DOGS. Hyunjii Song, Amy E. DeClue and Catherine E. Hagan (sponsor), Comparative Internal Medicine Laboratory and Department of Veterinary Pathobiology

Glucocorticoid resistance can lead to poor health outcomes; however, identifying glucocorticoid-resistant patients remains a challenge. Many studies have investigated cortisol and corticosterone as biomarkers for stress and glucocorticoid resistance, but discriminating between normal and pathologic ranges is complicated by individuals’ natural circadian fluctuations and adaptive responses to stress. Thus, it may be more useful to measure glucocorticoid resistance with a functional assay that mimics the effects of acute stress on immune function. In this study we examine cytokine production of whole blood from healthy dogs in the presence of variable dexamethasone concentrations in conjunction with an in vitro challenge with lipopolysaccharide (LPS). The sensitivity of immune cells to dexamethasone in blood is measured in the presence and absence of epinephrine, a hormone we hypothesize can recapitulate the effects of acute stress in vitro. We have adapted this assay from a study showing that acute stress sensitizes immune cells to dexamethasone in blood from healthy people, but not in blood from people with conditions associated with glucocorticoid resistance (Miller et. al., 2005). Our studies seek to determine whether results from this assay correlate with the prognoses of dogs with critical illness. We hypothesize that an in vitro “stressor” will sensitize immune cells to dexamethasone in blood from dogs with better prognoses, while dogs with poorer prognoses will have immune cells that either do not sensitize or become insensitive to dexamethasone. This assay for glucocorticoid sensitivity is expected to help identify glucocorticoid-resistant dogs and help inform the prognosis for dogs with critical illness.
Utilizing Antisense Oligonucleotides in Spinal Muscular Atrophy Gene Therapy. Marcella Springstead¹, Erik Osman²,³, and Christian Lorson²,³ (sponsor), ¹College of Veterinary Medicine, University of Missouri, ²Department of Molecular Microbiology and Immunology and ³Department of Veterinary Pathobiology.

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease that is the leading cause of infantile mortality worldwide. The disease causes degeneration of α-motor neurons in the anterior horn of the spinal cord leading to weakness of the lower limbs and eventual death. Patients affected with SMA suffer from a homozygous deletion of Survival Motor Neuron 1 (SMN1). A similar gene, Survival Motor Neuron 2 (SMN2), produces an identical protein to SMN1, but a single missense mutation of the SMN2 nucleotide sequence leads to formation of a functional SMN protein only 10% of the time. The truncated protein most commonly produced by the SMN2 gene, SMNΔ7, undergoes aberrant splicing leading to exclusion of the critical exon 7 from the protein. A regulatory region upstream of exon 7 named Element 1 (E1) was identified that acts as an intronic splice suppressor and represses exon 7 inclusion in SMN2 transcripts. Using Morpholino-based antisense oligonucleotides (ASOs), which bind to a specific nucleotide sequence in RNA, E1 was targeted for inhibition to promote full-length SMN expression from SMN2. ASOs were delivered to the central nervous system via intracerebroventricular injection in SMA mice. ASO administration resulted in a significant increase of SMN in the spinal cord, as well as increased life span and development of healthy neuromuscular junction pathology. Initial success in the murine model suggests that ASOs may be a potent therapy in the treatment of SMA in human patients.

Effect of Lithium Chloride on Inflammatory Processes in the Adult Horse: Ex Vivo PAMP-Induced Cytokine Responses in Whole Blood Culture. Jonathan Tomkovitch, Elizabeth Schroepfer, Mindy Wolfe, Charles Wiedmeyer, Alex Bukoski, Amy DeClue, Tim Evans, & Philip Johnson (sponsor), Veterinary Medical Diagnostic Laboratory, Department of Veterinary Medicine and Surgery.

Laminitis is a common and potentially devastating disease of the equine hoof for which effective preventive and therapeutic strategies are needed. Laminitis occurs following activation of the innate immune system and the degradation of basal epithelial cell attachments to both adjacent cells and to the underlying basement membrane. Recently, we showed that reduced expression of β-catenin and integrin-α4 in hoof lamellar basal epithelial cells is a component of laminitis resulting from activated innate inflammation. This finding may explain diminished cell-to-cell and cell-to-basement membrane attachment, and is possibly a consequence of suppressed canonical Wnt signaling pathways. Lithium chloride (LiCl) both supports Wnt signaling and inhibits innate inflammatory responses. Therefore, LiCl administration might prevent laminitis through support of canonical Wnt signaling pathways and inhibition of innate immune responses. As a first step toward employing LiCl for prevention of laminitis, we investigated the effect of systemically-administered LiCl on Pathogen-Associated Molecular Pattern (PAMP) motif-induced cytokine secretion in cultivated whole blood (Cwb) ex vivo. Blood was obtained from 8 healthy, adult horses before (time 0), during (+2 h), and at the conclusion of a 24-hour LiCl treatment at a titrated dose intended to maintain a steady state plasma Li concentration in the 0.8-1.2 mM therapeutic range. In order to ensure that the circulating Li concentration remained in the therapeutic range throughout the 24-hour treatment period, plasma Li concentration was measured every 4 h as a basis for adjustment of LiCl dose. PAMP-stimulated cytokine (IL-1 and TNF) production in Cwb was determined using methods developed in our laboratory.

Research and Student Support: University of Missouri College of Veterinary Medicine and The Animal Health Foundation of St Louis, MO.
YAP:EPHRIN INTERACTIONS IN SKELETAL MUSCLE. Alicia K. Tutino and DDW Cornelison, College of Veterinary Medicine, Division of Biology and Bond Life Sciences Center.

Yes-associated protein (Yap) is a transcriptional co-factor located in the Hippo signal transduction pathway. This pathway controls organ growth but also participates in control of actin cytoskeleton polarization and cell migration. In skeletal muscle, Yap promotes satellite cell proliferation thus inhibiting differentiation. Although the signals to which Yap responds are not completely understood, it is hypothesized that Yap senses mechanical properties of the cell niche and cell to cell contact. In response to these signals Yap regulates proliferation (low when cell-cell contact is high), differentiation (only when cell-cell contact is high), migration and apoptosis. Recent work in the Camargo lab has demonstrated that ephrins are a novel mediator for the Hippo pathway. Ephrins are molecules associated with cell migration during development with different members of the ephrin family found at different stages of muscle development or health (i.e. young, old, damaged, undamaged). Previous studies in the Cornelison lab have suggested that satellite cells are influenced by ephrin signaling. We plan to overexpress constitutively active hYAP1 (S127A) versus empty vector in these cells to test whether satellite cells with Yap hyperactivity ignore ephrin boundaries and/or proliferate excessively. Moreover, because both proteins exhibit cytoskeletal effects, questions arise asking whether Hippo and Eph/ephrin signaling act in concert to organize nascent myofibers into their stereotypical parallel arrays.

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ESTABLISHING IMMUNOHISTOCHEMISTRY STAINING PATTERNS FOR CANINE DEGENERATIVE MYELOPATHY. Sarah Weiss, Lisa Anderson, Joan Coates, Teresa Lever, Departments of Veterinary Medicine and Surgery and Biomedical Sciences

Canine Degenerative Myelopathy (DM) exhibits a phenotypic neurodegeneration with four established stages, beginning with hind limb proprioceptive ataxia leading to paralysis. The disease progresses to include difficulty with tongue movements and swallowing (dysphagia), general muscle wasting, and front limb paralysis. The causative gene mutation occurs in superoxide dismutase 1 (SOD1) and leads to the accumulation of aggregates in motor nuclei of the spinal cord and brain. The clinical signs associated with DM are similar to some forms of SOD1-associated human amyotrophic lateral sclerosis (ALS). Previous work in our lab used Hematoxylin and Eosin staining of DM-affected hypoglossal nuclei (motor nucleus for tongue movement) to identify subtle evidence of neurodegeneration, including shrunken nuclei, lipofuscin pigmenting, central chromatolysis, vacuoles, and neuronophagia. The goal of our current project is to better characterize neurodegeneration of the hypoglossal nucleus using immunohistochemistry (IHC). Specifically, we will immunostain for microglia (Ionized Calcium Binding Adapter Molecule 1, Iba1), astrocytes (Glial Fibrillary Acidic Protein, GFAP), mutant protein aggregates containing Nucleoporin P62 or SOD1, and Calcitonin Gene-Related Peptide (CGRP)/Choline Acetyltransferase (ChAT) colocalization in surviving motor neurons. Brainstem tissue from DM-affected dogs and control dogs (with other non-neurologic disease) was collected at the time of euthanasia and immersion-fixed in formalin. The brainstem at the level of the hypoglossal nucleus was embedded in paraffin and sectioned by microtome at 8 µm thickness. IHC analyses will allow us to evaluate for histopathological biomarkers of neurodegeneration in DM and further establish canine DM as a disease model for ALS.
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VALIDATION OF A HUMAN, AUTOMATED, ENZYMATIC ASSAY FOR DETERMINATION OF SERUM AND PLASMA LITHIUM CONCENTRATIONS IN HORSES. Mindy Wolfe, Philip Johnson, Elizabeth Schroepfer, Jonathon Tomkovitch, Charles Wiedmeyer (sponsor), Department of Veterinary Medicine and Surgery and Veterinary Medical Diagnostic Laboratory.

Lithium chloride (LiCl) is a therapeutic agent for the management of bipolar disorder in humans and more recently has been shown to have anti-inflammatory effects. As a result, LiCl therapy may be useful for the prevention and treatment of laminitis in horses. While the therapeutic dosage is known in other species, the therapeutic dose in horses is unknown. Also, in other species, the therapeutic verses toxic dose is very narrow. Therefore, an accurate assay is needed to measure LiCl levels in horse blood in order to assure that the plasma Li concentration does not exceed the target therapeutic range. For this study, an automated enzymatic lithium assay used for human clinical monitoring was validated to ensure the precise measurement of Li in horse plasma and serum. The assay was validated using a LiCl recovery study, assay linearity and determination of the assay’s coefficient of variation. Additionally, two horses were injected with a bolus of LiCl (600 mmol, IV) and blood drawn over a period of 24 hours at specific time points. The serum and plasma analyzed for Li concentrations. Assay stability was determined using a freeze/thaw procedure. This study resulted in a validated, automated assay that precisely measured the concentration of Li in horse blood. The assay was capable of distinguishing diminishing Li concentrations in horse serum and plasma in a pharmacokinetic manner. The validated Li assay should serve to enhance and facilitate further research using LiCl as a potential anti-inflammatory agent to aid in the prophylaxis and therapy of laminitis.

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VETERANS AND SHELTER DOGS INITIATIVE – ADOPTABILITY AMONG SHELTER DOGS.
Jaclyn Zangre, and Rebecca Johnson (sponsor), Research Center for Human Animal Interaction

Intro: Annually, tens of thousands of unwanted dogs arrive at animal shelters nationwide. It was proposed that regular training by a U.S. military veteran may facilitate shelter dogs’ adoptability, while providing constructive activity and enabling veterans’ stress and trauma relief. We expected dogs that received obedience training to become more adoptable due to their acquisition of obedience skills, and decreased stress levels attributed to positive social interactions.

Aim: The specific aims of this study were to identify the adoption rates among the canine participants and to determine to what extent dogs receiving basic obedience training from veterans had better behavior scores than the control group not receiving the training.

Methods: Ninety-one dogs from the Central Missouri Humane Society were matched for size and randomly assigned to the dog training or control group. Outcomes tracked for each group were: adoption, euthanasia, release to foster care, or breed rescue groups. Dogs in the training group received one hour of training, twice per week, for at least two weeks with a veteran. Each day, all study dogs (training or control) had their behavior assessed by an assessor blinded to group assignment using a standardized behavior assessment. Dogs were tested on skills such as recall (“come”), “sit,” “lie down,” “stay,” “heel,” “leave it,” and “off.” Dogs were scored 1-5 on each skill, with lower scores indicating better behavior.

Results and Conclusions: Preliminary findings showed that basic obedience training of shelter dogs increased the adoption rate and decreased the euthanasia rate of these dogs compared with those in the control group. Behavior scores of dogs in the training group improved while scores of dogs in the control group did not. However this change did not reach statistical significance.
CHRONIC CYCLOSPORINE TREATMENT DOES NOT REDUCE TOTAL LV COLLAGEN AND FIBROSIS IN MINI-SWINE WITH HEART FAILURE. Pamela J. Zgoda*, Noelany Cruz Rivera*, Jessica A. Hiemstra, Melissa S. Cobb, Jan R. Ivey, Christopher P. Baines, Craig A. Emter
*Co-first authors, †Biomedical Sciences and ‡Dalton Cardiovascular Research Center

Cardiac extracellular matrix remodeling is a pathological process that may negatively affect the mechanical properties of the heart in patients with heart failure with preserved ejection fraction (HFpEF). The remodeling process is partially regulated by the loss of cardiomyocytes through cell death pathways mediated in part by the mitochondria. Our laboratory previously showed low intensity interval exercise training attenuates mitochondrial dysfunction, characterized by increased mitochondrial permeability transition (MPT). Conventional treatments have failed to improve the prognosis of HFpEF patients, and there is a critical need for generating novel treatment options for those diagnosed with the disease. Therefore we hypothesized a reduced, non-immunosuppressive dose of the drug cyclosporine (CsA; a general cyclophilin inhibitor) would block MPT via inhibition of cyclophilin D, a key component of the MPT pore, and attenuate the development of HFpEF via inhibition of cell death pathways and subsequent fibrotic myocardial remodeling. The purpose of this study was to examine the effects of CsA on extracellular matrix remodeling in aortic-banded mini-swine divided into three groups (n=5); control non-banded (CON), HFpEF non-treated (HF), and HFpEF treated with CsA (HF-CsA; 2 mg/kg/day). CsA treatment began 6 weeks after banding and continued for 14 weeks. Tissue was isolated from the left ventricle (LV). Picosirius Red Stain was used to determine total LV collagen and Masson’s Trichrome Stain was used to determine total LV fibrosis. Fibrotic remodeling was assessed as percent area and density. The percent area of both collagen and fibrosis increased by approximately 35% in both aortic banded groups regardless of treatment. Collagen staining density was increased only in the HF group. In conclusion CsA treatment did not decrease total LV collagen or fibrosis in HF. Future directions include examination of regulators of fibrotic remodeling, including Matrix Metalloproteinases (MMPs) and their Tissue Inhibitors (TIMPs).
VERTEBRAL ABNORMALITIES CHARACTERIZED IN JAPANESE BOBTAILS. Delia M. Bouhan¹, LA Lyons¹,², B Gandolfi¹, RE Pollard², ¹MU College of Veterinary Medicine and ²University of California-Davis School of Veterinary Medicine.

The Japanese Bobtail is an ancient breed, said to have arrived in Japan around 600-700 A.D. with the Buddhist monks. Phenotypically, the breed is distinguishable by its shortened, kinked tail. This trait was introduced into a colony and was determined to have autosomal dominant inheritance with possible genetic modifiers accounting for the variability in tail presentation. Full radiological examination of Japanese Bobtail cats shows variation from the normal feline vertebral formula, including transitional vertebrae, hemi-vertebrae, and abnormal rib placement. Determining the genetic area of the mutation could provide insight to how the spinal column is developed in all vertebrates, including humans. A genome wide association study was attempted using 20 Japanese Bobtail cases and 80 controls of different breeds. The selection of breeds was based after assessing the available samples by multidimensional scaling. Genome wide signature of selection was attempted across the genome looking for reduced heterozygosity. A strong association has not yet been identified for this trait. This is likely due to the mutation being very ancient, possibly having different genetic backgrounds, low linkage disequilibrium within the breed, and low density of SNPs on the array. However, nine cats have now been whole genome sequenced; including five cats with the bobtail trait and four controls. Variant scanning of the sequencing data will hopefully identify candidate genes for the bobtail trait.

LONGITUDINAL MICROBIOME ANALYSIS IN A RAT MODEL OF COLON CANCER

Susheel Bhanu Busi, and James M. Amos-Landgraf (sponsor), Department of Veterinary Pathobiology

Colon cancer is the third most common cancer diagnosed in men and women. In addition to heritable genetic changes that affect an individual’s predisposition, environmental factors including the gut microbiota may play an important role in disease susceptibility. Using the Polyposis in the Rat Colon (Pirc) model we followed microbiome diversity and its effect on cancer development. We collected fecal pellets from 19 F344N/Tac rats (12 male and 7 female) at 3 different points over 3 months, including both Pirc and wild type. In addition, we characterized the microbiota of two congenic derivatives of the Pirc rat: one on a sensitive August-Copenhagen Irish genetic background and the other on a resistant Brown-Norway background. Mutant and wild-type Pirc rats were used to understand the diversity and stability of the gut microbiota over time. We also compared male and female rats helping shed light on the possible role of sex in disease sensitivity. Using the Illumina MiSeq platform through the University of Missouri DNA core, we sequenced the hypervariable V4 region of the bacterial 16S rRNA gene. With an average number of 86,000 sequence reads per sample, we compared the abundance of 107 operational taxonomic units (OTUs). We observed difference between the inbred genetic strains whose disease susceptibility varies. Our study provides insight into the gut microbiota as a modulator of cancer susceptibility and may also serve as a non-invasive diagnostic tool in determining the development of colon cancer based on microbiome composition and the genetics of the host.
GENOME-WIDE ASSOCIATION OF CONGENITAL HYDROCEPHALUS IN THE ORIENTAL SHORTHAIR CAT. Erica K Creighton¹, B Gandolfi¹, DP O’Brien¹, MK Keating², LA Lyons¹,²,¹ MU College of Veterinary Medicine and ²University of California-Davis School of Veterinary Medicine.

Human congenital hydrocephalus (CH) is a fairly common and often disabling disorder, however, little is known about the genetic causes of human CH. Disease models, offered by naturally occurring mutations in animals, can provide insights regarding the genetics and etiology of brain damage. To date, at least nine genes associated with hydrocephalus have been identified in model organisms, such as mouse and zebrafish. A better understanding of the disease pathway can be provided by the feline model. The domestic cats’ first known report of an inherited CH was identified in a group of closely related Oriental cats. Bred for their miniature ears and rounded heads, hydrocephalic status was discovered after a MRI was conducted on a young kitten. Detailed clinical evaluations confirmed early onset of disease in utero and variation in severity. A pedigree, genetically verified by parentage testing, confirmed an autosomal recessive and fully penetrant mode of inheritance. A genome-wide association study (GWAS) was performed using the Illumina Infinium Feline 63K iSelect DNA array data from sixteen affected cases and twenty unaffected controls from within the pedigree. The GWAS identified a 10 Mb region on cat chromosome A3 associated with CH. Visual screening of the region identified about 100 genes and several candidate genes that will be evaluated through whole genome sequencing of a trio from the Oriental pedigree. Subsequent breeding has led to a possible recombination event where hydrocephalus is observed without the small ear set. This sample will be added to the GWAS to possibly reduce the critical region. Understanding of the genetic components of heritable hydrocephalus may offer acumen into brain development and pathogenesis of developmental anomalies.

MICROBIOTA-INDUCED LYMPHOCYTE ELECTROTAXIS. Daniel J. Davis, Aaron C. Ericsson, Craig L. Franklin, and Catherine E. Hagan (sponsor), Department of Veterinary Pathobiology.

Lymphocytes traffic throughout the body from blood to secondary lymphoid tissues such as peripheral and mesenteric lymph nodes, spleen, and Peyer’s patches surveying for antigen. Upon activation, lymphocytes then undergo specific changes which allow them to leave the secondary lymphoid tissue and return to sites of inflammation or other effector sites. This complex trafficking pattern is thought to be primarily controlled through soluble chemokine gradients (chemotaxis). However, recent evidence suggests that lymphocytes have an electrotactic property which allows them to migrate toward electric fields (electrotaxis). Our research aims to determine novel pathways by which the gut microbiota alter immune function. Here we show that some microbiota compositions have distinctive electrogenic properties in vitro. Specifically, we measured the current-generating capacity of fecal samples from mice using microbial electrolysis cells, which are small-scale bioelectrical systems used to study biofuel energy applications. We found that increased electricity production from fecal samples correlates with an increased capacity to recruit lymphocytes to the gut independent of changes in chemokine gradients. The data suggest that exoelectrogenic metabolic strategies among gut microbiota may have important implications on physiology, and that electrotaxis may be an important mechanism involved in lymphocyte trafficking.
Economical and injectable antibiotics are beneficial when clinical manifestations of disease in research animals prevent the use of oral antibiotics. Ceftiofur crystalline-free acid (CCFA) is an injectable, sustained release form of ceftiofur, a third generation cephalosporin. Administered subcutaneously or intramuscularly, it is currently approved for use against susceptible respiratory tract pathogens in swine, cattle, and horses, and infectious pododermatitis in cattle. Since CCFA is an economical, injectable antibiotic that could be of value for use in MU research dogs, the objective of this study was to determine the pharmacokinetic properties of CCFA in apparently healthy dogs. Five, one-year-old dogs (24.7-26.9 kg) were accepted to be apparently healthy after no abnormalities were found on physical exam, complete blood count, and chemistry panel. Dogs were given 5.0 mg/kg of CCFA subcutaneously, and blood samples were collected prior to drug administration and 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hrs after injection. The plasma ceftiofur and desfuroylceftiofur-related metabolites were derivitized to desfuroylceftiofur acetamide (DCA) and measured by using mass spectrometry. The plasma DCA concentration versus time data were analyzed based on noncompartmental pharmacokinetics using PK Solutions 2.0®. The C<sub>max</sub> was 1.98 (SD±0.40) μg/ml, the T<sub>max</sub> was reached at 22.3 (±8.9) hrs, the half-life was 56.6 (±16.9) hrs, and the AUC<sub>0-last</sub> was 124.98 (±18.45) μg-hr/ml. Based upon MICs from common veterinary bacterial isolates cultured by the MU Veterinary Diagnostic Laboratory, 54% of respiratory pathogens, 79% of skin and wound pathogens, and 86% of the urinary tract pathogens would be susceptible to a single dose of CCFA. Based upon multiple-dose pharmacokinetic predictions, CCFA would be effective against 54% of respiratory isolates re-dosing every 72 hrs, 75% of skin and wound infections re-dosing every 96 hrs, and 84% of urinary tract isolates re-dosing every 168 hrs.

**GENIPIN EFFECTS ON CELL VIABILITY AND MECHANICAL PROPERTIES OF A BIOLOGICAL SCAFFOLD INTENDED FOR MENISCAL TISSUE ENGINEERING.**

**Farrah A. Monibi,** Pfeiffer FM, Stoker AM, Kuroki K, Sherman SL, Cook JL (Faculty Sponsor), Departments of Veterinary Medicine and Surgery and Veterinary Pathobiology.

**Hypothesis:** The purpose of the present study was to determine the effects of genipin cross-linking on a cell-seeded biological scaffold. We hypothesized that genipin would be associated with significantly higher compressive stiffness of cell-seeded scaffolds without detrimental effects on cell viability.

**Methods:** Canine meniscal fibrochondrocytes were encapsulated in 4% agarose hydrogel (1:1) to create 8 mm cylindrical constructs. Samples were maintained in media with or without continuous genipin supplementation (0.22 microM) for 42 days. Constructs (n=7) were collected from each group at days 14, 28, and 42. Samples were analyzed for compressive stiffness, cell viability, proteoglycan (GAG) content, and total collagen (hydroxyproline, HP) content. Cellular morphology and the microstructure of constructs were evaluated at days 0 and 14 using scanning electron microscopy. Histological analysis was performed at day 42. Data were compared between groups using SigmaPlot with significance set at p<0.05.

**Results:** There was nearly complete loss of cell viability in the genipin-treated constructs over the course of the study period. There was a significantly lower GAG concentration in genipin-treated constructs than in controls at day 14 (p=0.00157). There was a significant decrease in GAG concentration in both the control and genipin-treated groups between days 14 and 42 (p<0.001 and p=0.008, respectively). No HP was detected in any sample at any time point. Histological and biomechanical data analyses are pending.

**Conclusions:** Based on these data, genipin has cytotoxic effects on cell-seeded agarose constructs following 42 days of culture. These effects may be time-dependent, concentration-dependent, or both. Ongoing research in our laboratory is seeking to further elucidate the safety and efficacy of genipin prior to potential use as a cross-linking agent in future tissue engineering applications.
SELECTION AND CULTIVATION OF THE ANAEROBIC MURINE GUT MICROBE SEGMENTED FILAMENTOUS BACTERIA ON A HUMAN CELL LINE. Lisa Montoya, Turner, G., Franklin, C., and A. Ericsson (sponsor), Department of Veterinary Pathobiology.

The use of animal models in biomedical research is pivotal to advancing the field of both human and animal medicine. Unfortunately, researchers encounter changes or loss of model phenotypes associated with differences in environment or vendors. One explanation for such changes is potential differences in gut microbiota. Segmented filamentous bacteria (SFB) are anaerobic, spore-forming Gram-positive bacteria found in the intestinal tracts of several species, including humans. Once thought to be a non-pathogenic member of the commensal microbiota, SFB has been shown to modulate the development and maturation of the mucosal immune system. More importantly, the presence or absence of SFB can alter many mouse model phenotypes ranging from intestinal to systemic disease models. However, the inability to culture SFB in vitro is a hindrance to studying the mechanisms of these changes. The purpose of this study was to establish a reproducible method to culture SFB in vitro. We hypothesize that SFB requires a low oxygen environment and cells to mediate attachment, sporulation, and replication outside the host. We tested two candidate cell lines: Caco-2, a human colonic carcinoma, and 3T3, a mouse fibroblast line paired with several incubation conditions including aerobic, anaerobic, and two microaerophilic environments. Cell lines were inoculated with either serial dilutions of mouse ileal scrapes with or without chloroform treatment to isolate spore forming species or pure SFB spores. We found that inoculation of SFB spores onto an established monolayer of Caco-2 cells while providing a low oxygen environment for incubation resulted in germination and survival for up to seven days in culture. These data describe a cell culture technique that shows great promise for in vitro propagation of SFB for use in studies of intestinal and systemic diseases.

OPTIMIZATION OF MORPHOLINO MODIFIED ASO VARIANTS TARGETING INTRONIC REPRESSOR ELEMENT 1 IN A MOUSE MODEL OF SMA. Charles W. Washington III, Erik Y. Osman, Christian L. Lorson (sponsor), Department of Veterinary Pathobiology.

Spinal Muscular Atrophy (SMA) is the second most common autosomal recessive disorder with an incidence of ~1:6000 and a carrier frequency of ~1:35. A neurodegenerative disease, SMA is characterized by the degeneration of motor neurons within the anterior horn of the spinal cord, leading to skeletal muscle weakness, atrophy, and even death. SMA is caused by the loss of the Survival Motor Neuron gene, SMN1. SMN2 is a nearly identical copy of SMN1 and present in all SMA patients. Although the SMN2 coding sequence has the potential to produce full-length SMN protein, about 90% of SMN2-derived transcripts encode a truncated protein lacking exon7. Previously, an intronic region called Element 1 (E1) has been characterized as a repressor of SMN2 exon7 inclusion. Earlier, the Lorson lab developed a morpholino-based ASO sequence against E1, resulting in inhibition of repressor function and significant phenotypic improvements in two mouse models of SMA. Our previous work has shown promising results with delivering specific morpholino modified ASOs targeting the E1 repressor. In order to optimize inhibition of E1, we have designed multiple morpholino chemistry ASOs specifically targeting various lengths and segments of the intronic repressor region. In this study, we have developed twelve variants of the original ASO sequence to determine which ones have the most effective impact on SMA phenotype. We tested these ASOs in a severe SMA mouse model (mSmn−/−; hSMN2+/+; SMNΔ7+/+) via intracerebroventricular (ICV) injections. Animals were injected on P1 and were monitored daily for changes in SMA phenotype. We observed different degrees of phenotypic differences after ASO delivery. Life-span extension and weight gain varied. Molecules that block or inhibit the repressive activity of Element 1 could be envisioned as potential therapies for SMA if they relieve the repression and allow for high levels of full-length SMN expression from the SMN2 gene.
Spinal muscular atrophy (SMA) is a neurodegenerative disorder, traditionally characterized by the loss of motor neurons in the ventral horn of the spinal cord. Research in mouse and fly models of SMA showed that neither restoration nor depletion of full length SMN exclusively in motor neurons elucidated the expected phenotypes, suggesting that SMA may be a multi-system disorder. More recently, it was demonstrated that the early disruptions in induced pluripotent stem cell (iPSC)-derived astrocytes may contribute to the SMA pathology. We investigated whether astrocytes contribute to SMA pathogenesis. Using the SMNΔ7 mouse model, we restored full length SMN to astrocytes via intraventricular (IV) injections. We found when SMN is restored to astrocytes there is a significant improvement in phenotype. Survival was increased by approximately 20 days which correlated to an increase in weight gain and to a better performance in time-to-right, which tests for gross motor function. These results indicate that SMA is not solely a disease affecting lower motor neurons, but astrocytes may also have a contribution towards the pathogenesis of SMA. This may be important to consider when choosing targets for future gene therapy in patients.
MECHANISMS UNDERLYING HIGH MTOR LEVEL IN BREAST CANCER. Mohamed Alalem and Bimal Ray (sponsor), Department of Veterinary Pathobiology.

Mammalian target of rapamycin (mTOR) is a central regulator of various intracellular and extracellular stimuli. mTOR is a serine/threonine kinase which transduces signaling from growth factors, such as insulin, to stimulate mRNA translation. Insulin activates mTOR through PI3K-Akt-mediated phosphorylation and consequent activation of protein machinery to promote protein synthesis as well as to inhibit protein degradation. Increased protein synthesis in cancer cells contributes to cancer cells proliferation and progression. mTOR protein level is higher in breast cancer cells compared to normal breast epithelial cells. But the exact mechanism(s) underlying the high level of mTOR in breast cancer cells are not fully understood. High mTOR level in breast cancer cells could be attributed to an increase in its expression and/or a decrease in its degradation. Protein degradation could be either proteasome-mediated or autophagy-mediated processes. The objective of this study was to investigate some of the potential mechanisms which could be implicated in the increased mTOR level in breast cancer cells. Moreover, some novel potential therapeutic agents were tested for decreasing mTOR level in breast cancer cells. We found that the therapy-induced decrease in mTOR level was correlated with a decrease in viability and proliferation of breast cancer cells.

GENERATION OF TETRACYCLINE-INDUCIBLE EQUINE HOOF KERATINOCYTE AND FIBROBLAST CELLS TO STUDY EQUINE HOOF WOUND HEALING AND LAMINITIS.

Lori Gutzmann, Phillip J. Johnson and Rajiv Mohan (sponsor), Department of Biomedical Sciences and Department of Veterinary Medicine and Surgery

Hypothesis: Equine hoof keratinocyte (EHK) and equine lamellar fibroblast (ELF) are difficult to isolate from hoof tissue and grow in culture. We sought to immortalize EHF and ELF cells using tetracycline-inducible lentivirus vector system to study the role of epithelial-mesenchymal-transformation (EMT) in hoof wound healing and laminitis development in horses.

Methods: Normal and laminitic equine hoof tissues collected from horses were cultured or snap frozen in liquid nitrogen. Thoracic limb hoof tissues of normal horses were used to generate primary ELF cultures using 2 methods. In one method tissues were dropped in DMEM containing antibiotics immediately after collection, minced, incubated in dispase, washed, placed in culture dishes containing DMEM medium with 10% serum and incubated in a humidified CO2 incubator at 37°C. In the second method, tissues were cut into small pieces with a surgical blade, placed in culture dishes wetted with DMEM medium containing 10% serum, and incubated in a humidified CO2 chamber at 37°C. Cryofrozen equine hoof (developmental, acute and chronic stages) tissues were sectioned and immunostained for myofibroblasts (α-smooth muscle actin) and extracellular matrix proteins (F-actin, fibronectin and β-4 integrin) using appropriate antibodies. Fluorescence microscopy was used to quantify proteins. Student's t-test or Wilcoxon rank sum test was used for statistical analysis.

Results: A statistically significant increase in myofibroblast, F-actin and fibronectin expression was detected in laminitic equine hooves compared to the normal controls (p<0.001). Highly disorganized β-4 integrin immunostaining was detected in laminitic hoof tissues compared with normal hoof tissues (quantification and statistical analysis pending). In vitro EHK and ELF immortalization to understand molecular mechanisms is underway.

Conclusions: Myofibroblasts are likely to play an important role in laminitis pathophysiology.
DEVELOPMENT OF AN EQUINE MICROCHIMERISM ASSAY. Sarah Hansen, James Amos-Landgraf, Senthil Kumar, Jeffrey Bryan (sponsor), Departments of Veterinary Pathobiology and Veterinary Medicine and Surgery.

Fetal microchimerism (FMc) is the presence of fetal cells persisting in maternal tissue immediately following and for years after pregnancy. These cells are thought to be stem-like progenitor cells that become actively engaged in the mother’s body throughout her life. This is an interesting phenomenon that is being widely studied at this time, investigating both the positive and negative health effects of the cells as well as the potential therapeutic implications. Current research shows that the presence of these fetal cells may trigger an autoimmune reaction in the mother later in her life, but also that they may provide a form of cancer protection, as well as contribute to wound healing in tissues such as the brain, heart, skin, liver and kidney. With the wide-reaching potential activity of these cells, animal models are being identified to allow for study of disease progression in a natural, time-condensed setting. Humans, rodents, cattle, and dogs are known to experience FMc. We hypothesize that we will identify the presence of Y-chromosome specific DNA sequences in the peripheral blood of mares following pregnancy with a colt. We are developing multiple highly-sensitive, Y-chromosome-specific, nested PCR assays to test DNA extracted from the peripheral blood of parous mares. At this time we have collected blood samples and extracted DNA from the buffy coat and plasma of geldings, nulliparous mares and parous mares. We have identified a Y-chromosome-specific sequence and validated its presence/absence by testing 10 geldings and 4 nulliparous mares. In addition, we have sequenced a previously-deposited X/Y amelogenin polymorphism for development of an additional Y-specific assay. We anticipate testing parous mares within the upcoming month as more foals are born.

LIKELIHOOD OF PREDIABETES IN NHANES 2007-2012 FROM CHEMICAL EXPOSURES.
Steven Hanson and Chuck Wiedmeyer (sponsor), Veterinary Medical Diagnostic Laboratory and Department of Pathology and Anatomical Sciences

The increasing prevalence of diabetes in humans and companion animals has lead to the hypothesis of a shared exposure to chemicals which interact with glucose homeostasis mechanisms leading to dysglycemia. Data from the National Health And Nutrition Examination Survey (NHANES) 2007-2012 was used in this study. Multivariate logistic regression was performed using SAS to determine odds ratios for the prediabetic state (%HbA1c > 5.6 for subjects with normal fasting plasma glucose and two-hour oral glucose tolerance test levels and not diagnosed with diabetes) in subjects with elevated (defined as greater than the median or inflection point if this was greater than the median) levels of the measured chemicals in blood or urine as well as anxiety levels as a measure of allosteric load. Since the purpose of this study is not to assert significance without further study, but rather to help direct research projects better designed to detect possible pathophysiological interactions resulting in dysglycemia, p values close to but not exceeding 0.05 are reported as warranting further investigation. Significantly altered odds ratios (OR) were found with elevated levels of cadmium (OR 1.745, p=0.0317), urinary benzophenone-3 (OR 0.554, p=0.0044), perfluorooctanoic acid (OR 0.625, p=0.0013), perfluorohexane sulfonic acid (OR 0.662, p=0.0589), perfluoroheptanoic acid (OR 2.267, p=0.0280), and perfluorononanoic acid (OR 1.756, p=0.0032), as well as high anxiety, defined as feeling anxious more than 15 days each month, (OR 1.352, p=0.0538). The results of this study are meant to guide the development of model organism experiments, as well as shape potential human studies using continuous glucose monitoring and glycated albumin. Further statistical analysis will be done to assess the interaction of these same covariates with insulin resistance observed in the NHANES study.
IMPACT OF REDERIVATION ASSOCIATED MICROBIOME CHANGES ON A MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE. Marcia L Hart; J Cornelius-Green; A Goerndt; AC Ericsson; C Franklin (sponsor), Department of Veterinary Pathobiology.

Rederivation of mice is a common practice used to render animals free of unwanted infectious diseases. This also occurs when mutant mouse strains are maintained as cryopreserved germplasm and subsequently recovered for use in research. While rederivation eliminates unwanted pathogens, it may also result in changes in microbiota that impact model phenotypes. To this end, we investigated whether rederivation of common mouse models of inflammatory bowel disease (IBD) onto different recipient strains of mice would result in changes in microbiota that correlate with changes in disease severity. C57BL/6 and C3H/HeJBir mice with targeted mutations in the IL-10 gene were rederived onto CD-1 or C57BL/6 (B6) recipient mothers with distinct microbial compositions. To induce inflammatory bowel disease, pups were inoculated with Helicobacter hepaticus at 3 and 5 days post weaning. Cecal lesion scores and changes in microbiota were evaluated at 90 days post inoculation using histopathology and 16S rRNA gene sequencing respectively. Differences in both the microbiota and inflammatory lesion scores were seen between mice derived onto either CD1 or B6 recipients. These findings suggest that intestinal microbiota that is obtained during rederivation, can alter mouse model phenotypes.

SAXAGLIPTIN AND TADALAFIL DIFFERENTIALLY ALTER LEFT VENTRICULAR MECHANICS IN A TRANSLATIONAL MINIATURE SWINE MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION. Jessica A. Hiemstra, Melissa S. Cobb, Jan R. Ivey, Craig A. Emter (sponsor), Department of Biomedical Sciences

We previously demonstrated pathological cardiac remodeling in a swine model of heart failure with preserved ejection fraction (HFpEF) was associated with altered left ventricular (LV) mechanics. The inhibitory effect of cGMP (cyclic guanosine 3',5'-monophosphate) on pathological hypertrophic signaling is well established. Reduced cGMP signaling in HFpEF may occur as a result of dysfunctional production or enhanced catabolism via increased phosphodiesterase (PDE) activity. We hypothesized preservation of cGMP expression would attenuate pathological remodeling and improve LV mechanics in HFpEF. Thus, the purpose of this study was to promote cGMP signaling via two mechanisms: 1) the DPP4 inhibitor saxagliptin; and 2) the PDE5 inhibitor tadalafil. We assessed LV mechanics with 2D strain echocardiography 1 and 3 months post-aortic banding in mini-swine divided into four groups (n=5); control non-banded (CON), HFpEF (HF), HFpEF saxagliptin-treated (HF-SAX) and HFpEF tadalafil-treated (HF-TAD). Tadalafil attenuated compensatory increases in systolic strain and early diastolic strain rate observed in the HF and HF-SAX groups. In contrast, saxagliptin prevented increases in late diastolic strain rate associated with enhanced atrial systole observed in HF and HF-TAD animals. Saxagliptin increased torsion transiently (1 mo.), although at 3 mo. both apical and early diastolic rotation rate were increased only in the HF-TAD group. In conclusion, LV mechanics were distinctly altered in response to separate methods of pharmacological cGMP regulation. Our results suggest the mechanism by which cGMP signaling is promoted may play a role in LV mechanical adaptations to developing HFpEF.
Heart failure with preserved ejection fraction (HFpEF) is difficult to diagnose given the compensated state of resting cardiac function, and conventional treatments have failed to improve the prognosis of this HF sub-group. A critical need exists for novel treatment options and effective non-invasive mechanisms of clinical diagnosis. We hypothesized a reduced, non-immunosuppressive dose of cyclosporine (CsA; inhibiting only cyclophilin D, a key component of the mitochondrial permeability transition (MPT) pore, and not calcineurin) would attenuate the development of HF via improved myocardial energetics. The purpose of this study was to assess left ventricular (LV) mechanics in aortic-banded mini-swine divided into three groups; control non-banded (CON), HFpEF non-treated (HF), and HFpEF treated with CsA (HF-CsA). LV mechanics were measured acutely and chronically (2 & 14 weeks post-treatment) using 2D strain echocardiography. CsA acutely reduced atrial systole evident by a decrease in late diastolic longitudinal and radial strain rate compared to CON. At 14 weeks, both measures were increased in HF and HF-CsA groups despite observing elevated LV end diastolic pressure (LVEDP) in HF-CsA animals only. CsA treatment decreased torsion and global apical systolic rotation rate to a greater extent than seen in the HF group at both 2 and 14 weeks, and depressed systolic mechanics in general including longitudinal, circumferential, and radial global systolic strain and displacement compared to CON. In conclusion, CsA treatment alters mechanical properties of the LV that reduce atrial systole acutely, but chronically accelerates mechanics associated with decompensated HF. While CsA does not appear to be a viable therapeutic alternative for HFpEF, our results indicate 2D strain is an effective noninvasive diagnostic tool for evaluating therapies in these patients. Further, 2D strain detected impaired LV mechanics indicative of diastolic dysfunction prior to a significant increase in LVEDP, demonstrating its clinical relevance as an early diagnostic tool for HFpEF.
**THE MITOCHONDRIAL PROTEIN FASTKD1 PROTECTS CELLS FROM OXIDATIVE STRESS INDUCED DEATH INDEPENDENTLY OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE.** Kurt D. Marshall and Christopher P. Baines (sponsor), Department of Biomedical Sciences and Dalton Cardiovascular Research Center

The mitochondrial permeability transition pore (MPTP) is a non-specific pore in the inner-mitochondrial membrane that can open during pathological states including ischemia/reperfusion injury, muscular dystrophy and doxorubicin cardiotoxicity. Excluding the accessory protein Cyclophilin D (CypD), the makeup of the MPTP is unknown. The purpose of these experiments was to identify MPTP components or modulators by analyzing proteins that associate with CypD. Utilizing a yeast-two hybrid system, we identified the mitochondrial protein Fas-activated serine/threonine phosphoprotein kinase domain-containing protein 1 (FASTKD1) as a novel CypD interacting protein. We therefore hypothesized that FASTKD1 modulates the function of the MPTP and therefore cell death by interacting with the pore sensitizing protein CypD. To test this hypothesis, we knocked down FASTKD1 with small-interfering RNA (FASTKD1si) in mouse embryonic fibroblasts (MEFs) or over expressed FASTKD1 using an adenovirus (adFASTKD1) in both MEFs and neonatal rat cardiac myocytes (NRCMs). Neither FASTKD1si nor adFASTKD1 altered the expression of purported MPTP components in MEFs, although we detected a significant decrease in adenine nucleotide translocase 1/2 (ANT1/2) in NRCMs. FASTKD1si sensitized MEFs to H$_2$O$_2$ but not ionomycin induced cell death. Additionally, FASTKD1si increased mitochondrial membrane potential ($\Delta\Psi_m$) at baseline but not following H$_2$O$_2$ treatment and did not alter cellular reactive oxygen species (ROS) or mitochondrial oxygen consumption (mVO$_2$). AdFASTKD1 protected MEFs and NRCMs from H$_2$O$_2$ induced cell death but did not protect MEFs from ionomycin-induced death. AdFASTKD1 uncoupled mVO$_2$ at complex I, decreased $\Delta\Psi_m$ and decreased ROS following H$_2$O$_2$ treatment in MEFs. In NRCMs, adFASTKD1 also decreased $\Delta\Psi_m$ and decreased ROS at baseline. Finally, adFASTKD1 did not alter the calcium retention capacity of MEFs, and adFASTKD1 was still able to protect CypD-deficient MEFs against H$_2$O$_2$ induced cell death. In conclusion, FASTKD1 protects cells from oxidative stress induced death, but this protection appears to be independent of the MPTP.

**A NOVEL METHOD FOR TARGETED CELL ABLATION IN MULTIPLE SPECIES UTILIZING HUMAN CD59 AND INTERMEDILYSIN.** Marina R. McCoy, Jennifer Cornelius-Green, Suman Gurung, Anand Chandrasekhar, Xuebin Qin, Elizabeth C. Bryda (sponsor), Life Sciences Center and Department of Veterinary Pathobiology

The ability to selectively ablate cell types in model organisms is a powerful tool for understanding mechanisms of disease. Current methodologies have limitations with respect to the types of cells that can be targeted, the lack of specificity of ablation and the lack of appropriate methodologies to facilitate their use across species. The goal of our study is to provide proof of concept that intermedilysin (ILY) administration to ablate cells expressing human CD59 (hCD59) provides a sensitive, specific, and versatile tool for cell ablation in rats and zebrafish. While effective cell ablation using this system has been demonstrated previously in mice, it has not been tested in other model organisms. We generated a new transgenic rat line which expresses human CD59 (hCD59) specifically on erythrocytes rendering them susceptible to lysis by administration of the otherwise inert bacterial toxin, intermedilysin (ILY). Erythrocytes from a transgene positive founder rat found to express hCD59 via flow cytometry, were susceptible to lysis in a dose-dependent manner by ILY using an *in vitro* hemolysis assay. This new erythrocyte ablation model provides a valuable tool for studying hemolytic anemias. We have initiated experiments to create a rat pancreatic beta cell ablation model to confirm that the hCD59-ILY system is effective for cell ablation within whole organs. To demonstrate applicability in non-rodent models, we are testing the efficacy of the hCD59-ILY system in zebrafish. The use of hCD59-ILY will have wide application for any studies in any species that can benefit from selective cell ablation *in vivo*. 
LOSS OF CFTR RESULTS IN INTESTINAL STEM CELL HYPERPROLIFERATION. Ashlee M. Williams, Liu J, Walker NM, Clarke LL (Sponsor), Department of Biomedical Sciences and Dalton Cardiovascular Research Center.

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (Cftr) and CF patients have a 6-fold increased risk of gastrointestinal (GI) cancer (NEJM 332:494-99, 1995). Cftr is the major anion channel expressed in intestinal crypts and regulates intracellular pH (pHi) via HCO\textsubscript{3} efflux. Cftr loss results in an alkaline pHi, which can facilitate proliferation through cell cycle progression and Wnt/β-catenin signaling (J Biol Chem 278(45):44645-9, 2003; Nat Cell Biol 11(3):286-94, 2009). CF mouse models also exhibit increased intestinal proliferation (Am J Physiol 281:G681-87, 2001). To test the hypothesis that Cftr normally suppresses intestinal stem cell (ISC) proliferation, we crossed CF mice with mice expressing Lgr5-eGFP which marks ISCs. Crypts from CF-Lgr5-eGFP mice showed a greater number of Lgr5+ ISCs as compared to wild-type (WT). ISCs sorted from intestinal epithelium expressed Cftr and, using intestinal organoids, Lgr5-eGFP ISCs underwent alkalization during Cftr inhibition. To determine if the effect of Cftr on ISC proliferation is cell-autonomous, crypt cells from WT and CF mice were cultured as enterospheres, which are essentially a pure population of ISCs. Volume was markedly increased in WT enterospheres and could be reduced using Cftr inhibitors and increased by Cftr activation. CF enterospheres showed greater proliferation than WT as measured by EdU. Quantitative RT-PCR found altered transcription of acid-base transporters and ISC markers in CF enterospheres. We conclude that Cftr normally suppresses proliferation of ISCs possibly by modifying pHi. Therefore, loss of Cftr may contribute to intestinal hyperproliferation in CF patients.
STRESS ON A PLATE: AN IN VITRO ASSAY TO PREDICT EFFECTS OF ACUTE STRESS ON GLUCOCORTICOID SENSITIVITY OF DOG LEUKOCYTES. **Lydia C. Cook**, Ava Song, Celia F. Cowan, Amy E. DeClue & Catherine E. Hagan (sponsor), Departments of Veterinary Medicine and Surgery and Veterinary Pathobiology.

Although the physiologic response to stress can vary significantly between individuals, the cells and tissues of “healthy” people typically become sensitized (more responsive) to the effects of glucocorticoids after acute stress. Glucocorticoids are a hormone used by the body to maintain homeostatic control over immune responses and can be administered therapeutically to control inflammation. However in some disease states such as major depression, the effect of acute stress has the opposite effect with cells and tissues being desensitized to glucocorticoids. In both human and animal patients, the degree of glucocorticoid responsiveness of the immune system has potentially significant implications with respect to vulnerability to disease and prognostic health outcomes. We sought to validate an assay utilized in humans to measure leukocyte sensitivity to glucocorticoids in dogs, and to determine whether we could predict the effects of an acute stressor *in vivo* with epinephrine, a hormone released in the fight or flight response. Our data suggest that healthy dogs are also sensitized to the effects of glucocorticoids after exposure to an acute stressor (an overnight stay in the hospital), and that we can mimic the effects of acute stress on leukocytes using epinephrine in culture. Future studies using this functional assay of glucocorticoid sensitivity in dogs will determine the extent to which it could be a useful prognostic tool for predicting prognosis in critical care patients such as dogs vulnerable to sepsis or in dogs with abnormal behavior (e.g., dogs at risk of euthanasia due to chronic behavioral problems).

INJECTION OF *YERSINIA PESTIS* –YOPK MODULATES INNATE IMMUNE RESPONSES IN THE LUNG TO SET UP THE EARLY BIPHASIC INFLAMMATORY RESPONSE OF PNEUMONIC PLAGUE. **Miqdad O. Dhariwala**, Kristen N. Peters and Deborah M. Anderson (sponsor), 1Department Of Veterinary Pathobiology

*Yersinia pestis* is the causative agent of Pneumonic Plague, a primary bronchopneumonia characterized with a unique biphasic inflammatory response. *Yersinia pestis* uses its type 3-secretion system to manipulate immune responses in the lung via killing immune cells early post-infection. Previously, we demonstrated that the secreted effector protein yopK modulates the progression of pneumonic plague via the induction of apoptosis in macrophages. Here we demonstrate that the dynamics of the biphasic inflammatory response in early pneumonic plague are altered with the loss of yopK. Infection with ΔyopK leads to a global increase in inflammatory cytokine production and a decrease in bacterial titers as early as 12 hours post-infection. This loss of the classical suppressive environment seen in early pneumonic plague is supported by an increase in the frequency of immune cell populations seen in ΔyopK-infected lungs. Levels of MCP-1, a neutrophil chemoattractant, are progressively increased in ΔyopK-infected lungs, which lead to an early increase in neutrophil frequencies. These data suggest that yopK suppresses early inflammatory responses, possibly by decreasing recruitment of immune cells to the lung. We therefore, investigated the role of the receptor for MCP-1; CCR2 in early pneumonic plague. Indeed, the absence of yopK led to an increase in the frequencies of CCR2+CD11c+ and CCR2+B220+ cells. The alternative hypothesis that yopK suppresses early inflammation by killing of immune cell populations, may also be true as yopK contributed to enhanced caspase-3 cleavage in vivo in CD11c+ cells. Therefore, yopK helps moderate the critical biphasic inflammatory response in early pneumonic plague.
THE AAV-MEDIATED SELECTIVE AND TARGETED SMAD7 GENE DELIVERY INTO STROMA ATTENUATES CORNEAL SCARRING. Suneel Gupta and Rajiv Mohan (Sponsor), Department of Veterinary Medicine and Surgery.

Hypothesis: Our gene silencing and overexpression in-vitro studies revealed that TGFβ1 primarily uses Smad signaling to cause corneal fibrosis. Smad7 is an inhibitory Smad that has been shown to block TGFβ-induced profibrotic Smad2/3 signaling and NFκB pathway. We sought to test the hypothesis that selective Smad7 gene delivery into keratocytes of the stroma with our recently defined topical AAV5 gene transfer method would attenuate corneal scarring in rabbits in-vivo without side effects.

Methods: New Zealand White rabbits were used. Corneal scarring was induced by 9 diopter photorefractive keratectomy using excimer laser. Smad7 into keratocytes was delivered via AAV5 (100μL; 2.67X10^{12} vg/mL) utilizing customized topical technique. Slitlamp biomicroscopy analyzed ocular health and corneal fibrosis levels in live rabbits. Real-time PCR, southern blotting, western blotting, histological immunofluorescence and confocal microscopy were used to determine delivered-gene and fibrosis parameters (α-smooth muscle actin (α-SMA), f-actin, tenacin and collagens expression) in harvested corneal tissues. In-vitro studies were performed for mechanistic studies.

Results: Slitlamp biomicroscopy showed that targeted AAV5-Smad7 gene therapy into rabbit keratocytes significantly decreased corneal scarring compared to the naked-vector control eyes (1.8±0.4; p<0.01). Densitometric analysis of southern blot showed delivery of 10^7-10^8 genomic copies in rabbit corneas. Ongoing histological immunofluorescence, western blotting and quantitative PCR analyses show notable reduced levels of α-SMA, f-actin, fibronectin and collagens in Smad7-delivered rabbit corneal tissues. In-vitro mechanistic studies showed that Smad7 overexpression inhibits corneal scarring modulating Smad2/3 levels.

Conclusions: The AAV5-Smad7 has a potential for treating corneal scarring in-vivo. Toxicity studies are warranted.

GENETIC MANIPULATION OF CARDIAC MITOCHONDRIAL PHOSPHATE CARRIER DOES NOT AFFECT PERMEABILITY TRANSITION. Manuel Gutiérrez-Aguilar, Diana L. Douglas, Anne Gibson, Timothy L. Domeier, Jeffery D. Molkentin, Christopher P. Baines (sponsor), Dalton Cardiovascular Research Center, Department of Biomedical Sciences, Department of Medical Pharmacology and Physiology, University of Missouri and Department of Pediatrics, Cincinnati Children’s Hospital Medical Center, University of Cincinnati, Howard Hughes Medical Institute.

Opening of the Mitochondrial Permeability Transition (MPT) pore is known to instigate necrotic cell death following diverse cardiac insults. Although its biochemical and pharmacological features have been profoundly studied, its molecular identity has remained an enigma. Frontier models suggest that the MPT pore regulator cyclophilin D (CypD) activates the MPT pore by binding to either the F_{0}F_{1}-ATP synthase subunit OSCP or the mitochondrial phosphate carrier (PiC). Here we validate that CypD, through its N-terminus, can directly bind PiC. We consequently generated cardiac-specific mouse strains overexpressing or with decreased levels of mitochondrial PiC to assess the functionality of such interaction. While PiC overexpression had no observable phenotypic pathology, PiC knockdown resulted in cardiac hypertrophy along with decreased ATP levels. Mitochondria isolated from hearts of these mouse lines and their respective non-transgenic controls had no divergent phenotype in terms of oxygen consumption and Ca^{2+}-induced MPT, as assessed by swelling and Ca^{2+}-retention capacity readouts. Cardiomyocytes isolated from the PiC knockdown strain and its non-transgenic counterpart showed an undistinguishable MPT pore response in vitro. Further PiC knockdown (>90%) in Slc25a3^{-/-} mouse embryo fibroblasts treated with a selective PiC siRNA showed an intact MPT pore response to Ca^{2+} and phenylarsine oxide. These results provide strong evidence indicating that the mitochondrial PiC is not a key component of the MPT pore.
BIPHENOTYPIC B/MAC CELLS IN MURINE LYME ARTHRITIS. Carrie Lasky, Carmela Pratt and Charles R. Brown (sponsor), Department of Veterinary Pathobiology.

Mice infected with the bacterial spirochete Borrelia burgdorferi develop an inflammatory arthritis that localizes in the large joints and spontaneously resolves. Using flow cytometry, we have identified a unique cell type in both joint and heart tissue which possesses both B cell and macrophage cell surface markers. Biphenotypic B/Macrophage (B/Mac) cells in murine joints and hearts are CD19*, B220*, F4/80*, CD11b*, and IgM*. B/Mac cell numbers were tracked throughout an infection timecourse, and it was discovered that numbers peaked at the beginning of resolution. This indicates that B/Mac cells may be important in the resolution of inflammation, but exact function has yet to be elucidated.

5-LIPOXYSGENASE METABOLITES AND INNATE IMMUNE CELL FUNCTION. Rachel Olson and Charles Brown (sponsor), Department of Veterinary Pathobiology

Treatment refractory Lyme arthritis occurs in approximately 10% of patients infected with Borrelia burgdorferi, the causative agent of Lyme disease, despite antibiotic treatment and bacterial clearance. The mouse model of Lyme disease recapitulates many aspects of the human disease. We have found that in the absence of the 5-lipoxygenase (5-Lo) enzyme arthritis does not spontaneously resolve as it does in wild type mice. We know Lyme arthritis to be a disease of innate immune cell dysfunction requiring neutrophils for its induction and macrophages for clearance. As such we investigated the role of 5-Lo products in neutrophils and macrophages to delineate the role of this pathway in the contribution of neutrophils and macrophages to disease resolution and clearance. We used a variety of in vitro techniques for our experiments. Our results indicate that the 5-Lo enzyme is required for normal macrophage function and is implicated in the neutrophil cell death pathway. We hope to extrapolate our results and so develop new treatments not just for patients with antibiotic refractory arthritis but other disease of innate immune cell function as well.
MURINE MICROBIOTA TRANSFER AND STABILITY ANALYSIS. Kari Chesney; Craig Franklin, Aaron Ericsson (sponsor), Mutant Mouse Regional Resource Center and Department of Veterinary Pathobiology.

Recent data show that commensal microbiota of an organism plays a key role in the development of its immune system and susceptibility to infectious and immune-mediated diseases. Mutant mice preserved as cryo-preserved germplasm are typically resuscitated using an outbred surrogate dam, resulting in colonization of pups with a different microbiota than that of the original line. This begs the question of whether the model phenotype is influenced by the differing microbiota. To address this, we have set out to create lines of an outbred stock that differ in their intestinal microbial composition, or “enterotype”. These lines will be used to assess the impact of commensal microbiota on model phenotypes. Creating these lines will require microbiota transplantation. While many studies show the microbiota of an organism can be transferred between individuals, few have looked at long-term stability of these transfers. Our study aims to characterize the microbiota of recipient mice prior to transfer, and at 2, 30 and 60 days post-transfer, using automated ribosomal intergenic spacer analysis (ARISA), to determine whether stable transfer is possible. Briefly, weanling recipients received a single dose of streptomycin to reduce the number of established bacteria in the gut, followed by gastric lavage of a fecal slurry collected from an adult donor mouse of a different background strain. We expect to show that this method will result in a permanent shift from the endogenous microbiota of the recipient to that of the donor and provide invaluable surrogate mice for future studies designed to assess the impact of commensal microbiota on mouse models of disease.

IDENTIFICATION OF COAGULASE-NEGATIVE STAPHYLOCOCCUS SPECIES IN DAIRY HEIFER CALVES AND THEIR ENVIRONMENTS. Alicia Finger, Rachel Webster, Pamela Fry, John R. Middleton (sponsor). Department of Veterinary Medicine and Surgery.

Subclinical mastitis caused by coagulase negative staphylococci (CNS) is a major contributor to increased bulk tank somatic cell counts on dairy farms. Although recent studies have demonstrated variability in pathogenicity among CNS in cows’ mammary glands, there is a lack of understanding as to the origin of intramammary infection in heifers. The aims of this study were to 1) develop efficient methods for identification of CNS in dairy heifers and their environment and 2) use these methods to characterize the ecology of CNS in dairy heifers. Samples were collected from four individually housed Holstein heifers (< 8 weeks of age) and their environments. Body site samples included perineum, inguinal regions, teat skin, hair coat, and muzzle. Environmental samples included feed bucket, water bucket, hutch, hay, and bedding. Samples were collected with a sterile gauze or electrostatic duster, and samples were incubated using various enrichment methods and growth conditions. Species were identified using the rpoB gene sequence. A total of 1,280 samples yielded 628 CNS isolates, and 283 isolates have currently been speciated. The rpoB sequence did not yield a definitive species identification for 197 isolates (69.6%). Of those isolates definitively speciated (n = 86), S. chromogenes was most prevalent (30.2%) followed by S. haemolyticus (26.7%). Data suggests that the rpoB gene sequence is not an ideal single-gene protocol for CNS speciation. Future work will include identification of an accurate speciation method, and data will be analyzed to determine which isolation and in vitro growth conditions are optimal.
THE EFFECTS OF VARIOUS HYDRATION SOLUTIONS AND METHODS ON CHONDROCYTE VIABILITY AND WATER CONTENT, A SURGICAL MODEL. W. Dane Foxwell, Aaron Stoker (sponsor) and James L. Cook, Comparative Orthopaedic Laboratory.

Currently there are no standard procedures used for hydrating exposed cartilage during surgery and no research exists regarding the potential damage done to cartilage during this exposed period. Given these limitations in light of the critical importance for cartilage preservation during joint surgery there is an obvious opportunity for improvement of methods for maintaining chondrocyte viability (CV) and cartilage water content (WC) during surgery. This research had two goals. First, to determine if a significant difference in cartilage CV or WC exists between non-hydrated exposed cartilage (NHC) and hydrated exposed cartilage (HC). Second, to determine if significant differences in CV or WC would occur based on differences in various solutions used to hydrate exposed cartilage and, if so, to determine which hydration solution is optimal for retaining maximum CV and physiologic WC.

In order to compare various hydration solutions, canine femoral condyles (n=7) treated with saline, culture media, hyaluronan, or left untreated, were exposed to surgical lights for 2 hours. Two outcome measures were used: 1) percent chondrocyte viability measured by LIVE-DEAD assay, and 2) percent water content measured by determining weight and dry weights of tissues. After completing the experiments, data analyses suggested that technical errors in sample and data processing resulted in inaccurate and unusable data. Several factors contributed to the study’s failure including tissue processing for WC, quality of CV images, and poor time management and prioritization on the part of the student researcher. Examples of data, and the associated problems, will be presented and discussed.

THE TRPV1 AGONIST CAPSAICIN IS AN INEFFECTIVE BRONCHOPROVOCANT IN AN EXPERIMENTAL MODEL OF FELINE ASTHMA. Stacy Krumme, Megan Grobman, John R. Dodam, Carol R. Reiner (sponsor), Department of Veterinary Medicine and Surgery.

Airway hyperresponsiveness (AHR), defined as an excessive narrowing of the airways in response to a stimulus, is a key feature of feline asthma. Limitations of both direct and indirect bronchoprovocants evaluated in experimental feline asthma has led to a search for a more specific indirect bronchoprovocant (i.e., one which relies on existing inflammatory cells or activated neural pathways in diseased but not healthy airways). Capsaicin is an agonist to the transient receptor potential cation channel subfamily V member 1 (TRPV1). TRPV1 stimulation contributes to bronchospasm and neurogenic inflammation through release of Substance P (SP) and Neurokinin A (NKA). This study hypothesized that in experimentally induced asthmatic cats, aerosolized capsaicin would lead to dose-responsive increases in airway resistance measured by ventilator-acquired pulmonary mechanics. Five experimentally asthmatic cats were enrolled in the study. Cats were premedicated with ketamine (30 mg IV), and induced and maintained with propofol (6 mg/kg IV and 0.3 mg/kg/min IV, respectively). Cisatracurium (0.1 mg/kg IV with additional doses as needed) was administered for neuromuscular blockade. Ventilator-acquired pulmonary mechanics was performed in mechanically ventilated cats. Pulse oximetry measured hemoglobin desaturation. Ten-fold increases of capsaicin (0.4-4000 µM) aerosolized for 30 seconds were administered; each dose was followed by 4 minutes of data collection. The study endpoint was a doubling of baseline airway resistance (Raw), halving of compliance or oxygen desaturation <75%. All cats completed the trial reaching the highest dose of capsaicin without reaching any of the aforementioned endpoints. Capsaicin did not result in concentration-dependent increases in Raw in asthmatic cats and is an ineffective bronchoprovocant in the feline asthma model. This may be due to a modulated receptor environment in asthmatic cats resulting in depletion of SP and NKA.
EVALUATION OF THE IN VITRO DOSE-DEPENDENT EFFECTS OF RESVERATROL ON INNATE IMMUNE FUNCTION IN DOGS. Rowena Woode, Sandra Bechtel (sponsor), Yan Zhang, Juliana Amorim, Kaoru Tsuruta, Amy DeClue, Department of Veterinary Medicine and Surgery.

Resveratrol, a naturally-occurring phytophenol, has dose-dependent immunomodulatory activity in rodents and humans. Although safety and pharmacokinetic studies have been completed in dogs, no study to date has investigated the immunologic effects of resveratrol in dogs. The objective of this study was to determine the effect of resveratrol on canine innate immune cell function in vitro. We hypothesized that resveratrol would demonstrate a dose-dependent effect on immune cell function in dogs similar to other species, with low concentrations stimulating the innate immune system and high concentrations inhibiting innate immune system function. Whole blood samples were collected from 6 healthy, adult, client-owned dogs. Immediately after collection, each blood sample was incubated with high (6,000ng/mL), intermediate (3,000ng/mL) or low (1,000ng/mL) concentrations of resveratrol or control solution for 4 hours. Following incubation, leukocyte phagocytosis and oxidative burst were assessed using commercially available test kits and flow cytometry. Stimulated leukocyte cytokine production (TNF-α, IL-6 and IL-10) was evaluated using whole blood culture and a canine-specific multiplex bead assay. Phagocytosis was not altered by resveratrol at any concentration compared to control. While no difference in the percentage of cells performing oxidative burst was found, the robustness of Eschericia coli-and PMA-induced oxidative burst was significantly less in the resveratrol group compared to control, which appeared to be a concentration-dependent effect. Incubation with resveratrol also resulted in increased pro-inflammatory (TNF, IL-6) and decreased anti-inflammatory (IL-10) cytokine production in canine leukocytes. These data suggest that resveratrol has different innate immune system effects in dogs than other species and further study is indicated prior to routine supplementation.
DECREASING MORBIDITY ASSOCIATED WITH DIAGNOSTIC AIRWAY LAVAGE IN CATS. Christa Bernhard, Masseau I, Dodam J, Outi H, Krumme S, Grobman M, Kerl M, Reinero C (sponsor), Department of Veterinary Medicine and Surgery.

Introduction: Bronchoalveolar lavage (BAL) may induce hypoxemia and anesthesia-induced atelectasis. We hypothesized that lung function and CT evidence of atelectasis would be modified by altering inspired oxygen concentration and applying positive end expiratory pressure (PEEP) in cats undergoing BAL.

Methods: Six experimentally asthmatic cats underwent BAL, each under four randomized treatment conditions: (1) 100% oxygen, no PEEP, (2) 30% oxygen, no PEEP, (3) 100% oxygen, PEEP=2 cmH\(_2\)O and (4) 30% oxygen, PEEP=2 cmH\(_2\)O. Pulse oximetry was used to measure oxygen saturation. Baseline ventilator-acquired pulmonary mechanics and CT scans were collected prior to BAL, and at 1, 5, and 15 minutes post-BAL.

Results: While receiving 100% oxygen, no cat had SpO\(_2\) below 91% during or after BAL. Although cats treated with 30% oxygen had substantial desaturation, nearly all (22 out of 24 trials) had SpO\(_2\) greater than 90% by 1 minute post-BAL. Following BAL, all cats in all treatment groups had increased airway resistance, decreased lung compliance, and CT evidence of increased attenuation and decreased lung volume. Treatment (2) had significantly lower minimum compliance following BAL than treatments (3) and (4) (p<0.05). For maximum airway resistance and percentage increase of airway resistance over baseline, there was no significant difference between treatments (p=0.40 and 0.12, respectively). Preliminary CT data suggested less attenuation and higher lung volumes with the addition of PEEP.

Conclusion: Pulse oximetry may not correlate with expected changes in pulmonary mechanical function or lung anatomy. Addition of PEEP may improve lung compliance and decrease CT evidence of atelectasis after BAL.

THE ROLE OF FETAL MICROCHIMERISM IN MATERNAL CORNEAL WOUND HEALING. Michael K. Fink\(^1\), Elizabeth A. Giuliano\(^2\), Jeffrey N. Bryan\(^2\) (sponsor), and Rajiv R. Mohan, Ph.D\(^2\) (sponsor), Departments of Veterinary Pathobiology\(^1\) and Veterinary Medicine and Surgery\(^2\).

Hypothesis: Cells can traffic bidirectionally between offspring and mother during gestation and can persist within the mother for extended periods following parturition, a phenomenon known as fetal microchimerism (FMC). Fetal microchimeric cells (FMCs) acquired during pregnancy have been shown to contribute to the maternal wound healing response in several organ systems. We predict that FMCs participate in the maternal corneal wound healing response. Specifically, that FMCs can be detected in the wounded corneas of parous mice but not in the wounded corneas of nulliparous mice, and that FMCs will not be found in naïve corneas of parous or nulliparous mice.

Methods: Wild-type C57BL/6 female mice were mated with C57BL/6-Tg(CAG-EGFP)1Os/J male mice expressing green fluorescent protein (GFP) to provide experimental animals possessing GFP-labeled FMCs. Wild-type C57BL/6 females paired with wild-type C57BL/6 males served as controls. 14 days post-parturition, female mice were anesthetized and underwent corneal epithelial scraping of the right eye. Mice were serially imaged through brightfield and fluorescent stereomicroscopy. Mice were euthanized 10 days post-wounding, eyes were prepared for histological and immunohistochemical evaluation and FMCs were quantified through RT-PCR.

Results: We expect to provide evidence of GFP-labeled FMCs participating in the corneal wound healing response of parous wild-type female mice that have been mated with transgenic male mice expressing GFP.

Conclusions: We expect to demonstrate the FMCs actively participate in the maternal corneal wound healing response. Further in-vivo studies will be warranted for phenotypic characterization of the observed FMCs.
ACUTE NEUROKININ-1 RECEPTOR ANTAGONISM FAILS TO DAMPEN AIRFLOW LIMITATION OR AIRWAY EOSINOPHILIA IN ASTHOMATIC CATS. Megan Grobman, John R. Dodam, Carol R. Reinero (sponsor), Hilton Outi, Stacy Krumme, Department of Veterinary Medicine and Surgery.

Introduction/Hypothesis: Feline allergic asthma is a chronic inflammatory disorder of the lower airways. Tachykinins released from sensory nerves and immune cells binding NK1, NK2 and NK3 receptors have been implicated in asthma pathogenesis in humans and rodent models. There is evidence that blockade of NK1 receptors alleviates airway inflammation and bronchoconstriction. Maropitant (Cerenia) an NK-1 receptor antagonist marketed as an anti-emetic is being used to treat asthma in pet cats without supporting scientific studies. We hypothesized that as single dose of maropitant would blunt clinical signs after allergen challenge, reduce airway hyperresponsiveness (AHR) and diminish eosinophilic airway inflammation in experimental chronic allergic feline asthma.

Methods: Cats (n=7) induced to have an asthmatic phenotype using Bermuda Grass Allergen (BGA) were enrolled in a prospective, placebo-controlled cross-over design study. Subjects were randomized to receive maropitant (2mg/kg SC) or placebo (PBS SC) after BGA challenge; after a 2 week washout, cats were crossed-over to the alternate treatment. Study endpoints included a visual analogue scale (VAS) to score clinical signs, ventilator-acquired pulmonary mechanics to assess AHR after bronchoprovocation with methacholine and collection of bronchoalvelolar lavage fluid (BALF) to quantify airway eosinophilia. Statistical analysis was performed using a Mann-Whitney Rank Sum Test with P<0.05 considered significant.

Results: A single injection of maropitant failed to diminish VAS scoring (P = 0.710), AHR (P=0.456) or airway eosinophilia (P=0.165) compared with placebo.

Conclusions: A single injection of maropitant was ineffective at blunting clinical signs, AHR and airway eosinophilia associated with the early phase response of asthma after allergen challenge. Although this study failed to show benefit of a single injectable dose of maropitant in a model which mimics an asthmatic crisis, effects of chronic administration of maropitant for treatment of feline asthma are unknown and deserve further study.

MOLECULAR MECHANISMS OF SUBEROYLANILIDE HYDROXAMIC ACID (SAHA) IN THE INHIBITION OF CANINE CORNEAL FIBROSIS. Kristina M. Gronkiewicz, Giuliano EA (co-sponsor), Sharma A, Mohan RR (co-sponsor), Department of Veterinary Medicine and Surgery.

Hypothesis: The study aim was to investigate molecular mechanism(s) of the anti-fibrotic effect of SAHA. We hypothesized that SAHA will attenuate corneal fibrosis by modulating Smad-dependent/Smad-independent signaling pathways activated by TGF-β1 and matrix metalloproteinase (MMP) activity.

Methods: Cultured canine corneal fibroblasts (CCF) were incubated in media +/-TGF-β1 (5ng/ml) +/-SAHA (2.5μM) for 24hrs. Standard techniques were used to prepare total cell lysates and cDNA. Western blot analysis was used to quantify NF-κβ and non-phosphorylated/phosphorylated isoforms of p38-, ERK-, and JNK-MAPKs, Smad2/3 and Smad7. Real-time PCR and zymography were utilized to quantify MMP1, MMP2, MMP8 and MMP9 mRNA expression and MMP2 and MMP9 protein activity, respectively.

Results: TGF-β1 treatment caused an increase in phospho-Smad2/3 and phospho-p38-MAPK. SAHA reduced TGF-β1-induced phosphorylation of Smad2/3 but not of p38-MAPK. Phospho-ERK and phospho-JNK were detected in non-treated CCF; TGF-β1 did not cause any further increases in these protein levels. SAHA caused a reduction in phospho-JNK and phospho-ERK levels regardless of concurrent TGF-β1 treatment. TGF-β1 caused an increase in MMP1, MMP2 and MMP9 mRNA but did not alter MMP8. SAHA treatment attenuated TGFβ-induced MMP2 and MMP9 mRNA expression and an increase in MMP1 mRNA. SAHA inhibited TGF-β1-induced MMP9 activity.

Conclusions: SAHA inhibits canine corneal fibrosis through the modulation of TGF-β1-induced activation of pro-fibrotic Smad2/3 pathway and attenuation of MMP9 activity. The inhibition of ERK and JNK phosphorylation induced by other regulatory involved in corneal wound healing serves as an additional anti-fibrotic mechanism of SAHA. Support: MU Phi Zeta grant and ACVO Vision for Animals Foundation grant.
PERIOPERATIVE RED BLOOD CELL TRANSFUSION REQUIREMENT FOR VARIOUS SURGICAL PROCEDURES IN DOGS. Adrienne L. Haley, F. A. Mann (sponsor), John Middleton and Courtney A. Nelson, Department of Veterinary Medicine and Surgery.

Objective – To compare perioperative red blood cell (RBC) transfusion requirement among dogs undergoing liver lobectomy, splenectomy, partial gastrectomy, rhinotomy, thyroidectomy, perineal herniorrhaphy, and intrathoracic surgery.

Design – Retrospective case series

Animals – 48 client-owned dogs treated by liver lobectomy, splenectomy, partial gastrectomy, rhinotomy, thyroidectomy, perineal herniorrhaphy, and intrathoracic surgery that received perioperative RBC transfusion and 159 client-owned dogs treated by the above surgeries that did not receive perioperative RBC transfusion.

Procedures – Patient signalment, weight, perioperative whole blood, packed RBC or oxyglobin transfusion, and survival status were compared among liver lobectomy, splenectomy, partial gastrectomy, rhinotomy, neoplastic thyroidectomy, perineal herniorrhaphy, and intrathoracic surgery groups.

Results – Patients undergoing splenectomy and liver lobectomy were significantly more likely to require RBC transfusion when each was compared to all other procedures. There was a significant association between body weight and perioperative RBC transfusion with a greater odds of transfusion as body weight increased. Dogs receiving perioperative RBC transfusions were significantly less likely to survive.

Conclusions and Clinical Relevance – Dogs undergoing splenectomy and liver lobectomy are at risk for excessive hemorrhage, and may require RBC transfusion perioperatively. Veterinarians who perform these procedures should plan accordingly and have packed RBC or whole blood donors readily available.

LARYNGEAL PARALYSIS IN BLACK RUSSIAN TERRIER. Jeremy Shomper and Dennis O’Brien (sponsor), Department of Veterinary Medicine & Surgery

Introduction-Juvenile onset laryngeal paralysis (LP) of Black Russian Terriers (BRT) was described in 2 littermates in a 2011 review article by Nicolas Granger, but has not been well characterized.

Hypothesis-We hypothesized that LP of BRT is heritable and part of a generalized polyneuropathy with primary axonal degeneration. We expect that the left recurrent laryngeal nerve (RLN) will demonstrate more pathology than the right and appendicular nerves due to the longer length of the nerves, and electrodiagnostic and pathologic findings will reflect axonal rather than myelin changes.

Methods-Pedigrees, physical exam, neurologic exam, electromyography (EMG), nerve conduction velocity (NCV), swallow studies, and muscle and nerve histopathology were compiled in varying combinations from 4 affected BRT and 1 age-matched control.

Results- Segregation analysis suggests autosomal recessive inheritance. All 3 examined dogs presented at 3 months of age with stridor, LP, ataxia, proprioception deficits, hyporeflexia, and ocular abnormalities. Swallow studies showed oropharyngeal and esophageal, sensory and motor abnormalities. EMG demonstrated abnormal spontaneous activity in 2/3 dogs. Tibial NCV was slowed in 2 dogs evaluated. Pelvic limb muscle histopathology was normal in 3 samples. In 2 dogs evaluated, cricoarytenoideus dorsalis muscles contained neurogenic atrophy and poorly myelinated intramuscular nerve fibers and RLN biopsies demonstrated diminished number, thinly myelinated fibers compared to control, both worse on the left. Patchy fiber loss occurred in hind limb nerves. Conclusion- LP of BRT is part of a heritable polyneuropathy with sensorimotor deficits and mixed axonal and myelin pathology most pronounced in the left > right RLN followed by hind limb nerves.
IDENTIFICATION OF STAPHYLOCOCCUS AUREUS GENOTYPE B AMONG STAPHYLOCOCCI ISOLATED FROM CASES OF SUBCLINICAL BOVINE MASTITIS IN THE USA. Pamela R. Fry¹, Middleton JR¹ (Faculty Sponsor), Fox LK². ¹MU Department of Veterinary Medicine and Surgery and ²Washington State University College of Veterinary Medicine.

*Staphylococcus aureus* is the most common cause of contagious bovine mastitis. Previous studies done in Switzerland found *S. aureus* isolates with specific genotypes and virulence factors to be highly contagious and have increased pathogenicity. Specifically, this pathogenic genotype, called Genotype B, was characterized by the presence of enterotoxin genes A (*sea*) and D (*sed*), and by a polymorphism within the leucotoxin E gene (*lukEB*). Based on the findings of these previous studies, the hypothesis of the present study was that the presence of *sea*, *sed*, and *lukEB* genes will be correlated with highly contagious and pathogenic strains of *Staph. aureus* isolated from cow's milk on dairy farms in the USA. Banked isolates from a previous study with known strain-type, prevalence within a herd, mean milk somatic cell count, and mean milk N-acetyl-beta-D-glucosaminidase (NAGase) activity were studied. Polymerase chain reaction (PCR) was used to detect the presence of the *sea*, *sed*, and *lukEB* genes using previously described primers. A total of 78 *S. aureus* isolates belonging to 40 different strain types from 8 dairy farms were included. To date, PCR detection of the *sea*, *sed*, and *lukEB* gene has been completed on 31/78 isolates. Of those tested, 71% (22/31) were positive for *lukEB*, 6.5% (2/31) were positive for *sea*, and 0% (0/31) were positive for *sed*. Two isolates of the same strain-type were found to be positive for both *lukEB* and *sea*. Further work to fully characterize toxin profiles and genotypes is being completed. This research could identify a simple method to determine if a *S. aureus* strain identified within a herd is likely to be highly contagious and pathogenic enabling a farmer to implement control measures and potentially prevent spread within a herd.

ISOLATION AND CHARACTERIZATION OF URINARY EXOSOMES IN RODENT MODELS OF DISEASE. Tamara S. Hancock and Charles E. Wiedmeyer (sponsor), Veterinary Medical Diagnostic Laboratory.

Exosomes are membrane bound microvesicular bodies generated through regulated physiologic processes initiated by a variety of stimuli. Isolation of urinary exosomes can minimize the innately complex protein milieu, which could allow for detection of subtle pathophysiologic cellular signals leading to early disease detection or more nuanced monitoring of disease progression. Studies utilizing small volumes of urine from common rodent models of Type II Diabetes Mellitus and systemic hypertension are lacking. Using 1mL aliquots of Zucker and Ren2 rat urine, differential ultracentrifugation was employed to isolate exosomes. Detection of known exosomal markers, Alix and TSG-101, as well as electron microscopy demonstrating prototypical exosomal size and appearance, confirmed isolation. The presence of Aquaporin-1 (AQP1) indicates some isolated exosomes are of proximal tubular epithelial origin. Significant enzyme activity of DPP-IV was present and quantified in exosomal fractions of Zucker rat urine. Renal tubular epithelial derived exosomes are present in rodent urine samples and can be successfully isolated utilizing these techniques. Furthermore, enzyme activity of DPP-IV in exosomes could suggest a role of renal tubule derived exosomes in the progression of diabetic kidney disease. Further studies are needed to correlate these findings to other markers of disease progression and advance the use of exosomes as a biomarker.
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CHRONIC CYCLOSPORINE TREATMENT PRESERVES MITOCHLONDRIAL ENERGETICS BUT DOES NOT IMPROVE CARDIOMYOCYTE CONTRACTILE FUNCTION OR CALCIUM HANDLING IN A TRANSLATIONAL MINI-SWINE MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION. Jessica A. Hiemstra1, Manuel Gutierrez-Aguilar2, Kyle S. McCommis2, Kurt D. Marshall2, Melissa S. Cobb1, Anne K. Gibson3, Christopher P. Baines1,2, Timothy L. Domeier3, and Craig A. Emter1, 1Biomedical Sciences, 2Dalton Cardiovascular Research Center, and 3Medical Pharmacology and Physiology.

Conventional treatments have not reduced mortality for heart failure with preserved ejection fraction (HFrEF) patients, highlighting the critical need for novel treatment options in this HF sub-group. Our lab recently characterized a translational mini-swine model of HFrEF, in which impaired relaxation during early diastole and diminished contractile reserve was associated with mitochondrial dysfunction characterized by increased mitochondrial permeability transition (MPT). Early diastolic function is ATP and Ca2+-dependent, thus, we hypothesized a reduced, non-immunosuppressive dose of cyclosporine (CsA; inhibiting only cyclophilin D, a key component of the MPT pore, and not calcineurin) would improve individual cardiomyocyte function and Ca2+ handling via improved myocardial energetics. Individual LV cardiomyocytes were isolated from aortic-banded mini-swine divided into three groups; control non-banded (CON), HFrEF non-treated (HF), and HFrEF treated with CsA (HF-CsA; 2 mg/kg/day). Ca2+ transients and contractile properties were monitored in fluo-4-loaded myocytes electrically stimulated at frequencies of 0.25, 0.5, & 1.0 Hz. CsA attenuated functional uncoupling of the respiratory chain and ATP synthesis seen in the HF group evident by a significant reduction in Complex-I dependent respiration. However, Ca2+ transient amplitude (F/F0) was reduced and time to peak Ca2+ release increased in HF and HF-CsA groups compared to CON at all pacing frequencies, indicative of impaired Ca2+ handling. Recovery of the Ca2+ transient (i.e. tau) increased in HF and HF-CsA at 1 Hz, consistent with impaired diastolic function common in HFrEF. Cardiomyocyte shortening and shortening rate were decreased in HF and HF-CsA groups, and decreased LV systolic rotation rate and longitudinal transverse displacement (measured via 2-D speckle tracking) demonstrate functional coherence of whole heart and cellular contractile data. In conclusion, attenuation of mitochondrial dysfunction following CsA treatment did not improve cardiomyocyte Ca2+ handling of contractile function, suggesting preservation of myocardial energetics alone is not sufficient to improve myocardial function in HFrEF.

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AN EVOLUTION-GUIDED MINIMAL-DOMAIN SEARCH FOR THE DISEASE-RELEVANT FEATURES OF SMN. Madeline Miller and Chris Lorson, Department of Veterinary Pathobiology.

1. Problem: The SMN protein has several known activities in vivo. These functions include assembly of the spliceosome and axonal transport. However, the function responsible for the specific loss of motor neurons and resulting atrophy in Spinal Muscular Atrophy (SMA) is unknown. The objective is to identify functional domains that are responsible for SMA development. 2. Methods: To identify a functional minimal-domain SMN protein, we’ve chosen five model organisms exhibiting a wide range of Smn conservation. As each species becomes more distantly related to humans, their sequences include fewer interacting domains relevant to SMA. Further, we’ve synthesized a construct consisting of only SMN exons 2, 3, and 6—the most highly conserved regions of SMN. To deliver these in vivo, we prepare virus that will express the protein from each of these different "minimal domains" and perform ICV injection into neonatal SMA model mice. Observing gross phenotypic improvements demonstrates differential rescue effects. 3. Results: Smn derived from D. rerio and Xenopus greatly improve weight and survival while those from C. elegans, Drosophila & S. pombe do not. Further, SMN236 improves survival but not weight. Further experiments will identify which domains are responsible for these differing effects. 4. Conclusions: Several striking differences can be observed between Xenopus (which rescued) and C.elegans (which did not). Notably, C. elegans and all invertebrate species lack the profilin-interacting domain, the key domain involved in SMN’s putative role in mRNA transport across axons. Further, the Tudor domain, involved in snRNP biogenesis shows structural divergence that could render it less functional. The mild rescue achieved with SMN236 suggests the Tudor domain (found in exons 2 and 3) is sufficient to provide functional, though incomplete improvement in SMA.
CHARACTERIZATION OF BIPHENOTYPIC B/MACROPHAGE CELLS IN PNEUMONIC
FRANCISSELLA AND ACUTE LUNG INJURY (ALI). Carmela L. Pratt, Jerod A. Skyberg, Carrie Lasky,
and Charles R. Brown (sponsor).

Biphenotypic B/Macrophage cells are a unique cell type derived from B-cells that co-express both B
and macrophage cell surface markers (B220, CD19, IgM, F4/80, and CD11b). Following treatment with
M-CSF and GM-CSF, splenic B-2 B cells can be induced to transition into biphenotypic cells. As the
cells transition, the expression of CD19 becomes down-regulated, CD11b and F4/80 become up-
regulated, and B220 and IgM remain static. RNA microarray analysis of in vitro biphenotypic cells
demonstrates the expression of several monocyte and lymphocyte chemotactic receptors. To evaluate
the presence of these cells in an infectious model, B6 mice were intratracheally inoculated with the LVS
strain of Francisella tularensis, a pulmonary pathogen. Biphenotypic cells within the pulmonary tissue
were significantly elevated as compared to uninfected mice on days 10 and 14 (p<0.0001, p<0.001,
respectively) post-infection, which corresponded to the resolution of disease. Similar elevations
were appreciated during LPS induced ALI, however the elevations were not as dramatic. Further functional
studies are underway to elucidate the role of biphenotypic cells in disease, which may provide the
premise for devising therapies to enhance or attenuate their development.

PERITONEAL B CELL INTERACTION WITH COXIELLA BURNETII DURING PRIMARY INFECTION.
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Pathobiology.

Coxiella burnetii is an obligate intracellular bacterium that causes acute Q fever and occasional chronic
infections in humans. It is known that T cells and interferon gamma production are crucial for disease
clearance during primary infection. It has been suggested that B cells may play a role in regulating Th1
responses during acute infection, but this remains unclear. We sought to determine the effect of B cells
during primary infection with C. burnetii. For these experiments, we used peritoneal B cells, as these
cells can secrete large amounts of regulatory cytokines such as interleukin-10 (IL-10) and are also
capable of phagocytosing and killing bacterial cells. Peritoneal B cells were cultured with virulent NMI
and avirulent NMII. Indirect immunofluorescence indicated that peritoneal B cells can take up both NMI
and NMII. NMII is taken up into LAMP-1 positive vesicles in peritoneal B cells. However, these cells do
not appear to kill C. burnetii after uptake. A subset of peritoneal cells, known as B1a cells, secreted IL-
10 during stimulation with both NMI and NMII. To determine the role of these cells in vivo, SCID mice
were adoptively transferred with 1x10^5 purified peritoneal B cells 24 hours prior to challenge with
virulent NMI. Adoptive transfer did not impact splenomegaly or bacterial burden 14 days post infection.
These data indicate that B cells alone are not sufficient for protection during primary infection, but likely
play a role in regulating T cell responses in vivo via production of IL-10.
LONGITUDINAL EVALUATION OF EFFECTS OF INTRAVENOUS MESENCHYMAL STEM CELLS IN A FELINE MODEL AFTER ESTABLISHMENT OF CHRONIC ASTHMA. Julie E. Trzil\textsuperscript{1,2}, IMasseau\textsuperscript{2}, TL Webb\textsuperscript{3}, CH Chang\textsuperscript{4}, JR Dodam\textsuperscript{2}, H Liu\textsuperscript{1}, JM Quimby\textsuperscript{3}, SW Dow\textsuperscript{3} and CR Reinero\textsuperscript{1,2} (sponsor). 1. MU Comparative Internal Medicine Laboratory, 2. MU Department of Veterinary Medicine and Surgery. 3. Colorado State University Center for Immune and Regenerative Medicine, 4. Washington State University Department of Veterinary Clinical Sciences.

Studies in murine and acute feline asthma models suggested that intravenous allogeneic, adipose-derived mesenchymal stem cells (MSCs) could decrease airway eosinophilia, airway hyperresponsiveness (AHR), and remodeling. We hypothesized that MSCs would improve these asthma characteristics in a chronic asthma model. Nine cats with experimentally-induced asthma for 9 months prior to enrollment were selected for study. Five cats received six i.v. infusions of MSCs (0.36–2.5X10E7 MSCs/infusion) every two weeks, while four cats received placebo. Cats were evaluated at baseline, day 3, week 6, months 8 and 12. Outcome measures included: bronchoalveolar lavage (BAL) cytology to assess airway eosinophilia; ventilator-acquired pulmonary mechanics to assess AHR; immunologic tests including BGA-specific IgE concentration, BAL cell IL-10 production, and BGA-specific lymphocyte proliferation to assess mechanisms of MSC action. Airway remodeling was evaluated via thoracic CT scans at month 8 and 12 using a scoring system for lung attenuation (LA) and bronchial wall thickening (BWT). All variables were assessed statistically using a two-way repeated measures ANOVA except CT data which were assessed with a t-test or Mann-Whitney test; p<0.05 was considered significant. No difference was noted in airway eosinophilia, AHR, and immunologic assays. Lung attenuation and bronchial wall thickening scores were significantly lower in MSC-treated compared to placebo-treated cats at month 8 (LA p=0.0311; BWT p=0.0489), but not month 12 (LA p=0.406; BWT p=0.077). We concluded that therapy slows airway remodeling in chronic asthma; however, the effect is not sustained long term. Further study of MSC therapy is warranted in cats with naturally occurring disease.
REMOTE MONITORING OF THE PROGRESSION OF PRIMARY PNEUMONIC PLAGUE IN BROWN NORWAY RATS IN HIGH-CAPACITY, HIGH-CONTAINMENT HOUSING. Eric A. Coate, Andrew G. Kocsis, Kristen N. Peters, Paul E. Anderson, Mark R. Ellersieck, Deborah M. Fine, and Deborah M. Anderson (sponsor), MU Regional Biocontainment Laboratory, Department of Veterinary Pathobiology, Division of Animal Sciences, Department of Veterinary Medicine and Surgery.

Development of new vaccines, diagnostics and therapeutics for biodefense or other relatively rare infectious diseases is hindered by the lack of naturally occurring human disease on which to conduct clinical trials of efficacy. To overcome this experimental gap, the U.S. Food and Drug Administration established the Animal Rule, in which efficacy testing in two well-characterized animal models that closely resemble human disease may be accepted in lieu of large scale clinical trials for diseases with limited natural human incidence. In this work, we evaluated the Brown Norway rat as a model for pneumonic plague and describe the natural history of clinical disease following inhalation exposure to Yersinia pestis. In high-capacity, high-containment housing, we monitored temperature, activity, heart rate and rhythm by capturing electronic impulses transmitted from abdominal telemeter implants. Using this system, we show that reduced activity and development of fever are sensitive indications of disease progression. Furthermore, we identified heart arrhythmias as contributing factors to the rapid progression to lethality following the fever response. Together these data validate the Brown Norway rat as an experimental model for human pneumonic plague and provide new insight that may ultimately lead to novel approaches in post-exposure treatment of this devastating infection.

TLR7- MEDIATED INDUCTION OF TYPE I INTERFERON BY INTRACELLULAR YERSINIA PESTIS ENHANCES PLAGUE PATHOGENESIS. Miqdad O. Dhariwala and Deborah M. Anderson (sponsor), Department of Veterinary Pathobiology.

Yersinia pestis causes plague, a rapidly progressive and lethal disease. Extracellular Y. pestis relies on the type III secretion system to prevent activation of innate immune cells and induce programmed cell death in order to establish a replicative niche. Intracellular Y. pestis survives and replicates within a membrane-bound vacuole, and how this impacts the innate immune response is unknown. We recently demonstrated that respiratory infection of mice by Yersinia pestis induces type I interferon (IFN), a pro-inflammatory cytokine that is required for defense against viral infections, but causes increased susceptibility to plague. In fact, based on the pathogen, induction of type I IFN during bacterial infection leads to different outcomes, some of which result in clearance while others enhance pathogenesis. Data suggests that the cellular pathway exploited for the induction of type I IFN may influence its downstream effects. We therefore studied the mechanism whereby Y. pestis induces type I IFN in macrophages with the goal to understand how this cytokine enhances plague pathogenesis. We found that intracellular bacteria are recognized by toll-like receptor 7 (TLR7), leading to activation of type I IFN and increased host susceptibility in a murine model. TLR7 is known to localize to the endolysosome where it recognizes ssRNA and signals through the adaptor protein MyD88, which can activate NF-kB, IRF-3 and/or IRF-7, the latter two being transcription factors for type I IFN. We found that type I IFN production during Y. pestis infection was dependent on both NF-kB and IRF-3 suggesting both transcription factors are required for full induction of this response. Together the data suggest that, following phagocytosis, ssRNA of Y. pestis may be detected by TLR7 leading to a type I IFN response that enhances the pathogenesis of plague.
CHARACTERIZATION OF PORCINE VASCULAR TISSUE AND GOLD NANOPARTICLES AS A VASCULAR REPAIR. Allison Ostdiek, Raja Gopaldas, Jan Ivey, Sarah Hansen, Sheila Grant (sponsor), Departments of Veterinary Pathobiology, Biological Engineering, Biomedical Sciences, and Surgery.

Synthetic and biologic patches; the standard for cardiac and vascular reconstruction, have problems with rupture, calcification, and re-stenosis. The aim of this project was to perform an in vivo study of the feasibility, remodeling, and biologic effects of a nanostructured vascular patch to improve the effectiveness of repair materials. A porcine vascular tissue patch was conjugated with gold nanoparticles (AuNP) and evaluated to determine if enhanced integration occurred while avoiding rupture, calcification, and neo-intimal hyperplasia when compared to a currently used biologic patch material. Adult swine underwent a bilateral patch angioplasty of the carotids with the experimental patch on the right and a control of bovine pericardium on the left. Evans blue dye was administered before sacrifice. The patency of the arteries was checked using ultrasound and the vessels harvested. The carotid was examined grossly, with Evans blue for neoendothelial targeting, and with microscopy using Trichrome and H&E stains. Doppler ultrasound was performed every 3 weeks to evaluate the flow rates of the blood through the carotid arteries at the site of patch implant. There was a 100% success rate of implantation and a 0% mortality rate in survival animals. All patches were patent on ultrasound. At 3 weeks, regenerating endothelial cell growth was noted on the experimental patches. Histology showed normal inflammatory and healing response in all the experimental and control groups. At 9 weeks, the experimental groups showed better integration with the host tissue grossly. Histology showed cellular ingrowth into the experimental patches, particularly the carotid patch and no major foreign body reactions. We demonstrate the feasibility of a novel nanomaterial vascular patch for aortic, vascular and cardiac reconstructions. There was no evidence of rupture, pseudo aneurysm, or rejection. Superior reintegration and equivalent patency was demonstrated by the use of nanoparticle cross linking. Longer studies will be conducted to further evaluate the biologic reactions to the patch material and durability.

THE RESTORATION OF MINOR SPlicing PATHWAY IN A SMA MOUSE MODEL. Pei-Fen Yen, Francesco Lotti, Zhihua Feng, Chiara Mazzasette, Chien-Ping Ko, Livio Pellizzoni, Christian Lorson (sponsor), MU Bond Life Sciences Center, MU Department of Veterinary Pathobiology, MU Department of Molecular Microbiology and Immunology, Columbia University Center for Motor Neuron Biology and Disease, University of Southern California Section of Neurobiology

Spinal muscular atrophy (SMA) is a genetic disorder caused by reduced levels of the survival motor neuron (SMN) protein, resulting in the loss of lower motor neurons and the atrophy of voluntary muscles. Currently, there is no cure for SMA. Therefore, it is crucial to understand the pathogenesis of low SMN-caused defects in order to develop therapeutic strategies for the disease. SMN plays a vital role in snRNP biogenesis, which is the process to assemble snRNA-protein complex (snRNP) for both major and minor splicing pathways. Previous studies show SMN deficiency preferentially decreases functional minor splicing snRNPs, which further causes aberrant splicing of genes which are essential for motor circuit function. To verify the role of minor splicing in SMA development, we utilize a viral delivery system to globally restore the minor splicing pathway in a SMA mouse model. The goal of this project is to determine whether we could functionally restore an important cellular pathway, without altering SMN levels. We showed phenotypic improvements in survival, weight gain as well as motor performance. Aberrant splicing events of U12-intron containing genes were partially corrected and no change of SMN protein levels confirmed that these observed effects are SMN-independent. Results show that our treatment is able to rescue a portion of phenotypes of SMN-deficient mice through the restoration of the minor splicing pathway, suggesting that defects in minor splicing pathway contribute to the pathogenesis of SMA.