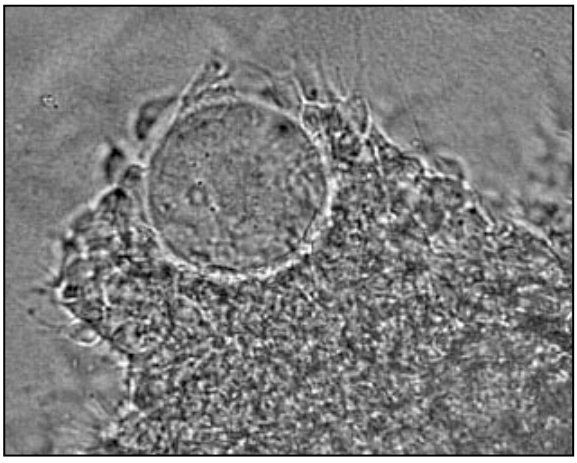
Colorado State University

College of Veterinary Medicine and Biomedical Sciences

12TH ANNUAL CVMBS RESEARCH DAY
SCIENTIFIC PROCEEDINGS

The Hilton Hotel
March 5, 2011



CVMBS Research Day 2011

Schedule Of Events		Room
11:30-12:00	Poster set up	Salon II, III
12:00	Opening remarks – Dr. James Graham	Salon I
12:05	Pfizer Research Award Winner – Dr. Scott Earley	Salon I
	"Functional Roles of Transient Receptor Potential Channels in the Vasculature"	
12:45	Break	
1:00-5:00	Oral Presentation I: Clinical Sciences	Salon I
1:00-5:00	Oral Presentation II: Basic Sciences	Salon V
1:00-5:00	Oral Presentation III : Basic Sciences	Salon IV
1:00-3:00	Poster Session I Judging: Basic Sciences	Salon II, III
3:15-5:00	Poster Session II Judging: Clinical Sciences	Salon II, III
5:00-6:00	Social Hour, Remove Posters	Salon II, III
5:30	Awards	Salon II, III

Oral Presentation: - Please limit to a 12 minute talk with 1-3 minutes for questions and changeover. Oral presentations will be in the Idaho and Michigan Rooms.

Poster Presentation: - Please hang your posters on Feb. 16 from 11:30-12:00 in the Oklahoma room. Individuals presenting the poster must be in attendance to discuss their materials with judges as listed above.

PFIZER RESEARCH AWARD WINNER

CVMBS Research Day
Saturday, March 5, 2011

Dr. Scott Earley, Ph.D.

"Functional Roles of Transient Receptor Potential Channels in the Vasculature"

Dr. Scott Earley received bachelors and master's degrees from the University of Maine and his PhD in Biomedical Sciences from the University Of New Mexico School Of Medicine. He completed postdoctoral training in physiology and biophysics at the University of Vermont chool of Medicine. Dr. Earley is currently an Assistant Professor of Biomedical Sciences at Colorado State University. His research is focused on identifying functional roles of members of the transient receptor potential (TRP) superfamily of cation channels in the vasculature. TRP channels are primary receptors for stimuli such as light, pressure, temperature, osmolality and certain chemicals, and are a fundamental means of detecting and responding to these signals at the cellular level. This research employs an array of experimental techniques, including qRT-PCR, Western blotting, immunolabeling, siRNA-mediated gene silencing, patch clamp and intracellular electorphysiology, high-speed, high-resolution live cell confocal imaging, total internal reflection fluorescence (TIRF) microscopy, and resistance artery pressure myography. Most experiments use freshly-isolated cells and arteries. The ultimate goal of these efforts is the identification of novel targets for the treatment of cardiovascular diseases such as stroke, hypertension, and atherosclerosis.

Salon I
The Hilton Hotel
Fort Collins, CO

Oral Presentations

SESSION 1: CLINICAL SCIENCE 1:00-4:45PM Salon I			
1:00	Adams	Equine bone marrow-derived mesenchymal stem cells: Comparing the sternum and the ilium	CS
1:15	Bradley	Cyclosporine Therapy for Canine Chronic Hepatitis – A Retrospectivee Study	CS
1:30	Cadmus	The Effect of Preoperative Planning Method Upon the Recommended Tibial Tuberosity Advancement Cage Size	CS
1:45	Dern	Development of a broad range qPCR assay to detect and identify fungal DNA in equine endometrial samples	CS
2:00	Kane	Preliminary Assessment of the Fecal Occult Blood Test in Blue and Gold Macaws (Ara ararauna)	CS
2:15	Linke	Effective small interfering RNA cocktails targeting viral and avian genes as an alternative to vaccination for avian influenza	CS
2:30	Mann	Bovine tuberculosis slaughter surveillance system in the USA and the ability to trace infected cattle back to the herd of origin	
2:45	BREAK		
3:15	Neary	The clinical signs and pathologic features of summer pneumonia in calves raised on a high altitude ranches	CS
3:30	Beckwith	Osteosarcoma of the canine head: a retrospective analysis of 136 cases (1991-2008)	CS
3:45	Neves	Pulmonary function of calves at one, three and six months of age in a high altitude environment	CS
4:00	Niyom	Evaluation of the analgesic efficacy of ABT-116, a TRPV-1 antagonist in dogs – comparison with buprenorphine	CS
4:15	Scorza	Prevalence of Giardia and Cryptosporidium co-infections in dogs in the United States	CS
4:30	Virgin	Incidence of support limb laminitis in horses treated with a half or full limb casts: a retrospective study of 113 horses (2000-2009)	CS
4:45			

Oral Presentations

SESSION 2: BASIC SCIENCE 1:00-4:45PM Salon V			
1:00	López	The RNA-binding protein CUGBP1 coordinates expression of secretory pathway mRNAs in muscle cells	MIP
1:15	Moore	Statistical modeling of risk factors for Escherichia coli O157:H7 in a beef packing plant	CS
1:30	Webb	Mesenchymal stromal cells protect allergen-sensitized mice from airway challenge	CS
1:45	Wiedenheft	African Horse Sickness: The Knowledge Base of U.S. Equine Veterinarians and the Effectiveness of Online Education	CS
2:00	Antoniazzi	Interferon-tau has endocrine action on the ovine corpus luteum during early pregnancy that is independent of its paracrine effect on endometrium	BMS
2:15	Bosco-Lauth	Threat assessment of Chikungunya virus in domestic and wild animals in the U.S and development of a hamster model of infection	BMS
2:30	Searcy	Mechanisms of gonadal steroid influences on the development of sex differences in the preoptic area	BMS
2:45	BREAK		
3:15	Silveira	Equine ovarian aging and differential control of gene expression	BMS
3:30	Angala	Reconstitution of functionally active mycobacterial AftC enzyme	MIP
3:45	Calvert	Human Monoclonal Antibodies to West Nile Virus Identify Epitopes on the prM Protein	MIP
4:00	Dickson	The Cellular Protein HuR Relocalizes to the Cytoplasm and Stabilizes Viral Transcripts during Sindbis Virus Infection of Human Cells	MIP
4:15	Henao-Tamayo	Vaccine inhibition by highly virulent strains of tuberculosis	MIP
4:30	Lee	CUGBP1 binds and regulates decay of mRNAs essential for muscle function	MIP
4:45			

Oral Presentations

SESSION 3: BASIC SCIENCE
1:00-4:45PM

Salon IV

1:00	Moon	A potential role for a flavivirus-derived non-coding RNA in modulating cellular RNA decay	MIP
1:15	Palusa	Rabies virus glycoprotein mRNA persists in human cells by recruiting cellular RNA binding proteins to the 3'UTR to repress mRNA decay	MIP
1:30	Rholl	Deleting the Undeletable: A novel approach to make previously unattainable gene knock-outs in Burkholderia pseudomallei	MIP
1:45	Soffler	Development of a Caprine Model of Melioidosis	MIP
2:00	Wyckoff	Development of a Novel Detection Assay for Chronic Wasting Disease Prions in Soil	MIP
2:15	Koesterich	Assessment and Prioritization for an Occupational Health and Safety Management System for a Veterinary Teaching Hospital	ERHS
2:30	Troy	Liposomes Combined with TLR9 Agonist Produce Effective Mucosal Vaccine against *Mycobacterium tuberculosis*	MIP
2:45	BREAK		
3:15	Carlsten	Hypofractionated Radiation Therapy Plus Toceranib for Unresectable Canine Mast Cell Tumors	CS
3:30	Dregalla	Role of pADPr modification in DNA-PK-mediated end-joining	ERHS
3:45	Eby	Evaluation of the Effects of a GnRH Immunocontraceptive Vaccine on the Behavioral Ecology of Wild Horses (*Equus caballus*) at Theodore Roosevelt National Park, North Dakota.	BMS
4:00	McGrew	Are gastrointestinal helminths of ringed seals (*Phoca hispida*) and spotted seals (*Phoca largha*) capable of mercury (THg) uptake within their definitive host?	MIP
4:15	Ochola	The effect of low-dose ionizing radiation in lungs of recombinant congenic strains of mice	ERHS
4:30	Krafsur	Baseline histological health assessment of subsistence harvested arctic marine mammals from the North Slope Borough villages of Barrow and Wainwright, Alaska	MIP
4:45			

Departmental Abbreviations

BMS: Biomedical Sciences
CMB: Cell and Molecular Biology Program
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
MIP: Microbiology, Immunology, and Pathology

Poster Presentations

Session 1-Odd Numbered Posters 1:00-2:45PM
Session 2-Even Numbered Posters 3:15-4:45PM

#1	Allaband	Influences of Mouse Parvovirus on Cytokine Production in Mice
#2	Autenrieth	Occupational Exposure to Noise from Personal Stereos
#3	Benedict	Associations between antimicrobial use and antimicrobial resistance in Escherichia coli sampled from individual feedlot cattle
#4	Birkenheuer	The retro-viral cyclin of walleye dermal sarcoma virus enhances transcript levels of serum response and cell cycle genes
#5	Blauvelt	Validation of an Equine Back Profiling System to Optimize Saddle Fitting
#6	Burgess	Disinfectant comparison and exam room general cleaning effectiveness of the Small Animal Clinic at the James L. Voss Veterinary Teaching Hospital
#7	Carvalho-Netto	Gene expression profiling of an industrial bioethanol yeast strain during the fermentative process
#8	Dailey	Expression of HES-1 in Canine Osteosarcoma
#9	Duffy	Stimulation of Intestinal Immunity by Diets Containing Rice Bran
#10	Enriquez	The Role of LIN28 and MicroRNAs in Ovarian Cancer Biology
#11	Feirer	Laboratory Mice Experience Minimal Hematologic and Cytokine Changes When Moving from Sea Level to High Altitude
#12	Ficociello	Comparison Of Commercially Available PCR Assays For the Amplification Of Ehrlichia Canis and Anaplasma Phagocytophilum DNA From The Blood Of Naturally Infected Dogs
#13	Fiege	Survey of large colon volvulus therapy in horses and impact on prognosis
#14	Forster	Canine Digestibility of navy bean powders: Novel dietary strategy for disease prevention in companion animals
#15	Fowles	Comparative Pathway Analysis of Human and Canine Melanoma
#16	Fox	Paranasal Sinus Masses of Rocky Mountain Bighorn Sheep (Ovis canadensis canadensis)
#17	Gillette	Equine infectious disease priorities based on a 2010 on-line survey
#18	Goodyear	Burkholderia pseudomallei Persistently Colonizes and Disseminates from the Gastrointestinal Tract Following Oral or Intranasal Inoculation
#19	Gwynn	An indolent aerosol mouse model for Acinetobacter baumannii-induced pneumonia
#20	Habenicht	Urinary Cytokine Concentrations in Cats with Kidney Disease
#21	Hafeman	Tumor associated macrophages increase proliferation of osteosarcoma cells
#22	Hansen	Comparison of Current Veterinary Sonography Techniques to Human Medial Sonography Techniques Recommended by OSHA
#23	Harms	The AMPA cleft-pore linker mutant: a new target for cognition enhancing drugs

#24	Hart	Flow cytometric determination of the immune-mediated component of the anemia seen with Mycoplasma haemofelis infection in a cat
#25	Hemphill	scAAV Transduction Efficiencies in Joint Tissue Monolayer and Explant Cultures and the Effects of Synovial Fluid Neutralization
#26	Johnson	Immune Enhancement of Antimicrobial Therapy for Treatment of *Salmonella enterica* infection.
#27	Kirk	Differences in Antibiotic Susceptibility of Corynebacterium pseudotuberculosis Grown Planktonically or as a Biofilm
#28	Kumar	Reduced Susceptibility to Salmonellosis by Dietary Rice Bran
#29	Lagana	Evidence of cross species transmission of feline immunodeficiency virus between bobcats and pumas
#30	Lee	Investigating Mechanisms of Cross-Species Transmission of Feline Immunodeficiency Virus between Bobcats (Lynx rufus) and Mountain Lions (Puma concolor)
#31	Lishnevsky	Comparative Analysis of Bleomycin In Pulmonary Disease Susceptible PECAM Deficient Mice
#32	Lord	Combined Immunotherapy and Antimicrobial Therapy for Treatment of Chronic Staphylococcal Osteomyelitis
#33	Magden	Rapid development of lymphomas in cats with virulent FIV infection
#34	McKenna	Aerosol Deposition System for Lung Epithelial Cell Culture
#35	McLeland	A comparison of biochemical and histopathologic staging in cats with renal disease
#36	McQuinn	The Role of Nitric Oxide in Immune-Mediated Hemolytic Anemia Coagulopathy
#37	Mehlman	Myocardial structural changes in canine obesity
#38	Meyerett	Monitoring the Development of Behavioral and Cognitive Effects of Prion Protein Deficient Mice
#39	Michel	Monitoring Immune Cells Trafficking Fluorescent Prion Rods Hours after Intraperitoneal Infection
#40	Mitchell	Myeloid Suppressor Cell Depletion Augments Cancer Vaccine Effectiveness
#41	Myers	Comparison of Two Serological Tests for Antibodies to Toxoplasma gondii in Feral Swine
#42	Nakayama	Detection of vaccine derived canine parvovirus DNA via polymerase chain reaction assays
#43	Neff	Changes in the stability of cell-cycle regulated mRNAs occur when cells achieve pluripotency
#44	Nguyen	Efficacy of Coffee Makers at Removing Contaminants
#45	Penman	Osteogenic potential of bone marrow-derived mesenchymal stem cells from Equine Sternum and Ilium
#46	Podell	Hyperglycemia increases disease severity and bacterial burden in Mycobacterium tuberculosis infected guinea pigs
#47	Sagawa	Deposition of the oncoprotein nucleophosmin on mRNAs influences poly(A) tail length and mRNA export
#48	Schow	Vascular development and sex differences in the region of the paraventricular nucleus of the hypothalamus

#49	Seabrook	MYC-mediated LIN28 activation regulates let-7 expression in human trophoblast cells
#50	Seelye	Cellular Localization of the Nup210l protein
#51	Shaughnessy	Comparison of Third Metacarpal Condyle Density Pattern and Shape to Histologic Characteristics of the Osteochondral Tissues
#52	Shoeneman	Expression and Function of Survivin in Canine Osteosarcoma
#53	Sishc	Measuring stand-specific telomere length using two-color Chromosome-Orientation Quantitative Fluorescent in situ Hybridization
#54	Sonius	Association between Feline Antibody Responses to Alpha-Enolase and Azotemia in Privately-Owned Cats
#55	Stone	The cellular distribution of GluA2 flip AMPA receptors and stargazin changes upon application of glutamate
#56	Sullivan	Comparison of tissue oxygen saturation in ovariohysterectomized dogs recovering on room air versus nasal oxygen insufflation
#57	Sullivan	Glial interactions and neuroinflammation in manganese neurotoxicity
#58	Tangtrongsup	Intestinal parasites of dogs in Chiang Mai, Thailand
#59	Torres	Role of Mycoplasma spp. in Feline Cat Bite Abscesses
#60	Walker	Risk Factors for Dehydration and Stress in Horses Participating on a High Altitude Week-Long 100-Mile Ride
#61	Walton	Immuno-Antimicrobial Therapy for Treatment of Chronic Staphylococcal Infection with Pre- and Post-Exposure Vaccination
#62	Wolf-Ringwall	Identification of metastasis-related microRNAs in osteosarcoma
#63	Wood	Development of a microsphere immunoassay for the detection of cytokines in plasma/serum from the domestic cat (Felis catus)
#64	Yates	A Retrospective Echocardiographic Study of Left Atrial Size in Horses as a Prognostic Indicator for Mitral Valve Disease
#65	Zeh	Comparison of Immunocytochemical and Immunohistochemical c-KIT Expression Pattern in Canine Cutaneous Mast Cell Tumors
#66	Zeidler	Development of a reporter system for the study of gene Copy Number Variation (CNV)

Thank you moderators and judges!!

Thank you to our sponsors:

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Oral Presentations

Session I ~ Salon 1
1:00-5:00PM

CLINICAL SCIENCE

Equine bone marrow-derived mesenchymal stem cells: Comparing the sternum and the ilium

MK Adams, LR Goodrich, S Rao, FJ Olea-Popelka, N Phillips, JD Kisiday, CW McIlwraith

Purpose: Bone marrow-derived mesenchymal stem cells (BMDMSCs) have been shown to improve healing of cartilage, bone, and soft tissue defects in horses and other species. The two sites of BMDMSC harvest in the horse are the sternum and ilium, and site selection is based primarily on practitioner preference. The goal of this study was to determine the effects of harvest site and harvest fraction on stem cell quantity and rate of growth. We hypothesized that there would be a higher concentration of cells in the sternum compared to the ilium, and that the first fraction of marrow from either site would yield the greatest concentration of cells. Furthermore, we hypothesized that growth rates of cells from each site would not differ. Materials/methods: Seven horses from the Equine Orthopedic Research Center were sampled prior to euthanasia. Two sequential 5-ml marrow samples were taken from the sternum and ilium of each horse. Nucleated cell counts were obtained for all samples pre and post marrow processing. Cells were expanded in culture for three passages and cell counts were obtained at each passage. Results: There was no significant difference ($p>0.05$) between the nucleated cell counts of the first sternum aspirate and first ilium aspirate. However, the nucleated cell counts of the first 5-mL aspirate were significantly higher than the second 5-mL aspirate for both sites (p0.05). Conclusions: These data should give practitioners confidence that cell numbers and growth rate characteristics do not vary between sternal and ilial sites and that the first 5 ml aspirate yields the highest concentration of stem cells for both sites. Depending on clinician preference both sites offer a rich supply of BMDMSCs that have similar growth rate characteristics. Further studies will reveal the importance of marrow volume in regard to cell counts and growth rate characteristics

Cyclosporine Therapy for Canine Chronic Hepatitis – A Retrospective Study

AM Bradley, DC Twedt

Cyclosporine Therapy for Canine Chronic Hepatitis – A Retrospective Study. AM Bradley, DC Twedt. Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

Purpose: Chronic hepatitis (CH) in dogs is a progressive condition without clearly defined treatment. Glucocorticoids are commonly used to stop progressive inflammation and fibrosis but are associated with significant side effects including a steroid hepatopathy that complicates enzyme monitoring. Cyclosporine is proposed as an alternative therapy, but there are no published reports of its use for canine CH.

Materials/Methods: Patient records at the CSU Veterinary Teaching Hospital were searched for histologically confirmed cases of CH treated with cyclosporine. Data were compiled on cyclosporine dosing, concurrent medications, clinical course and biochemical parameters.

Results: 13 patients over a 50-month period were identified. Serum alanine aminotransferase (ALT) decreased by an average of 71% in 12 dogs. The ALT normalized completely in 6 of 10 dogs treated for >60 days. In 5 of 6 dogs on >9 mg/kg/day, the ALT also normalized. Five of the 6 patients that exhibited clinical signs prior to treatment showed measurable improvement (weight gain, fewer gastrointestinal signs). Eight patients had hyperbilirubinemia or ascites prior to treatment; these resolved in 7. Post-treatment histopathology, available in one patient, revealed decreased severity of CH. Five patients exhibited adverse effects including gastrointestinal signs (3), gingival hyperplasia (1), and papillomatosis (1). Cyclosporine was discontinued in 2 dogs with gastrointestinal signs.

Conclusions: Cyclosporine was an effective therapy for many cases of CH and should be considered for patients who are refractory to or cannot tolerate glucocorticoids. Prospective clinical trials with histological documentation are needed to better define cyclosporine's effectiveness in CH.

The Effect of Preoperative Planning Method Upon the Recommended Tibial Tuberosity Advancement Cage Size

JC Cadmus, RH Palmer, C Duncan

INTRODUCTION: Tibial tuberosity advancement (TTA) seeks to stabilize the cruciate ligament-deficient stifle by orienting the patellar tendon angle (PTA) to =90° during weight-bearing. Preoperative TTA planning in clinical practice uses various different techniques to determine the advancement required to attain PTA =90°. Our hypothesis was that different preoperative planning methods lead to variable TTA cage size recommendations. MATERIALS & METHODS: Medio-lateral radiographs were made of 14 large-breed canine stifles. TTA was planned on each radiograph using 2 sets of tibial plateau landmarks (anatomic – [A] vs. femoro-tibial common tangent – [T]) and 2 advancement measurement techniques (transparent overlay – [O] vs. simulated TTA using imaging software – [S]) for a total of 4 different methods (AO, AS, TO and TS). Data was tabulated and statistically analyzed. RESULTS: The mean recommended advancements (± SD) were: TO* -- 7.5 ± 2.0mm; TS --10.0 ± 3.2mm; AO --10.1 ± 3.3mm; and AS* – 11.5 ± 3.6mm (* denotes statistical difference). Larger advancement was associated with use of anatomic landmarks (vs. common tangent) and imaging software to simulate TTA (vs. overlays). In the 14 stifles studied, these preoperative planning methods would have led to four different cage size recommendations in 1 stifle, three different recommendations in 8, two different recommendations in 3, and one recommendation in 2 stifles. DISCUSSION/CONCLUSION: Preoperative planning techniques currently practiced lead to variable TTA cage size recommendations and may be a source of inconsistent functional outcomes. ACKNOWLEDGEMENT: Funded by CSU Center for Companion Animal Studies PVM Student Grant. Orthoplan provided by Sound-Eklin™.

Development of a broad range qPCR assay to detect and identify fungal DNA in equine endometrial samples

K Dern, R Ferris, J Veir, J Hawley, MR Lappin, P McCue

Purpose: Fungal endometritis is an important cause of subfertility in the mare. Unfortunately, traditional culture of a fungal pathogen is often slow or unrewarding. The aim of this study was to develop a broad range 28S rDNA qPCR assay for detection of fungal pathogens in the equine uterus.

Materials and Methods: The qPCR assay was optimized using A. fumigatus (ATCC 1022), and C. albicans (ATCC 10231). Samples were heat-shocked, and then the DNA extracted, amplified, detected, and the sequence analysis of amplicons performed. Identification of fungal pathogens was compared between biochemical analysis in the CSVDL and the qPCR assay from 12 agar plates from clinical samples submitted to the CSVDL. Fungal elements were analyzed by the optimized qPCR assay on 3 different runs.

Results: The broad spectrum primers were able to detect 2 x 10^-14 g of DNA/mcL from A. fumigatus, and C. albicans. The inter-assay coefficient of variation over 12 runs from one single extraction was 6%. Fungal DNA was detected in all 12 clinical samples using the qPCR assay. The intra-assay coefficient of variation was

Conclusions: The qPCR assay has an inter-assay coefficient of variation less than 10%, and the ability to identify 94% of fungal pathogens through DNA sequencing. Additionally the time for sample submission to obtaining PCR results was reduced by 2 hours. The development of this diagnostic technique to detect fungal endometritis may allow for rapid identification of fungal organisms when traditional culture and biochemical classification have been unrewarding.

Preliminary Assessment of the Fecal Occult Blood Test in Blue and Gold Macaws (Ara ararauna)

NG Kane, MS Johnston, B Nevitt, AE Hill

Gastrointestinal (GI) disease accounts for many presentations seen by avian veterinarians. Assessing for GI hemorrhage can be difficult in avian patients without the use of endoscopy. Given the unique anatomy of the avian GI tract and small size of most companion birds, a non-invasive screening test for GI hemorrhage could be a valuable tool. This study aimed to determine whether the guaiac-based fecal occult blood test designed for use in humans could detect avian fecal occult blood. Ten apparently healthy adult blue and gold macaws (Ara ararauna) of both sexes were used. Once determined healthy based upon review of medical records and a physical examination, each bird was placed in a separate cage. Fruits, vegetables, red meat, and supplements were withheld from the diet for four days prior to data collection per Hemoccult (Beckman Coulter) product insert instructions. Fecal samples were collected from each subject's cage and applied to two separate Hemoccult cards. Each bird was then restrained for venipuncture of the right jugular vein, from which two mL of blood were withdrawn and then gavage-fed to the bird. Over the following four hours, fecal samples from each bird were collected and pooled, applied to the Hemoccult cards, and run in duplicate. All ten birds tested positive for fecal occult blood following ingestion of blood. Two of the birds tested positive for fecal occult blood prior to being gavage-fed, and were excluded from statistical analysis as they no longer met our criteria of healthy individuals. All paired samples were confirmatory for each bird. In our study, eight of eight (100%) birds with occult blood in their feces tested positive on the Hemoccult test, indicating a test sensitivity of 100% (95% CI: 63-100%). The results demonstrate that the guaiac-based fecal occult blood test can detect avian blood. With further investigation, this test may prove valuable in screening for GI hemorrhage in birds.

Effective small interfering RNA cocktails targeting viral and avian genes as an alternative to vaccination for avian influenza

L Linke, J Triantis, F Olea-Popelka, MD Salman

Purpose: Avian influenza virus (AIV) is a high consequence, economically relevant disease of poultry. The lack of vaccine that confers complete immunity and the pressures disease behavior could place on promoting the spread of virulent strains, underlines the urgency to accelerate the development of more effective control methods for AIV in poultry. The goal of this study is to use RNA interference (RNAi) to develop a unique antiviral application for AIV in an avian tissue model using small interfering RNAs (siRNAs). There are no published reports indicating research targeting avian host genes with RNAi technology or the use of siRNA cocktails has been considered. Our approach considers siRNAs targeting viral and chicken genes both individually and in combination and is novel in terms of providing a more comprehensive prevention strategy so optimal effectiveness in prevention of AIV is achievable. Methods: The AIV genes chosen for siRNA knockdown were the NP, PA, PB1, PB2, and NS1 segments. Chicken hepatocellular carcinoma epithelial cells were transfected with each viral siRNA and infected with AIV. In addition to mono-siRNA transfection, a combined viral siRNA cocktail was optimized. AIV infection and the production of infectious viral particles were assayed via immunocytochemistry staining and the TCID50 assay. Selection criteria were used to select 9 chicken genes. SiRNAs were designed against each mRNA sequence and are being evaluated for optimal knockdown efficiency. Results: Individual and siRNA cocktails targeting specific viral genes significantly reduced AIV infection in vitro. A list of testable chicken targets is being generated for further evaluation with siRNA transfection and AIV infection assays. Conclusions: In future work, these chicken siRNAs will be evaluated for their ability to inhibit AIV infection in vitro. Targeting these viral and host genes could constitute an alternative and novel approach for future potent antiviral use in poultry.

Bovine tuberculosis slaughter surveillance system in the USA and the ability to trace infected cattle back to the herd of origin

H Mann, K Orloski, M Salman, R Basaraba, FJ Olea-Popelka

The detection of gross bovine tuberculosis (bTB) lesions in cattle at slaughter and the successful trace-back to the herd of origin is crucial to the detection of infected herds and for the success of the national bTB eradication program in the United States (USA). The objectives of this study are to: 1) quantify the successful trace-back of bTB cases detected during slaughter inspection back to their herd of origin and 2) identify whether at least one affected herd was found as a result of the traceback investigation and 3) identify factors associated with the probability of identifying additional bTB infected cattle after bTB cases were detected at slaughter surveillance. Descriptive statistics will be conducted to complete this study. During 2001-2010, 386 lesioned cattle were classified as bTB positive in the USA. Of these, 345 (90%) were in young (fed) cattle predominantly originating from feedlots, and 39 (10%) were culled adult beef and dairy cattle. Preliminary results show that of the 345 fed cattle, 262 (76%) originated from Mexico, where Mexican officials conducted epidemiologic investigations, 67 (19%) were of undeterminable origin and 11 (3%) originated from the USA with 6 (55%) of these successfully traceable to a herd in the USA. Of the 39 adult lesioned cattle, 35 (90%) originated from the USA with 30 (86%) successfully traceable to a domestic herd of origin. Domestic herds with at least one additional animal confirmed bTB infected in the herd were found in 4 (67%) out of the 6 fed cases and in 20 (67%) out of 30 adult cases. The factors impacting successful trace-backs of slaughter bTB cases to the herd of origin and the ability to identify additional bTB infected animals are currently under investigation. Our preliminary results highlight the importance of slaughter surveillance and trace-back of bTB cases in the overall efforts to eradicate bTB in the USA.

The clinical signs and pathologic features of summer pneumonia in calves raised on a high altitude ranches

J Neary, B Neves, A Knight, D Gould, D Dargatz and F Garry

Purpose: On high altitude ranches (>8000ft) in the Rocky Mountains pre-weaned calves summer pastured commonly experience high mortality rates. Herd records from ranches in Gunnison, CO show that 10-20% mortality of the calf crop is common. Ranchers attribute this mortality to a problem known as 'summer pneumonia'. In comparison, 5-6% mortality in similar age calves is reported in national surveys of the cow-calf industry. The objective of this study was to characterize the clinical signs and pathologic features of calves affected by summer pneumonia.

Materials/methods: Calves (n=3,200) born on ranches in the Gunnison valley, CO and turned out onto summer grazing over 9,000ft in June were systematically monitored for signs of illness until weaning (October). Postmortem examinations were performed, gross pathology recorded and tissue samples submitted for bacterial culture or histopathology. Samples taken for histopathology included: right and left ventricle, interventricular septum, pulmonary artery, aorta, liver, kidney, spleen and diaphragmatic, middle and cranio-ventral lobes of the right lung. Hematoxylin and eosin and von Kossa staining were performed on slides.

Results: Nineteen necropsy examinations were performed. Nine calves (47%) died of Pasteurella pneumonia. Ten calves (53%) died of high mountain disease (HMD). Clinical signs for infectious pneumonia and HMD overlapped: high respiratory rate, lethargy, roughened hair coat and droopy ears. Coughing was more pronounced in the nine pneumonia cases. Evidence of tissue mineralization was seen on histopathology in all ten HMD calves in all tissue samples except the liver.

Conclusions: 'Summer pneumonia' is an inappropriate term for death of pre-weaned calves at high altitude as both infectious pneumonia and high mountain disease are contributors. Both diseases have similar but not identical clinical signs. This knowledge will allow greater diagnostic accuracy and highlights important areas for future research.

Osteosarcoma of the canine head: a retrospective analysis of 136 cases (1991-2008)

KA Beckwith, JS Eickhoff, WS Dernell, KJ Kazmierski, MH Lafferty, SJ Withrow, SE Lana

Purpose: To describe the biologic behavior of canine osteosarcoma (OSA) of the head and determine what factors impact outcome. Materials and Methods: Medical records at the VTH were searched for cases of canine OSA over a 17 year period. Cases were included if they had a histologic diagnosis of OSA, location in the head (mandible, maxilla, calvarium) and adequate follow-up. Information collected from medical records included signalment, primary treatment, time and location of metastasis, disease-free interval, and overall survival. Results: One hundred thirty-six cases met inclusion criteria. Of these, 46 had OSA of the mandible, 51 of the maxilla, 39 of the skull. Eight (6%) were confirmed to have metastasis at the time of diagnosis. Surgery alone was the primary form of treatment in 100 dogs; surgery in conjunction with radiation therapy in 7 dogs; curative intent radiation therapy alone in 4 dogs; palliative radiation therapy alone in 11 dogs. Of the 107 dogs that underwent surgery, clean margins were obtained in 63. Seventy-six dogs underwent chemotherapy in addition to their primary form of treatment. The overall metastatic rate for the 122 patients who received treatment was 43%. For all cases, median progression-free interval was 151 days and median survival time was 218 days. Of the 111 dogs that underwent curative intent therapy, the median progression-free interval was increased to 224 days and median survival time to 305 days. Factors determined to significantly improve outcome included disease localized to the mandible or maxilla, treatment with surgery, administration of chemotherapy. Cause of death was due to local disease in 60 dogs, distant disease in 36 dogs, both in 13 dogs. Seventeen dogs died of other causes; 10 were lost to follow up. Conclusions: OSA of the head is a locally aggressive form of neoplasia and appears to have a lower overall metastatic rate than appendicular OSA. Aggressive local therapy is warranted to improve outcome.

Pulmonary function of calves at one, three and six months of age in a high altitude environment

B Neves, J Neary, A Knight, D Gould, D Dargatz and F Garry

Purpose: Pre-weaned calves summer pastured at high altitude (>8000ft) in the Rocky Mountains commonly experience 10-20% mortality of the calf crop. Much of this mortality is attributed to a problem known as 'summer pneumonia'. In comparison, 5-6% mortality in similar age calves is reported in national surveys of the cow-calf industry. Pneumonia impairs lung function which may have severe consequences at altitude such as pulmonary hypertension, heart failure and death. In order to evaluate the consequences of pneumonia on lung function baseline data from healthy calves at altitude must be established. The objective of this study was to document the changes in calf pulmonary function associated with high altitude grazing; at 1 month (May), 3 months (July) and 6 months of age (October).

Methods: Samples were collected from calves (n=49) that were born at 8,000ft and summer grazed over 9,000 ft. Arterial blood from the coccygeal artery was taken for blood-gas analysis using a handheld analyzer (iSTAT-1). Pulmonary arterial pressure (PAP) was measured by connecting an intravenous catheter to a pressure transducer and oscilloscope. The lung function of the calves' dams was determined in July only (n=20). Parameters measured include: pH, PCO2, HCO3, TCO2, base excess (BE), PO2, sO2 and lactate.

Results: Both cows and calves exhibited respiratory alkalosis. Calves were consistently hypocapnic (range of means (ROM) 32.2-36.2 mmHg) and hypoxic (ROM 46.2-50.3 mmHg). Calf blood pH increased significantly from 7.46 (May) to 7.52 (Oct.) (p

Conclusions: In summary, calves raised over 9,000 ft showed respiratory alkalosis and elevated PAP scores; both trending upwards from one month to six months of age. This explains the higher incidence of HMD cases in the fall. It is purposed that the higher ventilation rate is a predisposing factor for infectious pneumonia.

Evaluation of the analgesic efficacy of ABT-116, a TRPV-1 antagonist in dogs – comparison with buprenorphine

S Niyom, ML Rezende, FL Balzano, KR Mama

Introduction: The analgesic efficacy of ABT-116, a TRPV1 antagonist was evaluated and compared to buprenorphine, a partial Mu agonist opioid, using mechanical and thermal nociceptive thresholds. Buprenorphine was selected as a control because of our prior experience evaluating its analgesic efficacy in dogs using similar methodology. Methods: Each treatment was administered to six, 7 month old dogs (3 males, 3 females, 16 – 23 kg) using a balanced crossover design; treatments separated by one week. Temperature (T), heart (HR) and respiratory rate (RR) were recorded prior and 15 min, 1, 6, and 24 hours after drug administration. Thermal and mechanical (forearm and c-clamp) nociceptive thresholds were assessed prior to and 15 min, 1, 2, 4, 6, 12, 18 and 24 h after drug administration. Data were summarized as mean ± standard deviation. Overall effects between treatments were evaluated using an ANCOVA (baseline values were treated as the co-variate). A RM ANOVA was used to evaluate changes over time within a treatment group. Post-hoc evaluations used pair wise comparisons ($p < 0.05$). Results: Buprenorphine resulted in higher overall thermal ($P = 0.035$) and forearm ($P = 0.0165$) nociceptive thresholds than ABT-116. C-clamp thresholds were elevated from baseline in both groups, but magnitude of change was greater for the buprenorphine group; e.g., 74.1 ± 12 vs. 53.2 ± 11.5 Newton's at 1 hour post drug. While HR and RR varied sporadically T increased from a baseline value of 39 ± 0.3 to a peak of 40.6 ± 0.2 °C at 6 hours post ABT 116 administration. Conclusions: ABT-116 did not consistently result in elevation of nociceptive thresholds. Clinically relevant changes in body temperature were noted following ABT-116 administration.

Prevalence of Giardia and Cryptosporidium co-infections in dogs in the United States

A Scorza, MR Lappin

Giardia spp. and Cryptosporidium spp. cause infections in dogs and humans in the United States. Prevalence rates for dual infection in dogs had not been widely reported. In this study, fecal samples from dogs from a northern Colorado animal shelter (n = 121),dogs owned by veterinary students in northern Colorado (n=132),and dogs from the Pine Ridge reservation in South Dakota (n=85) were collected. Samples were assayed with a fluorescent antibody assay that detects Giardia spp. and Cryptosporidium spp. Those samples that were positive for Giardia spp. or Cryptosporidium spp. with adequate DNA available for sequencing were genotyped by the glutamate dehydrogenase [gdh] and by the heat shock protein-70 [HSP-70] genes, respectively. Among the total samples, the prevalence of Giardia, Cryptosporidium, dual infection or either infection were: 32(9.4%), 4 (1.1%),9 (2.6%) and 45 (13.3%)respectively. From the student dogs, sequencing was successful for the three Giardia isolates (assemblage D from 2 dogs; assemblage C from one dog) and one Cryptosporidium isolate (C. canis).From the reservation dogs, sequencing was successful for nine Giardia isolates (assemblage D from 4 dogs; assemblage C from 5 dogs) and one Cryptosporidium isolate (C. canis).Cryptosporidium and Giardia co-infections are commonly detected in dogs; in this study dual infections were more common than Cryptosporidium infections alone. Although the Giardia and Cryptosporidium isolates that were sequenced were the dog specific assemblages/genotypes, more samples should be analyzed in order to assess the potential for zoonotic transmission of either parasite.

Incidence of support limb laminitis in horses treated with a half or full limb casts: a retrospective study of 113 horses (2000-2009).

J Virgin, L Goodrich, G Baxter, S Rao

Support limb laminitis is a potentially fatal complication in horses suffering from severe lameness in a fore or hind limb that results in excessive unilateral weight bearing on the contralateral limb. This study examined medical records of 113 horses that received half-limb, full-limb, or transfixing casts at Colorado State University Veterinary Medical Center from 2000 to 2009 to determine the prevalence of support limb laminitis and identify associated risk factors. Bivariable and multivariable logistic regression analyses were performed to evaluate the association of each risk factor to the development of support limb laminitis and the adjusted effect of risk factors in the model. Of the horses included in this study, 12% developed support limb laminitis. The probability of developing support limb laminitis was not significantly different in horses that were casted due to a fracture compared to other conditions. Weight bearing capacity on presentation, breed, and limb affected did not significantly increase the likelihood of developing support limb laminitis. The duration of casting, the type of cast, and body weight were significantly associated with the development of support limb laminitis. The probability of support limb laminitis was 1.2 times higher for each week increase in duration of casting and 1.01 times higher for each 1 kg increase in weight. Horses cast with full limb and transfixation casts were more likely to develop support limb laminitis than those that were cast with a half limb cast and of the 37 cases that were casted for more than 4 weeks, 18.9% developed support limb laminitis. To our knowledge, there are no previous studies investigating the prevalence of support limb laminitis in horses that receive casts. This study should assist practitioners in determining prognosis and risk of developing support limb laminitis in surgical cases requiring casts.

Oral Presentations

Session II ~ Salon V
1:00-5:00PM

BASIC SCIENCE

The RNA-binding protein CUGBP1 coordinates expression of secretory pathway mRNAs in muscle cells

CM López, J Wilusz, CJ Wilusz

CUGBP1 is an RNA binding protein known to have roles in the post-transcriptional control of gene expression: it is an mRNA destabilizing factor it also affects translation rates. We previously identified mRNAs of genes encoding several secretory pathway proteins as probable binding targets of CUGBP1 suggesting that CUGBP1 may coordinate the activity of this pathway. Through RNA immunoprecipitation experiments using anti-CUGBP1 antibody and extracts from C2C12 myoblasts, we verified that all six mRNAs encoding protein subunits of the signal recognition particle (SRP; an essential mediator of the first step of the secretion process) are indeed associated with CUGBP1. Our preliminary results show that recombinant CUGBP1 is binding the 3'UTR of these mRNAs. In order to determine which aspect of mRNA metabolism CUGBP1 is affecting, we next measured the half-lives of the SRP mRNAs. While these mRNAs decayed relatively slowly even in control cells (half lives ranging from 2.75 to 16.2 hours), the half-lives were all significantly extended (1.3 to 2.7 fold) in CUGBP1 knock-down cells. These results are consistent with the transcript destabilizing function reported for CUGBP1 when it binds the 3' UTR of its target mRNAs. We conclude that expression of SRP protein subunits may be coordinated through association of their mRNAs with CUGBP1, therefore we are currently assessing abundance of the SRP protein subunits in our CUGBP1 knock down versus control cells to determine if there are any differences. We are also investigating the efficiency of secretion of a luciferase reporter to characterize the secretory capabilities of the CUGBP1 knock down cells. We hypothesize that secretion will be defective in this cell line if the SRP complex is affected. This project was funded by the NIH, the MDA, and a College Research Council Award, all to CJW.

Statistical modeling of risk factors for Escherichia coli O157:H7 in a beef packing plant

JEB Moore, D Rice, D Morrow, A Hill

Purpose: Enterohemorrhagic E. coli O157:H7 causes serious foodborne illness. By analyzing routine data from a beef packing plant, we hoped to find new risk factors for E. coli O157:H7 contamination and new prevention strategies. Materials/Methods: A dataset covering 484 process days was compiled. Variables included day of week, number of lots per day, number of organ condemnations, processing errors and carcass quality measures. Using counts of positive E. coli tests as our dependent variable, a negative binomial model was fitted to the data. After selecting the initial variables, interactions and confounders were analyzed. Results: Three variables were included in the initial forward selection: percentage of bagging errors, percentage of liver condemnations, and percentage of choice carcasses. No significant interaction between these variables was found. Two additional variables (number of lots per day and percentage of heads condemned by dentition) were added into the model as confounders because they changed one or more variable coefficients by >10%. Final model: 1. On days with bagging errors, the rate of positive test results increased by 2.26 [1.12-4.55, p = 0.022]. 2. On days where >75% carcasses grade choice, the rate of positive test results increased by 2.57 [1.01-6.57, p = 0.048]. 3. All categories of increased liver condemnations were associated with an increased rate of positive test results; 19-22% increased rate by 3.28 [1.31-8.22, p = 0.011], 22-27% increased rate by 3.89 [1.50-10.08, p = 0.005], and >27% increased rate by 3.58 [1.31-9.77, p = 0.013] Conclusions: Bagging errors can result in fecal contamination of the carcass, so association with increased positive tests is expected. Increased positives associated with condemned liver percentages as well as grade is interesting and suggests risk factors in management at the pre-harvest level. With carcass level data, this mode of analysis might clarify even more control points for E. coli O157:H7 contamination.

Mesenchymal stromal cells protect allergen-sensitized mice from airway challenge

TL Webb, K Takeda, Y Shiraishi, S Ashino, S Dow, EW Gelfand

Purpose: Asthma is characterized by reversible airflow obstruction and airway inflammation, most often linked to T-helper 2 cell activation and specific cytokine release, including interleukin (IL)-4, IL-5, and IL-13. However, treatments aimed at blocking these cytokines have had limited clinical success. Mesenchymal stromal cells (MSCs) have been shown to exhibit potent anti-inflammatory effects in immune-mediated disease models and are therapeutically attractive as MSC possess low immunogenicity and can be adoptively transferred into autologous or allogeneic hosts. In this study we investigated the effects of MSC transfer on allergen-induced airway hyperresponsiveness (AHR) and inflammation when administered prior to or after allergen challenge of sensitized mice. Materials/methods: C57Bl/6 mice were sensitized to ovalbumin (OVA) followed by 3 consecutive days of OVA inhalation. Forty-eight hours after the last OVA challenge, mice were assessed for airway responsiveness to inhaled methacholine and bronchoalveolar lavage fluid cell composition. Preventive effects of MSC transfer were assessed by administering MSC either intravenously or intra-tracheally into sensitized mice 2 days prior to the first OVA challenge (pre-challenge treatment). “Curative” effects of MSCs were assessed by performing MSC transfer 2 hours after the last OVA challenge (post-challenge treatment). Results: Pre-challenge treatment with MSCs significantly inhibited the development of AHR and airway eosinophilia. Post-challenge MSC treatment inhibited the development of AHR but did not affect the numbers of eosinophils in the airways. Conclusions: These results suggest that treatment with even small numbers of MSC exhibit both preventive as well as potent ameliorative effects on the development or reversal of allergen-induced AHR in mice. Treatment with MSCs may represent a novel therapeutic approach in the treatment of established asthma, either alone or in conjunction with other therapies.

African Horse Sickness: The Knowledge Base of U.S. Equine Veterinarians and the Effectiveness of Online Education

A Wiedenheft, J Traub-Dargatz, MD Salman, S Gillette, G O'Keefe

Equine veterinarians need to be prepared to identify and report suspect FADs. This study will:

1. Assess the African horse sickness (AHS) knowledge among U.S. equine veterinarians
2. Access the use of an online educational system for improving disease awareness
3. Educate U.S. equine veterinarians about indentifying and reporting AHS

Study Design: A one online survey will be given to AAEP veterinarians.

Section 1: What is the U.S. veterinarians' baseline knowledge level on recognizing and reporting AHS? The U.S. has a deficiency in FAD awareness (Thurmond et al 2003) and U.S veterinarians are inadequately prepared to identify and report a FAD (Merryman 2008). A case scenario will be used to determine participant's AHS baseline knowledge based on the number of prompts it takes to correctly suspect and report the FAD (scores between 0-10).

Section 2: Two types of on-line educational systems about AHS will be randomly distributed to the participants: a webinar-type presentation and a textbook-type document.

Section 3: Are webinar lectures more effective as an educational system then text documents? Multimedia summaries with less text may enable students to learn more effectively than lengthy textbook passages (Mayer1996). Multiple choice questions about AHS will be used to test educational effectiveness. The number of incorrect answers will be averaged for the webinar and text group (scores between 0-10).

Scoring: The scores in section 1 will be compared with scores in section 3 both for the individuals and the two educational groups. The effectiveness of the education will be measured by determining the differences and the direction of the differences between the webinar and the text groups' respective survey scores.

Project timeline: Survey distributed in 1/2010; results analyzed in 3/11.

Interferon-tau has endocrine action on the ovine corpus luteum during early pregnancy that is independent of its paracrine effect on endometrium

AQ Antoniazzi, RL Ashley, FW Bazer, TE Spencer and TR Hansen

The ovine conceptus secretes interferon-tau (IFNT) with greatest release on Days 14-16 of pregnancy. IFNT acts in a paracrine manner to silence transcription of endometrial estrogen receptor alpha (ESR1) and oxytocin receptor (OXTR), preventing luteolytic pulses of prostaglandin F2 alpha (PGF). Endocrine release of IFNT into the uterine vein occurs on Day 15 of pregnancy, which induces IFN-stimulated genes (ISGs) in extrauterine tissues. Our hypothesis is that endocrine release of IFNT occurs earlier than Day 15 of pregnancy. We also hypothesized that 72h infusion of roIFNT starting on Day 10 of the estrous cycle induces ISGs in the CL, liver and endometrium. Semi-quantitative RT-PCR was used to examine ISG15 in the CL, liver and endometrium; and ESR1 and OXTR in the CL and endometrium. Tissues were collected on Days 12-15 of the estrous cycle (NP) and pregnancy (P) and also 72h following infusion of BSA or roIFNT into the uterine vein starting on Day 10 of the estrous cycle. All differences described are significant at $P<0.05$.

Threat assessment of Chikungunya virus in domestic and wild animals in the U.S and development of a hamster model of infection

AM Bosco-Lauth, NM Nemeth, AE Tolnay, RA Bowen

Purpose: Chikungunya virus (CHIKV) is an arbovirus that can cause severe arthralgic illness in humans but it is unknown how the virus affects other species. This experiment investigates the potential for common domestic animals and wildlife to become infected by CHIKV. Materials/Methods: Domestic or wild animals were infected subcutaneously with one of two African strains of CHIKV, one historic and one recent isolate. Blood samples were collected daily for up to 7 days and clinical observations were taken up to twice daily for 14 days. When applicable, rectal temperatures were recorded for up to 14 days, at which time animals were euthanized and necropsied. Muscle and joint-associated tissues were saved for histopathology. Blood samples were tested for viremia and antibodies against CHIKV. Results: Eight species of domestic animals and eleven species of wild animals have been tested so far, including a variety of birds, mammals and reptiles. None of the birds or mammals became viremic or displayed clinical illness following inoculation of CHIKV. Ball pythons, however, developed viremias for both strains of virus, with the titers of the historic isolate being much higher than the newer strain. Similar results were seen in hamsters. H&E staining of hamster muscle tissues 14 days post-infection did not reveal any distinct lesions. Conclusions: CHIKV is a mosquito transmitted virus of zoonotic origin, but is now generally considered a human pathogen with no known animal reservoirs. This study indicates that many mammals and birds are refractory to the virus, but that snakes may be a potential zoonotic host. In addition, hamsters are susceptible lab animal hosts and may be used to study pathogenesis and help characterize the different lineages and strains of CHIKV.

Mechanisms of gonadal steroid influences on the development of sex differences in the preoptic area

BT Searcy, P Kumar, MS Stratton, SA Tobet

The preoptic area/anterior hypothalamus (POA/AH) is critical to sex specific behaviors. Correspondingly, sex differences in the density and location of POA/AH neurons have been identified. In the embryonic mouse POA/AH estradiol rapidly modulates cell movements and could be a regulator of neuron position during normal development. We have characterized the response of neurons in the POA/AH to S-diarylpropnitrile (S-DPN), an estrogen receptor-beta selective agonist. S-DPN induced a rapid 50% reduction in the rate of cell movement in the rostral POA/AH. One mechanism through which estradiol could transduce this signal is through induction of nitric oxide (NO). Neuronal nitric oxide synthase (nNOS) is the primary enzyme required for de novo synthesis of NO in the brain and has been characterized with a distinct cellular distribution in POA/AH. To investigate the impact of nNOS on neuronal movement in the POA/AH the distribution of immunoreactive (ir)-calbindin was compared between nNOS-knockout and wild type mice on postnatal day 0. Calbindin, a calcium binding protein, is a useful biomarker to identify sex differences in the POA/AH. Because NO is a signaling molecule, the locations of neurons with ir-calbindin might illuminate a role for NO in directing neuron movements. To determine the spread of cells containing ir-calbindin, a 6x6 grid of 100µm2 boxes were digitally overlaid on top of an image of one side of the POA/AH. The total number of grid squares containing dense ir-calbindin (ir-coverage above 10%) was more than 50% greater in wild-type animals than in nNOS knockout mice ($p<0.05$).

Equine ovarian aging and differential control of gene expression

JC Silveira, EM Carnevale, QA Winger, GJ Bouma

The mare is a good model to study oocyte quality, as follicular waves and hormone profiles are very similar to the women. Oocyte competence depends on communication between the oocyte and somatic cells contributing to development and competence of the oocyte. Follicular fluid (FF) provides an important environment of oocyte development and serves as a reservoir for products from surrounding cells. Our goal is to identify factors associated with oocyte quality using the mare as a model. MicroRNAs (miRNAs) are small RNAs that can regulate gene expression and function. Cathepsin ß (CTSB), is involved in apoptosis, and recently has been correlated with low oocyte quality and competence in bovine. CTSB mRNA is a predicted target of miRNA 186 (mir-186). We postulated that mir-186 and CTSB expression could correlate with low oocyte quality in mares. Thus, CTSB expression was determined in cumulus cells (CC) of young (good oocyte quality) and old (poor oocyte quality) mares. Expression of miRNAs was examined in FF and CC. Ovarian follicles from 22 young, and 18 old mares were aspirated at three different time points 23-25mm, 30-33mm prior to des/hCG, and 35mm 32-34hs after des/hCG. Real time PCR analysis of CTSB in CC demonstrated a significant (P0.10). These results: 1) Indentify CTSB as being significantly higher expressed in CC from old mares, suggesting it plays a role in decreased oocyte quality observed in old mares. 2) Demonstrate the presence of differentially expressed miRNAs in FF, which could serve as novel diagnostic tool to assess oocyte quality.

Reconstitution of functionally active mycobacterial AftC enzyme

Shiva K Angala, Jian Zhang, Pradeep Pramanik, Dean Crick, Delphi Chatterjee

Treatment of tuberculosis is prolonged and multidrug resistant (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) cases are ever increasing. Efforts to discover and develop new drugs have increased in recent years and improvement over the existing therapies is urgently needed. The cell wall of Mycobacterium tuberculosis (M.tb) with its unique physiological properties has historically been an important and valid drug target. Arabinosyltransferases (AraTs) are a small family of membrane bound glycosyltransferases involved in the biosynthesis of the arabinan portion of two major polysaccharides, arabinogalactan (AG) and lipoarabinomannan (LAM) found in the mycobacterial cell wall. In this study, Mycobacterium smegmatis was used as a model organism to study AraTs and the biosynthesis of cell wall arabinofuran. Studies have shown that AftC is a bifunctional AraT with alpha (1-3) branching activity in both AG and LAM, which contribute toward proinflammatory activity of Mycobacterium species. Technical bottlenecks in designing enzyme assays for screening for inhibitors of AraTs are: the enzymes are membrane proteins and refractory to isolation; there are no commercial substrates and the sole arabinose donor, decaprenylphosphoryl-D-arabinofuranose (DPA) is sparsely produced and difficult to isolate. In this study we have been able to express, solubilize and purify M.tb AftC. We have developed an in vitro transferase assay using purified recombinant AftC and demonstrated that AftC retains transferase activity only when reconstituted into proteoliposomes prepared from M.tb total lipids. In addition, we were also successful in synthesizing an alternate arabinose donor Z-farnesylphosphoryl-D-arabinose (Z-FPA), which has shown better solubility in the assay buffer than compared to native donor DPA. This work originally opens up many avenues to explore AraTs and related applications.

Human Monoclonal Antibodies to West Nile Virus Identify Epitopes on the prM Protein

AE Calvert, GF Kalantarov, G-J J Chang, I Trakht, CD Blair, and JT Roehrig

West Nile virus (WNV) is an emerging global pathogen causing a range of illness in humans from a mild fever to lethal encephalitis. An effective therapy to treat the more severe forms of the disease is needed. In this study, hybridoma cell lines (2E8, 8G8 and 5G12) producing fully human monoclonal antibodies (hMAbs) specific for the pre-membrane (prM) protein of West Nile virus (WNV) were prepared using a human fusion partner cell line, MFP-2, and human peripheral blood lymphocytes from a blood donor diagnosed with WNV. Using site-directed mutagenesis of a WNV-like particle (VLP) we identified 4 amino acid residues in the prM protein unique to WNV and important in the binding of these hMAbs to the VLP. Residues V19 and L33 are important epitopes for the binding of all three hMAbs. Mutations at residue, T20 and T24 affected the binding of hMAbs, 8G8 and 5G12 only. While these hMAbs did not significantly protect AG129 interferon-deficient mice or Swiss Webster outbred mice from lethal WNV infection, their isolation from the human antibody repertoire increases our understanding of the significance of the prM protein during a human flavivirus infection.

The Cellular Protein HuR Relocalizes to the Cytoplasm and Stabilizes Viral Transcripts during Sindbis Virus Infection of Human Cells

AM Dickson, K Sokoloski, CJ Wilusz, J Wilusz

Background: Sindbis virus (SinV) stabilizes its mRNAs by utilizing the cellular HuR protein, a known stability factor. SinV induces the dramatic relocalization of HuR from the nucleus to the cytoplasm during infection of human cells. We hypothesize that HuR relocalization is either a result of a cellular stress response to viral infection or is actively induced by a virus-specific mechanism. Results: SinV replication is required to induce HuR relocalization as adsorption of SinV to the cell is not sufficient to induce HuR relocalization. Nuclear-associated SinV nsp2 and nsp3 proteins were also not sufficient for HuR relocalization. Additionally, removal of the high affinity HuR binding sites from the SinV genome still induces HuR relocalization. HuR movement is species-specific, as it does not occur in SinV infections of mosquito cells. Infections with measles virus do not cause HuR movement, suggesting that relocalization is not a generalized stress response of the cell to a viral infection. Changes in the phosphorylation state of HuR during SinV infection may present vital clues to the underlying mechanism of its relocalization. Conclusion: HuR protein movement out of the nucleus is selective for alphavirus infections and requires active viral gene expression. Based on the need for alphaviruses to use HuR to stabilize mRNAs and promote a productive infection, interfering with the induction of HuR relocalization could represent a novel avenue for antiviral therapeutics.

Vaccine inhibition by highly virulent strains of tuberculosis

MI Henao-Tamayo, S Shang, A Obregon-Henao, L Nold, M Caraway, C Shanley, IM Orme and DJ Ordway

The global epidemic caused by the bacterial pathogen Mycobacterium tuberculosis continues unabated. The only available vaccine against tuberculosis, the BCG vaccine, is extremely variable in efficacy, from 80% down to zero in various trials, but the reason for this remains very unclear. In this study, we evaluated the impact of prior BCG vaccination on exposure of mice with a low dose aerosol of the W-Beijing family Mycobacterium tuberculosis strains HN878 and SA161. BCG vaccinated mice demonstrated reduced bacterial loads 30 days after infection compared to controls indicating vaccine efficacy. However, as these animals entered into chronic infection, vaccine efficacy waned as bacterial loads increased and lung pathology become similar to controls animals. Tracking the Foxp3+ T regulatory cells in these mice demonstrated clearly that mice infected with the W-Beijing strains induced expansion of more regulatory T cells at day 60, a time when the inhibition of BCG vaccination occurs. Expansion of regulatory T cells was associated with reduction of IFNgamma protective immunity required for bacterial killing. Given that new clinical trials using new recombinant forms of BCG are beginning in areas of the world where W-Beijing strains are prevalent, our research has very serious implications.

CUGBP1 binds and regulates decay of mRNAs essential for muscle function

JE Lee, JY Lee, B Tian, J Wilusz, CJ Wilusz

The RNA-binding protein CUGBP1 associates with GU-rich elements (GREs) and is an important regulator of mRNA metabolism at the levels of splicing, translation and mRNA decay. CUGBP1 appears to play a unique role in muscle cells as it is aberrantly expressed in several neuromuscular diseases including myotonic dystrophy. Altered CUGBP1 expression in myotonic dystrophy leads to inappropriate splicing of clinically relevant mRNAs, but we suspect that there may be additional impacts on decay of additional CUGBP1 mRNA targets. We measured the rates of decay for over 7000 transcripts in mouse C2C12 myoblasts and determined that GREs and AU-rich elements (AREs) are significantly enriched in the 3'UTRs of short-lived mRNAs. Further analysis indicated that GREs have more impact on mRNA decay in myoblasts than in other cell types. Microarray analysis of mRNAs associated with CUGBP1 revealed that transcripts bound by CUGBP1 are likely to contain GREs in their 3'UTRs. The enriched mRNAs encoded factors linked with cell cycle control, and protein secretion to name a few. Of note, several mRNAs encoding factors required for efficient myogenesis, including MyoD1, Myog and Cdkn1a, are bound by CUGBP1 in myoblasts. Intriguingly, these three mRNAs were previously shown to be regulated at the level of RNA stability during myogenesis, and we find that they are significantly stabilized in CUGBP1 KD cells. Further comparison of wild type and CUGBP1 KD cells suggests that decay of many more transcripts is regulated by CUGBP1 in muscle. Overall, our data demonstrate that the influence of CUGBP1 in muscular disease is potentially wide-ranging with regards to the number of processes and transcripts impacted.

Oral Presentations

Session III ~ Salon IV
1:00-5:00PM

BASIC SCIENCE

A potential role for a flavivirus-derived non-coding RNA in modulating cellular RNA decay

SL Moon, JR Anderson, CJ Wilusz, J Wilusz

Purpose: All insect-borne flaviviruses generate a series of short noncoding RNAs (sfRNAs) derived from the 3' untranslated region (UTR) of the virus genome. Although these noncoding RNAs have been implicated in cytopathogenicity, the function of these sfRNAs remains unknown. Recent work in our laboratory and others has shown that sfRNAs are RNA decay products generated from the 3' UTR of the virus by the exonuclease XRN1. We hypothesize that sfRNA acts as a specific repressor of 5' to 3' mRNA decay mediated by XRN1. Materials/methods: Radiolabeled sfRNAs were generated from the 3' UTR of DENV2 by in vitro transcription and incubated in HeLa or C6/36 cytoplasmic extracts, or with recombinant XRN1. Activity of XRN1 was assessed using labeled pGEM reporter transcripts. Transfections, northern blots, infections and PCR were performed using standard techniques. Results: We have reproduced the generation of DENV2 sfRNA in three ways: insertion of DENV2 3' UTR sequences into transfected reporter constructs, incubation of RNA substrates containing DENV2 3' UTR sequences in cell extracts from HeLa and C6/36 cells, and incubation with recombinant XRN1 protein. Several stem loop structures at the proximal side of the 3' UTR are associated with sfRNA generation. Intriguingly, in cell extract systems, the generation of sfRNA is also strongly associated with the repression of XRN1 enzymatic activity. The implied sequestration of XRN1 would shut down a major pathway of RNA decay in DENV2 infected cells and likely have dramatic effects on cellular mRNA stability during infection. We are currently assessing the relative stability of cellular mRNAs in mosquito and human cells during DENV2 infection. Conclusions: The highly structured 3' UTR of DENV2 appears to repress a major pathway of cellular mRNA decay by inhibiting the exonuclease XRN1. These findings demonstrate that a virus-derived, non-coding RNA may contribute to pathogenicity by altering host mRNA stability.

Rabies virus glycoprotein mRNA persists in human cells by recruiting cellular RNA binding proteins to the 3'UTR to repress mRNA decay

SG Palusa, CA Ndaluka, RA Bowen, CJ Wilusz, J Wilusz

Rabies virus (RV) is a negative - sense RNA virus belonging to the family Rhabdoviridae. The RV genome encodes five genes that are transcribed sequentially resulting in a gradient of decreasing transcript abundance as the polymerase moves towards the 3' end. Thus, it is generally thought that RV gene expression is controlled primarily at the level of transcription. However, we hypothesize that, like cellular mRNAs, RV mRNAs are also subject to mRNA decay which could impact the relative levels of the 5 transcripts. To address this question, we first analyzed the abundance of all 5 rabies viral mRNAs through qRT-PCR. Our results show that the glycoprotein mRNA is expressed at higher levels than predicted by the simple transcription gradient model consistent with mRNA decay playing a role. We next generated RNAs containing rabies viral 3'UTR sequences and used them in in vitro decay assays. We found that rabies glycoprotein mRNA 3'UTR is significantly more stable than the four other RV 3'UTRs. Excess specific glycoprotein 3'UTR competitor RNA activated decay of the glycoprotein 3'UTR RNA and this correlated with competition of a 38kD protein. Through in vitro affinity purification we have identified the 38kD protein as poly (rC) binding protein2 (PCBP2). PCBP2 is an RNA-binding protein that has been shown previously to have a stabilizing effect on mRNAs and also is utilized by other virus families, such as poliovirus, to promote productive infection. In summary, these results suggest that PCBP2 may bind the 3'UTR of RV glycoprotein mRNA and stabilize it to increase glycoprotein production during infection. We are currently investigating the effects of depleting PCBP2 from cells on viral growth and RV glycoprotein mRNA stability.

Deleting the Undeletable: A novel approach to make previously unattainable gene knock-outs in Burkholderia pseudomallei

DA Rholl, HP Schweizer

Burkholderia pseudomallei is a potential biowarfare agent and the etiological agent of melioidosis, a rare but serious tropical disease. The bacterium has a large, constantly evolving genome. Because of this genetic variability, clinically observed mutations resulting in increased virulence or antibiotic resistance strain must be confirmed in defined genetic backgrounds. Recently a group of 6 patients all failed treatment with ceftazidime. Initial isolates were ceftazidime sensitive, but secondary isolates were resistant and had decreased fitness. Each ceftazidime resistant strain had large deletions from chromosome 2, all including a group of 49 genes. To identify the responsible gene(s), we attempted to delete three candidate genes possibly associated with ceftazidime's target (peptidoglygan synthesis) within lab strain 1026b. Two genes were readily deleted using an allele exchange system. The system uses sucrose counter-selection to identify mutants that have undergone homologous recombination, which results either in the mutation or a reversion to the wild-type. One gene, BPSS1219, could not be deleted in this way. Spontaneous sucrose resistance kept us from isolating slow-growing mutants. We designed a new strategy involving chromosomal insertion of a rescue copy of BPSS1219 flanked by loxP sites, allowing us to use highly efficient Cre- lox site-specific recombination to excise the rescue copy after deleting the endogenous BPSS1219. This allowed us to delete BPSS1219, which resulted in drastically decreased cellular fitness, filamentous growth and ceftazidime resistance; the same phenotype observed in the clinical strains. Using the same rescue construct, we were able to restore near wild-type phenotypes in ceftazidime resistant clinical mutants. By employing these strategies we demonstrated that of the 49 deleted genes, BPSS1219 alone was responsible for the clinical observations. This approach will be a valuable tool in the study of B. pseudomallei.

Development of a Caprine Model of Melioidosis

C Soffler, TA Aboellail, RA Bowen

Purpose: Melioidosis results from infection with the gram-negative saprophyte Burkholderia pseudomallei and is an important cause of sepsis in southeast Asia and northern Australia. Rodent models have been central in the study of the pathogenesis of melioidosis. However, there are significant limitations to these models. In addition to the fact that rodents are not naturally infected with B. pseudomallei, rodent models do not readily allow for serial assessment of clinical signs/vital parameters, clinical pathology, and immunologic events because of the small size and limited blood volume. This greatly limits how the models can evaluate disease progression on a human-relevant scale. This study describes the development of an aerosol model of caprine melioidosis, which avoids many of the limitations of the rodent models.

Materials and Methods: Goats were infected with a goat isolate of B. pseudomallei via an intra-tracheal aerosol at one of three doses (10^8, 10^6, and 10^5 CFU). Disease progression was monitored until humane euthanasia was clinically indicated or for a maximum of two weeks post infection. Gross necropsy, histopathology, and organ burden measurement was performed for all goats.

Results: Goats at the highest dose level developed acute respiratory distress and were euthanized by 48h post-infection. Gross necropsy revealed severe pulmonary edema and hemorrhage. All other goats became acutely and persistently febrile. Goats infected with10^6 had more severe fever, oculonasal discharge, and cough than goats infected with 10^5 CFU. Gross necropsy and histopathology revealed pulmonary abscessation and extrapulmonary dissemination in goats infected with 10^5 and 10^6 CFU. The extent of extrapulmonary dissemination was greater in goats in the higher dose group.

Conclusions: Goats infected with an intratracheal aerosol of B. pseudomallei can be used as reproducible model of human melioidosis.

Development of a Novel Detection Assay for Chronic Wasting Disease Prions in Soil

AC Wyckoff , KC VerCauteren, V Gilman, T Truong, MD Zabel

Studies suggest that environmental deposits of Chronic Wasting Disease (CWD) prions, proteinaceous particles lacking a genome, play an important role in the transmission and persistence of CWD among captive and wild cervids. Furthermore, studies indicate that the prion molecule forms a close association with clays and other types of soil, enhancing its persistence and surprisingly, enhancing the transmissibility of the infectious agent. Successful detection and quantification of prion deposits in soil are essential for progress in understanding the disease ecology and developing management strategies for CWD. Unfortunately, prion persistence in soil has been a particularly challenging aspect to study due to limited sensitivity of existing laboratory assays. In collaboration with InfoScitex, the study develops a novel detection assay that uses small oligonucleotides, referred to as aptamers, to selectively bind prions. These aptamers can then be amplified by qPCR and used as a proxy to determine presence or absence of prions. The objective is to develop this assay to allow for an increased detection limit of prions in soil samples. CWD positive elk brain homogenate was used to spike samples of whole soil. The soil samples were then incubated with the selected aptamer, digested unbound aptamer, and tested samples for presence of the aptamer by qPCR. Preliminary results indicated aptamers are capable of detecting soil bound prions. Future directions include testing serial dilutions to determine detection limit and testing of naturally CWD prion exposed soil samples.

Assessment and Prioritization for an Occupational Health and Safety Management System for a Veterinary Teaching Hospital

M Koesterich, W Brazile, K Blehm, and D Hendrickson

There are many hazards that could cause injuries in veterinary hospitals, yet there are no published reports of any veterinary hospital in the U.S. of successfully implementing an Occupational Health and Safety Management System (OHSMS). In 2005, the voluntary standard, Occupational Health and Safety Management Systems (AIHA/ANSI Standard Z10-2005) was published to assist organizations in implementing an OHSMS. This research is focused on the development of an OHSMS at the Colorado State University (CSU) Veterinary Teaching Hospital (VTH), following the AIHA/ANSI Standard to help improve health and safety (H&S) performance. The VTH was audited in 2003 and 2009 revealing significant hazards, yet showed little improvement. The rationale for this study is to improve the H&S performance at the VTH as well as provide a template that other veterinary hospitals may use to develop and implement an OHSMS. Through a safety and health assessment and employee interviews at the VTH, the researcher will: review relevant business management systems; identify hazards; conduct a comprehensive hazard analysis and risk assessment of tasks; identify applicable regulations, standards, and requirements; prioritize issues based on risk; and recommend protective strategies for each job task to lower risk to acceptable levels. Data on the hazardous operations and conditions in the VTH are being collected and range from minimal risk to serious risk. These results will be prioritized and provided to VTH management. Anticipated results include the documented ANSI planning elements. Preliminary conclusions are: the H&S assessment will reveal the need for the implementation of an OHSMS; and that an OHSMS can be successfully implemented in veterinary hospitals and will improve H&S performance. Please note that although results are not yet complete, results and discussion will be presented at the Research Day.

Liposomes Combined with TLR9 Agonist Produce Effective Mucosal Vaccine against Mycobacterium tuberculosis

AR Troy, S Dow, AA Izzo

The battle against Mycobacterium tuberculosis (M. tb) has been prevalent since a time when the pharaohs ruled Egypt but the disease continues to remain the dominant cause of death from infectious disease to this very day. Despite decades of vaccine development the human race is still tragically vulnerable to the disease. Current vaccine strategies now extend beyond vaccine components and an equal amount of focus has been placed on the route of administration as well. Due to the particular virulence of M. tb when aerosolized and inhaled, we have developed a vaccine strategy which addresses not only the stimulation of the immune system but also the priming of cells in the local pulmonary environment. We have determined that CpG Oligodeoxynucleotides (ODN), in particular the sub-type CpG-C ODN, stimulates a robust Th1 cellular immune response capable of providing significant protection against pulmonary bacterial burden following aerosol challenge. This CpG-C ODN was used in combination with a strongly stimulating M. tb antigen, ESAT-6 to elicit the induction of antigen-specific T-cells. Lastly, when the antigen vehicle consisting of cationic liposomes was added to the CpG ODN adjuvant and the ESAT-6 antigen the cellular immune response was significant. When this vaccine formulation was administered intra-nasally the mice developed a robust cellular immune response within the local mucosa and developed a significant reduction in bacterial burden when compared to saline controls. This vaccine containing CpG ODN, ESAT-6, and cationic liposomes may prove to be a promising vaccine capable of limiting the infection and dissemination of disease following exposure to aerosolized M. tb.

Hypofractionated Radiation Therapy Plus Toceranib for Unresectable Canine Mast Cell Tumors

K Carlsten, D Thamm, C London, S Haney

Purpose: Mast cell tumors (MCT) represent the most common cutaneous tumor in dogs and those that are nonresectable present a therapeutic challenge. The aims of this study were to determine the efficacy, tolerability and adverse effect profile of combined treatment with toceranib and hypofractionated radiation therapy (RT) in dogs with MCT.

Materials and Methods: Seventeen client-owned dogs were enrolled in this study. All dogs had measurable MCTs amendable to RT. All dogs received prednisone, omeprazole, and diphenhydramine prior to starting toceranib. Toceranib was administered (2.75 mg/kg PO M, W, F) for 1 week prior to starting RT A total of 24 Gy was delivered in 3 fractions of 8 Gy on a 0, 7, 21 day schedule or 4 fractions of 6 Gy weekly. Response was determined by serial tumor measurements and evaluated using the RECIST scoring system. Adverse events were evaluated using the modified VCOG criteria and the VRTOG acute radiation scoring system.

Results: On an intent-to-treat basis, the overall response rate was 76% (13/17). Of the patients that responded 10 experienced a complete response and 3 had a partial response. Additionally, 2 patients had stable disease. Median time to best response was 22 days (range, 7 to 156). 73% of dogs required a drug holiday and 40% received a dose reduction. The most common toxicities were grade I gastrointestinal, grade III elevation in liver enzymes, and grade I anemia. At the time of this abstract, 1 dog experienced local recurrence, 2 had De Novo MCTs, 1 had metastatic disease, and 1 had recurrence outside the radiation field. Two patients were removed from the study prior to starting toceranib.

Conclusions: The combination of hypofractionated RT and toceranib was well tolerated and demonstrated short-term efficacy in the majority of dogs. This combination may be a viable option for treatment of nonresectable MCTs in the future.

Role of pADPr modification in DNA-PK-mediated end-joining

RC Dregalla, D Maranon., SM Bailey

Recent research from our laboratory has provided the first evidence for Poly(ADP-ribosyl)ating Polymerase (PARP) activity in the intracellular stability of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) protein (Dregalla, 2010). We found that the telomeric PARP family member PARP-5a, better known as tankyrase 1, operates as a regulator of DNA-PKcs protein stability via poly(ADP-ribosyl)ation (pADPr/PAR) modification of DNA-PKcs. In this modified form, DNA-PKcs becomes proteasome-resistant and is dynamically regulated by the contributions of tankyrase 1, poly(ADP-ribose) glycohydrolase (PARG) and the proteasome. When tankyrase 1 is depleted or catalytically inhibited, intracellular DNA-PKcs is de-PARsylated (via PARG activity) and becomes a substrate for proteasome-mediated degradation, resulting in the rapid and significant reduction of DNA-PKcs protein levels. Identification of tankyrase 1 as a regulator of DNA-PKcs protein stability provides intriguing insight to the intricate mechanisms and function of posttranslational modifications. Interestingly, 'traditional' pADPr protein modifications results in destabilization of the modified protein. In the case of DNA-PKcs, pADPr addition is a positive regulator, joining a select few other proteins under a similar classification (Olabisi, 2008, Chang P., 2009). Although not the first protein to be stabilized via pADPr addition, the story regarding DNA-PKcs and pADPr -modification is just emerging and the story is incomplete.

It is clear that pADPr modification of proteins can have multiple purposes. pAPDr addition can result in subsequent: modification by an additional enzyme (i.e. E3 ubiquitin ligase), recruitment of additional proteins electrostatically (i.e. PARP-1), non-covalent pADPr-dependent protein interactions (i.e. NuMA) or protein activation (i.e. NFAT & DNA-PKcs). Thus, the story of DNA-PKcs PARsylation likely extends well beyond protein stabilization. Currently, we understand modification of DNA-PKcs via tankyrase 1-dependent PARsylation has a dynamic, stabilizing role by protecting DNA-PKcs from proteasome-mediated degradation. However, the functional implications of this modification have yet to be established. We hypothesize DNA-PKcs in a PARsylated form plays an essential role in DNA-dependent protein kinase (DNA-PK) –mediated non-homologous end-joining (NHEJ) which is not achievable by unmodified forms of DNA-PKcs. Our overall goal is to move forward from our initial observation of tankyrase 1-dependent PARsylation of DNA-PKcs being required for proteasome resistance, to pADPr modification being critical for appropriate recruitment, scaffolding and activation of DNA-PK and 'classic' NHEJ. We aim to investigate the involvement of pADPr-modified forms of DNA-PKcs in DNA-PK holoenzyme assembly and function. Further, we aim to provide evidence of PARP-1 as a critical accessory protein to DNA-PK assembly, supporting studies that identify PARP-1 as a DNA-PK activator.

Evaluation of the Effects of a GnRH Immunocontraceptive Vaccine on the Behavioral Ecology of Wild Horses (Equus caballus) at Theodore Roosevelt National Park, North Dakota.

LJ Eby, JI Ransom, AT Kirby, HM Garbe, MW Oheler, JG Powers, TM Nett, DL Baker

Overabundant and rapidly expanding wild horse (Equus caballus) populations present a challenging dilemma in western North America. There is widespread concern among federal and state agencies, that unregulated wild horse populations are severely altering native plant communities. Controlling the fertility of female horses offers an alternative to existing methods of population control. A promising new approach to contraception involves active immunization against gonadotropin-releasing hormone (GnRH) to suppress reproductive hormones and ovulation. A field study began in October 2009 to investigate the effect of this agent on reproduction, behavior, and side-effects in female wild horses at Theodore Roosevelt National Park, North Dakota. Fifty-seven adult mares were captured during a scheduled roundup and treated with either GnRH vaccine (n = 29) or saline solution (n = 28). During the following breeding season (March to November 2010), we evaluated and compared the effects of GnRH vaccine on reproductive behaviors, social dynamics, and activity patterns of experimental horses. We used a combination of instantaneous scan sampling procedures to record time budget data and all-occurrence sampling to record reproductive behaviors of treated and control mares. During the early breeding season (April 10 to May 10, 2010) a total of 314 20-minute observations were made during this sampling period. We found no difference ($P \geq 0.248$) in activity budgets between experimental groups. Likewise, social interactions between treated and control mares were similar. There was no difference in stallion aggression between groups ($P = 0.475$), herding ($P = 0.158$), or reproductive behaviors ($P = 0.342$). Aggression from stallions to mares was primarily in the form of threats (63.3%) followed by chases (31.8%), and kick/bites (4.5%). Reproductive behavior of stallions toward mares was expressed primarily as tending (80.0%) with copulation (20%) infrequently observed. Based on the initial evaluation of the early breeding season, we are hopeful that the complete data will show promising results suggesting GnRH vaccine has no short-term effect on behavioral ecology of wild horses during the breeding season. The definitive data set is in the process of evaluation representing data collected April 8 to November 15, 2010.

Are gastrointestinal helminths of ringed seals (Phoca hispida) and spotted seals (Phoca largha) capable of mercury (THg) uptake within their definitive host?

AK McGrew, TM O'Hara, MD Salman, LR Ballweber

Parasites have been recognized for their role in evaluating ecosystem health, and certain parasites are known to bioaccumulate heavy metals in concentrations exceeding those in host tissues. To evaluate the role of helminths in mercury (Hg) distribution in seals, we sampled subsistence-harvested ringed seals (Phoca hispida; n=15) and spotted seals (Phoca largha; n=4) from Kotzebue Sound in the fall of 2009 and 2010. Livers, gastrointestinal tracts (GI), and kidneys were obtained. Helminths were removed from GI tracts, enumerated, and frozen for total mercury (THg) analysis. All 19 animals were parasitized. In ringed seals, prevalence of cestodes, nematodes, and acanthocephalans was 6.7%, 46.7%, and 100%, respectively. Nematode counts ranged from 2-19 (x = 7.0). Acanthocephalan counts ranged from 2-247 (x=89.8). Among spotted seals, prevalence of nematodes and acanthocephalans was 75.0%, and 100%, respectively. Cestodes were not present. Nematode counts ranged from 3-17 (x=10.7), whereas acanthocephalan counts ranged from 362-1818 (x=874.3). Acanthocephalan counts were significantly higher in spotted seals ($p<0.05$) than in ringed seals. There was no significant difference in nematode counts. Both liver and kidney THg concentrations (ppb, wet weight) were significantly higher in spotted seals than in ringed seals ($p<0.05$). In ringed seals, THg in acanthocephalans ranged from 9.0-173.5 (n=14) ppb wet weight (ww), and nematode THg ranged from 12.1-160.3 (n=7) ppb ww. In spotted seals, acanthocephalan and nematode THg ranged from 25.5-120.5 (n=4) and 21.7-59.2 (n=2) ppb ww, respectively. THg in the cestodes of ringed seals (n=1) was 103.8 ppb ww. Helminths are clearly demonstrating THg uptake in these animals, and total biomass may be an important factor in determining the effect of toxicant-parasite interactions on host health.

The effect of low-dose ionizing radiation in lungs of recombinant congenic strains of mice

D Ochola, M Weil, J Bedford

Medical exposures to ionizing radiations carry risks as well as benefits. One of the risks is that of radiation induced cancer, including lung cancer. Some inbred mouse strains are more susceptible than others to radiation-induced lung cancer, which suggests that genetic background may be an important contributor to risk. Inbred mouse strains also differ in their ability to efficiently repair DNA double strand breaks caused by radiation exposure. We suspect that strains of mice that are unable to efficiently repair radiation-induced DNA double strand breaks (DSB) in lung tissue will also be susceptible to radiation-induced lung cancer.

Many studies on effects of radiation-induced DNA damage relied on cultured cells and cell lines, with very little available data on induction and processing of radiation-induced DNA damage repair in complex normal tissues during and after radiation exposure. We decided to investigate the impact of low-dose irradiation on DSB repair efficiency in a complex tissue setting using special inbred recombinant congenic strains of mice, and to correlate the repair efficiency with known cancer susceptibility of sets of these strains of mice. While the study is being done with mice, humans also have inter-individual differences in their abilities to repair DNA DSBs. Thus the development of assays to measure radiation-induced DNA damage repair in complex normal tissues may allow us to predict outcomes of radiation exposures in the general population and provide more clinically relevant data that can be used as a foundation for future decisions on radiation use in diagnosis, treatment or practices where individuals encounter radiation exposures.

Preliminary observations using merged images of H2AX and 53BP1 foci as markers of DSB repair response in the lung shows a clear difference in the number of foci in the bronchiolar epithelium on the one hand, and the cells in the lung parenchyma on the other. In the epithelium, cells in the basal layer appear to have more foci than those in the luminal layer. Within the parenchyma compartment, there are isolated cells having more foci than other cells surrounding them. Future studies will reveal the identity of these apparently unique parenchyma cells.

Baseline histological health assessment of subsistence harvested arctic marine mammals from the North Slope Borough villages of Barrow and Wainwright, Alaska

GM Krafsur, TR Spraker, B Adams, C George, C Hanns, R Suydam, T Hepa, H Brower Jr.

Routine histological analysis of formalin- fixed tissues obtained from subsistence harvested seals and walruses taken near the North Slope Borough villages of Wainwright and Barrow, Alaska was conducted in order to survey these important subsistence resources for evidence of contaminant exposure, disease surveillance and infection with zoonotic pathogens. Mild exertional rhabdomyolysis with loss of sarcoplasmic detail, fragmentation, myositis, repair and healing was consistently observed. Sarcocysts were identified in the skeletal muscles of seals, but not walruses. Endoarteritis in ringed seals with moderate to severe fibrointimal and fibromuscular proliferation, fragmentation of the internal elastic membrane, and thrombi formation indicative of Acanthocheilonema spirocauda infection was repeatedly observed. Parafilaroides spp were positively identified in sections of lung from ringed seals and were implicated in the presence of verminous pneumonia in bearded seals. A consistent finding among all sections of liver examined from seals and walruses was focal areas of hypertrophied Kupffer cells, occasional neutrophils and lymphoplasmacytic infiltration suggestive of parasitic migration through the liver parenchyma. Positive identification of eggs and flukes consistent with Orthosplanchnus spp infection along with moderate to severe chronic active cholangitis, cholangiohepatitis and hepatocellular necrosis caused by fluke presence was a common finding. The same flukes were responsible for inflammation and fibrosis of the pancreatic ducts and periportal fibrosis. One seal had a focus of severe chronic granulomatous pancreatitis with focal peritonitis. There was no histopathogical evidence of renal colonization with Leptospira spp. Histological evidence of immunosuppression or endocrine disruption associated with environmental contaminant exposure was not observed.

Poster Presentations

Influences of Mouse Parvovirus on Cytokine Production in Mice

CF Allaband, DJ Ordway, S VandeWoude, KS Henderson, LV Kendal

Mouse parvovirus (MPV) is a continual concern in mouse colonies. The presence of the virus can negatively impact research results through its immunosuppressive effects on T cells, but its serologic detection remains inconsistent. In order to determine a more reliable way to detect MPV in mouse colonies, a better understanding of the host immune response during the course of infection is necessary. The aim of this project is to evaluate the T cell responses in MPV infected mice to determine if regulatory T cells influence the host immune response. Toward this aim, T cell responses in BALB/c, C57BL/6, and Swiss Webster mice inoculated with MPV1e were compared to sham inoculated mice. Infection was confirmed by fecal PCR. Ten mice from each strain were euthanized after five days, representing an acute infection, and ten mice from each strain were euthanized after six weeks, representing chronic infection. Mesenteric lymph nodes and spleen samples were taken from all of the mice and their cellular contents analyzed by flow cytometry to evaluate Th1, Th2, Th17 and regulatory T cell responses. All strains had an increase in CD4+ and CD8+ T cells and Dec205+ dendritic cells, and decrease in Foxp3+CD4+ T cells, IL17+ T cells and IL-10 production. There were variable strain dependent changes in CD11c, CD11b, and NK1.1 cells, and TNF-alpha and IFN-gamma production. These results demonstrate the complexities of the host immune response to MPV and how it may potentially impact the antibody response and subsequent serologic detection of MPV. With a better understanding of the host immune response and the role of regulatory T cells, more reliable diagnostic assays could be developed to enhance detection and minimize the research effects of MPV infection in mice.

Occupational Exposure to Noise from Personal Stereos

D Autenrieth and WJ Brazile

With recent improvements in portable digital media players (PDMPs) such as increased media storage and prolonged battery life, individuals can enjoy music more conveniently and for longer periods during recreation and work. Contemporary studies have evaluated the risk of hearing damage from PDMP use and found that a small proportion of recreational users listen at levels that may increase the risk of noise-induced hearing loss (NIHL), though none have considered the occupational use of these devices. This pilot study examines the contribution of PDMP use in the workplace to overall employee noise exposures. We will assess workers at a local manufacturing facility who are using PDMPs while on the job. Our approach is to administer a questionnaire to evaluate listening behaviours and measure PDMP output using a KEMAR Ear and Cheek Simulator, and background noise using a sound level meter. Sound levels recorded with the Ear and Cheek Simulator will be converted to free-field equivalent, A-weighted averages for comparison to occupational exposure limits. The self-reported duration of use from our questionnaire will be applied to PDMP and background noise levels to construct a daily noise dose. Workers who use PDMPs in high (manufacturing floor) and low (office areas) background noise environments will be evaluated to compare the listening level to background noise ratios. We suspect that PDMP use in the workplace may significantly increase the risk of NIHL and anticipate that our results will be used to educate employers on the risks of PDMP use and the prevention of NIHL. This study is funded by the NIOSH Mountain and Plains Education and Research Centre (MAP ERC) Pilot Research and Community Projects Program in Occupational and Environmental Health and Safety.

Associations between antimicrobial use and antimicrobial resistance in Escherichia coli sampled from individual feedlot cattle.

KM Benedict, SP Gow, CW Booker, TA McAllister, and PS Morley

Purpose: The objectives of this study were to 1) estimate the prevalence of antimicrobial resistance in the study population and 2) to investigate the associations between exposures to antimicrobial drugs and antimicrobial resistance in fecal non-type specific E. coli (NTSEC) recovered from individual feedlot cattle.
Materials/Methods: Two-stage random sampling was used to identify cattle for enrollment at 4 western Canadian feedlots. A fecal sample was collected per rectum from each individual at arrival and in the middle of the feeding period when cattle were rehandled as part of standard feedlot protocol. From samples collected at this second time point, a total of 2,133 NTSEC isolates were tested for susceptibility to antimicrobial drugs by disk diffusion. Parenteral and in-feed exposures to antimicrobial drugs were recorded for each individual enrolled in the study. The least square means estimates and 95% confidence intervals for the prevalence of resistance at each time point were modeled using Poisson regression. Multivariable logistic regression was used to investigate associations between antimicrobial resistance and exposure to antimicrobial drugs. Regression models were adjusted for clustering of observations among individuals and pens.
Results: The most common resistances identified in arrival samples were sulfisoxazole (7.5%; 95%CI: 6.1-9.2), streptomycin (7.7%; 95%CI: 6.3-9.5) and tetracycline (20.0%; 95%CI: 17.7-22.6). At the second sampling point, resistance prevalence was 25.6% (95%CI: 23.5-28.0) for sulfisoxazole, 25.0% (95%CI: 22.8-27.3) for streptomycin, and 72.7% (95%CI: 70.5-75.1) for tetracycline. Logistic regression modeling identified weak associations of exposures to tetracycline and macrolide classes of drugs with antimicrobial resistance at the second time point.
Conclusions: Exposures to tetracyclines and macrolides were common yet associations between resistance and exposure to antimicrobials were not of great magnitude.

The retro-viral cyclin of walleye dermal sarcoma virus enhances transcript levels of serum response and cell cycle genes

C Birkenheuer, S Quackenbush, J Rovnak

Walleye dermal sarcoma is a seasonal dependent tumor found in walleye fish. It is caused by the complex retrovirus, walleye dermal sarcoma virus (WDSV). Expression of the accessory proteins encoded by this virus differs throughout the tumor life cycle. Retro-viral cyclin (rv-cyclin) is one of these accessory proteins, and its expression correlates with tumor development. Rv-cyclin interacts with different components of the transcriptional machinery such as cyclin dependent kinase 8 (Cdk8), and TATA-binding protein associated factor 9 (Taf9), as well as with cyclin dependent kinase 3 (Cdk3), a regulator of the cell cycle.
Cdk8 has been previously demonstrated to play a positive role in transcription of the serum response genes. These genes are referred to as immediate response genes (IEG), and some examples include fos, egr1, and jun. Knowing that rv-cyclin interacts with CDK8, we were interested in testing the IEG transcript levels in cells expressing rv-cyclin when compared to cells that were not. Here we demonstrate, in transiently transfected HeLa cells and serum starved HCT 116 cells that the presence of rv-cyclin increases the amount of mRNA transcripts from the immediate early genes as much as 65 fold. Additionally, previous gene array experiments had shown a possible activation of other cell cycle genes. Two of these genes encode cyclin D, and p19INK4d, an inhibitor for cyclin dependent kinase 4 (Cdk4). However, it had not been demonstrated that rv-cyclin acted on these genes directly, or if the activation of these genes was due to a down-stream effect of the IEGs. We show that transcript levels from these cell cycle genes also increase between 2 and 4 fold when rv-cyclin is present. This increase is coincident with the stimulation of the IEGs. In summary, this data suggests that rv-cyclin's role in tumor development may be to alter gene expression patterns of IEGs and some cell cycle genes aiding in the development of tumors.

Validation of an Equine Back Profiling System to Optimize Saddle Fitting

S Blauvelt, K Haussler, A Hill, K Kawcak

Few objective and easy-to-use methods exist for capturing dorsal trunk contours in an attempt to improve saddle fit or to design custom-fitting saddles. The Dennis Lane Equine Back Profiling System (EBPS) consists of a series of contoured cards that provide a user-friendly method for quantifying the dorsal contours of the equine trunk. The objective of this study was to validate EBPS's ability to quantify transverse and parasagittal contours of the dorsal saddle region in different breeds and uses of horses. Our hypothesis was that EBPS could capture the majority of the dorsal trunk contours of several breeds of horses used in different athletic activities. The transverse and parasagittal dorsal contours were mapped at 5 thoracolumbar vertebral levels in the saddle region of 211 adult horses consisting of Warmbloods, Thoroughbreds, Quarter Horses and Morgans involved in jumping, dressage, cutting, reining and show, respectively. The EBPS was easy to use and readily quantified the dorsal trunk contours of a large horse population. The cards proved to be more precise on well muscled horses compared to those with muscle atrophy. Quarter Horses had the widest transverse contours at the level of the withers and the croup. Morgans were the widest throughout the thoracolumbar area while Thoroughbreds consistently had the narrowest transverse contours. The Warmbloods and Thoroughbreds had the smallest parasagittal concavities while the Quarter Horses had the deepest. Each breed was asymmetric (by at least 20%) with respect to the cranial-caudal parasagittal contour, with Morgans being most significant at 60%. Comparing athletic use, the reining Quarter Horses were less symmetric than the cutting Quarter horses. The EBPS provided an easy-to-use method for quantifying the size and shape of a horse's back, which is a significant advancement in methods used to standardize saddle fit.

Disinfectant comparison and exam room general cleaning effectiveness of the Small Animal Clinic at the James L. Voss Veterinary Teaching Hospital

BA Burgess, NR Noyes, PS Morley

Purpose: The quaternary ammonia disinfectant product used by the James L. Voss Veterinary Teaching Hospital (JLV-VTH) has been discontinued. Two brands were identified which were equivalent to the discontinued product. The objectives of this study were to evaluate overall reduction in bacterial count when using these disinfectants and to evaluate the effectiveness of the general daily cleaning of the Small Animal Clinic (SAC) at the JLV-VTH.
Materials/methods: Fifteen rooms in the SAC were purposefully selected for study (11 exam rooms, 2 urgent/intermediate care rooms, 2 treatment rooms), and four general sampling locations were identified (floors, tables, counters, doors). Within all possible sampling locations, sampling sites were randomly chosen such that 32 sites were sampled before and after cleaning, for both disinfectants (n=128) on randomly selected days. Using blood agar contact plates, surface bacterial counts were estimated after 24 hours of incubation. Data were analyzed using linear regression with the outcome of bacterial reduction at 24 hours of incubation while controlling for baseline colony count, site, and crew member.
Results: Disinfectant 2 had a greater reduction in colony count at 24 hours of incubation when compared to Disinfectant 1 with an average difference of 1.6 colonies per 10 cm^2 when controlling for differences attributable to baseline colony count, site, and crew member (p=0.05). Cleaning and disinfection had greater impact on floor cleanliness when compared to other surfaces; 3 crew members were found to have greater average reductions in colony counts when compared to other crew members.
Conclusion: These disinfectants have similar practical efficacies in reducing bacterial counts on surfaces in the SAC. The findings of this study suggest that cleaning and disinfection practices will have a larger effect on environmental cleanliness than can be attributed to differences between disinfectants.

Gene expression profiling of an industrial bioethanol yeast strain during the fermentative process.

OV Carvalho-Netto, J L Argueso

The bioethanol production system based on sugarcane feedstock is one of the most successful and sustainable models of renewable energy production currently available. This system is based on the alcoholic fermentation of sucrose by highly adapted strains of the yeast Saccharomyces cerevisiae. We have recently described the complex genome structure of one of the most productive and widely adopted industrial strains: PE-2 / JAY270 (Genome Research 19:2258). PE-2 is a heterothallic diploid, and its genome is characterized by a high degree of hetorozygosity, both at the nucleotide sequence and karyotype levels. This intrinsic genetic diversity is likely a key factor in PE-2's extraordinary ability to thrive in the stressful environment found in large industrial fermentation tanks. However, the molecular mechanisms that contribute to this adaptation are largely unknown. This gap in basic biological knowledge about this strain represents a significant barrier to its genetic improvement and the full exploitation of its biotechnological potential. In this study we are taking advantage of the available PE-2 genome sequence to investigate the molecular physiology of sugarcane bioethanol fermentation through genome-wide transcription profiling using cDNA sequencing of samples harvested from industrial fermentation tanks. The results to be presented here offer new insight into the biology of the PE-2 strain, including the expression behavior of novel genes absent in well-characterized laboratory strains, and the identification of the stress response mechanisms active in PE-2 that could explain its superior fitness and competitiveness during bioethanol production.

Expression of HES-1 in Canine Osteosarcoma

DD Dailey, L O'Donoghue, KP Anfinsen, JB Charles, EJ Ehrhart, DL Duval

HES-1, a bHLH (basic helix-loop-helix) transcriptional repressor, is a downstream target of the Notch signaling pathway. Additionally, Notch-independent HES-1 expression has been reported some human tissues. Notch signaling and HES-1 expression have been linked to growth and survival in a variety of human cancer types. Increased expression of HES-1 has been shown to be associated with increased metastasis and invasiveness in human osteosarcoma. Objectives for this study included confirmation and exploration of HES-1 expression in canine osteosarcoma (OSA). Quantitative RT-PCR was utilized to quantify HES-1 gene expression in tumor and normal bone samples taken from dogs treated for appendicular OSA at the Colorado State University Veterinary Teaching Hospital with surgical amputation of the affected limb and adjuvant chemotherapy. HES-1 gene expression was elevated in tumor samples relative to matched normal bone, but decreased in tumor samples from dogs with a disease free interval (DFI) of less than 100 days relative to those with a DFI of greater than 300 days. Immunohistochemistry was utilized to confirm translation of mRNA and expression of HES-1 protein in a subset of the same tumors analyzed by RT-PCR. Protein expression of HES-1 varied across tumors and within individual tumors with neoplastic cells showing predominantly nuclear and less frequently diffuse cytoplasmic immunostaining. Immunostaining appeared to correlate with quantitative RT-PCR results. Changes in HES-1 gene and protein expression within these tumor samples suggest that alterations in the Notch signaling pathway occur in canine OSA. Furthermore an inverse relationship of HES-1 expression and DFI warrants additional exploration of the correlation of HES-1 expression with patient survival in canine OSA.

Stimulation of Intestinal Immunity by Diets Containing Rice Bran

AJ Duffy, A Kumar, ER Ryan, SW Dow

Rice bran contains a number of bioactive compounds, some of which have been shown in vitro to activate immune cells such as macrophages. We hypothesized therefore that feeding rice bran might be capable of non-specifically enhancing mucosal immunity. Therefore, mice were fed either a control diet or a 10% rice bran supplemented diet and the effects on systemic and local immune responses were assessed. Immune responses examined included systemic and mucosal antibody responses (IgA and IgG) and T and B cell responses. Cytokines and antigen presenting cells infiltration into the GALT as well as the effect of rice bran on the microbial flora of the gut was also studied. We found that feeding a 10% rice bran diet elicited a significant increase in mucosal, but not systemic, concentrations of IgA. In addition, we found that there was a significant increase in the number of IgA+ B cells in the Peyer's patches of rice bran fed mice. We also found a significant increase of dendritic cells in the lamina propria following the intake of rice bran diet. We conclude therefore that feeding rice bran can non-specifically stimulate intestinal immune responses and may therefore be an efficient means of inducing non-specific protection against enteric pathogens.

The Role of LIN28 and MicroRNAs in Ovarian Cancer Biology

VE Enriquez, MA Spillman, QA Winger, GJ Bouma

Ovarian cancer is the fifth most deadly cancer among women in the nation and the second most common gynecological malignancy in the Western World. Although treatment of early stage ovarian cancer has a survival rate of ≥90% after 5 years of remission, diagnosis at early stages is often overlooked leading to widely disseminated peritoneal disease and increased risk of relapse even after treatment with chemotherapy and surgery. The treatment for patients with recurring ovarian cancer is not well defined; therefore, discovering novel regulatory factors that influence recurrence of ovarian cancer can lead to insights into cancer progression and proliferation. Pluripotency stem cell factor LIN28 is an evolutionary conserved RNA binding protein, which negatively regulates mature let-7 miRNA expression thereby preventing stem cell differentiation. Assessment of the LIN28-let-7 miRNA loop in ovarian cancer can provide insight into cells in ovarian tumors that possess cancer stem cell qualities. Tumor cells have been shown to release exosomes containing tumorigenic factors. Exosomes are endosome-derived organelles (50-100nm) containing bioactive materials, including miRNAs that are transported through the bloodstream. The objective of this study was to: 1) detect LIN28 expression in IGROV-1, OV420, and SKOV3 human epithelial ovarian cancer (EOC) cell lines and 2) identify miRNAs expressed these three EOC cell lines. LIN28 mRNA was significantly higher expressed in IGROV-1 EOC cells compared to OV420 and SKOV3 EOC cells. In addition, qRT-PCR analysis revealed that let-7 miRNA was significantly lower expressed in IGROV-1 EOC cells compared to SKOV3 EOC cells. To assess the presence of miRNAs in exosomes, IGROV-1, OV420, and SKOV3 EOC cells qRT-PCR was performed. Let-7f-1 miRNA was significantly lower expressed in exosomes from IGROV-1 cells along with miR-200-b and miR-200-c. These results indicate circulating exosomes secreted by cancer cells contain miRNAs that are potential molecular regulators of tumorigenesis. The findings from this study suggest a potential regulatory role of LIN28-let-7 miRNA loop in development and proliferation of ovarian cancer in humans and identify a novel mechanism of ovarian cancer metastasis involving exosomes, containing miRNAs, secreted into a patient's bloodstream.

Laboratory Mice Experience Minimal Hematologic and Cytokine Changes When Moving from Sea Level to High Altitude

MR Feirer, C Konrad, B Wood, W Burgess, C Olver, S Vandewoude

Purpose: Many physiologic changes occur when mammals are transported from low to high altitude. Little is known on the effects in mice. Since the major vendors of laboratory rodents exist at or near sea level, the length of time required to acclimatize laboratory mice to high altitude settings should be a concern for all researchers. Known changes in other species include increases in erythrocyte production, cytokine levels, and acute phase proteins. As these changes have the potential to significantly affect a wide variety of research, we felt it vital to investigate their presence, magnitude, and duration due to altitude exposure in the mouse. Our goal in this study was to ascertain exactly how many days after arrival at altitude mice take to become “normalized”.
Materials/Methods: This was undertaken by monitoring various hematological parameters, cytokines, and hormone levels in ICR and C57BL/6J mice. Our first goal was to identify the hematologic changes that occur during altitude adjustment and the length of time for those parameters to stabilize. We hypothesized that we would see reticulocytosis and a gradually increasing hematocrit (HCT) during acclimatization. Reticulocyte counts would then decrease and HCT would stabilize by 3 weeks post arrival from sea level. Our second aim was to identify the degree and duration of changes in IL-6, TNF, CRP, IL-10 and erythropoietin during acclimatization; with our hypothesis being that all factors will increase and then level off within 3 weeks.
Results: Erythrocyte indices did not significantly change during the monitoring period. While CRP levels for both strains remained constant following shipping, C57 mice had consistently higher levels over all intervals. The remaining cytokine levels remained stable or below detectable limits.
Conclusions: Based on these findings, standard acclimatization practices for animals shipped from sea level to a higher altitude such as Colorado are sufficient.

Comparison Of Commercially Available PCR Assays For the Amplification Of Ehrlichia Canis and Anaplasma Phagocytophilum DNA From The Blood Of Naturally Infected Dogs.

J Ficociello, S Moroff, M Brewer, MR Lappin

Anaplasma phagocytophilum and Ehrlichia canis are two of the most common vector borne disease agents that infect dogs and cats. While PCR assays that amplify the DNA of these agents from blood are currently available, there is minimal information concerning the performance of these assays in different commercial laboratories that utilize different techniques. The purpose of this study was to compare the E. canis and A. phagocytophilum results of two different laboratories on the same samples collected from client-owned animals.
Veterinarians in 3 states (AZ, MD, CT) were recruited to participate in the study based on high prevalence rates for E. canis or A. phagocytophilum infection. Blood in EDTA was collected from dogs or cats with fever, thrombocytopenia, or clinical evidence of polyarthritis and an equal volume of the same blood sample was simultaneously shipped on cold packs by overnight express to Colorado State and to Antech Diagnostics. Standard operating procedures at each laboratory were followed for total DNA extraction and amplification of GAPDH as the DNA control. At Colorado State University, a previously published PCR assay that amplifies the DNA of Ehrlichia spp., Anaplasma spp., Neorickettsia spp., and Wolbachia was performed on each sample with positive amplicons sequenced to determine the species. At Antech Diagnostics, a proprietary real time PCR assay (FastPanel™) that amplifies the DNA of Anaplasma phagocytophilum, A. platys, Ehrlichia canis, E. chaffeensis, and E. ewingii was performed.
In the study to date, samples from 35 animals (30 dogs and 5 cats) have been assayed at both laboratories. DNA of A. phagocytophilum (2 cats and 2 dogs) and E. canis (1 dog) were amplified at both laboratories with a percentage agreement between laboratories of 100%.
The results to date suggest that the assay results of the two laboratories for A. phagocytophilum and E. canis are comparable.

Survey of large colon volvulus therapy in horses and impact on prognosis

JK Fiege, ES Hackett, S Gillette

Purpose: Large colon volvulus occurs spontaneously in horses and has a high morbidity and mortality rate. The purpose of this study was to determine how large animal surgeons are treating large colon volvulus in horses and to assess the success rate of treatments. Materials/methods: A survey was created utilizing Qualtrics survey programming in a subpopulation of veterinary surgeons with American College of Veterinary Surgeon (ACVS) board credentials in the large animal specialty. Email addresses were compiled using the public domain ACVS search directory. Surgeons were then contacted by email to complete a survey. The survey contained 4 demographic questions which concerned type and location of the respondent's practice, case load variation and year of board certification through the ACVS. There were 4 medical questions concerning treatment of the specific type of colic and associated prognosis for the animal. Questions in the survey consisted of multiple choice or fill in the blank where necessary. After completion of the survey every respondent was given the opportunity to provide feedback or discussion through an open text box. The survey had an estimated completion time of 5 minutes. Survey responses were anonymous. Results: 450 veterinary surgeons with ACVS board credentials in the large animal specialty were identified. Of those 66 general equine, 228 equine general and orthopedic, 26 equine orthopedic, 22 large animal general, 70 large animal general and orthopedic, and 3 large animal orthopedic had email addresses listed. Surgeons provided both demographic information and medical opinions based on their experiences with surgical treatment of large colon volvulus. Conclusions: Surgical preference can be successfully determined through anonymous polling techniques. Information gained from this survey may assist surgeons in training and promote a consensus among ACVS diplomates on the surgical treatment of this challenging disease.

Canine Digestibility of navy bean powders: Novel dietary strategy for disease prevention in companion animals

G Forster, D Hill, G Gregory, K Weisheir, S Lana, JE Bauer, EP Ryan

Dry beans (Phaseolus vulgaris), when incorporated into a diet provide excellent sources of protein, fiber, minerals, and essential vitamins in higher concentrations then cereal grains such as wheat and corn. Numerous health benefits from rodent and human studies have shown that cooked dry beans are a low glycemic index food, are low in fat and contain no cholesterol. In addition to these properties, beans have been identified as a functional food or nutraceutical, containing bioactive compounds that can inhibit cancer, prevent diabetes, reduce risk of cardiovascular disease, and improve intestinal health. Furthermore, epidemiological studies have contributed to recent claims of legumes as an important dietary predictor of survival in humans. Although dry bean consumption has been shown to promote health in humans and rodents, dry beans have yet to be incorporated into commercial canine diet, or even tested in dogs, even though veterinary nutritionists have recommended the inclusion of cooked dry beans as an ingredient in home cooked canine diets. The objective of this study was to establish the digestibility and effect on potential health biomarkers in dogs of one dry bean cultivar, the Navy bean. This cultivar was chosen for its availability in the U.S. and relatively low cost. A 25% Navy bean powder diet was formulated to meet all macro and micro nutrient requirements for maintaining the health and weight of an adult dog. A placebo control diet containing no beans was also formulated to match the Navy bean diet as closely as possible. 21 healthy CSU employee/student owned dogs with healthy Body Condition scores were recruited to participate in the 4 week study. Blood, urine, and fecal samples were collected at baseline, 2 weeks, and 4 weeks. Dogs were randomly divided into either the bean group or control group. After 10 days on the diet a 96 hour pooled fecal sample was collected and analyzed. We found no significant differences in the apparent digestibilities of the two diets, suggesting that beans are fully digestible by dogs and the macronutrients are readily absorbed. Furthermore, the inclusion of dry beans into canine diets may help modulate inflammation, thereby preventing many chronic diseases. There were no reported incidences of increased flatulence, extreme changes in stool consistency or lack of palatability of the bean diet. These data suggest that beans are safe, digestible ingredient for dogs, may promote health and chronic disease prevention, as well as providing an important alternate protein source to be used in commercial canine diets.

Comparative Pathway Analysis of Human and Canine Melanoma

JS Fowles, CL Denton, A Ptitsyn, DL Gustafson

Intro: Melanoma is the deadliest skin cancer in humans and dogs. The over activation of the MAPK and PI3K/AKT pathways is well documented in human melanoma, but not sufficiently investigated in canine melanoma. Highly predictive animal models are needed in cancer research, especially in the anti-cancer drug development process. The purpose of this study was to perform pathway analysis of human and canine melanoma in order to advance our knowledge of these diseases as well as validate the increased use of companion animal models of cancer for human research.
Methods: Gene expression microarrays were used for pathway analysis of normal and tumor tissues of human and canine melanoma. Western blot, proliferation assays, and cell cycle analysis were used with melanoma cell lines to study protein expression, drug sensitivity and mechanism of action of AZD6244 and rapamycin, inhibitors of MAPK and PI3K/AKT pathways.
Results: Pathway analysis of microarray data for canine melanoma revealed a heavy involvement of MAPK and AKT pathways in tumor samples compared to normal tissue. This trend is documented in human melanomas by others. Results with our human data are pending. Constitutive activation of MAPK and AKT pathways was observed in human and dog cells after serum starvation. Cell lines from human and dog were similarly sensitive to AZD6244 and rapamycin with IC50 values ranging from 5.7-391 nM and 0.027-12 nM, respectively. The drugs combined increased inhibition in all lines, with synergy measured in all but one human line. AZD6244 and/or rapamycin caused a G1 cell cycle block in human and dog.
Conclusions: Human and canine melanoma have over active MAPK and PI3K/AKT pathways, and they respond similarly to pathway inhibitors. These results suggest that pet dogs with melanoma may serve as a highly predictive model to be utilized for human research via veterinary clinical trials. Combining our efforts with human and canine melanoma research may prove equally beneficial for both species.

Paranasal Sinus Masses of Rocky Mountain Bighorn Sheep (Ovis canadensis canadensis)

KA Fox, SK Wootton, SL Quackenbush, LL Wolfe, IK LeVan, MW Miller, TR Spraker

The purpose of this study is to describe the gross features, histological features, and preliminary diagnostic findings for a previously undescribed lesion in bighorn sheep: Paranasal sinus masses. From February, 2009 through December, 2010, we identified 21 cases of paranasal sinus masses in Rocky Mountain bighorn sheep (Ovis canadensis canadensis) that presented for routine necropsy. We examined 58 bighorn sheep carcasses from a total of 20 herds in Colorado, USA. Of these, 21/58 (36%) sheep including individuals from 8/20 (40%) herds had paranasal sinus masses. Affected animals included 14/31 (45%) of the females and 7/27 (26%) of the males, ranging in age from approximately 2 years to greater than 10 years. Defining gross features of these masses included unilateral or bilateral diffuse thickening of the respiratory linings of the maxillary and/or frontal sinuses, with abundant seromucinous exudate in the affected sinus cavities. Defining histological features of these masses included chronic inflammation, and proliferation of both epithelial and mesenchymal cells of the mucosa and submucosa. Epithelial changes included hyperplasia of the mucosal surface epithelium, hyperplasia of submucosal glands and ducts, and neoplasia (adenocarcinoma). Mesenchymal changes included submucosal myxedema, submucosal fibroplasia/fibrosis, bone destruction, and neoplasia (myxomatous fibroma). We examined tissue samples using polymerase chain reaction and immunohistochemistry for Jaagsiekte sheep retrovirus and enzootic nasal tumor virus, with no evidence of these viruses in the tissue samples. We also performed polymerase chain reaction using degenerate primers for herpesviruses and identified a novel herpesvirus associated with the masses, and also present in tissues acquired from bighorn sheep lacking sinus masses.

Equine infectious disease priorities based on a 2010 on-line survey

S Gillette, JK Fiege, J Traub-Dargatz

Purpose: The purpose of this study was to determine equine infectious priorities. Materials and methods: Respondents were asked to provide their input via an on-line survey instrument (Qualtrics) that allowed for branching from lead in questions to response for subsequent questions. We solicited participation by announcing the survey to equine associations (breed and discipline), equine press contacts, horse councils and equine practitioner associations. The survey consisted of 12 questions and took an average of 10 minutes to complete. Questions regarding respondent demographics such as state of residence, role in equine industry, and type of equine ownership were included as well as questions that allowed the respondent to indicate their concerns and priorities related to equine infectious diseases. Results: The majority of our respondents replied to our survey based on seeing an announcement on “The Horse” website or through a horse publication. Survey respondents represented all regions of the United States. Internal parasites were of highest concern to respondents followed by sleeping sickness (encephalitis such as West Nile Virus, Eastern Equine Encephalitis, etc.) strangles, rabies, influenza, equine herpesvirus (EHV1) and Equine Infectious Anemia. Conclusions: Our online survey had limitations due to the nature of the online target population which was disparate and largely unknown. Therefore, it is difficult to determine the representativeness of our sample. However, we were able to attain answers to our most pertinent questions. Internal parasites were of highest concern to respondents. The diseases that were not of concern to respondents were also more likely to be diseases that a large proportion of respondents had not heard of yet. Respondents indicated that it was important for government to address death rates related to diseases and less important to address cost concerns. Results were provided promptly through a report to the APHI website.

Burkholderia pseudomallei Persistently Colonizes and Disseminates from the Gastrointestinal Tract Following Oral or Intranasal Inoculation

AW Goodyear, H Bielefeldt-Ohmann, HP Schweizer, SW Dow

Purpose. Burkholderia pseudomallei is a soil and water bacterium found in tropical and subtropical regions of the world that causes human disease (melioidosis), which may manifest as acute, overwhelming sepsis or as chronic subclinical or recurrent infections. At present, it is unclear where the B. pseudomallei organism resides in infected patients during chronic, subclinical infection. Therefore we used a mouse model to test the hypothesis that the gastrointestinal (GI) tract was a potential site of persistent infection following oral inoculation with B. pseudomallei.
Methods. BALB/c mice were inoculated orally (p.o.), intranasally (i.n.), intraperitoneally (i.p.), or subcutaneously (s.c.) with B. pseudomallei and selective media was used to quantitate infection in various regions of the GI tract, feces and in other organs following inoculation. GI and systemic tissues were fixed and sectioned for histopathological observation. We also investigated the relationship between intestinal colonization with B. pseudomallei and dissemination to extra-intestinal sites.
Results. B. pseudomallei readily and persistently infected the GI tract following p.o. or i.n. inoculation, followed by sustained low-level fecal shedding. Though mice maintained active enteric infection with B. pseudomallei, bacterial numbers in the feces were low relative to infection with well-known GI pathogens such as Escherichia coli, Shigella spp. and Salmonella spp. Moreover, there was a complete absence of lesions throughout the GI tract during either acute or chronic infection with B. pseudomallei. Oral or i.n. inoculation was also much more likely to lead to disseminated infection in the liver and spleen than was s.c. inoculation.
Conclusions. We conclude that B. pseudomallei can readily establish low-level persistent colonization of the GI tract, and that the chronically colonized GI tract may serve as a reservoir for dissemination of infection to extra-intestinal sites.

An indolent aerosol mouse model for Acinetobacter baumannii-induced pneumonia

AD Gwynn, NL Marlenee, A Tolnay, BK Podell, RA Bowen

Acinetobacter baumannii is a gram negative bacterium responsible for both community and nosocomial infections. Due to its increasing antimicrobial resistance over the last decade, it has re-emerged as a serious pathogen of interest. Previously, immuno-compromised, fulminantly-infected mice produced via intratracheal inoculation have served as the pneumonia research model. Unfortunately, producing this mouse model is very labor- intensive and limited in its research capacity since untreated mice rarely survive longer than 72 hours. We hypothesized that exposure to a low-dosage A. baumannii aerosol will establish a non-acute, progressive pneumonia which can serve as an alternate and more realistic model. We exposed immuno-competent Balb/c and ICR mice to a relatively low dose of A. baumannii (ATCC strain) via aerosolization in a GlasCol Aerosol Exposure System for 30 minutes. Over the 10 day period following exposure, ICR mice failed to show clinical illness and Balb/c mice exhibited only minor disease. However, upon necropsy the Balb/c mice showed gross signs of lung inflammation and congestion suggestive of pneumonia. Histological evaluation of the lungs and CFU counts are currently being evaluated for additional confirmation. Production of a low dose aerosol pneumonia model is, ultimately, less labor intensive and promises to provide a model that closely mimics human disease. It can also further extend the current research boundaries for evaluating therapeutic outcomes and for elucidating differences in the virulence of isolates. Questions regarding routes of transmission and optimal environmental conditions for transmission can also be explored via this technique.

Urinary Cytokine Concentrations in Cats with Kidney Disease

L Habenicht, JM Quimby, TL Webb, S Dow

Chronic kidney disease (CKD) is a major cause of illness and death in cats, and develops in virtually all older animals. The hallmark of CKD in cats is chronic tubulointerstitial nephritis, and unrelenting inflammation is believed to play a key role in the progressive nature of renal fibrosis in cats with CKD. At present, there is no effective means of assessing the degree of intrarenal inflammation in cats with CKD, nor is there an efficient means of assessing response to therapy other than through routine blood chemistry screening. Therefore, we hypothesized that urine concentrations of pro-inflammatory cytokines (eg, MCP-1, IL-8, IL-6) and pro-fibrotic cytokines (eg, TGF-b) might be a more sensitive means of directly assessing the degree of renal inflammation and fibrosis. To address this question, we used specific cytokine ELISAs to measure cytokine concentrations in serum and urine from healthy cats, cats with CKD, and cats with other inflammatory disorders. We found that IL-8, MCP-1, TGF-b, and IL-10 could be detected in the urine of both healthy and CKD cats. Interestingly, the concentrations of IL-8 and MCP-1 were suppressed by a putative binding factor (EDF) in the urine of healthy cats, such that dilution of the urine paradoxically increased cytokine concentrations. Serum cytokine concentrations were not different between healthy cats and cats with CKD. However, when cytokine concentrations in healthy and CKD cats were compared, we found significantly higher concentrations of TGF-b in the urine of cats with CDK compared to healthy cats. Therefore, we concluded that urinary cytokine measurement may be a much more efficient and sensitive means of measuring intra-renal inflammation in cats with CKD.

Tumor associated macrophages increase proliferation of osteosarcoma cells

SD Hafeman, SW Dow

Purpose: Increased numbers of tumor associated macrophages (TAMs) have been associated with a poor prognosis in many different tumor types. These cells make up a large percentage of the tumor stroma, and have been associated with a number of pro-tumor properties. However, the direct effects of TAMs on tumor cells are not fully understood, and there have been very few in vitro studies to elucidate their mechanisms of tumor growth promotion. We therefore set out to develop an in vitro assay to study the effects of TAMs on osteosarcoma tumor growth. Materials/Methods: Osteosarcoma cells of multiple lines were stained with carboxyfluorescein succinimidyl ester (CFSE). They were then allowed to grow alone or were incubated for 24 hours with different concentrations of either canine macrophage cell lines (DH82, MH-1, or MH-2) or with monocyte derived macrophages from the peripheral blood of canine cancer patients. The cells were then analyzed via flow cytometry to determine the percentage of dividing tumor cells. The macrophage cells were stained with fluorescent CD45 antibody so that they could be excluded from the analysis. Results: Incubation with macrophages led to a significant increase in tumor cell proliferation. This increase in proliferation was dose dependent. Monocyte derived macrophages from the peripheral blood of canine donors caused significantly more tumor proliferation than canine macrophage cell lines. Conclusions: Tumor associated macrophages cause an increase in tumor cell proliferation in vitro. While immortalized cell lines are capable of producing this effect, it is much more pronounced using monocyte derived macrophages. Therefore depletion of these macrophages by liposomal clodronate or other macrophage targeted therapies may directly decrease tumor proliferation in vivo. Consequently, combination therapy with standard chemotherapy and macrophage depletion may work synergistically to decrease tumor growth.

Comparison of Current Veterinary Sonography Techniques to Human Medial Sonography Techniques Recommended by OSHA

C Hansen, E Randall, D Gilkey, A Patil, J Rosecrance, D Douphrate

Veterinary sonography involves similar risk factors as human medical sonography, which leads to similar musculoskeletal symptoms (MSS) within each group. However, the risk factors in human medical sonography have been recognized by Federal OSHA and the Society of Diagnostic Medical Sonography (SDMS). As a result, guidance to control the risk factors in human medical sonography has been published by OSHA/SDMS. Similar guidance for veterinary sonographers does not currently exist. Phase 1 of this research involved a cross-sectional study of 246 veterinary sonographers to explore the incidence of ultrasound related MSS and their association with risk factors presented during veterinary sonography. Musculoskeletal pain related to performing ultrasound exams was reported by 62% of the respondents. Also, MSS were significantly associated with several veterinary sonography risk factors. Veterinary sonographers that perform ultrasounds on small animals (i.e. dogs and cats) are a similar exposure group to medical sonographers that perform ultrasounds on humans. Therefore, veterinary sonographers may be able to benefit from following the OSHA/SDMS guidance when performing small animal ultrasounds. Phases 2 and 3 of this research will aim at directly comparing current veterinary sonography techniques against the techniques recommended in the OSHA/SDMS guidance. Exposures to force, repetition, and awkward postures in the upper extremities will be quantified and comfort data will be recorded while each technique scenario is performed in a veterinary setting. The data sets will be evaluated and compared. Our hypothesis is that there will be differences between the two technique scenarios. If statistical differences are demonstrated, this would indicate that the OSHA/SDMS techniques were more protective than current veterinary techniques.

The AMPA cleft-pore linker mutant: a new target for cognition enhancing drugs

JE Harms, M Benveniste, L Stone-Roy, KM Partin

Glutamatergic signaling has been a suspect of many debilitating cognitive disorders, including schizophrenia, epilepsy, Alzheimer's disease, Parkinson's disease, and mood disorders such as depression. Consequently, the ionotropic glutamate receptors, which play a fundamental role in strengthening synaptic connections, have become a prime target for drug development. For example, allosteric modulators of the glutamate-gated AMPA receptor, GluA2, are available as dietary supplements, undergoing successful clinical trials, and being investigated for their potential neuroprotective action. Unfortunately, many of these drugs have an underlying problem of leading to over-excitable synapses with susceptibility to seizures. As such, basic research exploring the biophysical properties of GluA2 and its kinetics of gating is essential to producing more efficacious drugs with less detrimental side effects. We have been studying AMPA receptor kinetics by using targeted mutagenesis; a technique that allows us to probe how specific domains of the intact receptor contribute to gating and function. One mutant that has been of particular interest is the arginine 628 to glutamate mutant (R628E). Unlike previous studies that have focused on residues directly involved in agonist binding, the R628E mutant lies along the M3-S2 linker region, which connects the glutamate binding domain to the channel pore. We transiently-transfected human embryonic kidney cells with either wildtype GluA2 DNA or R628E mutant GluA2 DNA. Using ultra-fast perfusion exchange, we performed electrophysiological recordings in response to pulses of glutamate. Our results show that the R628E mutant slows the kinetics of both receptor deactivation and desensitization. As such, this study implicates the M3-S2 linker region as a new and promising target for the development of cognition enhancing drugs. Our future studies aim to investigate how presently available modulatory drugs interact with this region.

Flow cytometric determination of the immune-mediated component of the anemia seen with Mycoplasma haemofelis infection in a cat.

ML Hart & CB Webb

One proposed mechanism for the pathogenicity of M. haemofelis is the development of autoantibodies that trigger an immune-mediated disorder. Based on positive Coombs' test, it is thought that the host's immune response to the hemoplasma organisms exacerbates an acute hemolytic episode. Flow cytometry and fluorescent antibodies directed at feline IgG and IgM immunoglobulins were used to directly identify the immune-mediated component of the hemolytic anemia seen with acute M. haemofelis infection in a 2-year old DSH cat. The cat presented with a complaint of acute lethargy and anorexia. A complete blood count showed a packed cell volume (PCV) of 10% (normal 28.0 – 43.0%) and a reticulocyte count of 57,050/µl (0.0 – 50,000/µl). The sample was icteric with negative saline agglutination and marked mycoplasma haemofelis infection noted. The cat's total bilirubin was 4.3 mg/dL (0.0 – 0.2 mg/dL). Fine-needle aspirate of the cat's liver revealed microagglutination and hemophagocytosis by mononuclear cells. Large numbers of organisms were seen as single or short chains of small, basophilic cocci on the erythrocyte membrane, compatible with M. haemofelis. Flow cytometry showed that 19.7% of the cat's erythrocytes (RBCs) were positive for the presence of IgM anti-feline RBC antibodies, and 15.5% of the RBCs were positive for the presence of IgG anti-feline RBC antibodies. Controls included samples from non-anemic cats run in parallel. The cat was treated with a blood transfusion and corticosteroids. Three days later the cat's PCV had increased to 21.0% with a reticulocyte count of 229,840/µl and no mycoplasma observed, while the owner reported significant clinical improvement. This sample had a decrease in positively labeled RBCs for anti-feline IgM antibodies to 2.2% and anti-feline IgG antibodies to 0.52%. This assay could be used to identify the need for immunomodulation in the treatment of anemic cats, as well as for monitoring the response to therapy.

scAAV Transduction Efficiencies in Joint Tissue Monolayer and Explant Cultures and the Effects of Synovial Fluid Neutralization

D Hemphill, CW McIlwraith, RJ Samulski, LR Goodrich

Within the joint there are two major cell types currently targeted by gene therapy vectors to treat osteoarthritis: synoviocytes found in the synovial membrane and chondrocytes found in the cartilage layer. Although transduction efficiency has been well quantified in chondrocyte and synoviocyte monolayers, transduction in explants of cartilage and synovium has not been thoroughly investigated. AAV-specific neutralizing antibodies have been identified in the SF of human, but this has not been studied in horses. The purpose of this study was to compare self complimentary adeno-associated virus (scAAV) genetic modification (transduction) in monolayer to explant tissue of synovium and cartilage and to investigate whether the presence of synovial fluid (SF) inhibits efficient transduction in joint tissue.
Cartilage and synovium were isolated postmortem from three horses 2-5 years of age, sectioned into explants and digested for monolayer plating. Samples were transduced with 8000 virus particles per cell (VPC) of scAAV-GFP serotypes 1, 2, 2.5, 3, 4, 5 and 6, and GFP expression evaluated by flow cytometry performed 35 days post transduction. SF samples were taken from four horses and serial dilutions (1:12.5, 1:25, 1:50, 1:100, 1:200) were incubated with 8000 VPC of scAAV-GFP serotypes 2, 2.5, 3, 5 and 6 for one hour prior to transduction of monolayer synoviocytes. Plate readings of fluorometric data were gathered every other day to quantify GFP expression.
No difference was found in transduction efficiency between monolayer and explants when analyzing the percentage of cells transduced. The mean fluorescence intensity of the vector gene expression revealed that monolayer cells appeared more fluorescent than explant cells. Serotype 2 resulted in the highest transduction efficiency of both chondrocytes and synoviocytes. Synovial fluid showed graded neutralizing power against AAV serotypes.
scAAV provides sufficient transduction of joint tissue explants when compared with cell monolayers. Additionally, there is neutralization of AAV from synovial fluid, indicating the possibility of AAV-specific antibodies.

Immune Enhancement of Antimicrobial Therapy for Treatment of *Salmonella enterica* infection

VA Johnson, E Silva, K Mosovsky, S Dow

Salmonella typhimurium is an enteric pathogen responsible for disease in a wide range of host species. It is a common cause of nosocomial infections in large animal hospitals and can be difficult to eradicate due to the presence of chronic asymptomatic infections and antimicrobial resistance. Recent studies have shown increased efficacy of immunotherapy combined with conventional antibiotic therapy for the treatment of resistant, intracellular bacterial infections. To ascertain whether this therapy would be effective against S. typhimurium, an in vitro macrophage infection assay was used to assess the efficacy of a combination therapy using gamma interferon (IFN-?) with ceftazidime. IFN-? is a cytokine that has been demonstrated to provide protection against intracellular bacterial infections. Macrophages were infected in vitro with Salmonella enterica 14028s serovar Typhimurium utilizing a multiplicity of infection of 100. The infected cells were subsequently treated with ceftazidime (1 ug/ml), IFN-? (10ng/ml), and combination therapy. Infected macrophages treated with combination immunotherapy demonstrated a 100 fold reduction in bacterial growth when treated with subtherapeutic concentrations of antibiotics in combination with IFN-?. The demonstrated synergy between IFN-? and ceftazadime in S. typhimurium infection may contribute to more effective eradication of subclinical infection and simultaneously allow a reduction in antibiotic use. Experiments are currently underway to investigate the mechanism of the synergistic interaction between IFN-? and ceftazidime. Control of chronic infection and decreased antibiotic use would have a major impact in nosocomial infection control while concurrently lowering the risk of further antibiotic resistance.

Differences in Antibiotic Susceptibility of Corynebacterium pseudotuberculosis Grown Planktonically or as a Biofilm

NM Kirk, DF Ackart, J Haugen, L Hascall-Dove, S Turner, RJ Basaraba

Purpose: Corynebacterium pseudotuberculosis (Cptb) is the causative agent of caseous lymphadenitis in sheep and goats. Infections are difficult to control due to lack of diagnostic assays and poor response to antibiotic therapy. The purpose of this study was to demonstrate that Cptb expresses phenotypic drug resistance by forming extracellular biofilm-like communities in vivo and in vitro. Materials/Methods: Isolates of Cptb from infected sheep were grown in tryptic soy broth (TSB) both with (planktonic) and without (attached) 0.05% Tween-80 overnight. The planktonic culture was treated separately with different concentrations of ampicillin and rifampin to determine Minimum Inhibitory Concentration (MIC). This method of determining drug susceptibility utilizes planktonic cultures and is not designed for attached colonies. In these studies, attached cultures were treated with a range of ampicillin and rifampin concentrations centered around the MIC of the planktonic culture in 96-well plates. We compared the susceptibility of attached and planktonic cultures to ampicillin and rifampin by determining and comparing the Colony Forming Units (CFU) of each on TSB+5% blood agar plates Results: Attached cultures showed an increased antibiotic resistance to both ampicillin and rifampin compared to planktonic cultures. Additionally, in either culture condition, Cptb is significantly more susceptible to rifampin than ampicillin. Conclusions: When grown attached to a 96-well plate, Cptb shows a phenotypic antibiotic resistance to both ampicillin and rifampin. This attached state mimics biofilm communities seen in vivo, indicating that Cptb can form a biofilm and that this biofilm formation significantly impacts antibiotic susceptibility. This study suggests that Cptb is more susceptible to rifampin and that current methods of determining antibiotic susceptibility based on planktonically grown bacteria may not reflect in vivo drug susceptibility.

Reduced Susceptibility to Salmonellosis by Dietary Rice Bran

A Kumar, G Forster, J Leach, S Dow, E Ryan

Salmonella enterica is a gram negative, food born pathogen that affects approximately 1.3 billion humans around the world annually. Little is known regarding dietary strategies to prevent Salmonellosis. Salmonellosis is primarily treated with synthetic antimicrobial drugs, but these can exhibit deleterious side effects, and are not globally available to mitigate infection. Rice bran is a large agricultural waste byproduct and a rich source of bioactive compounds with immune stimulatory properties. Pilot data from our laboratory has shown that a methanolic extract from rice bran stimulates production of IL-6 by RAW264.7 cells. These findings led to the hypothesis that rice bran reduces the susceptibility of mice to oral challenge with Salmonella enterica typhimurium. Dietary intake of rice bran at a dose of 10, 20 and 50% significantly reduced fecal shedding of Salmonella after 2 days. The rice bran mediated reduction in Salmonella fecal shedding persisted for 5 days post infection in mice fed the10% rice bran diet group and 7 and 9 days for mice fed 20 and 50% rice bran in their diet, respectively. A significant reduction ($p<0.05$) in Salmonella tissue invasion was detected in ileum (90, 96 and 77%) at day 14-post infection. Mice consuming a rice bran diet also decreased invasion of Salmonella by 88% (50% rice bran diet group) in mesenteric lymph nodes as compared to control diet group ($p<0.05$). An in vitro Salmonella entry assay was developed in mouse small intestinal epithelial (MSIE) cells to evaluate if rice bran from different varieties showed equal protection against Salmonella entry. These findings have important implications for the incorporation of dietary rice bran for prevention of Salmonellosis and further studies are underway to evaluate varietal differences in mucosal immune induction properties.

Evidence of cross species transmission of feline immunodeficiency virus between bobcats and pumas.

D Lagana, J Lee, S Bevins, J Lewis, L Sweanor, K Crooks, S VandeWoude

Purpose: Feline immunodeficiency virus (FIV) is an enveloped RNA retrovirus of the family Lentiviridae. Previous research shows that each felid species harboring an FIV-like virus appears to have a species specific strain that is highly divergent from other FIVs, demonstrating that most infections occur from intra-species transmission. FIV sequences from pumas (Puma concolor) cluster into two distinct monophyletic groups: FIV-Pco clade-A and FIV-Pco clade-B. FIV Pco clade-B is the most widely described lentiviral type infecting pumas. The much rarer and highly divergent clade, Pco clade-A, was previously detected in Florida panthers and California pumas. FIV isolates sequenced from infected bobcats in California clustered with Pco clade-A sequences as did an archived Florida bobcat FIV sequence. This was the first documented report of a strain of FIV that infects more than one species in the wild, providing possible evidence of cross species transmission from bobcats to pumas or vice versa. Materials/Methods: DNA was isolated from peripheral blood mononulcear cells from 26 bobcats from the Western Slope of Colorado and 25 bobcats from Florida. All bobcats were tested for the presence of FIV using DNA PCR analysis. Results: No FIV was detected in any of the Colorado bobcats we tested. Five samples from the Florida bobcats were FIV-positive by PCR. Sequence analysis demonstrates that the FIV-isolates amplified from Florida bobcats are FIV-Pco-clade A. Conclusion: Our data supports the hypothesis that bobcats in Florida and California are infected with FIV-Pco clade-A, and contact with pumas in these areas has lead to cross-species transmission. In areas where bobcats are not infected with FIV- Pco clade-A, or where infection rates with this pathogen are low (such as Colorado), pumas are infected with FIV-Pco clade B.

Investigating Mechanisms of Cross-Species Transmission of Feline Immunodeficiency Virus between Bobcats (Lynx rufus) and Mountain Lions (Puma concolor)

J Lee, E Boydston, L Lyren, J Troyer, S Franklin, S Bevins, K Crooks, S VandeWoude

Feline Immunodeficiency Virus (FIV) is a lentivirus with clinical and biological similarities to HIV. Highly divergent species-specific strains of FIV naturally infect at least 9 species of felids, including bobcats (Lynx rufus) and mountain lions (Puma concolor). The strain of FIV which infects mountain lions, FIVpco, is composed of two highly divergent clades. FIVpco clade B infects mountain lions throughout their entire geographic range which encompasses most of North and South America. FIVpco clade A has only been documented to infect mountain lions in southern California and Florida. Interestingly, sequences of the FIV genome obtained from bobcats in these two geographic areas are monophyletic with FIVpcoA. Phylogenetic analyses of sequences from the pol region of the FIV genome suggest FIVpcoA is frequently transmitted between bobcats and mountain lions. Furthermore, the relatedness of viral isolates is highly correlated to the sampling location, suggesting sympatry and contact rates are important factors in cross-species transmission of FIV. We hypothesize that FIVpcoA was historically a bobcat specific strain which has broadened its host range to now infect mountain lions. This is the only documented strain of FIV which has been found to readily infect two species of felids under natural conditions. We are characterizing full-length viral genome sequences from FIVpcoA isolates in bobcats and mountain lions to investigate viral mechanisms of cross-species transmission. Specific analyses will evaluate: 1) recombination between viral isolates, 2) diversity among key gene segments known to play a role in cross-species restriction, and 3) evidence of positive selection (adaptation) in mountain lions. This work will describe evolutionary mechanisms of cross-species lentiviral transmission in natural populations of wild felids.

Comparative Analysis of Bleomycin In Pulmonary Disease Susceptible PECAM Deficient Mice

M Lishnevsky, SJ Woods, WA Muller, DWH Riches, AR Schenkel

Platelet Endothelial Cell Adhesion Molecule (PECAM/CD31) deficient mice in the FVB/n background spontaneously develop a chronic interstitial pneumonia. It has similarities to human interstitial pneumonia, including early alveolar collapse, subsequent development of fibrosis, and proliferation of myofibroblasts. This disease affects mice of all ages but is not fully penetrant, developing in about 40% of the mice. We show here that there appears to be leakage of blood into the lung. Further, this implies that these mice would be susceptible to pulmonary insults. Surprisingly, these mice are resistant to infection. They also do not show any disease after intratracheal histamine instillation to induce vascular leakage. Bleomycin was used to induce lung injury and subsequent disease in PECAM deficient and wild type mice in order to compare the disease process in this well-characterized model of pulmonary fibrosis. As shown with other pulmonary insults, the FVB/n strain is highly resistant to bleomycin, paradoxically suggesting that rather than being more susceptible to damage, these mice may have highly active repair mechanisms that may contribute to subsequent fibrosis.

Combined Immunotherapy and Antimicrobial Therapy for Treatment of Chronic Staphylococcal Osteomyelitis

A Lord, R Richmond, E McQuinn, L Kendall, S Dow

Background. The high prevalence of Staphylococcus aureus nosocomial infections, combined with rapidly developing resistance to traditional antibiotic regimens, suggests a need for novel therapies against this common pathogen. We found recently that combined immuno-antimicrobial therapy with IFN-g and penicillins generated strong synergistic killing of intracellular Staphylococcus. Therefore, we investigated the effectiveness of combined therapy in a mouse model of chronic osteomyelitis. Methods. Osteomyelitis was induced in CD1 mice by placing a 1 mc steel pin pre-coated with a luciferase-expressing S. aureus biofilm in the tibia. At various time points after implantation, mice (n = 4-5 per group) were treated with subtherapeutic doses of piperacillin, combined with a once weekly heat-killed S. aureus vaccine. Group 1 animals received piperacillin (50 mg/kg; i.p.) every 12 hours, while Group 2 animals received 3 S. aureus vaccines. Group 3 animals received both piperacillin and vaccine, while Group 4 animals were untreated controls. Bone infection was quantitated every 3 days using an IVIS Imaging System and bacteria present upon the pins were cultured and quantified. Results. Preliminary results indicated that combined vaccination and antibiotic therapy did not improve resolution of osteomyelitis, regardless of timing of therapy. Unexpectedly, we also noted an increase in luciferase expression in vaccinated mice compared to untreated mice. Experiments are currently underway to quantitate bacterial numbers at the infection site and correlate these results with IVIS imaging. Conclusions. Our preliminary studies indicate that vaccination may not improve the efficacy of antimicrobial therapy for treatment of chronic osteomyelitis.

Rapid development of lymphomas in cats with virulent FIV infection

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Eighteen two-month-old cats (SPF) were experimentally infected with FIV strain C36 as part of a study to evaluate anti-retroviral therapies. All animals developed high viral loads, significant CD4 depletion and neutropenia within four weeks of inoculation, signifying a highly virulent infection. In addition, four animals developed neoplastic changes in lymph nodes and/or bone marrow within four months of inoculation. Two cats developed B cell follicular lymphoma confirmed by immunohistochemistry and detection of clonally rearranged immunoglobulin genes. One cat developed leukemia, and the fourth cat demonstrated pre-neoplastic changes in the mesenteric lymph node. FeLV antigenemia was ruled out using a commercially available ELISA assay. Tissue samples were screened for a concurrent herpes virus infection via PCR using degenerate primer sequences designed to amplify the DNA pol region of herpesviruses, but no herpesviral sequences were amplified. Age at inoculation and relative virulence of the infection, or other co-pathogens not yet identified are potential co-factors resulting in this unusual presentation of FIV infection.

Aerosol Deposition System for Lung Epithelial Cell Culture

D McKenna, J Volckens, AJ Marchese

Purpose: Studies of aerosol-lung interactions are important for the fields of toxicology, infectious disease, and inhalation therapy. The objective of this work was to develop a more physiologically and environmentally relevant model of aerosol deposition onto lung epithelial cell culture. Our prototype uses bi-polar charging in conjunction with electrostatic force to push particles onto cultures grown at an air-liquid interface (ALI) in a humidified, heated exposure chamber. Materials/methods: A carbon fiber ionizer charges incoming aerosol, which then flows into an exposure chamber where an applied electric field forces charged particles to migrate onto the culture plate. The chamber was designed to accept standard culture plates, enabling increased throughput and ease of use. The device was designed to promote uniform and reproducible deposition of airborne particles onto cell cultures. Experiments are underway to determine the uniformity and reproducibility of particle deposition onto different regions of the culture plate. Results: A prototype has been assembled. We have demonstrated that the ionizer can impart multiple charges onto spherical nanoparticles. We plan to test deposition efficiency, as a function of particle size, flow rate, and applied voltage. We will also test particle deposition uniformity across the culture plate using fluorescein-tagged aerosol. Finally we will quantify the effect of accumulating either a positive or negative charge on the cultures through gene expression and cell viability assays. Conclusions: Improved models of aerosol-lung interactions are needed at the in vitro level in the fields of environmental health, medicine, and infectious disease. This research developed an aerosol deposition system for lung epithelial cell culture that is a more physiologically and environmentally relevant model of particle interaction with the lung epithelium in vitro.

A comparison of biochemical and histopathologic staging in cats with renal disease

SM McLeland, CG Duncan,and JM Quimby

Feline chronic renal failure (CRF) is a common disease, especially among aged cats. Loss of functional renal mass can result from a variety of etiologies and in cases of CRF is progressive and irreversible. The most reliable, non-invasive method of evaluating renal function is plasma creatinine and blood urea nitrogen. Typically these laboratory parameters are not elevated until a substantial proportion of renal mass has been lost. This retrospective study reviews clinical, biochemical, and post-mortem renal histopathology in cats that presented to Colorado State University Veterinary Teaching Hospital between 2000-2009. Renal histology samples were evaluated via light microscopy and assigned a qualitative grade based on severity of lesions present within glomeruli, tubules, interstitium, vasculature and pelvises. Histologic grades were then compared to ante-mortem plasma creatinine levels and stage, according to the International Renal Interest Society (IRIS), to evaluate if there was an association between the clinical stage, characterization and severity of renal lesions. A total of 56 cats were included in this study representing IRIS stage 1 (n=8), stage 2 (n=19), stage 3 (n=9) and stage 4 (n=20). Of all the components evaluated, histological grade of interstitial fibrosis, and tubular degeneration and loss were the only lesions that statistically predicted clinical stage. Lesions present within glomeruli, inflammation within the interstitium, vasculopathy or pyelonephritis did not statistically correlate with clinical stage. Progressive renal disease leading to elevations in serum creatinine is best characterized primarily by interstitial fibrosis accompanied by loss of tubules. Insight into the pathogenesis of renal fibrosis may assist in monitoring and management of feline renal disease in the future.

The Role of Nitric Oxide in Immune-Mediated Hemolytic Anemia Coagulopathy

ER McQuinn, A Avery, S Dow, J Perry

Immune-mediated hemolytic anemia, (IMHA) is a devastating disease in which the immune system attacks a dog's red blood cells causing them to be destroyed. Though these dogs can experience severe anemia, many times case fatality results from thromboembolic complications secondary to the disease process. To date, no one has determined why these dogs are so prone to forming blood clots. Additionally, given the prevalence of the disease and our lack of effective treatments our goal was to investigate the possible role of nitric oxide in canine IMHA associated coagulopathy. We propose that erythrocyte lysis leads to nitric oxide depletion which puts the patient at increased risk for blood clot formation. Upon red blood cell destruction, hemoglobin and arginase are released into the bloodstream. Hemoglobin is able to scavenge nitric oxide at diffusion-limited rates. Arginase converts the nitric oxide precursor, arginine, to ornithine and urea rendering it useless in nitric oxide production. With these 2 proposed deficits to the nitric oxide system, we predicted loss of hemostatic function, activation of platelets and a coagulable state favoring thromboembolism in dogs with IMHA. We observed that arginase was significantly higher in IMHA than control dogs. Additionally, arginase was significantly higher in IMHA dogs that experienced pulmonary thromboembolism (PTE) than those dogs that did not. With our testing methods, we were not able to demonstrate increased free hemoglobin in dogs with IMHA compared to dogs without the disease; additionally, we were unable to observe a significant decrease in nitric oxide levels in dogs with IMHA compared to controls. Alternative avenues for measuring scavenging of nitric oxide by hemoglobin need to be considered. Direct measurement of nitric oxide did not prove valuable.

Myocardial structural changes in canine obesity

E Mehlman, M Frye, J Bright, J Boon

Human obesity is increasing in prevalence globally. Evidence suggests that the prevalence of overweight and obesity in canine patients approaches 40%, and will parallel the rise in the human population. Increased body weight in humans predicts greater morbidity and mortality attributed to cardiomyopathy and heart failure. Little is known about the prevalence and pathology of cardiomyopathy in obese dogs. This study partly addresses this deficit by comparing gross myocardial structure and function in lean and obese dogs. Obese normotensive dogs belonging to CSUVMC faculty and students were recruited via facility-wide electronic communication. Body condition score (BCS) was quantitated using a 9-point system, and dogs with a BCS = 7 were considered obese. Echocardiographic measures of myocardial structure and systolic and diastolic function were determined in 19 dogs and indexed to body surface area. Obese subjects were matched by age, ideal weight and body type to lean controls. Obese dogs had increased left ventricular (LV) wall thickness during systole ($p < 0.01$) and diastole ($p < 0.05$). Septal thickness, LV chamber diameters, E:A (indicator of diastolic function) and fractional shortening (indicator of systolic function) were similar in lean and obese dogs. The findings of this study suggest that obese dogs develop LV wall thickening without concomitant functional changes. This structural change is observed in obese humans and often precedes functional decline. It is possible that dietary composition and duration of obesity play a role in progression to myocardial dysfunction. Future studies will examine the contribution of these factors; additionally, myocardial tissues from lean and obese dogs are currently being studied for processes known to occur in obese humans, including lipid accumulation, extracellular matrix remodeling and myocyte hypertrophy. Collectively, these data may identify candidate lesions to serve as the focus of future mechanistic studies.

Monitoring the Development of Behavioral and Cognitive Effects of Prion Protein Deficient Mice

C Meyerett, H Bender, B Wyatt, MD Zabel

Prion diseases are classified as transmissible spongiform encephalopathies (TSEs) due to their unique clinical pathology and transmissibility of the proposed etiological agent, a protein. It is hypothesized that the disease is caused by the recruitment of normal, cellular prion protein (PrPC) by a protease K – resistant abnormally conformed isomer (PrPRes). It has been established that PrPC knock-out mice (PrP-/-) are incapable of amplifying PrPRes upon inoculation and therefore, are resistant to prion infection. Despite decades of research using PrP-/- mice, the normal function of PrPC still remains unclear. It has been reported that PrPC is widely distributed throughout the olfactory system in wild-type mice. Recently, Pichon et al. (2008) demonstrated that PrP-/- mice were deficient in odor-guided behavioral tasks as compared to wild-type mice; however, this study only examined behaviors at a single time point. Using 3-month old, age-matched mice, we conducted a longitudinal study (~12 months) to monitor the development of behavioral and cognitive deficiencies of PrP-/- mice compared to wildtype mice. Using an object recognition task and odor-guided behavioral tasks, we determined that there is a significant deficiency in the olfaction of PrP -/- mice throughout time ($P < 0.05$). However, there is no difference in spatial recognition between PrP-/- and wild-type mice. In light of these results, we will be conducting a longitudinal study to determine the effects of prion disease (e.g., scrapie) on the olfaction of mice expressing PrPC, and to compare these results to those observed with PrP-/- mice. If prion-inoculated mice exhibit the same olfaction deficiency as PrP -/- mice, then odor-guided behavioral tasks could be used as a pre-clinical behavioral test of prion diseases in mice.

Monitoring Immune Cells Trafficking Fluorescent Prion Rods Hours after Intraperitoneal Infection

BA Michel TE Johnson MD Zabel

The lymphoreticular system plays an important role in prion pathogenesis. To investigate the role of lymphoid cells in the uptake and trafficking of prions, tg5037 mice were inoculated intraperitoneally (I.P.) with prion rods derived from the brain of an elk naturally infected with chronic wasting disease (CWD). These mice were sacrificed at time points 2, 6, 16, and 36 hours post inoculation (HPI). Peritoneal cells and lymphoid tissues including the mediastinal lymph node, mesenteric lymph node, and spleen were collected and analyzed using flow cytometry. After 2, 6, 16, and 36 hours post inoculation a significant number of prion positive dendritic cells, macrophages, and monocytes were collected from the peritoneal cavity. Low levels of prion rods were detected in the mediastinal lymph node at all time points tested, whereas no prion rods could be detected in the mesenteric lymph node or the spleen. Based on these results, we show that immune cells play an important role in the uptake and trafficking of prion rods from the peritoneal cavity to the mediastinal lymph node hours after inoculation.

Myeloid Suppressor Cell Depletion Augments Cancer Vaccine Effectiveness

L Mitchell, A Duffy, S Hafeman, S Dow

Myeloid suppressor cells (MSC) are a population of immature and immunosuppressive myeloid cells (monocytes and neutrophils) that are rapidly mobilized from the bone marrow by inflammatory stimuli, including cancer and infection. We observed recently that vaccine administration induced rapid recruitment of MSC to vaccine-draining lymph nodes and we hypothesized therefore that depletion of MSC at the time of vaccination could improve vaccine responses. To test this hypothesis, we vaccinated mice and concurrently administered liposomal clodronate (LC), an agent that efficiently depletes MSC in the blood and bone marrow. Vaccine responses (antibody titers, T cell responses, tumor responses) were assessed in animals vaccinated with or without LC. We found that MSC depletion by concurrent administration of LC markedly increased antibody titers and CD4 and CD8 T cell responses to vaccination. In addition, tumor responses in mice vaccinated with an autologous tumor vaccine were significantly augmented by combined LC treatment. We concluded therefore that MSC depletion was an effective and widely applicable means of enhancing overall vaccine effectiveness.

Comparison of Two Serological Tests for Antibodies to Toxoplasma gondii in Feral Swine

E Myers, S Swafford, JA Baroch, LR Ballweber

Disease risks associated with an estimated 5 million feral swine (Sus scrofa) occupying 38 states need to be investigated and validated to thoroughly address human health issues. However, approved tests for the serological detection of Toxoplasma gondii in feral swine are currently not available, and testing choices for the detection of T. gondii in feral swine are limited. Toxoplasma gondii, an obligate intracellular protozoan that utilizes felids as a definitive host, may be harbored as tissue cysts in many mammals, including feral swine. Transmission to humans can occur through ingestion of undercooked meat, with congenital infections being of particular concern. Therefore, the Modified Agglutination Test and Enzyme-Linked Immunosorbent Assay are compared for agreement on 250 serum samples collected from feral swine across the U.S. Although the diagnostic tests use different methodologies, they are expected to agree when serum is screened as recommended by the manufacturers. Both tests demonstrated good specificity and sensitivity using Bayesian statistics in the absence of a “gold standard.” However, a kappa index showed only fair concordance agreement. Labor intensiveness and testing time are also important considerations when screening feral swine. Study findings may potentially guide testing choices for T. gondii in feral swine and help evaluate risks to human health.

Detection of vaccine derived canine parvovirus DNA via polymerase chain reaction assays

Y Nakayama, JK Veir

The most commonly used methods for clinically detecting canine parvovirus (CPV) from fecal samples are ELISA and hemagluttinin (HA) assays, which are both simple and rapid, but have relatively low sensitivity. Conventional end-point PCR (cPCR) has been developed as a diagnostic method for detecting CPV DNA with higher sensitivity; however, neither the effects of recent vaccination on the results of clinical samples nor the effect of PCR conditions has been explored. This current study addresses both issues by qualitatively assessing PCR efficacy after amplifying varying amounts of CPV vaccine derived DNA in PBS, whole blood, and feces with a previously described cPCR method. Serial 10-fold dilutions of a modified live CPV vaccine in PBS, canine whole blood, and feces was prepared, and DNA was extracted, amplified, and visualized by agarose gel electrophoresis. The conventional PCR detected CPV DNA derived from the vaccine in all three sample series, but the presence of PCR inhibitors required significant dilutions prior to amplification. Therefore, a positive PCR result in a canine derived sample may reflect shedding of vaccine virus rather than infection with wild-type virus, complicating the diagnosis of canine parvovirus in vitro. Further studies to evaluate the ability of conventional PCR and quantitative PCR to allow for a DIVA (Differentiating Infected from Vaccinated Animals) strategy for canine parvovirus diagnosis are ongoing.

Changes in the stability of cell-cycle regulated mRNAs occur when cells achieve pluripotency

AT Neff, JY Lee, B Tian, J Wilusz, CJ Wilusz

We investigated how post-transcriptional control of gene expression at the level of mRNA stability might be altered in human induced pluripotent stem cells (hiPSCs) as compared to the fibroblasts (HFFs) they were derived from. Interestingly, expression levels of several important mRNA decay factors were significantly changed in pluripotent cells. Using microarray analysis, we determined the half-lives of 4,002 HFF mRNAs and 2,538 iPSC mRNAs with an overlap of 1,617 transcripts whose half-life was established in both cell lines. On a global level, we find that iPSC mRNAs are generally less stable than HFF mRNAs. We identified 278 genes whose transcripts decay at significantly different rates in the two cell lines. This set is enriched for mRNAs encoding factors required for cell cycle progression and gives several interesting insights into how mRNA stability may affect pluripotency and the abbreviated cell cycle of pluripotent cells. Compared to HFFs, mRNAs encoding histones are stabilized from 1.8 to more than 5 fold in iPSCs with corresponding increases in mRNA abundance of up to 12 fold. These histone transcripts may be up-regulated to facilitate the rapid cell cycle progression seen in iPSCs and we are currently investigating possible mechanisms for how stability may be increased. Finally, we have observed that several transcripts known to be targets of ES cell specific miRNAs, including WEE1 and DKK1 mRNAs, are destabilized in iPSCs. We are now searching for new miRNA targets amongst transcripts that are specifically destabilized in iPSCs. In summary, this global analysis has helped to establish an important role for regulated mRNA decay in iPSCs.

Efficacy of Coffee Makers at Removing Contaminants

VT Nguyen, GP Dooley, BL Brattin, HS Ramsdell, TE Johnson

Studies that examine the intake of water impurities frequently assume that the most exposed individual drinks water in their home. However, some people do not consume any water in their home, but instead drink coffee or purchased beverages. According to E-Imports statistics, over 50% of people in the U.S. drink coffee everyday – that's more than 150 million daily drinkers – averaging 3.1/3.2 cups per day, with an average cup size of 9 ounces. This study was performed to examine coffee makers as a decontamination device. A previous study found that coffee makers are able to remove about 40-50% of inorganic contaminants such as manganese and yttrium, and rare earths with 50-80% loss of radioactivity in the brewing process (Denham and Soldat 1974). The ability of a coffee maker to remove common organic and inorganic contaminants was examined. Organic contaminants such as atrazine, p-dichlorobenzene, methoxychlor, and alachlor were selected according to an EPA water regulation list and the possibility of high removal. Inorganic contaminants such as uranium, arsenic, and cadmium were chosen from the same EPA listing with water concentration concerns, like soil absorption of uranium leading to uranium contaminated water being consumed, and increasing the risk of cancer and kidney toxicity. The water used to make coffee was spiked from ½mL to 1mL, then 5mL (about 10 times the public health goal) of the inorganic contaminants, while organic contaminants went from 10 micrograms to 50, then 100 micrograms. Organic contaminants were ascertained by gas chromatography-mass spectrometry (GC-MS) and inorganic contaminants by inductively coupled plasma mass spectrometry (ICP-MS). Preliminary results indicate that greater than 50% removal of inorganic contaminants could be achieved. Organic contaminant analysis was complicated by the particulates generated during the brewing of the coffee. Prior to organic analysis, filtering was required, and no preliminary results are yet available. Overall, it appears that coffee makers could alter the quantity of water contaminants consumed by individuals.

Osteogenic potential of bone marrow-derived mesenchymal stem cells from Equine Sternum and Ilium

KG Penman, LR Goodrich, JD. Kisiday, CM. Lee, J Phillips

Osteoarthritis presents a major challenge for both humans and companion animals. In horses, it is a major cause for loss of athleticism, chronic pain and ultimately, death. Repairing cartilaginous defects has been the focus of orthopedic researchers for many years. Damaged cartilage rarely reforms a functional hyaline cartilage surface; however, mesenchymal stem cells (MSC) have the capacity to differentiate into chondrocytes, osteocytes and other connective tissue precursors. MSC are derived from bone marrow, and are generally harvested from equine sternum or ilium. Previous research indicates MSC have repair potential but tend to vary widely in their quality and quantity within marrow aspirates. The purpose of this study was to examine bone marrow aspirates extracted from equine sternum and ilium. As these two sites are the most common for aspiration in equine medicine, it is critical that MSC here be examined, and their therapeutic potential assessed.

Materials and Methods: Marrow was extracted from seven horses between 3-5 years of age using aseptic technique. Two 5mL aspirated were taken from each site without needle redirection. Marrow was spun down and serum was collected. Cells were grown in monolayer with DMEM until confluent, trypsonized and grown to passage 3, at which point they were stored in liquid nitrogen. Osteogenic Differentiation was conducted in 24 well plates with a cell density of 80,000 cells per cm2. Cells were allowed to grow to confluency within wells (24 hours) with only aMEM and fetal growth factor (FGF). Cells were then treated, for 7 days, with aMEM supplemented with dexamethasone, ascorbic acid and glycerol phosphate to encourage osteogenic development. Chondrogenic Differentation was conducted by pelleting live MSCs in a low melting agarose gel surrounded with chondrogenic differntation media (high glucose DMEM + 1%ITS, dexamethasone, ascorbate 2 phosphate and TGF beta). Cells were allowed to grow within wells for 21 days with media changes every 3rd day.

Findings: Initial observations indicate there may be no difference in MSCs from equine sternum or ilium with regard to their propensity to differentiate into osteocytes or chondrocytes. Further, cells from the first of the 2 aspiates appeared more prolific in general. These initial results indicate clinicians can draw from either site without concern that one is more appropriate than another, but moreover, concern themselves with the quality of the aspirate collected.

Hyperglycemia increases disease severity and bacterial burden in Mycobacterium tuberculosis infected guinea pigs

B Podell, F Ackart, N Kirk, S Eck, R Basaraba

Many risk factors are recognized for the development of active pulmonary tuberculosis (TB) but recently, attention has been drawn to diabetes mellitus as an important and poorly understood risk factor for TB. Type 2 Diabetes was previously limited to a Western lifestyle, but it is rising at an alarming rate in countries where risk for exposure to Mycobacterium tuberculosis is highest. Since uncontrolled hyperglycemia is the hallmark of diabetes, we aimed to initially study the impact that hyperglycemia has on Mtb infection in non-diabetic guinea pigs (GP).
In this study, 20 GPs were randomly assigned to 4 groups. Two groups were fed 400 mg per day of sucrose (+sucrose) and the other two were fed an equal volume of water (-sucrose). All 4 groups were infected by low dose aerosol with Mtb H37Rv (+Mtb). Serum glucose levels, tissue bacterial burdens and lesion severity were assessed on day 30 and 60 of infection. Lung cell homogenates from each of the 4 groups were subjected to flow cytometric analysis on day 30, 60 and 90 of infection to evaluate the impact of hyperglycemia on the cellular response to Mtb.
Median serum glucose levels were elevated by 37.7 and 92.2 mg/dl on day 30 and day 60 of infection in (+sucrose, +Mtb) GPs, respectively, and by 70.5 mg/dl on day 60 of infection in (-sucrose, +Mtb) Gps compared to levels on day 30 of infection in (-sucrose, +Mtb) GPs. Mean bacterial numbers were increased by 0.8 and 0.5 log 10 CFU/ml in the lung and spleen, respectively, in (+sucrose, +Mtb) GPs at day 60 compared to (-sucrose, +Mtb) GPs. Mean percentage of lesion:lung and lesion:spleen was increased by 20% and 15%, respectively, in (+sucrose, +Mtb) GPs compared to (-sucrose, +Mtb) GPs with increased lesion necrosis present in both as well. Flow cytometry showed greater numbers of macrophages, CD4+ and CD8+ lymphocytes and granulocytes in (+sucrose, +Mtb) compared to (-sucrose, +Mtb) GPs.

Deposition of the oncoprotein nucleophosmin on mRNAs influences poly(A) tail length and mRNA export

F Sagawa, H Ibrahim, A Morrison, CJ Wilusz, J Wilusz

Nucleophosmin (NPM) is over-expressed in essentially all cancers and has been directly implicated in the development of non-translocation lymphomas. The NPM protein appears to have multiple functions in cells. Previously, using a cell-free system we demonstrated that NPM is deposited on the 3' untranslated regions of mRNAs containing strong viral 3' end processing signals as a direct result of the polyadenylation event to form the 3' end of the transcript. We now show that in dividing cells NPM is also deposited on all endogenous human poly(A)+ mRNAs that we tested. Therefore NPM effectively 'marks' mRNAs that have undergone successful 3' end processing. Mechanistically, NPM deposition on mRNAs could take place during the initiation, extension or termination of poly(A) tail synthesis. Premature termination of poly(A) tail synthesis induced by cordycepin resulted in failure to deposit NPM on processed RNAs. This strongly suggests that the natural polyadenylation termination event is required for the successful NPM deposition. Co-immunoprecipitation assays indicate that NPM is interacting with other polyadenylation factors involved in poly(A) tail length regulation, including a direct interaction with the CPSF160 factor that recognizes the core AAUAAA polyadenylation signal. NPM knockdown by shRNA expression results in significantly extended poly(A) tails on mRNAs. This hyperadenylation phenomenon in the presence of reduced amounts of NPM can be also recapitulated in nuclear extracts and is consistent with a direct influence of NPM on the determination of poly(A) tail length. Finally, FISH assays demonstrate a failure of poly(A)+ mRNAs to be exported from the nucleus in NPM knock-down cells, suggesting a role for the NPM polyadenylation mark in mRNA nuclear export. In summary, these data suggest a model where NPM controls poly(A) tail length and its deposition influences the downstream fate of the transcript.

Vascular development and sex differences in the region of the paraventricular nucleus of the hypothalamus

MJ Schow, JG Knoll, KA Frahm, Q Zhang, SA Tobet

The Paraventricular Nucleus of the Hypothalamus (PVN) contains a high density of blood vessels at the top of the third ventricle, mirroring cell density in this region. While the majority of angiogenesis occurs prenatally, additional vascularization occurs in the PVN postnatally. The vessel density increases from postnatal day (P) 4 through weaning age (P19), and only increases slightly more into adulthood. Immunoreactive platelet endothelial cell adhesion molecule (PECAM) was used to identify blood vessels in males and females. A bilateral region of interest containing the densest area of vasculature within a 50µm thick section containing the PVN was used to quantify blood vessel branches. Preliminary data indicates that males and females have a different rate of angiogenesis over this postnatal time course, though all animals reach a similar level of vascularization by adulthood. Thus, angiogenesis in the region of the PVN is developmentally regulated and dependent upon sex. In starting to search for molecular aspects of the vasculature that may play functional roles in the difference, we examined mRNA levels for the protein encoded by vascular endothelial growth factor (VEGF). Using real-time reverse transcriptase PCR there was a trend towards a greater level of VEGF mRNA in female than male mice in a dorsal hypothalamic dissection that included the area of the PVN at P10. In adult animals, there was no significant difference in mRNA levels. This result is consistent with the higher level of vessel branching in the female PVN during development. The existence of this dense vascularization has been known for over 70 years; however, the current findings suggest that the vascularization may be highly regulated. The existence of a sex difference in vascularization may be related to sex differences in the etiology and prevalence of a number of disorders related to the hypothalamic-pituitary-adrenal axis, including major depressive disorder.

MYC-mediated LIN28 activation regulates let-7 expression in human trophoblast cells

JL Seabrook, RV Anthony, GJ Bouma, QA Winger

Purpose: Disrupted trophoblast cell proliferation and invasion is a major factor contributing to placental dysfunction. We previously reported an inverse relationship between LIN28 and let-7 miRNA expression in mouse trophoblast stem cells. The c-MYC transcription factor (MYC) is known to down-regulate miRNA expression, including let-7 miRNA. Recently, Myc has been reported to act as a post-transcriptional repressor of let-7 miRNA through the up-stream induction of Lin28B expression in mouse lymphoma cells.
Materials/Methods: To investigate the role of MYC and LIN28 in regulating let-7 miRNA in human trophoblast, we used BeWo cells as a model for syncytiotrophoblast, and ACH-3P cells as a model for extravillous cytotrophoblast. LIN28 knockdown (KD) in ACH-3P cells was achieved using lentiviral infection of shRNA, designed against the human LIN28 sequence. LIN28 and MYC gain-of-function ACH-3P cells were created by lentiviral infection of plasmid expressing LIN28 or MYC under the control of the CMV promoter.
Results: Western blot analysis showed higher LIN28 and MYC expression in ACH-3P cells compared to the BeWo cells suggesting that the Myc-Lin28 pathway of let-7 miRNA regulation is not functioning in BeWo cells. LIN28 KD ACH-3P cells had decreased LIN28 confirmed by Western blot and real-time RT-PCR analysis (23, 20 percent of WT expression, respectively), and preliminary analysis confirmed that let-7 expression is increased. Preliminary data suggest that LIN28 levels are increased in MYC gain-of-function ACH-3P cells, indicating MYC regulation of LIN28 expression in these cells.
Conclusions: Establishing the Myc-Lin28-let-7 regulatory pathway in the progression from undifferentiated placental cells in the preimplantation embryo to various trophoblast cell lineages will improve our knowledge of the molecular mechanisms controlling trophoblast proliferation and invasion, which is important for determining causes of placental dysfunction and disease.

Cellular Localization of the Nup210l protein

S Seelye, E Bryda

The FVB-Tg(Gt(ROSA)26Sor-EGFP)130910 Eps mouse strain (M366) carries a random genomic insertion of a transgene within the *Nup 210l* gene on Chromosome 3. This results in an insertional mutation that leads to infertility in male mice that are homogygous for the mutation. Because of the similarities between spermatogenesis in mice and humans, characterization of *Nup210l* and its role in male infertility in mice may provide insight into potential genetic causes of human male infertility. Our working hypothesis is that Nup210l is a novel nucleoporin important for spermatogenesis. The specific aim of this summer project was to determine the cellular location of the Nup210l protein which we expect to be predominantly associated with the nuclear pore complex. A DNA construct that codes for a recombinant form of the Nup210l protein fused to a fluorescent protein, ptdTomato will be transiently transfected into a Sertoli cell line. Expression of the epitope tagged Nup210l recombinant protein will be visualized by fluorescent microscopy. This in vitro analysis will allow the cellular localization of the recombinant protein to be examined. By characterizing the protein expression pattern of wild type Nup210l, we can propose to explore the effect of the mutant version of Nup201l on its cellular localization to begin to better understand the role of Nup210l in spermatogenesis.
We were unsuccessful in creating our DNA construct of the *Nup210l* gene and our selected vector, tdTomato-N1. We were able to recover 5 transformed *E. coli* colonies. It was determined that none of these carried our DNA construct; instead, they carried the recircularized vector. Subsequent control experiments indicate that the *Age1* digest was not efficient. Due to the time constraints of the program, we were unable to perform further experiments to localize the reason for this failure.

Comparison of Third Metacarpal Condyle Density Pattern and Shape to Histologic Characteristics of the Osteochondral Tissues

ML Shaughnessy, CE Kawcak

Purpose: The distal third metacarpal bone (MC3) in racehorses is prone to osteochondral disease, including catastrophic fracture often necessitating euthanasia. New evidence suggests that bone density pattern and bone shape, as characterized using Computed Tomography (CT), may be determinants of fracture. This led to the hypothesis that those MC3 bones with higher grade of bone density gradient and abnormal shape have histologic characteristics of abnormal osteochondral remodeling.
Materials/Methods: CT images of the metacarpophalangeal joints of twelve 18-month-old horses were exported to a 3-D software platform and analyzed in the palmar 30 degree plane and graded using two different density gradient scales. Nondecalcified histological sections of the same area were stained with Basic Fuchsin and histologic characteristics of abnormal osteochondral remodeling were graded. The shape of each condyle was also graded based on flatness of the condylar surface. Histologic results were compared between density gradient and shape groups using a mixed model ANOVA (SAS 9.2, Cary, NC).
Results: Condyles with higher grade of bone density gradient and abnormal shape were associated with more severe osteochondral changes, including horizontally-oriented trabeculae, stacking of horizontal trabeculae and vascularization.
Conclusion: The results of this study demonstrate abnormal histologic characteristics in condyles with abnormal shape and density patterns, thus providing a better understanding of the findings commonly seen using CT. These findings will further justify the use of CT for prevention of fracture in racehorses.

Expression and Function of Survivin in Canine Osteosarcoma

JK Shoeneman, EJ Ehrhart, JC Eickhoff, JB Charles, BE Powers, DH Thamm

Canine osteosarcoma (OSA) is a disease with an extremely high mortality rate, and while current therapies can slow the progression of metastatic disease, new approaches to therapy are needed. Survivin is a protein with both anti-apoptotic and proproliferative activity; it is commonly elevated in human cancer. Survivin expression is a negative prognostic factor in dogs with lymphoma, and both canine lymphoma and OSA cell lines express high levels of survivin. We hypothesized that inhibition of survivin via siRNA knockdown and locked nucleic acid (LNA) antisense oligonucleotide (EZN-3042) in canine OSA cells would inhibit cell cycle progression, and increase apoptosis and chemotherapy sensitivity. We additionally hypothesized that elevated survivin expression in canine OSA tissue would correlate with poor patient outcome. Transient survivin knockdown via siRNA in OSA cells resulted in ~85% inhibition of survivin protein expression as well as a 9-20 fold decrease in endogenous survivin mRNA expression. When knockdown cells were compared to controls, total and viable cell numbers were decreased and apoptosis was increased. Cell-cycle analysis demonstrated both mitotic arrest and apoptosis. Survivin knockdown enhanced OSA sensitivity to carboplatin and doxorubicin. Similar results have been obtained using EZN-3042. Elevated survivin expression in canine OSA tissue correlated with a decreased disease free interval (DFI), and increased histologic grade, numeric mitotic index, and mitotic grade. Our results clearly demonstrate that survivin inhibition increases apoptosis and chemosensitivity and inhibits cell cycle progression. We also demonstrate a correlation between survivin expression in canine OSA tissue and poor patient prognosis. Thus, survivin may be a viable therapeutic target for the treatment of canine OSA.

Measuring stand-specific telomere length using two-color Chromosome-Orientation Quantitative Fluorescent in situ Hybridization

BJ Sishc, DG Maranon, EH Goodwin, SM Bailey

Telomeres are nucleoprotein complexes located at the terminal ends of chromosomes that play a critical role in maintaining genomic integrity. Due to the requirement of an RNA primer for lagging-strand DNA synthesis, replication cannot proceed to the very end of the chromosome, therefore telomeres shorten with every cell division, a phenomenon known as the end replication problem. Telomere shortening has long been recognized as a molecular mechanism of aging, as it triggers cellular senescence and contributes to replicative cell death. Additionally, misregulation of telomere maintenance can also lead to genomic instability and facilitate the development of cancer. Traditional techniques for measuring telomere length have only allowed for relative comparisons of the average telomere lengths in a cell population. However, in 1999 the development of Quantitative Fluorescent in situ Hybridization (Q-FISH) allowed for the specific measurement of telomeres on each arm of individual chromosomes in metaphase cells. We are developing an approach that combines Q-FISH technology with the strand-specific Chromosome Orientation FISH (CO-FISH) technique to facilitate the simultaneous and quantitative measurement of leading- and lagging-strand telomere lengths utilizing a two colored peptide nucleic acid (PNA) probe system.

Association between Feline Antibody Responses to Alpha-Enolase and Azotemia in Privately-Owned Cats

C Sonius, JC Whittemore, JR Hawley, L Schoenberg, MR Lappin

Purpose:Antibodies against alpha-enolase are associated with immune-mediated nephritis in people. It was previously shown that vaccinated cats commonly develop antibodies against alpha-enolase. The purpose of this study was to assess for associations between alpha-enolase antibodies and azotemia in privately-owned cats. Materials and Methods: Clinically stable privately owned cats > 10 years of age, with and without azotemia (creatinine >2 mg/dl), and with an available vaccine history for > 5 years were recruited for the study. Sera were assayed for creatinine concentrations and alpha-enolase antibodies by use of previously validated techniques. Results from cats with and without azotemia were compared by Student's 2-tailed t test or Fisher's exact test with significance defined as $p < 0.05$. Results: Median ages were 15 years (range: 10-18) and 12 years (range: 10-15) for cats with (n=35) and without azotemia (n=27), respectively. There was no significant difference in vaccine events (number, type, or route of administration) between groups. Azotemic cats (34.3%) were more likely than normal cats (12.5%) to be positive for antibodies against alpha-enolase ($p = 0.016$). In addition, alpha-enolase antibody concentrations were greater ($p = 0.041$) in azotemic cats (mean %ELISA=62.5%) than cats with normal creatinine concentrations (mean %ELISA = 47.2%). Conclusions: Results of this study suggest that antibodies against alpha-enolase in cats may be associated with renal disease. Additional prospective evaluation in a larger number of cats is indicated.

The cellular distribution of GluA2 flip AMPA receptors and stargazin changes upon application of glutamate

LM Stone, KM Partin

AMPA receptors are ionotropic glutamate channels that play a key role in fast excitatory neurotransmission. These receptors are essential for proper functioning of the nervous system; defects that alter their function lead to a number of disorders including Fragile-X mental retardation, schizophrenia, Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), depression and epilepsy (Bowie, 2009). The presence and composition of AMPA receptors at synapses is dynamic, changing in response to specific conditions and during development. These changes underlie synaptic plasticity, which is central to long-term potentiation and long-term depression. Mechanisms that interfere with AMPA receptor distribution or trafficking lead to pathological conditions. For example, in the absence of stargazin, a transmembrane AMPA receptor regulatory protein, AMPA receptors are not targeted to the membrane properly, leading to cerebellar ataxia and absence epilepsy in stargazin mutant mice. In culture, exposure of AMPA expressing cells to agonists influences receptor trafficking and provides a way to study the mechanisms behind this process. Biotinylation and immunoprecipitation studies indicate that in the presence of AMPA, stargazin and AMPA receptors separate; stargazin remains more stable at the membrane and the previously associated AMPA receptors are internalized (Tomita et al., 2004). To investigate the trafficking of AMPA receptors and stargazin more closely, we used confocal microscopy of GluA2i wildtype YFP tagged receptors (GluA2i-wt-YFP) and stargazin-CFP tagged protein to observe the movement of these proteins in HEK cells after a brief exposure to glutamate, mimicking synaptic events. Our studies indicate that the sub-cellular co-localization of GluA2i–wt and stargazin changes within 60 seconds and continues to change in the presence of agonist. Future experiments will address the impact of various AMPAR agonists and modulators on the trafficking of GluA2i and stargazin.

Comparison of tissue oxygen saturation in ovariohysterectomized dogs recovering on room air versus nasal oxygen insufflations

LA Sullivan, VL Campbell, SV Radecki, CB Webb

Purpose: To compare tissue oxygen saturation in ovariohysterectomized dogs recovering postoperatively on room air versus nasal oxygen insufflation.
Materials/methods: Prospective clinical study in a university teaching hospital, including twenty dogs undergoing ovariohysterectomy. Dogs were randomized to breathe either room air or 100 ml/kg/min of nasal oxygen insufflation for two hours postoperatively. Tissue oxygen saturation (StO2) was evaluated at 2 mm and 20 mm lateral to the surgical incision, as well as in the inguinal region using a non-invasive tissue oximeter.
Results: In dogs recovered on nasal oxygen insufflation (n=10), tissue oxygen saturation was significantly higher 20 mm from the surgical site (88.44 +/- 2.50%, P = 0.02) and in the inguinal region (83.56 +/- 1.91%, P = 0.032), compared to dogs recovered on room air (n=10, 79.11% +/- 2.50 and 77.12% +/- 1.91, respectively).
Conclusions: In ovariohysterectomized dogs, oxygen supplementation for two hours postoperatively improves tissue oxygen saturation 20 mm adjacent to the linea alba and in the inguinal region. Oxygen supplementation in postoperative dogs is an inexpensive and easily applicable method to improve tissue oxygen saturation. Additionally, tissue oximetry is a simple and non-invasive technology to monitor oxygenation status at the level of peripheral tissues.

Glial interactions and neuroinflammation in manganese neurotoxicity

KA Sullivan, RB Tjalkens

Microglia are the resident immune cells of the brain that help protect the brain from stress and infection, but chronic activation of microglia results in the production of chemokines and proinflammatory mediators such as tumor necrosis factor a (TNFa) and inducible nitric oxide synthase (NOS2). Previous studies have implicated activated microglia and astrocytes in neurodegenerative disorders of the basal ganglia such as manganism. Recent work from our laboratory identified activated microglia in the basal ganglia of mice exposed to manganese (Mn) before the appearance of activated astrocytes; however, the role of activated microglia in manganism is poorly understood. In this study we postulated that Mn directly activates microglia, resulting in increased expression of TNFa that ultimately lead to increased astrocyte activation. Primary microglia and astrocytes were isolated from C57Bl/6 mice for experiments. Immunofluorescence staining for microglia and astrocytes was performed using ionized calcium binding adaptor protein-1 (IBA-1) and glial fibrillary acidic protein (GFAP), respectively. This method yielded culture purities of 97% for microglia and 99% for astrocytes. Treatment of microglia with Mn induced a dose-dependent expression of TNFa, iNOS mRNA and protein. Furthermore, a quantitative PCR array revealed increased expression of proinflammatory mediators in microglia when treated with Mn. Treatment of astrocytes with conditioned media from Mn-treated microglia or via co-culture with microglia caused an activated phenotype characterized by increased iNOS, and TNFa expression that was inhibited by knockdown of TNFa in microglial cells. Collectively, these data indicate that Mn activates microglia in a dose-dependent manner resulting in increased production of proinflammatory mediators that enhance activation of astrocytes, suggesting a complex pattern of glial-glial interactions underlying a neuroinflammatory phenotype in this model.

Intestinal parasites of dogs in Chiang Mai, Thailand

S Tangtrongsup, AV Scorza, LR Ballweber, JS Reif, MR Lappin, MD Salman

Purpose: The current study was conducted to determine the prevalence of intestinal parasites in dogs visiting the Veterinary Teaching Hospital, Chiang Mai University, Northern Thailand. Materials/methods: Fecal samples (n=301) were collected and submitted by owners during August 2009 to February 2010. Demographic and geographic data were recorded. Intestinal parasitic infection was diagnosed by both microscopic examination after zinc sulfate centrifugation flotation and commercially available IFA for Giardia spp. and Cryptosporidium spp. Polymerase chain reaction and DNA sequencing were performed on all Giardia and Cryptosporidium positive samples to provide genotypic information. Results: Overall prevalence of intestinal parasitic infection in dogs in Chiang Mai was 38.9%. The most prevalent parasite was Giardia spp. (24.9%) followed by Ancylostoma spp. (12.0%), Cryptosporidium spp. (7.6%), Cystoisospora spp. (6.0%), Toxocara canis (2.7%), Trichuris vulpis (2.0%), coccidian-like parasites (1.7%), Toxascaris leonina (0.7%) and Strongyloides spp. (0.7%). The prevalence of having at least one parasite in dogs < 1 year, 1-7 years and > 7 years were 49.3%, 39.8% and 27.4%, respectively. Of these infected dogs, 59.0%, 34.2%, 5.1% and 1.7% were infected with one, two, three and four organisms, respectively. DNA sequencing analyses of Giardia spp. positive samples were dog specific – Assemblages C (4/18) and D (14/18). Only one adequate DNA sequence was available for Cryptosporidium spp., which was shown to be C. canis. Conclusions: The findings suggested that intestinal parasitic infection was common in dogs in Chiang Mai, Thailand. Dogs could be potential source for zoonotic intestinal parasitic infection since dogs in this area are allowed for free roaming. Regular deworming program is indicated to prevent not only transmission among dogs but also to human.

Role of Mycoplasma spp. in Feline Cat Bite Abscesses

C Torres, J Hesser, M Brewer, MR Lappin

Purpose: Mycoplasma spp. are common inhabitants of the feline oral cavity and so likely contaminate many cat bite abscesses. Mycoplasma spp. are cell-wall deficient and so do not respond to beta-lactam class antibiotics, the class most commonly used for the treatment of cat bite abscesses. The objective of this study was to determine whether Mycoplasma spp. are common contaminants of cat bite abscesses and are associated with beta-lactam resistant clinical disease. Materials and Methods: Privately owned cats with clinical evidence of an acute abscess suspected to be from a cat bite were included in the study. Participants were given a free aerobic and anaerobic culture as well as Mycoplasma spp. culture and polymerase chain reaction using Mycoplasma genus specific primers. Mycoplasma spp. amplicons were sequenced to determine the species. All cats were initially treated with appropriate wound management, were administered an antibiotic in the beta lactam class (amoxicillin-clavulanate or cefovicin), follow up was done 7 days after beginning treatment. Results: Of the 26 cats entered into the study to date, Mycoplasma spp. were amplified from 4 cats (15.4%). Of the 2 positive samples with adequate DNA for sequencing, one was consistent with M. felis and the other was consistent with M. equigenitalium. Of the 26 cats, 25 responded by Day 7 to the initial treatment, including 3 of the 4 Mycoplasma spp. positive cats. The cat that failed initial treatment was positive for M. equigenitalium on both Day 0 and Day 7 and ultimately responded to administration of a fluoroquinolone. Conclusions: The results suggest that while Mycoplasma spp. commonly contaminate cat bite abscesses, routine wound management and antibiotic therapy is adequate for control. However, as Mycoplasma spp. infections do not respond to beta lactam class antibiotic therapy, these organisms should be considered in cats with abscesses that fail treatment with this antibiotic class.

Risk Factors for Dehydration and Stress in Horses Participating on a High Altitude Week-Long 100-Mile Ride

WT Walker, DM Hassel, AE Hill, KB Tisher

Horses participating on long distance rides are prone to dehydration. Many risk factors have been proposed, however, little scientific validation is available. The purpose of this study was to determine the risk factors for dehydration and stress in horses participating on a 100-mile high altitude horseback ride. 48 horses arriving at a camp 2500 meters in altitude were ridden at a walk or trot approximately 20 miles a day for 5 days. Horses arrived either 1 or 2 days prior to the ride and all horses were rested after 3 days of riding. Baseline blood samples were collected prior to the first day of riding, as well as in the evenings of ride days 1, 2, 3, and 5. Samples were analyzed for PCV, cortisol, and plasma chemistry parameters. Demographic risk factors including breed, age, sex, arrival day, home environment and altitude, miles traveled to camp, and conditioning were analyzed to determine if any of the demographic factors predisposed horses to dehydration or stress. Cortisol and hydration parameters were highest initially and decreased on every subsequent day of the ride. Arrival to camp 2 days prior to the ride also seemed to improve baseline hydration and stress levels. Analysis of the demographic risk factors is pending. Initial results of this study suggest that travel to camp is more stressful and causes more dehydration than long term low intensity high altitude exercise.

Immuno-Antimicrobial Therapy for Treatment of Chronic Staphylococcal Infection with Pre- and Post-Exposure Vaccination

KD Walton, A Lord, LV Kendall, S Dow

Staphylococcus aureus is a common biofilm producing pathogen that is frequently involved in the development of nosocomial and community acquired infections. The prevalence and severity of staphylococcal infections is steadily increasing, and the emergence of new, highly resistant strains continues to impede efforts to reduce morbidity and mortality rates associated with the bacteria. The increased incidence of severe staphylococcal infections, in addition to the prevalence of antimicrobial resistance, requires that new therapeutic measures are developed against this pathogen. We hypothesize that the combination therapeutic regimen of antibiotics and vaccination will reduce the incidence and severity of staph-associated infections and implant failure. Mice were implanted with a subcutaneous catheter inoculated with luciferase expressing S. aureus. Five mice from each treatment group received either sub- therapeutic antibiotics, heat-killed S. aureus vaccine or combination therapy post exposure. Another 5 mice from each group received pre-exposure vaccine from S. aureus biofilm antigens 10 days prior to subcutaneous catheter implant. Biofilm antigens were collected at 2, 5 and 10 days post culture. Results indicated that combined vaccination and antibiotic therapy commencing post exposure did not improve bacterial resolution compared to non-treated controls. However, pre-exposure vaccination with biofilm antigens showed a reduction in bacterial numbers compared to sham vaccinated mice demonstrating the potential for vaccination with biofilm antigens to improve recovery of chronic S. arueus infections.

Identification of metastasis-related microRNAs in osteosarcoma

AL Wolf-Ringwall, VA Enriquez, GJ Bouma, DH Thamm

Purpose: Increasing evidence indicates that dysregulation of microRNAs (miRNAs) can be correlated with pathological processes in cancer, including metastasis. The purpose of this study was to examine the miRNA expression profiles in 2 isogenic mouse osteosarcoma cell lines with differing metastatic capacity and to identify miRNAs potentially involved in metastasis, also known as metastamiRs.
Materials/Methods: We used a quantitative real-time PCR array to detect differentially expressed miRNAs in a highly metastatic mouse osteosarcoma cell line, DLM8 and the parental Dunn mouse osteosarcoma cell line. Total RNA was extracted from 4 biological replicates of each cell line and miRNA expression was evaluated using primers for 44 murine miRNAs previously implicated in cancer. Relative expression levels were analyzed with a t-test of normalized values.
Results: Ten miRNAs were significantly altered in the metastatic DLM8 cell line compared to Dunn cells (p
Conclusions: Among the miRNAs identified, some have a well-characterized association with cancer progression, particularly members of the let-7 family and miR-200b. Our results suggest that specific miRNAs may be involved in the metastasis of mouse osteosarcoma; however functional assays are needed to confirm specific roles of these candidate miRNAs in the metastatic phenotype. We are currently using real-time qPCR to identify the global expression pattern of all 709 known miRNAs of the mouse genome in these cell lines, which will allow us to identify a potential metastatic miRNA signature in mouse osteosarcoma.

Development of a microsphere immunoassay for the detection of cytokines in plasma/serum from the domestic cat (Felis catus)

BA Wood, S VandeWoude

Cytokines are small proteins secreted by cells to coordinate cellular communication in response to inflammation or infection, and contribute to the activation of the adaptive immune response. Recently developed microsphere immunoassays (MIAs) allow rapid and accurate evaluation of cytokine levels in plasma/serum samples from several species, including humans, dogs, and mice. However, this technology is not available for the evaluation of cytokines in domestic cat (Felis catus) plasma/serum samples. We describe the development of a MIA for detecting domestic cat cytokines interferon-gamma (IFNg), interleukin-10 (IL-10), and IL-12/IL-23 p40 in plasma/sera samples. This assay was modified from a MIA developed in our laboratory for the quantification of these three cytokines in cell culture supernatant. The validated lower and upper limits of quantitation for the detection of these cytokines in plasma/sera are: 31 and 1000 pg/ml for IFNg, 63 and 2000 pg/ml for IL-10, and 20 and 625 pg/ml for IL-12/IL-23 p40. Preliminary studies suggest that circulating IL-10 and IFNg in domestic cats fall below the lower limits of quantitation. Ongoing studies will address cytokine levels in plasma from cats infected with immunodeficiency viruses, from which we have historically measured increases in mRNA transcripts for IFNg in peripheral blood cells. This assay could be used to evaluate cytokine levels in other disease states of the domestic cat.

A Retrospective Echocardiographic Study of Left Atrial Size in Horses as a Prognostic Indicator for Mitral Valve Disease

TL Yates, JA Keen, KJ Blissitt, LE Young

Purpose: Mitral valve regurgitation (MR) is the most common valvular disease associated with poor performance in the horse and it is the most common pathology associated with congestive heart failure in horses. Regurgitation of the mitral valve causes dilation and volume overload of the left atrium. When the left atrium can no longer compensate for increased volume, LA pressures rise leading to pulmonary congestion and the development of left sided heart failure. LA enlargement is also considered a significant risk factor for the development of atrial fibrillation, which is the most common arrhythmia associated with poor performance in horses. The goals of this study are: 1) to identify any relationship between the severity of MR, as assessed by murmur grade and color-flow Doppler echocardiography, and LA size and mechanical function determined by two-dimensional echocardiography, and 2) to determine if LA measurements made using standard two-dimensional echocardiography can be used as a prognostic tool in cases of MR. Materials and Methods: This is a retrospective study of horses presenting to the Royal "Dick" School of Veterinary Studies (RDSVS) and to Dr. L.E. Young for standardized echocardiographic examinations. Four variables from the right parasternal long-axis views and five variables from the short-axis view recommended for the evaluation of LA size and function were measured. Color-flow Doppler images for each horse were analyzed and the severity of MR was graded on a 1-9 scale. Statistical analysis is currently in progress grouping horses based murmur grade and color-flow Doppler grade. Results: Statistical analysis is still in progress and results are not yet available. Conclusion: Based on analysis of preliminary statistics, there are several measurements analyzed in this study that may be reasonable prognostic indicators in cases of MR.

Comparison of Immunocytochemical and Immunohistochemical c-KIT Expression Pattern in Canine Cutaneous Mast Cell Tumors

KA Zeh, JB Charles, EJ Ehrhart, DA Kamstock

Mast cell tumors (MCTs) are the most common cutaneous tumor of dogs and demonstrate highly variable biologic behavior. Biologic behavior can be predicted by histologic grade and tumor-associated molecular parameters. The expression pattern of the tyrosine kinase receptor c-kit (CD117) in formalin fixed paraffin embedded (FFPE) MCTs has been shown to correlate with time to local recurrence and overall survival. C-kit is normally expressed on the cell surface but may demonstrate aberrant cytoplasmic localization in MCTs. This study evaluated c-kit expression in cytological samples via immunocytochemistry (ICC) and compared the expression pattern to that observed in the corresponding FFPE tumor biopsies via immunohistochemistry (IHC). Surgical biopsies and FNAs were obtained from 9 dogs with cutaneous MCT. ICC and IHC for c-kit was performed on all cytological samples and corresponding histological specimens when immunopositivity was identified cytologically. C-kit immunoreactivity and localization was evaluated. Expression patterns were characterized as Type I, II, or III and corresponding cytological and histological expression patterns were compared. Positive c- kit immumoreactivity was identified in 5 of the 9 cytological specimens with variable expression patterns. Positive c-kit immunoreactivity was identified in all corresponding histological specimens and all cases (5/5; 100%) demonstrated similar ICC and IHC c-kit expression patterns. ICC can be successfully used to identify c-kit protein expression in cytological samples of canine cutaneous MCTs and, moreover, the expression pattern correlates with that observed via IHC in histological samples. The use of ICC to determine the expression pattern of c-kit would provide the clinician with prognostic information more rapidly, with a less invasive procedure, and at an earlier time point which may impact therapeutic decisions and overall case management.

Development of a reporter system for the study of gene Copy Number Variation (CNV)

A Zeidler, J Stanton, JL Argueso

Multiple studies have shown that in different individuals, specific genomic segments can occur at a variable copy number relative to the reference human genome. Chromosomal rearrangements resulting in Copy Number Variations (CNVs) have long been recognized as contributing factors in carcinogenesis, and more recently, spontaneous de novo CNVs have been implicated in other sporadic diseases, most notably in Autism Spectrum Disorders. The molecular mechanisms underlying the formation of CNVs are not completely understood, although it is likely that both environmental and intrinsic cellular factors affect this process. Industrial residues, pharmaceuticals, pesticides and other environmental contaminants may be in part responsible for the occurrence of de novo CNVs in the exposed population. The goal of our research is to investigate the environmental factors and molecular mechanisms associated with de novo CNV formation. We have developed a CNV detection assay using the budding yeast Saccharomyces cerevisiae as an experimental model system. Our CNV reporter contains two yeast genes, CUP1 and SFA1 that confer gene dosage-dependent tolerance to copper and formaldehyde, respectively. This system enables the detection of rare clones containing a duplication of the chromosomal segment containing the reporter by selection in media containing high levels of copper and formaldehyde, allowing us to calculate the rate of CNV formation and to test whether specific environmental exposures, genetic defects, or genomic context affect the baseline CNV rate. In addition, we are subjecting individual CNV clones to karyotyping and array-CGH analyses to establish the qualitative chromosomal rearrangement signature associated with different stimulatory conditions. We will present details on the development of the CNV reporter system and initial results from the CNV stimulating conditions assayed so far.