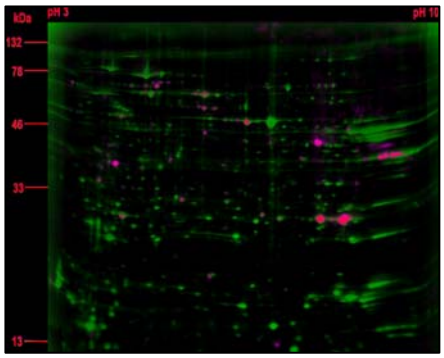
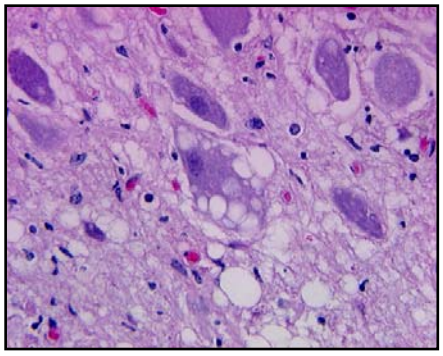
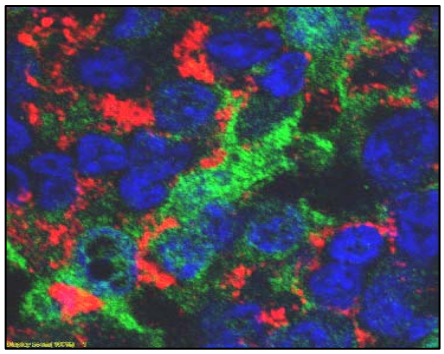




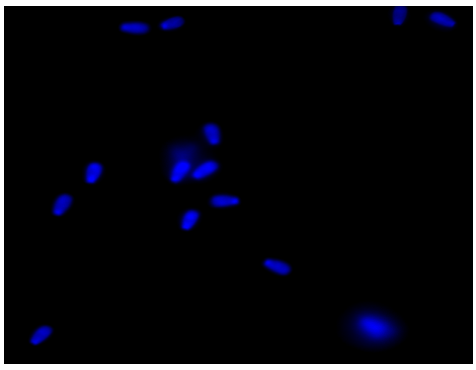


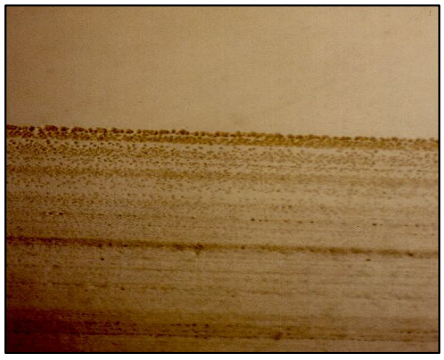
Colorado State University

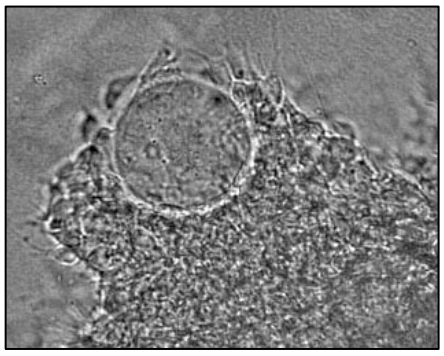
College of Veterinary Medicine and Biomedical Sciences

11TH ANNUAL CVMBS RESEARCH DAY
SCIENTIFIC PROCEEDINGS

The Hilton Hotel
January 23, 2010







CVMBS Research Day 2010

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. Daniel L. Gustafson "Pharmacokinetic Modeling in Dog Populations"	Salon I
12:45	Break	
1:00-5:00	Oral Presentation I: Basic Sciences	Salon I
1:00-5:00	Oral Presentation II: Clinical Sciences	Salon V
1:00-5:00	Oral Presentation III: Clinical 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, January 23, 2010

Dr. Daniel L. Gustafson, Ph.D.

"Pharmacokinetic Modeling in Dog Populations"

Dr Daniel Gustafson, received a bachelor's degree in Biology from Santa Clara University and his Ph.D. in Cell and Molecular Pharmacology and Physiology from the University of Nevada, Reno. He completed postdoctoral training in Radiation Biology and Pharmacology at Colorado State University. He is currently the Director of Research at the Colorado State University Animal Cancer Center and coordinates the cancer research programs at CSU. Dr. Gustafson is an Associate Professor of Pharmacology in the College of Veterinary Medicine and Biomedical Sciences. His research focuses on developing therapeutic treatment modalities for cancer with an emphasis on the pharmacology of agents used in combination cancer treatment, including characterizing optimum drug combinations and treatment schedules for new molecularly targeted drugs with cytotoxic chemotherapy and/or ionizing radiation. His research initially utilizes animal cell tissue culture, rodent models, and with collaborators utilizes human and veterinary cancer patient populations.

Salon I
The Hilton Hotel
Fort Collins, CO

Oral Presentations

SESSION 1: BASIC and CLINICAL SCIENCE
1:00-5:00PM
Salon I

1:00	Benson	Development of a mouse model for the chronic disease states induced by Coxiella burnetii.	MIP
1:15	Bosco-Lauth	Vector competence of some common North American mosquito species for Japanese encephalitis virus and transmission to horses	MIP
1:30	Goodyear	Oral Burkholderia pseudomallei infection results in persistent enteric infection and dissemination to systemic organs	MIP
1:45	Pecoraro	Comparison of primary equine and canine respiratory epithelial cells inoculated with equine and canine influenza viruses in vitro	MIP
2:00	Propst	Immunotherapy Markedly Increases the Effectiveness of Antimicrobial Therapy for Treatment of Burkholderia pseudomallei Infection	MIP
2:15	Warry	Pharmacokinetics of cyclophospahmide given by intravenous verses oral routes in canine lymphoma patients.	CS
2:30			
2:45			
3:00	BREAK		
3:15	Quintana	Immune responses of equine respiratory epithelial cells to equine herpesvirus-1	MIP
3:30	Seelig	Trafficking of CWD prions via the autonomic & enteric nervous systems in cervidized mice.	MIP
3:45	Shang	Cigarette smoke exposure increases susceptibility to tuberculosis – evidence from in vivo infection models	MIP
4:00	Troy	Innate immune modulation as a means of regulating adaptive immunity.	MIP
4:15	Deford	Assessing complex cognition in the mouse: Development and validation of the Morris water maze task for mice	CVMBS
4:30			
4:45			

Oral Presentations

SESSION 2: BASIC and CLINICAL SCIENCE
1:00-5:00PM

Salon V

1:00			
1:15	Linke	An alternative to the avian influenza vaccine: Preliminary assessment of small interfering RNAs targeting viral and avian genes associated with avian influenza infection	CS
1:30	Perry	Pretreatment monocyte chemotactic protein-1 concentrations and peripheral monocyte counts independently predict a poorer outcome in canine lymphoma	CS
1:45	Premashthira	Quantitative Spatial Methods to Identify Foot-and-mouth Disease of Centrally-originating Potential Outbreak Area in Central United States	CS
2:00	Wilson	Mechanical comparison of the 3.5mm broad limited contact dynamic compression plate and the 3.5mm broad locking compression plate	CS
2:15	Okunaka	Evaluation of cyclosporine blood levels in eight cats after administration of metoclopramide	CS
2:30	Tuohy	Investigating pulmonary regional lymph node classification to improve staging in canine primary lung cancer	CS
2:45			
3:00	BREAK		
3:15	Ashley	DNA-PKcs Roles in the Cellular Replication Stress Response	ERHS
3:30	Rao	The Furosemide Treatment and Performance in Standardbred Race Horses	CS
3:45	Steneroden	The effect of training on the infection control and zoonotic disease awareness knowledge of animal shelter workers and volunteers	CS
4:00	Guth	Depletion of Myeloid Suppressor Cells with Liposomal Clodronate Elicits Spontaneous Activation of Systemic Immunity to Control Tumor Growth	CS
4:15	Bevins	Modeling animal movement, landscape connectivity, and disease transmission in fragmented urban habitats	MIP
4:30			
4:45			

SESSION 3: CLINICAL SCIENCE
1:00-5:00PM

Salon IV

1:00	Barrell	Seroprevalence of Canine Influenza Virus in Household Dogs in Colorado.	CS
1:15	Beckwith	Retrospective analysis of canine osteosarcoma of the head: 136 cases (1991-2008).	CS
1:30	Benedict	Metrics for quantifying antimicrobial use in the beef industry	CS
1:45	Bennett	Evidence of Toxoplasma gondii and Bartonella spp. infections of cats in Scotland	CS
2:00	Burgess	Methicillin resistant Staphylococcus spp in commercial pigs used in veterinary student training	CS
2:15	Burton	Optimizing Metronomic Chemotherapy Using Tumor Biomarkers in Dogs with Soft Tissue Sarcoma	CS
2:30			
2:45			
3:00	BREAK		
3:15	Bybee	Effect of the probiotic, Enterococcus faecium (FortiFlora®, Nestle Purina), on diarrhea in cats housed in a Northern Colorado animal shelter.	CS
3:30	Congdon	Evaluation of sedation scores and cardiovascular variables during dexmedetomidine sedation with and without atropine	CS
3:45	Gingrich	Prevalence of methicillin-resistant Staphylococci in a northern Colorado animal shelter	CS
4:00	Lori	Doxorubicin and Cyclophosphamide for the Treatment of Canine Lymphoma: A Randomized, Placebo-Controlled Study	CS
4:15	Marcus	Characterization of Canine Brain Tumor Stem Cells	CS
4:30			
4:45			

Poster Presentations

Session 1- Odd Numbered Posters 1:00-3:00 PM
Session 2- Even Numbered Posters 3:15-5:00 PM

#1	Alvillar	Epidural use of NK-1 receptor antagonist “maropitant” to elicit analgesia	CS
#2	Assarasakorn	Prevalence of Bartonella species, haemoplasma species, and Rickettsia felis DNA in blood of cats in Bangkok, Thailand.	CS
#3	Bradbury	Survival of Bartonella henselae in the blood of cats used for transfusion.	CS
#4	Christakos	Migration of Bone Marrow Derived Stem Cells in a Dilute Fibrin Matrix on Cartilage and Meniscus	CS
#5	Clarke	Prevalence of Mycoplasma haemofelis, ‘Candidatus M. haemominutum’ and ‘Candidatus M. turicensis’ in feral cats and mosquitoes in Northern Colorado	CS
#6	Cochran	Evaluation of gastric transit time, motility, and pH during sevoflurane anesthesia in dogs	CS
#7	Ficociello	Detection of Bartonella henselae IgM in serum of naturally exposed cats	CS
#8	Hafeman	Liposomal clodronate for depletion of myeloid suppressor cells and treatment of cancer in dogs	CS
#9	Hart	Flow cytometric determination of anti-erythrocyte antibody-mediated anemia in cats.	CS
#10	Herman	Susceptible genotypes to brucellosis in American bison (Bison bison): preliminary assessment	CS
#11	Ireland	Detection of eubacterial DNA in blood of dogs by real time PCR assay	CS
#12	Mandel	Efficacy of Adeno-Associated Gene Therapy in Equine Bone Marrow Derived Mesenchymal Stem Cells	CS
#13	McCord	Methicillin-resistant Staphylococcus spp. contamination of stethoscopes in a small animal veterinary teaching hospital	CS
#14	McGreevey	Determination of eubacterial DNA in the peripheral blood of healthy neonatal foals from birth to 72 hours of age	CS
#15	McInnis	Prevalence of Bartonella spp. DNA in conjunctival cells collected from shelter cats with and without conjunctivitis	CS
#16	Mitchell	Metronomic dosing of cyclophosphamide reduces the ratio of T regulatory/TH17 cells and prevents growth of fibrosarcoma in mice	CS
#17	Oman	Outcome of Radioactive Iodine Therapy in Cats Receiving Prior Methimazole Therapy	CS
#18	Quimby	The Pharmacokinetics of Mirtazapine in Cats with Chronic Kidney Disease	CS
#19	Qurollo	The Effects of Curcumin on Feline Vaccine-Associated-Sarcoma Cells	CS
#20	Scorza	Prevalence of Selected Zoonotic and Vector-Borne Agents in Dogs and Cats on the Pine Ridge Reservation.	CS
#21	Scott	Treatment of Canine Lymphocytes with the immunotoxin, HA22.	CS
#22	Shaver	Regional analgesia of the pinna, ear canal, and soft tissues of the lateral face in mesocephalic canids	CS

#23	Shoemaker	Detection of calprotectin and its correlation to apoptosis within the equine gastrointestinal tract from horses with black walnut extract-induced laminitis	CS
#24	Sonius	Annexin A2 and alpha-enolase antibodies in cats with and without azotemia.	CS
#25	Strobel	Correlation Between Geometric Parameters Identified on Computed Tomographic Images and Radiographic Images of Horses	CS
#26	Tam	Correlation between right atrial and jugular pressures in lateral recumbent horses under anesthesia	CS
#27	Walker	The effects of administering oral powder electrolytes on the incidence of colic in horses participating in a week long 100 mile ride	CS
#28	Wang	Prevalence of enteric parasites in veterinary student owned dogs that frequent dog parks	CS
#29	Anema	Storage of Bovine Sperm for 20 h between Semen Collection and Sexing	BMS
#30	Antoniazzi	Endocrine action of interferon-tau on the corpus luteum in sheep: Implication for antiluteolytic and luteotrophic mechanisms.	BMS
#31	Ashley	The expression profile of the chemokine receptor CXCR4 and specific T-cell markers in peripheral blood and endometrium during early pregnancy in cows	BMS
#32	Barfield	MicroRNA regulation of genes in bovine oocytes and embryos	BMS
#33	Enriquez	LIN28 is a Regulator of Endocycles in Trophoblast Stem Cell Differentiation	BMS
#34	Gates	The role of proline-rich 15 in trophoblast cell migration and invasion	BMS
#35	Harper	Evaluating the Efficacy of Metronomic Cyclophosphamide using Regulatory T cell Populations and Markers of Angiogenesis in Canine Soft Tissue Sarcomas	BMS
#36	Kumar	Role of nitric oxide in the expression of gonadotrophin releasing and gonadotrophin inhibiting (RFamide related peptide) hormone in mouse development	BMS
#37	Mower	Mouse retinal expression of the Mu-opioid receptor and its preferential endogenous agonist ß-endorphin	BMS
#38	Schow	Paraventricular Nucleus of the Hypothalamus: Novel Vascular Development and Relation to Adjacent Cell Types	BMS
#39	Silveira	MICRORNAS IN EQUINE OVARIAN FOLLICULAR FLUID	BMS
#40	Stratton	GABA and the Developing Paraventricular Nucleus of the Hypothalamus	BMS
#41	Varland	Inducible photoreceptor degenerative model in goldfish	BMS
#42	Al Mubarak	New approaches for the identification of biochemical markers of infection and nerve damage in leprosy	MIP
#43	Best	Oral Bioavailability of a Novel Francisella tularensis Chemotherapeutic	MIP
#44	Caraway	Comprehensive evaluation of the effects of chemotherapy in the guinea pig model of tuberculosis	MIP
#45	Chaskey	Sindbis virus usurps the cellular HuR protein to stabilize its transcripts and promote productive infections in mammalian and mosquito cells	MIP

#46	Duffy	Liposome-Nucleic Acid Adjuvants Elicit Effective Mucosal Immunity	MIP
#47	Elmore	Epidemiological aspects of Neospora caninum in Alaskan canids	MIP
#48	Lee	Evidence of reduced gene flow among bobcats due to habitat fragmentation in southern California	MIP
#49	Li	Identification of new Mycobacterium leprae variable number tandem repeat loci for strain typing	MIP
#50	Linton	Intestinal macroparasites and mercury (Hg): the chemical ecology within the alimentary tract of a piscivore host	MIP
#51	Magden	Walleye Dermal Sarcoma Virus (WDSV) Orf C: a possible oncolytic therapy	MIP
#52	Miller	Strain-Specific Neuropathology of Feline Immunodeficiency Virus	MIP
#53	Rholl	Studies of Burkholderia pseudomallei beta-lactam resistance mechanisms	MIP
#54	Sagawa	A novel role of nucleophosmin in mRNA export and quality control	MIP
#55	Sakamuri	MOLECULAR EPIDEMIOLOGY OF LEPROSY IN CEBU, PHILLIPPINES	MIP
#56	Sprague	Temporal association of large granular lymphocytosis, neutropenia, proviral load, and FasL mRNA in cats with acute feline immunodeficiency virus infection	MIP
#57	Weiner	Chemokine Receptor 4 (CXCR4) is Up-Regulated in Bovine Peripheral Blood Mononuclear Cells following Infection with Bovine Viral Diarrhea Virus in vitro	MIP
#58	Wood	Development & optimization of a multiplex microsphere based feline cytokine assay	MIP
#59	Zheng	FIV transcription levels in tissues from single and co-infected cats	MIP
#60	Amerin	Neoamphimedine, a new Topoisomerase II alpha inhibitor, in Metnase-mediated DNA decatenation	ERHS
#61	Bushey	Evaluating an Air-Liquid Interface Culture of Normal Human Bronchial Epithelial Cells	ERHS
#62	Hawley	Using Direct Air to Cell Deposition of Cookstove Emissions to Evaluate the Inflammatory Response of Human Lung Cells In Vitro	ERHS
#63	Nie	Defining Metnase Function in Plasmid DNA Integration	ERHS
#64	Wischhusen	Role of Rad51 ATPase in Double-Strand Break Repair by Homologous Recombination	ERHS
#65	Shoeneman	Effects of Survivin and Survivin Inhibition in Canine Osteosarcoma	CMB

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

Thank you moderators and judges!!

Thank you to our sponsors:

CSU Office of Economic Development

PFIZER

for their generous support!

Oral Presentations

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

BASIC & CLINICAL SCIENCE

Development of a mouse model for the chronic disease states induced by Coxiella burnetii

JM Benson, RA Bowen, P Gordy, C Duncan

Purpose: To develop a mouse model that mimics chronic disease manifestations observed in humans infected with Coxiella burnetii in order to further characterize pathogenesis and provide a means for evaluating vaccine and drug therapies.

Materials/Methods: Forty-eight C57BL/6 female mice were used to evaluate two experimental parameters: age (4 weeks versus 9 months) and mild immunosuppression (induced by dexamethasone). All mice were inoculated intranasally with C. burnetii spleen homogenate from previously infected mice. Sixteen mice (8 juvenile and 8 geriatric) were necropsied 14 days post infection and tissues were evaluated for bacterial burden and histopathologic lesions. On day 28 post-inoculation, a subset of remaining mice (8 juvenile and 8 geriatric) were started on dexamethasone in their drinking water at a dose of 8 mg/L for 14 days. The remaining mice served as non-immunosuppressed controls. Terminal endpoints: All mice were weighed, terminally bled, and necropsies were performed. Spleen, liver, lungs, heart, and kidney were collected for histopathology. Splenic slip smears were performed for immunofluorescence (IFA) analysis; bone marrow and splenic tissue was collected for real time polymerase chain reaction (qPCR) analysis.

Results: None of the mice showed overt signs of disease throughout the 42 day time course. However, preliminary PCR results indicate bacterial presence in the bone marrow and splenic tissue of the majority of infected mice. PCR analysis was also found to be more sensitive than IFA for determining bacterial presence in the splenic tissue of infected mice.

Conclusion: The effect of age and immunosuppression on the development of chronic disease in the mouse is speculative. Comprehensive data analysis and future studies will be essential in determining the susceptibilities associated with the development of chronic Coxiella burnetii infection.

Vector competence of some common North American mosquito species for Japanese encephalitis virus and transmission to horses

AM Bosco-Lauth, RA Bowen

Purpose: To determine competency of several common North American mosquito species for Japanese encephalitis virus.

Materials/methods: Adult female mosquitoes were fed Japanese encephalitis virus (JEV) infectious blood meals using the Hemotek mosquito feeding system and allowed to incubate for 12-14 days at which time they were assayed for infectious virus. Legs were assayed separately to check for dissemination of virus in the mosquito. Species that were JEV positive were then used in horse inoculations, in which mosquitoes were intrathoracically inoculated with JEV and allowed to feed on horses. Horse infection was determined by virus isolation from the blood.

Results: Culex tarsalis, Cx. pipiens, and Aedes aegypti were all tested for JEV competence. Cx. pipiens fail to acquire infection from blood-feeding while small proportions of both Cx. tarsalis and Ae. aegypti could be infected and disseminate the virus. Both of these species were then intrathoracically inoculated with JEV and allowed to feed on horses after seven days incubation. While Cx. tarsalis refused to feed on horses, they did transmit virus into an uninfected bloodmeal. Ae. aegypti fed very well and were able to transmit the virus to three of four horses. None of the horses developed clinical signs of encephalitis, although fevers were observed. Viremias persisted for 6 days and horses were kept for 21 days post infection at which time necropsies were performed.

Conclusions: These experiments clearly indicate that there are at least two species of mosquitoes in the U.S. that can become infected with JEV and in the case of Ae. aegypti transmit to horses, which, like humans, are incidental hosts. Were this virus to reach the U.S, this evidence suggests that we need to prepare for it to become endemic and look for ways to mitigate the impact of potential epidemics, both in horses and in humans.

Oral Burkholderia pseudomallei infection results in persistent enteric infection and dissemination to systemic organs

A Goodyear, H Helle Bielefeldt-Ohmann, H Schweizer, S Dow

Purpose. B. pseudomallei is a soil bacterium which causes the disease melioidosis, and is endemic in Southeast Asia and Northern Australia. Due to its potential use as a bio-weapon the CDC has classified B. pseudomallei as a category B select agent. Currently accepted routes of infection include inhalation and subcutaneous (s.c.) infection. Because the bacterium is known to survive for years in distilled water, we investigated the ability of B. pseudomallei to cause disease following an oral infection.

Methods. Mice were infected orally (p.o.) with B. pseudomallei strain 1026b using a gavage needle. LD50 doses were determined in BALB/c, C57BL/6, and 129SvEv mice. Sub-lethal infection of BALB/c mice was used to investigate GI colonization, bacterial dissemination, and the minimal infectious dose necessary for bacterial persistence in the GI tract. B. pseudomallei clinical isolates obtained from human infections were used to confirm findings with strain 1026b.

Results. Lethality studies demonstrated that B. pseudomallei is a very efficient oral pathogen, with an LD50 dose comparable or lower than many known enteric bacteria. Quantification of bacterial burdens on days 3, 14 and 56 demonstrated that the bacteria is capable of persisting in the GI tract, disseminating to systemic organs, and is shed in the feces. Finally, all three clinical isolates tested were capable of infecting mice when delivered p.o., confirming the results obtained with strain 1026b.

Conclusions. B. pseudomallei is an efficient enteric pathogen. Following oral infection the bacterium is capable of persisting in the GI tract, disseminating to systemic organs, and ultimately causes lethal systemic disease. These studies describe a novel infection route for B. pseudomallei, and warrant investigation of potential GI infection of humans in regions where melioidosis is endemic.

Comparison of primary equine and canine respiratory epithelial cells inoculated with equine and canine influenza viruses in vitro

HL Pecoraro, AM Quintana, KM Annis, GS Hussey, GA Landolt

Prior to 2004 dogs were not considered to be natural hosts for influenza A viruses. In 2004, however, influenza was reported in Florida racing greyhounds and the virus has since been maintained in dog populations throughout the U.S. Phylogenetic analyses indicated that the canine isolates evolved from contemporary equine H3N8 influenza viruses. A recent study conducted by our laboratory suggested that, despite this close genetic relatedness, a canine influenza virus isolate (A/Ca/WY/86033/07 [Ca/WY]) was unable to infect, replicate, and cause clinical disease in horses. The objective of the present study was to compare the infection and growth characteristics of Ca/WY and a contemporary equine H3N8 isolate (A/Eq/CO/10/07 [Eq/CO]) in differentiated primary canine and equine respiratory epithelial cells. Cultured cells were infected at an MOI=10 with virus and incubated for up to 24 hours. Our results showed that the equine influenza virus was able to infect and replicate efficiently in both canine and equine respiratory epithelial cells. In contrast, while the canine isolate infected and replicated to high titers in canine respiratory cells, the virus exhibited restricted infectivity and replication efficiency in equine airway cells. Our results demonstrated that the infection and/or replication of the canine virus were strongly influenced by the species from which the primary respiratory epithelial cells were derived. While these findings mirror the results of the in vivo study, the host and viral factors contributing to the differences in infection are unknown and are still under investigation.

Immunotherapy Markedly Increases the Effectiveness of Antimicrobial Therapy for Treatment of Burkholderia pseudomallei Infection

KL Propst, RM Troyer, LM Kellihan, HP Schweizer, SW Dow

Burkholderia pseudomallei is classified as a category B Select Agent and is endemic to southeast Asia and northern Australia. This pathogen can cause both acutely lethal pneumonia as well as chronic systemic infections in humans. Effective treatment of infection with B. pseudomallei requires rapid diagnosis and prolonged treatment with high doses of antimicrobials. Even with appropriate antibiotic therapy, patient relapse is common. Thus, new approaches to treat B. pseudomallei infection are needed. In the present study, we investigated whether active immunotherapy could increase the effectiveness of conventional antimicrobial therapy for treatment of acute B. pseudomallei infection. Macrophage infection assays and in vivo pulmonary challenge models were used to assess the inhibitory effects of combined treatment with IFN-gamma and ceftazidime on B. pseudomallei infection. We found that treatment with even very low doses of IFN-gamma and ceftazidime elicited strong synergistic inhibition of B. pseudomallei growth within infected macrophages. In vivo, active immunotherapy markedly potentiated the effectiveness of low-dose ceftazidime therapy for treatment of infected mice in a pulmonary challenge model of B. pseudomallei. Combined treatment was associated with a significant reduction in mortality. In addition, combined therapy significantly decreased bacterial burden within the lungs and reduced dissemination to the spleen and liver. We conclude that immunotherapy with either endogenous or exogenous IFN-gamma could significantly increase the effectiveness of conventional antimicrobial therapy for treatment of acute B. pseudomallei infection.

Pharmacokinetics of cyclophospahmide given by intravenous verses oral routes in canine lymphoma patients

E Warry

Purpose: To determine and compare the plasma concentration of cyclophosphamide and its metabolite, 4-OHCP, within the plasma of lymphoma bearing dogs being treated with either oral or intravenous cyclophosphamide. Methods: In this prospective study, patients were randomly assigned to either receive oral or intravenous cyclophosphamide, at a dose of 250 mg/m2. Based on a priori power calculations, eight patients per treatment group will be enrolled. Plasma was obtained at times 0, 15, 30, 60 minutes, then at 2, 4, 6, 8 and 24 hours post administration for evaluation of 4-OHCP concentrations by liquid chromatography-dual mass spectrometry (LC/MS/MS).. Results: Currently six patients have been analysed; two within the IV cohort and four within the oral group. Average values were obtained for both cyclophosphamide and 4-OHCP concentrations within the plasma of both groups. The average values for cyclophosphamide in the intravenous group were: half life (HL) 34.68 minutes, time to maximum concentration (Tmax) 10 minutes, maximum concentration (Cmax) 27290 ng/mL, area under the curve (AUC) 537876 (min)ng/mL. When administered orally, the values were: HF 39.16, Tmax 97.5, Cmax 560.75, AUC 57217. The average values for 4-OHCP in the intravenous group were; HL 13.06, Tmax 10, Cmax 5180, AUC 133315. When administered orally the 4-OHCP values were: HL 16.64, Tmax 63, Cmax 1079.7, AUC 51236. Conclusions: Preliminary data suggests that maximum concentration of cyclophosphamide and 4-OHCP in plasma when cyclophosphamide was administered intravenously, was approximately five fold higher relative to orally administered cyclophosphamide. The AUC for the intravenous group verses the oral group was approximately 10 fold higher for cyclophosphamide in plasma and 2.5 times higher for 4-OHCP. These results suggest that there is better bioavailability when administered intravenously.

Immune responses of equine respiratory epithelial cells to equine herpesvirus-1

AM Quintana, GA Landolt, KM Annis, G Soboll.

Equine herpesvirus-1 (EHV-1) causes respiratory disease, abortion and myelitis in horses. EHV-1 infection is initially characterized by an upper respiratory tract infection however, immune responses to EHV-1 at the epithelial cell barrier remains poorly characterized. For this reason, we have recently established a primary equine respiratory epithelial cell culture (EREC) model to study immunity to EHV-1. In this study, four-week old differentiated ERECs were infected with EHV-1 strain Ab4 at a multiplicity of infection of 10. Major histocompatibility complex (MHC) I and MHC II as well as toll-like receptor (TLR) 3 and TLR 9 protein expression were examined at 24 and 48 hours using fluorescence activated cell-sorting analysis (FACS). Infection with Ab4 caused down-regulation of MHC-I as well as MHC-II, although MHC-II was down-regulated to a lesser extent. Lastly, changes in TLR 3 and TLR 9 expression were subtle and are currently being investigated further. Together these results provide crucial information regarding the initial immune response to EHV-1 at the epithelial cell barrier. Similarly to what has been reported for other ?-herpesviruses, there is strong evidence that EHV-1 evades the host immune responses by modulating crucial components of the immune system. In the future the EREC system may provide a valuable tool to further study the role of individual EHV-1 immunomodulatory genes using deletion mutants.

Trafficking of CWD prions via the autonomic & enteric nervous systems in cervidized mice

DM Seelig, GL Mason, GC Telling, EA Hoover

Purpose: As the pathway by which CWD prions efficiently transit from the periphery to the central nervous system remains incompletely understood, the chief objective of this work was to determine the role of the autonomic nervous system (ANS) in the trafficking of CWD prions by longitudinally mapping the PrPres tropism patterns in neural and non-neural tissues of multi-route inoculated Tg[CerPrP] mice. Materials/methods: Five groups of n=10 Tg[CerPrP] mice were inoculated with CWD prions via either the intracerebral (IC), intraperitoneal (IP), intravenous (IV), or oral (PO) route. Mice were sacrificed at specified time points post inoculation and at the onset of clinical disease. CWD infection was documented and prion trafficking patterns determined by detection of PrPres using sensitive amplified immunohistochemistry methods. Results: Early and progressive PrPres depositions were detected in the parasympathetic, sympathetic, and enteric nervous systems, in particular within the myenteric plexus, vagus nerve, dorsal motor nucleus of the vagus, solitary tract, lateral ventricular nuclei of the hypothalamus, periqueductal gray matter, and spinal cord. Moreover, PrPres was detected in intimate association with fibers and cells of the enteric nervous system, namely enteroglial cells, and sympathetically-innervated lymphoid organs, including the spleen, Peyer's patches, and mesenteric lymph nodes. Conclusions: We present evidence for a temporal-based pattern of PrPres accumulation in the parasympathetic and sympathetic elements of the autonomic and enteric nervous systems, implicating these elements as major pathways for CWD prion neuroinvasion and gastrointestinal prion shedding. These patterns of spread, including the accumulations in the enteric nervous system, may explain the ease by which CWD prions are taken up from and shed into the environment, and thereby its high degree of horizontal transmission.

Cigarette smoke exposure increases susceptibility to tuberculosis – evidence from in vivo infection models

S Shang, D Ordway, M Henao-Tamayo, C Shanley, IM Orme, RJ Basaraba

Cigarette smoke (CS) exposure is epidemiologically believed to be an independent risk factor for tuberculosis (TB) infection, however the precise reason for the correlation remains unknown. In this study, we evaluated the impact of prior CS exposure on the ability of mice to control the spread of an aerosol infection with Mycobacterium tuberculosis. CS exposed mice had a greater burden of M. tuberculosis in their lungs and spleens at 14 and 30 days post-infection, and CS exposure promoted the progression of lung lesions in M. tuberculosis infected mice. Analysis of host immunological function indicated that, compared to unexposed mice, CS exposed mice showed a decreased number of macrophages and dendritic cells that produced IL-12 and TNFa in the lungs and reduced influx of IFN?- and TNFa-producing CD4+ and CD8+ effector and memory T cells into the lungs following infection. These data indicated that CS exposure of mice increased their susceptibility to M. tuberculosis by impairing host protective immunity, and our study provided the only experimental evidence for the epidemiological link between CS exposure and TB.

Innate immune modulation as a means of regulating adaptive immunity

AR Troy, S Dow, AA Izzo

Tuberculosis (TB) is one of the leading causes of mortality due to an infectious disease causing 2 million deaths each year worldwide. Despite the current global use of the live attenuated Mycobacterium bovis BCG vaccine, TB continues to be one of the foremost causes of morbidity and mortality in developing countries. The development of a novel vaccine against TB is crucial. A limitation of BCG is its inability to provide long-lasting immunity, a hallmark that is essential for any vaccine. As a result, children receiving postnatal vaccinations become susceptible to infection as they grow older. The goal of our research is to understand the immune response generated by novel vaccines so as to better formulate them to provide longer lasting immunity. As a first step we examined the effect of several closely related adjuvant formulations to determine their ability to induce Th1 immunity. The immunological effects of three different CpG oligonucleotides were compared in vitro to determine which of these would provide signals capable of inducing a Th1 type immune response. Of these, one oligonucleotide was chosen and conjugated with cationic liposomes to create a vaccine formulation to be tested in a mouse model of tuberculosis. This liposome-based formulation appeared to provide a protective effect and although it was not better than BCG it was statistically significant when compared with saline. Further experiments will be performed to identify critical factors that will lead to enhanced immunity and greater long-term protection.

Assessing complex cognition in the mouse: Development and validation of the Morris water maze task for mice

SM DeFord, RJ Hamm

Purpose: Rats and mice are routinely used in scientific studies to model complex cognitive function. The variable start Morris water maze (MWM) task is commonly used to evaluate integrity of the hippocampus (brain region involved in complex visuo-spatial learning/memory). While the MWM task has been used with mice, modifications to the task for mice have not been validated. The purpose of the present study was to develop and validate a hippocampal-dependent MWM task specific for mice.

Methods: In general, standard variable start MWM procedures (developed for the rat) were used as well as common modifications used for mice. Briefly, subjects were placed in a 2m or 1m diameter pool and allowed 120s to locate a submerged platform. Subjects were evaluated on 4 trials per day for 5 days. Internal and external maze cues were assessed in both the 1m and 2m pools. C57/B6 and B6C3F1 mouse strains were evaluated in the 2m pool to determine potential for strain differences. Importantly, brain injury repeatedly shown to produce hippocampal dysfunction was used to validate hippocampal dependency of the task.

Results: Unlike the C57/B6 mice, the B6C3F1 mice demonstrated decreased latency (~40s) to find the hidden platform on the standard variable start MWM task, as used in rats. Thus, the B6C3F1 strain was used for all other assessments. While control group mice demonstrated a learning curve in all tasks (i.e., 1m external and internal maze cues, 2m external and internal maze cues), only the 2m pool with external maze cues and variable start MWM procedure was both sensitive to learning and to hippocampal damage.

Conclusions: While mice can be used to model complex cognition, evaluation of a specific strain's performance using wild-type controls prior to experimental subjects is critical to scientific integrity. Results demonstrate only the variable start MWM task using a 2m pool with external maze cues is appropriate for assessing hippocampal integrity in mice.

Oral Presentations

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

BASIC & CLINICAL SCIENCE

An alternative to the avian influenza vaccine: Preliminary assessment of small interfering RNAs targeting viral and avian genes associated with avian influenza infection

LM Linke, J Triantis, MD Salman

Purpose: Losses due to avian influenza (AI) outbreaks are devastating and estimates of potential loss are enormous. Due to insufficient vaccine-induced protection and limited practicality in the field, vaccination is not a highly effective control strategy. Consequently, research to develop more effective methods should be a priority. The aim of this study is to apply RNA interference and design small interfering RNAs (siRNAs) targeting viral genes required for successful replication, for transfection into a susceptible chicken cell line. SiRNAs will also be designed to target chicken genes hypothesized to play a specific role in host-viral interactions. Materials and Methods: The AI genes chosen for siRNA mediated knockdown were the nucleoprotein (NP) and polymerase acidic protein (PA) segments. H8N4 pathogenesis has been established in chicken hepatocellular carcinoma epithelial (LMH) cells, siRNA specific for the H8N4 NP and PA genes have been designed and transfection methods optimized. LMH cells were transfected with NP and PA siRNAs and infected with H8N4. Assessment of viral infection, replication and infectious titers were determined via Immunocytochemistry (NP antigen), RRT-PCR based on the AIV matrix gene, and the TCID50 assay. Using specific selection criteria, 9 chicken genes have been chosen as siRNA targets. Results: There are significant differences in mean m-gene copy# and TCID50 titers between transfected samples and positive controls ($P < 0.05$). Conclusions: NP and PA siRNA transfection correlates with decreased H8N4 infection and infectious viral titer production. Future work includes use of siRNA targeting chicken genes to determine if siRNA mediated gene knockdown at the host level has the potential to reduce AI replication. These are the first steps towards establishing resistance to AI replication in this avian tissue model and establishing proof of concept for utilizing key siRNAs that have the potential to prevent AI infection.

Pretreatment monocyte chemotactic protein-1 concentrations and peripheral monocyte counts independently predict a poorer outcome in canine lymphoma

JA Perry, D Thamm J Eickhoff, D Rice, and S Dow

Purpose: To determine if increased pre-treatment neutrophil and monocyte counts as well as serum MCP-1 concentration predicts a poorer outcome in canine patients being treated for lymphoma. Methods: Serum and CBC data were evaluated retrospectively from patients that enrolled into the CCOGC tissue archiving study with histologically confirmed high grade multicentric lymphoma who were treated with a CHOP based chemotherapy protocol as their first line of therapy. Twenty-six animals met the inclusion criteria. Healthy, age matched dogs were used as controls. Threshold values for MCP-1 concentration, monocyte count and neutrophil count were determined using the Classification and Regression Tree (CART) methodology. Based on these threshold values, univariate and multivariate analyses were performed to determine significance relative to response to treatment as measured by disease free interval (DFI). Statistical significance was achieved with $p < 0.05$. Results: Dogs with lymphoma had significantly higher concentrations of serum MCP-1 as well as higher numbers of circulating monocytes and neutrophils at the time of diagnosis compared to healthy control dogs. Peripheral monocyte and neutrophil counts as well as serum MCP-1 concentration were significantly predictive of a shorter DFI in the univariate model. Only MCP-1 concentration and monocyte count were independent predictors of prognosis in the multivariate model. Conclusions: This study suggests that circulating monocyte count, neutrophil count, and MCP-1 concentration may provide additional prognostic information at the time of diagnosis of patients with lymphoma. Further studies are necessary to elucidate the role of these biomarkers in lymphoma progression. Possible mechanisms include recruitment and activation of myeloid suppressor cells, stimulation of tumor angiogenesis or more likely, a multitude of mechanisms.

Quantitative Spatial Methods to Identify Foot-and-mouth Disease of Centrally-originating Potential Outbreak Area in Central United States

S Premashthira, AE Hill, DL Pendell, RM Reich, MD Salman

Purpose: Foot-and-mouth disease (FMD) is a highly contagious animal viral disease in cloven-hoofed animals. The USA has not experienced an FMD outbreak since 1929. Simulation modeling is used to prepare for FMD introduction in the USA. Our objectives are to outline a study conducted to identify the geographic region within which 95% of simulated outbreaks would be contained, in the event of FMD introduction into a major cattle-feeding region of the United States and to outline the methods of analysis to determine the spatial patterns of simulated FMD outbreaks in the study area. Materials/methods: The stochastic North American Animal Disease Spread Model (NAADSM) was used to simulate an outbreak originating in a large beef feedlot operation in southwest Kansas. Kernel density estimates (KDEs) that consider nonparametric estimates of the intensity of infected herds will be applied to identify the outbreak area, and point pattern analysis and spatial autocorrelation of infected herds will be examined. Expected results: The result of the outbreak area identified from this bivariate KDE will be illustrated as two and three dimension images by contour and perspective maps respectively. The point pattern analysis and spatial autocorrelation will be analyzed to describe the spatial characteristic of the study area. The findings of spatial occurrence will be used as the foundation of the epidemiologic and economic impacts of FMD in the central United States. This approach is a useful tool for informing policy makers in order to develop contingency plans to control FMD in the event of an outbreak.

Mechanical comparison of the 3.5mm broad limited contact dynamic compression plate and the 3.5mm broad locking compression plate

DM Wilson, A Bhattacharjee, BG Sanoni, N Ehrhart

Introduction: The recent availability of 3.5 mm broad locking plates has increased their utility in small animal orthopedics. The objective of this study was to compare the bending properties of the 3.5 mm broad locking compression plate (LCP) to the 3.5 mm broad limited contact dynamic compression plate (LC-DCP) and to analyze the mechanical effect of using threaded plugs in empty screw holes in the LCP.
Materials and methods: The following treatment groups were compared: 3.5 mm broad LC-DCP, 3.5 mm broad LCP, and 3.5 mm broad LCP with threaded plugs inserted in the four central screw holes. Each plate was subjected to four-point bending. Load-deformation curves were plotted to determine bending stiffness, structural stiffness, and bending strength.
Results: The 3.5 mm broad LC-DCP had significantly less stiffness ($p<0.001$), structural stiffness ($p=0.002$), and bending strength ($p<0.001$) when compared to the 3.5 mm broad LCP. The addition of threaded plugs did not significantly enhance mechanical parameters in the LCP.
Discussion: The 3.5 mm broad LC-DCP achieved 90% of the stiffness and 72% of the strength of the 3.5 mm broad LCP. The addition of threaded inserts into the locking component of four screw holes did not significantly improve the mechanical properties of the locking plate. Although this study provides fundamental material testing data about the 3.5 mm broad LC-DCP and LCP plates, additional investigation using in vitro bone construct models would provide better insight into their ultimate performance.
Acknowledgment: The implants were provided by Synthes Veterinary Incorporated.

Evaluation of cyclosporine blood levels in eight cats after administration of metoclopramide

N Okunaka, S Zabel, SF Hudachek, DL Gustafson, MR Lappin

Purpose. Cyclosporine A (CsA) has been shown effective in treating allergies and immune-mediated diseases. However, CsA is relatively expensive and potentially cost-prohibitive. Anecdotal reports state that metoclopramide, which is safe and inexpensive, may increase CsA blood levels in cats. No rigorous scientific evidence has yet been provided. We evaluated the efficacy of metoclopramide as an adjunct to CsA therapy in cats to determine whether the addition of metoclopramide will increase CsA blood levels.

Materials/Methods. The study cohort consisted of eight research cats with steady state CsA blood levels. CsA administration was continued throughout the study (7mg/kg q24hrs PO). Blood was collected from each cat on day 0, 3, and 7 to monitor CsA trough blood levels without metoclopramide treatment. Metoclopramide administration at 0.3mg/kg q24hrs PO was started on day 7. Pre treatment samples were collected on day 14, 21, and 28 and CsA blood levels were measured in all samples by LC/MS. Then metoclopramide dosage was increased to 0.6mg/kg q24hrs PO and the study was continued for an additional 21 days following the same metoclopramide/CsA administration and sample collection schedule.

Results. In 3/8 cats (38%) metoclopramide administration decreased cyclosporine levels. In two of these cats this decrease was statistically significant (p value

Conclusions. Administration of metoclopramide prior to CsA therapy did not significantly increase steady state CsA blood levels when given at 0.3mg/kg q24hrs PO or 0.6mg/kg q24hrs PO compared to steady state CsA blood levels prior to metoclopramide treatment. Since metoclopramide also decreased CsA levels in 3/8 cats, combining metoclopramide with CsA may not be advisable since it may decrease CsA levels and therewith increase the cost of treatment.

Investigating pulmonary regional lymph node classification to improve staging in canine primary lung cancer

J Tuohy, DR Worley

Purpose: A standardized lymph node classification has not been developed for canine lung tumors in contrast to an established human lung cancer lymph node classification scheme. A reliable method of surgically identifying canine thoracic lymph nodes is also lacking. The purpose of this study is to incorporate the recognized human standard of lymph node classification for surgical use in dogs with sentinel lymph node mapping technique.
Materials & Methods: Five healthy purpose-bred Walker Hounds involved in a concurrent project were maintained under general anesthesia. An intercostal thoracotomy was performed in 3 dogs to facilitate a subpleural methylene blue injection into the distal right middle, right caudal, or caudal part of the left cranial lobes. A bilateral intercostal thoracotomy was performed in 2 dogs to facilitate a similar injection into the cranial part of the left cranial and right accessory lobes, or the right cranial and left caudal lobes. Any draining node with visible dye uptake was noted. Any visible, palpable or blue lymph node ipsilateral to the lobar injection was removed in vivo. Following euthanasia, all remaining visible or palpable thoracic lymph nodes were harvested ex vivo and compared to the surgical yield and to the human nodal mapping system.
Results: Total number of in vivo and ex vivo lymph nodes found ranged 8-12 per dog. Total number of in vivo lymph nodes removed per dog ranged 1-4. Dye seen in only 7 of the 12 extirpated in vivo lymph nodes aided removal. Exclusively mediastinal lymph nodes were seen and no hilar nodes in these dogs which is dissimilar to human anatomic patterns.
Conclusions: Subpleurally injected methylene blue dye did not prove to be reliably efficacious in identifying the draining lymph nodes in this population. There is marked anatomic variability to the thoracic lymph nodes of these 5 dogs. Additional work is necessary to determine utility of the human thoracic lymph node classification in dogs.

DNA-PKcs Roles in the Cellular Replication Stress Response

AK Ashley, M Shrivastav, JA Nickoloff

DNA replication forks stall at DNA lesions caused by endogenous processes and by exogenous genotoxins including the topoisomerase I inhibitor camptothecin (CPT) and ribonucelotide reductase inhibitor hydroxyurea (HU). Stalled forks are initially stabilized by ATR/ATM, and BLM, but may collapse to a single-ended double-strand break (double-strand end; DSE). Homologous recombination (HR) is the primary system mediating fork restart, but non-homologous end-joining (NHEJ) proteins are also implicated. DNA-PKcs regulates DSB repair by NHEJ and HR, in part through functional interplay with ATM. ATM phosphorylates DNA-PKcs, and ATM levels are reduced in DNA-PKcs null cells. NHEJ is defective in mutant cells expressing kinase-dead (K3752R; “KR”) DNA-PKcs, but HR is enhanced, partly reflecting restoration of normal ATM levels. DNA-PKcs null and KR are mildly sensitive to the replication stressors, HU and CPT, but KR showed a dramatic hyper-HR phenotype after HU and CPT treatments. Flow cytometry revealed normal HU- and CPT-induced G2 arrest in WT and DNA-PKcs null, but KR showed abnormally high DNA content (>2C) that may reflect altered replication and underlie the high HR in these cells. Our hypothesis is that DNA-PKcs phosphorylates proteins that regulate HR efficiency and/or accuracy to preserve genome integrity. Both null and KR showed increased cytokinesis failure, amorphic nuclei, and formation of giant cells. CPT increased formation of large cells in null but not KR or WT cells. Cytokinesis failure may be caused by tangled chromosomes reflecting unregulated HR. Interestingly, DNA fiber analysis showed that null and KR cells restarted stalled or collapsed forks faster than WT, indicating DNA-PKcs regulates fork restart. Concomitant phosphorylation of replication checkpoint proteins RPA and Chk1 was observed only in wild-type cells. The more rapid fork restart in DNA-PKcs mutants may be less accurate leading to loss of viability, increased HR, and/or genome instability.

The Furosemide Treatment and Performance in Standardbred Race Horses

S Rao, PS Morley, K Hinchcliff

Exercise induced pulmonary hemorrhage (EIPH) is a common condition of horses experiencing high intensity exercise and is associated with bleeding from pulmonary parenchyma into lower airways. Furosemide (Lsx) is commonly administered prior to racing as a prophylactic drug in the US and Canada. However, the effect of Lsx on performance of Standardbred racehorses has not been studied extensively. Hence, the objective of this study was to determine the effect of Lsx treatment on performance in Standardbred racehorses.

The study design was retrospective and cross-sectional. Data from 20,720 standardbreds representing 9,349 races from 93 tracks between June 27 and July 11, 1998 were obtained from U.S. Trotting Association. The outcome variables evaluated using linear regression analysis were individual race times and the amount of money earned in that race. Outcome variables evaluated using logistic regression were whether the horse won the race, finished in top 3 positions, and earned any money in the race. The primary predictor variable of interest was 'Lsx administration' (yes/no). Other variables were included to control for potential sources of confounding. Because many of these potential counfounders were highly correlated, they were included in the models as principal components.
There were 37.4% females, 22.3% males and 40.3% geldings between ages 2 and 15 yrs, 22.4% of which received Lsx. The use of Lsx varied by gender and age. Females and males given Lsx had an average of 0.3 and 0.2 seconds faster individual race times compared to those not treated with Lsx, respectively. The average earnings in the current race were $629.6±2578.2, and Lsx treatment was not associated with significant differences in earnings. Females with Lsx had 1.2 times higher probability of earning when compared to those without Lsx. In conclusion, the horses with Lsx irrespective of gender raced faster, the affect on other performance variables varied by gender.

The effect of training on the infection control and zoonotic disease awareness knowledge of animal shelter workers and volunteers

K Steneroden, A Hill, MD Salman

Introduction: One of the greatest challenges facing animal shelters is the control of infectious and zoonotic diseases. Animal shelters experience high turnover of animals, high density, stressful housing conditions, and limited funding. These factors contribute to disease introduction and spread which may result in increased exposure of workers and the adopting public to zoonotic disease. The aim of this project was to develop and evaluate knowledge dissemination training for educating animal shelter workers on infection control and zoonotic disease awareness. Methods: Two training sessions were to be conducted in animal shelters in 6 western states. Training included pre-training knowledge assessments, presentation with discussion, and post-training knowledge assessment. Training topics included infectious and zoonotic disease concepts including, modes of transmission, clinical signs of disease, incubation periods, carrier states, hand washing, barrier protection, isolation principles, cleaning and disinfection, infectious and zoonotic diseases of concern to shelters, as well as information on zoonotic diseases and immune compromised persons. Pre and post tests were identical and included questions on infectious and zoonotic diseases of concern in shelters: feline upper respiratory disease, plague, ringworm, panleukopenia, rabies, parvovirus, canine respiratory disease, leptospirosis, internal parasites, MRSA and salmonella. Results: One hundred eleven participants were trained at 10 animal shelters in 4 states. A significant difference in scores between pre-test and post test was observed. The greatest changes were seen with leptospirosis, MRSA, rabies and plague, zoonotic diseases with potentially highly significant health consequences for humans. Discussion: In this study training was successful in transferring short term knowledge to animal shelter workers. Infection control and zoonotic disease awareness training is a valuable service to animal shelters.

Depletion of Myeloid Suppressor Cells with Liposomal Clodronate Elicits Spontaneous Activation of Systemic Immunity to Control Tumor Growth

AM Guth, SD Hafeman, JL Sottnik, SW Dow

Purpose. Liposomal clodronate (LC) has been widely used as an immunological tool for depleting macrophages in mice. However, more recent studies have also shown that repeated administration of LC can elicit therapeutic antitumor activity, an effect that has been attributed to depletion of tumor associated macrophages (TAM) and inhibition of tumor angiogenesis. However, we found that direct killing of TAM by LC was unlikely to be the primary mechanism of antitumor activity of LC. Instead, our studies suggested that LC treatment functioned primarily by eliciting systemic antitumor activity rather than through local tumor immune effects.
Materials and Methods. We used mouse tumor models to assess the effects of systemic LC treatment on phagocytic myeloid cells in addition to TAM and on T cell- and NK cell-mediated antitumor activity.
Results. We found that treatment with LC induced efficient depletion of immunosuppressive myeloid-derived suppressor cells and monocytes, both in circulation and the spleen, as well as decreased TAMs in tumor tissues. When RAG2-/- mice were treated with LC, we found that antitumor activity was almost completely abolished. Moreover, the effects of LC treatment on tumor growth were markedly reduced in CD8-/- mice, whereas the antitumor activity of LC treatment was not significantly inhibited in CD4-/- mice. NK cell depletion was also found to significantly reduce the tumor effectiveness of LC treatment.
Conclusions. We concluded that LC treatment generated antitumor activity through spontaneous activation of systemic T cell and NK cell antitumor activity, which was likely mediated by the effects of depletion of myeloid suppressor cells.

Modeling animal movement, landscape connectivity, and disease transmission in fragmented urban habitats

SN Bevins, JATracey, KR Crooks, SVandeWoude

The effect of habitat fragmentation on disease transmission has been discussed extensively in both ecologic and epidemiologic disciplines; however, there is limited consensus regarding patterns of pathogen dynamics in landscapes fragmented by urban development. We offer a novel approach -- an agent based model (ABM) -- in an attempt to understand habitat fragmentation and emergent disease patterns. Agent based models explore disease dynamics by focusing on interactions of discreet individuals in order to determine effects on the system as a whole and offers the possibility of understanding disease transmission on a large spatial scale. The model is based on feline immunodeficiency virus (FIV) infections in an ongoing study of carnivores in urban southern California. Results are striking and sometimes counterintuitive; habitat fragmentation often increased between patch transmission events. Animal behavior and movement are often not incorporated into traditional theoretical disease models, but ABM simulation results show them to have profound effects on pathogen movement across landscapes. These data represent a truly interdisciplinary attempt to understand transmission dynamics by incorporating anthropogenic landscape change, species of conservation concern, and a pathogen that can move between domestic and non domestic animal hosts.

Oral Presentations

Session III ~ Salon IV
1:00-5:45PM

CLINICAL SCIENCE

Seroprevalence of Canine Influenza Virus in Household Dogs in Colorado

EA Barrell, HL Pecoraro, C Torres-Henderson, S Bennett, GA Landolt

Since first isolation in 2004, Canine Influenza Virus (CIV) has spread throughout dog populations in the US. While studies have indicated that CIV is an important pathogen of dogs housed in shelters, little is known about its significance in the household animal. Our research aimed to determine the seroprevalence of CIV in Colorado pet dogs and to identify risk factors that may predispose these dogs to CIV infection. Serum samples were collected from dogs admitted to the Community Practice service at the Veterinary Medical Center at Colorado State University (CSU). To identify risk factors for CIV infection, owners completed questionnaires at the time of sample collection. Additional serum samples were obtained from submissions to the Veterinary Diagnostic Laboratory at CSU. Samples were tested with three genetically distinct viruses to account for any potential antigenic drift that may have occurred since 2004. At this time, four of 139 sampled dogs were positive for CIV for a seroprevalence of 3.07%. Additionally, 75 serum samples collected from dogs seen at CSU prior to 2004 tested negative for CIV. No differences were seen in titers between the three influenza viruses. Data from the questionnaires was analyzed and showed associations between CIV seroprevalence and dogs attending doggie day care or spending time in boarding facilities. Our results indicate that CIV seropositivity in household dogs in Colorado is low, although it has increased since 2004. Moreover, as antibody titers to all the canine isolates tested were comparable, it appears that measurable antigenic drift has not yet occurred. Finally, dogs boarded in kennels or attending doggie day care may be at an increased risk of CIV infection.

Retrospective analysis of canine osteosarcoma of the head: 136 cases (1991-2008)

KA Beckwith, 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, such as location and treatment, impact outcome.
Materials and Methods: Medical records at the VTH were searched for cases of canine osteosarcoma over a period of 17 years. 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, and 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 97 dogs; surgery in conjunction with radiation therapy in 11 dogs; curative intent radiation therapy alone in 4 dogs; palliative radiation therapy alone in 10 dogs. Of the 97 dogs that underwent surgery, clean margins were obtained in 63. Fifty-eight dogs underwent chemotherapy in addition to their primary form of treatment. Of the 122 dogs that underwent some form of definitive treatment, 80 developed progressive disease: 35 local, 32 distant, 13 both local and distant. The overall metastatic rate for the 122 patients who received treatment was 37%. For all cases, median disease-free interval was 191 days (range: 21 to 1882 days) and median survival time was 204 days (range: 0 to 2186 days). Cause of death was due to local disease in 60 dogs, distant disease in 36 dogs, and 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 may be warranted to improve outcome.

Metrics for quantifying antimicrobial use in the beef industry

KM Benedict, SP Gow, RJ Reid-Smith, PS Morley

Purpose: Accurate data is needed regarding antimicrobial drug use to inform the debate regarding the impact of these uses. The primary objective of this study was to investigate the preferences for reporting antimicrobial drug use data within the beef industry.
Materials/methods: Producers, veterinarians, industry representatives, public health officials, and other knowledgeable beef industry leaders were invited to fill out a voluntary, web-based survey. Participants were asked to provide background demographic information and to comment on the most appropriate portrayal of antimicrobial drug use data in different scenarios. The survey further explored perceptions on the presentation of antimicrobial drug use and the degree of concern with regard to antimicrobial resistance.
Results: A total of 156 participants in 33 US states, 4 Canadian provinces, and 6 other countries completed the online survey. Preference for methods of presenting antimicrobial use data varied and was influenced by the perception regarding clarity and accuracy of the method to represent antimicrobial drug use for large cattle populations. Antimicrobial drug use has most commonly been reported as mass of active compound or sales value; however, participants in this study indicated that these methods were two of the least appropriate for reporting data to the general public. Compared to 10 years ago, 40-60% of these participants have greater concern about antimicrobial resistance as a health issue for both humans and animals.
Conclusions: The method of reporting antimicrobial use data for ongoing surveillance efforts should appropriately represent this important health data and should be easily understood by user groups.

Evidence of Toxoplasma gondii and Bartonella spp. infections of cats in Scotland

AD Bennett, D Gunn-Moore, M Brewer, A Alberico, MR Lappin

Purpose. Toxoplasma gondii is an enteric coccidian that has been associated with a variety of polysystemic disease syndromes in cats and people. Bartonella spp. of cats are vectored by Ctenocephalides felis and are commonly associated with cat scratch disease and polysystemic illness in immune suppressed people as well as some clinical abnormalities in cats. Both of these infectious agents are common throughout the world, but the prevalence rates in cats in the United Kingdom are relatively unknown. The purpose of this study was to determine the regional occurrence of these agents in client-owned and shelter cats in Scotland.

Materials and Methods. Samples were collected from cats presented to the Hospital for Small Animals at the Royal Dick School of Veterinary Studies between June and August 2009. Blood was being collected for investigation of clinical disease or for health screening purposes in stray cats. Once aliquots were removed for the primary analyses, remaining blood was placed in a serum separator and EDTA containing plastic tubes. Toxoplasma gondii IgM and IgG and Bartonella spp. IgG were detected in serum by ELISA. Total DNA was extracted from the blood samples and was assessed in a PCR assay that amplifies the DNA of 6 Bartonella species.

Results. Bartonella spp. antibodies were detected in 8 of 52 samples (15.3%). Bartonella spp. DNA was amplified from 3 of 52 samples (5.8%%), each of which was also seropositive. Toxoplasma gondii IgG alone (5 cats), IgM alone (4 cats), or both antibodies (1 cat) were detected in 10 of 53 samples (18.7%).

Conclusion. The data documents that cats living in Scotland are commonly exposed to both agents. The findings support maintenance of flea control to lessen transmission of Bartonella spp. among cats and to limit hunting and feeding of raw meat to lessen prevalence rates for T. gondii. These agents should be on the differential list for cats exhibiting appropriate clinical signs of disease.

Methicillin resistant Staphylococcus spp in commercial pigs used in veterinary student training

BA Burgess, M Jacobsson, D Smeak, PS Morley

Purpose: Research groups from many countries have recently identified methicillin resistant Staphylococcus aureus (MRSA) as a common colonizing agent in pigs, and that people with frequent contact have increased risks for colonization and infection. MRSA has also been identified as one of the most common causes of outbreaks of nosocomial infections in veterinary hospitals. The College of Veterinary Medicine and Biomedical Sciences (CVMBS) utilizes commercial pigs in veterinary student training. These pigs are a potential source of environmental contamination as well as nosocomial and zoonotic infections. The objectives of this study were to estimate the frequency of MRSA colonization in young pigs used in teaching laboratories at the CVMBS and to evaluate associated environmental contamination.

Materials/methods: Environmental samples were collected twice weekly from walls and hand contact surfaces of the holding facility (n=28) and twice daily from floors and hand contact surfaces in the teaching laboratory (n=140) using a commercially available electrostatic dust collection wipe (Swiffer, Procter and Gamble). Additionally, swabs were collected twice from each pig (n=100) including nasal, perineal, and rectal samples. All samples were cultured for the presence of methicillin resistant Staphylococcus spp by a previously reported method.

Results: Overall, the frequency of MRSA colonization in young pigs at the holding facility and teaching laboratory was 65.6% and 80%, respectively. The frequency of colonization was markedly different between pig groups. There was environmental contamination as well as evidence of residual contamination between cleaning.

Conclusions: This study demonstrates a previously unrecognized risk associated with the use of pigs in training and research. Colonization of these pigs does not preclude their use rather it demonstrates the importance of biosecurity and hand hygiene measures to control the associated risk.

Optimizing Metronomic Chemotherapy Using Tumor Biomarkers in Dogs with Soft Tissue Sarcoma

JH Burton, D Rice, J Harper, L Strange, BJ Biller

INTRODUCTION: Metronomic chemotherapy has been shown in murine models and in humans to improve tumor control by inhibiting tumor angiogenesis and suppressing regulatory T cells (Treg). Tregs are a subset of T lymphocytes that function normally to prevent autoimmune diseases but have been demonstrated to be increased in humans and dogs with cancer and are thought to suppress cellular immune responses against tumors. The purpose of this study is to determine whether metronomic cyclophosphamide therapy depletes Tregs and/or exhibits antiangiogenic activity.

METHODS: Client owned dogs with grade I or II soft tissue sarcoma were eligible. Five dogs were administered cyclophosphamide 12.5 mg/m2/day orally for 28 days and the next three patients were administered 15.0 mg/m2/day orally for the same time period. Flow cytometric analysis of T lymphocyte subsets (CD4+ CD8+, and CD4+FoxP3+ Treg) was performed on peripheral blood sampled on day 0, 14, and 28. Tumor biopsies collected at the same time points were assessed for microvessel density (MVD) by performing immunohistochemistry (IHC) for CD146. Statistical analysis was performed using repeated measures ANOVA.

RESULTS: Eight of nine planned dogs have been enrolled in this trial to date. The first five dogs were treated with a mean cyclophosphamide dose 12.2 mg/m2/day (range, 11.2 to 13.9 mg/m2/day). For these dogs, there was no significant difference between the either the total number of Tregs or percentage of Tregs at any time point. The following three dogs received a mean cyclophosphamide dose of 15.8 mg/m2/day (range, 15.3 to 16.9 mg/m2/day). These dogs had a significant decrease in the percentage of circulating Tregs in the blood. MVD data has yet to be evaluated.

CONCLUSIONS: Daily low dose cyclophosphamide is well tolerated in canine patients with soft tissue sarcoma and doses of 12.5 mg/m2/day or greater for 28 days decreases the percentage of circulating Tregs.

Effect of the probiotic, Enterococcus faecium (FortiFlora®, Nestle Purina), on diarrhea in cats housed in a Northern Colorado animal shelter

SN Bybee, V Scorza, MR Lappin

Purpose. Numerous studies have evaluated the effects of probiotics in clinical settings. Although results have been conflicting, anecdotal reports describing the benefits of probiotics have resulted in the widespread use of probiotics as an auxiliary treatment for diarrhea and other diseases in dogs and cats. In previous studies, we showed that administration of a commercially available probiotic (Enterococcus faecium; FortiFlora, Nestle Purina) raised CD4+ lymphocytes counts in cats over time and lessened conjunctivitis in cats with chronic FHV-1 infection. The purpose of this study was to determine if administration of FortiFlora lessens diarrhea in cats housed in a shelter. Materials and Methods. Stray or feral cats housed in two separate rooms were all fed a standardized diet and were administered the probiotic or a placebo mixed with their food daily for four weeks. After a one week period when treatments were not administered, the treatment and placebo rooms were switched for four weeks to control for a possible room effect on the prevalence of diarrhea. A standardized fecal score system was applied to all samples daily and the percentages of cats with diarrhea of > 2 days duration were calculated. A generalized linear mixed model using a bionomial distribution with treatment being a fixed effect and the room being a random effect was used to assess for statistical differences between treatment groups. Significance was defined as $p < 0.05$. Results. The percentage of cats with diarrhea > 2 days was 7.7% for the probiotic group and 20.7% for the placebo group. This result was significantly different ($p = 0.0297$). Conclusions. The results of this study suggest that administration of this probiotic to cats housed in shelters may lessen the numbers of days with diarrhea. As this was a short term study, the positive effect was likely from probiotic effects on intestinal flora rather than from systemic immune enhancing effects.

Evaluation of sedation scores and cardiovascular variables during Dexmedetomidine Sedation with and without Atropine

J Congdon, M Marquez, M Niyom, P Boscan

Purpose: This study measured cardiovascular and sedation variables during intramuscular administration of dexmedetomidine alone or dexmedetomidine with atropine in dogs. Cardiac output during dexmedetomidine sedation with concurrent atropine use has not been previously reported.
Materials/Methods: Five adult Walker Hounds received dorsal pedal artery and lateral saphenous intravenous catheters. All dogs received either dexmedetomidine 10 µg/kg IM (DM) +/- atropine 0.02mg/kg IM (DM-At). Cardiac output (CO), mean arterial blood pressure (MAP), heart rate (HR), respiratory rate (RR), temperature, and sedation score (SS) were measured before drug administration ('baseline'), 5, 15, 30 and 60 minutes later.
Results: Data were analyzed with a multivariable linear regression model for repeated measures. CO decreased from baseline 5.07 ± 1.0 to 2.1 ± 0.9 L/min at 15 minutes, then plateaued to 60 minutes. However, CO was not different between DM and DM-At groups ($p=0.29$) at any timepoint. MAP was higher in the Dm-At group over 60 minutes ($p<0.0001$), increasing from 102.8 ± 17.1 to 179.8 ± 48.7 mmHg in 30 minutes. MAP in the Dm group did not change over time ($p=0.39$). HR was higher in the Dm-At group over 60 minutes ($p<0.01$). Mean HR in the Dm-At group was 96.3 beats/min (range: 69.6-122.4). HR in the DM group decreased from 110 ± 14.2 to 49.4 ± 10.4 beats/min at 15 min, then plateaued to 60 minutes. Arrhythmias in the Dm-At group included AV block, VPCs and bigeminy.
Conclusions: CO decreased after dexmedetomidine independently of atropine administration. Use of atropine with dexmedetomidine at 10 mcg/kg significantly increases arterial blood pressure and HR. Use of atropine with dexmedetomidine 10 µg/kg IM is not recommended.

Prevalence of methicillin-resistant Staphylococci in a northern Colorado animal shelter

EN Gingrich, T Kurt, R Ruch-Gallie, MR Lappin

Purpose: Methicillin-resistant Staphylococcus aureus (MRSA) and S. pseudintermedius (MRSP) are recognized as significant pathogens in veterinary medicine. To date there have been no studies examining MRSA or MRSP colonization rates in animal shelters in the United States. There is potential for animals to serve as a source of MRSA or MRSP re-infection in humans. The purpose of the study was to determine the prevalence of MRSA and MRSP colonization in 200 cats and 200 dogs in a northern Colorado animal shelter.
Materials/Methods: Nasal and perianal swabs were collected from 200 cats and 200 dogs in an open admission shelter. This included 100 cats and 100 dogs housed in respective stray wards and 100 cats and 100 dogs available for adoption. Samples from each animal were pooled and inoculated on selective media to isolate MRSA/MRSP. Antimicrobial sensitivity was confirmed via Kirby-Bauer disc diffusion. Overall MRSA/MRSP prevalence rates and prevalence rates by subgroup for MRSP were calculated.
Results: MRSA (0.5%) or MRSP (3%) samples were isolated from dogs. The MRSA dog was housed in the stray ward. Of the MRSP, 33% (2/6) were obtained from dogs housed in the stray ward while 66.7% (4/6) were obtained from dogs on the adoption floor. The length of stay at the time of sampling for the MRSP dogs ranged from 1 to 8 days. The MRSA (0.5%) isolated from a cat was from a 3 month old kitten housed in the stray ward.
Conclusions: Results reveal a low prevalence of MRSA and are consistent with previous findings of 1-2% prevalence in dogs and cats in animal hospitals. The MRSP results were slightly higher than the 0-2% prevalence reported in other canine studies. The 1 cat colonized with MRSA was housed with 4 MRSA negative littermates. These results suggest animals housed in a regularly cleaned shelter environment are not at greater risk of acquiring MRSA/MRSP and pose no more risk to adopters than animals in the general population.

Doxorubicin and Cyclophosphamide for the Treatment of Canine Lymphoma: A Randomized, Placebo-Controlled Study

JC Lori, TJ Stein, DH Thamm

Purpose: Median survival times (ST) for doxorubicin-treated canine lymphoma range from 5.7-9 months. Because dogs treated with multi-agent protocols have longer survival times, we sought to evaluate whether adding cyclophosphamide would improve outcome in canine lymphoma patients while maintaining an acceptable level of toxicity. Materials/Methods: This was a prospective study evaluating thirty two dogs with stage III-V multicentric lymphoma. Dogs were treated with doxorubicin (30 mg/m2) every three weeks for five total cycles and prednisone at a tapering dose for the first four weeks. Dogs were randomized to receive either cyclophosphamide (50 mg/m2 daily for three days) or placebo concurrently. Response, toxicity, PFI, and ST were evaluated. Results: 17 dogs received doxorubicin and placebo, while 15 dogs received doxorubicin and cyclophosphamide. There was no difference in the likelihood of achieving a clinical response, or the incidence of dose-limiting toxicity. Median PFI for the placebo dogs was 169 days, while that of cyclophosphamide dogs was 246. This difference was not statistically significant (P= 0.58). Median ST was 295 days for placebo dogs, and 423 days for cyclophosphamide dogs (P= 0.11). Conclusions: The combination of doxorubicin and cyclophosphamide for treatment of canine lymphoma was well tolerated, causing no increase in adverse events over doxorubicin alone. However, the addition of cyclophosphamide did not result in improved response, PFI or ST.

Characterization of Canine Brain Tumor Stem Cells

EC Marcus, LY Pang, DJ Argyle

Traditionally, tumorogenesis has been thought of according to the stochastic model whereby any somatic cell can undergo multistage carcinogenesis and become malignant. Recent studies suggest that cancer may instead be a stem cell disease.

The purpose of this study was to investigate a population of cells within the J3T canine glioma cell line that may exhibit primitive stem cell characteristics. The goals of this study were to characterize these cells in terms of expression of embryonic stem cell markers (Oct-4, Nanog, Stat-3), and to explore the radiation and chemosensitivity of these cells compared to the parent population.

It has been demonstrated that tumor tissues or cell lines grown as spheres retain the property of self renewal and are representative of cancer stem cells. In this study, glioma J3T sphere colonies were cultured in non adherent culture conditions using serum starved semi solid media. Cells expressing CD133, previously identified as a marker of neural and cancer stem cells, were isolated in this study using a magnetic cell sorting technique. These cells were tested for the expression of stem cell markers, Oct-4 and Nanog using rtPCR and immunoflourescent antibody staining. Colony formation assays were performed in order to subject cells to a radiosensitivity study, and chemotherapy sensitivity assays were performed in order to determine if these cells exhibited enhanced resistance to typical cancer therapies.

Preliminary data analysis revealed that CD133+ J3T glioma cells express increased quantities of embryonic stem cell markers Oct-3, Nanog, and Stat-3. Additionally, the glioma spheres and CD133+ cells exhibited enhanced resistance to radiation and chemotherapeutic agents.

These data suggest that stem cells may play a role in the development of canine brain tumors. Further investigation of the tumor stem cell model could lead to novel therapeutic targets for both canine and human brain tumor patients alike.

Poster Presentations

Epidural use of NK-1 receptor antagonist "maropitant" to elicit analgesia

B Alvillar, KR Mama, J Congdon, T Ferreira, P Boscan

To determine the anesthetic sparing effect of the NK-1 receptor antagonist maropitant when injected either intravenously or into the epidural space of dogs. Materials and Methods: Seven adult spayed-female adult dogs were used. Each dog was anesthetized and maintained with sevoflurane in oxygen and was mechanically ventilated during the duration of the study. Following anesthesia induction, an epidural catheter was placed with the assistance of fluoroscopy. The tip of the catheter was advanced to the 4th lumbar vertebrae. After equilibrating for 30 minutes, the baseline minimum alveolar concentration (MAC) was determined using the tail-clamp technique. The MAC for epidural saline, epidural maropitant (1mg/kg), and intravenous maropitant (5mg/kg) were then determined. The MAC values were adjusted to sea level and compared using student's t-test. Results: The baseline MAC for sevoflurane was 2.08 ± 0.25%. Intravenous maropitant decreased MAC by 16% (1.74 ± 0.17%, p=0.007). In contrast, epidural administration of either saline or maropitant did not decrease the MAC response (2.17 ± 0.34%, p=0.29; 1.92 ± 0.12%, p=0.08 respectively). Conclusion: Maropitant decreases the sevoflurane MAC when administered intravenously. The anesthetic sparing effect appears to be mediated outside the spinal cord.

Prevalence of Bartonella species, haemoplasma species, and Rickettsia felis DNA in blood of cats in Bangkok, Thailand

S Assarasakorn, JK Veir, MR Lappin

While Ctenocephalides felis infestations are common in Thailand, little is known about the prevalence of flea-associated pathogens. The purpose of this study is to determine the prevalence rates of Bartonella spp., hemoplasmas, and Rickettsia felis in client-owned cats in Bangkok, Thailand.

Materials/methods: Cats were randomly selected during June and August 2009 from a referral veterinary hospital and 2 private veterinary clinics in Bangkok. Blood (1ml) in EDTA was stored at -20C until being shipped to CSU for analysis. Total DNA was extracted and was assessed in conventional PCR assays that amplify the DNA of Bartonella spp., haemoplasmas, and Rickettsia felis.

Results: A total of 153 feline blood samples were collected. The overall prevalence rates for Bartonella spp., haemoplasma spp., and Rickettsia felis were 16.3% (25/153), 22.9% (35/153), and 0% (0/153), respectively. Bartonella henselae and Bartonella clarridgeiae were the common Bartonella spp. amplified. 'Candidatus M haemominutum' and Mycoplasma haemofelis were the haemoplasmas that were amplified. Combinations of Bartonella spp. and haemoplasma DNA were amplified from some cats. The cats with flea infestation were approximately 6 times more likely to have Bartonella spp. DNA than cats without flea infestation (OR 6.12; 95% CI, 2.42 to 15.49, P

Conclusions: The results demonstrate that Bartonella spp. and haemoplasma spp. infections are common in client-owned cats in Bangkok and suggest that further study of associations of these infections with clinical syndromes is indicated in the area. The association of Bartonella spp. infection with cat fleas reflects the fact that fleas are the primary vector. The lack of association of haemoplasmas with fleas adds to the growing evidence that C. felis is not a vector of these organisms.

Survival of Bartonella henselae in the blood of cats used for transfusion

CA Bradbury, M Green, M Brewer, M Lappin

Purpose. Bartonella henselae is a common pathogen of cats and is considered a significant zoonotic disease. While blood transfusions are commonly administered to cats and B. henselae is known to be transmitted by IV administration of infected blood, the ACVIM Consensus Statement on Canine and Feline Blood Donor Screening was equivocal concerning this organism. Blood used for transfusions is most often stored in citrate-phosphate-dextrose-adenine (CPDA-1) solution for up to one month and it is unknown if B. henselae remains viable in blood stored in this solution. The purpose of this study was to determine whether B. henselae survives after storage in blood preserved in CPDA-1.

Materials/Methods. A specific pathogen free cat was inoculated intravenously with a field strain of B. henselae that had been passaged twice through cats in a previous flea transmission study. When infection was confirmed via a Bartonella spp. PCR assay, blood was collected from the cat and placed into a commercially available sterile, closed-collection system that contained CPDA-1 and stored at 4°C. On days 0, 7, 14, 21, and 28, 2 ml samples were aseptically removed from the bag. The 2 ml were divided into 0.5 ml aliquots and each was plated separately onto four plates per time point. The plates were incubated and checked daily for 28 days. When colonies characteristic of B. henselae were identified, they were counted and confirmed as B. henselae by PCR.

Results. Bartonella henselae colonies were identified on all plates from each time point. The number of viable colonies diminished over time.

Conclusions. The results of this experiment confirm that B. henselae remains viable when stored for up to 28 days in CPDA-1 solution and suggests that cats used as blood donors should be screened for this organism, particularly in states endemic for the vector, Ctenocephalides felis.

Migration of Bone Marrow Derived Stem Cells in a Dilute Fibrin Matrix on Cartilage and Meniscus

J Christakos, LR Goodrich, B Hale, JN Phillips, J Kisiday, CW McIlwraith

Purpose: Mesenchymal stem cells (MSCs) have the ability to differentiate into all mesenchymal cell types and the potential to self-renew, making them ideal for use therapeutically in tissues with limited innate healing capacity and high incidence of injury, such as cartilage and meniscus. The use of a fibrin gel carrier to deliver MSCs to the site of injury makes clinical use practical by allowing the appropriate amount of time for cells to bind to damaged tissue while keeping cells at the desired location. Fibrin gel must provide adequate attachment to tissue while allowing cell migration out of the carrier onto the tissue defect, making concentration an important factor in effective delivery of cells. Additionally, the suspension of fibrin gel in platelet-rich plasma (PRP) may enhance the migration of cells by incorporating platelet-derived cytokines, producing a chemotactic effect. Materials/methods: In this study, varying concentrations of fibrin gels suspended in plasma or PRP were seeded with bone marrow derived equine MSCs genetically modified with green fluorescent protein (GFP) and placed on equine cartilage and meniscus tissue explants. Migration patterns of MSCs were studied using fluorescence microscopy. The ability of differing concentrations of fibrin gels to bind to tissue defects was compared macroscopically. Results: Fibrin concentrations as low as 25% bound strongly to tissue without compromising viability of MSCs. All fibrin concentrations appeared to be an acceptable environment for maintaining viability of cells over a 7 day period. However, migration of MSCs onto parent tissue was not demonstrated. Preliminary studies showed no observable effect of PRP on cell migration. Conclusions: These results will allow further development of MSC-seeded fibrin gels as a clinical treatment. Future studies will test lower concentrations of fibrin gels, aiming to determine the best dilution to provide both gel integrity and cell migration.

Prevalence of Mycoplasma haemofelis, 'Candidatus M. haemominutum' and 'Candidatus M. turicensis' in feral cats and mosquitoes in Northern Colorado

LL Clarke, P. Lin, Eisen, JR Hawley, MR Lappin

Purpose- All routes of transmission for feline hemoplasmas are still unknown. The prevalence rates for hemoplasmas in cats do not vary by geographical region suggesting either cat-to-cat transmission or the presence of a commonly occurring vector, such as a mosquito. This study was performed to determine if DNA of hemoplasmas are common in feral cats and mammal-feeding mosquitoes in Northern Colorado.
Materials/Methods- Mosquitoes were collected using CO2-baited CDC light traps near feral cat colonies around Fort Collins, CO, during June and August 2009. Aedes vexans (n = 21 pools) and Culex tarsalis (n = 30 pools) mosquitoes were identified and pooled in groups of 50 mosquitoes. Other mosquito species collected were pooled together (n = 25 pools). Cats (n = 81) in the area were captured during an ongoing trap/spay/neuter program and 1 ml of blood in EDTA was collected with permission. Total DNA was extracted from each feline blood sample and mosquito pool and assessed in a PCR assay that amplifies DNA of all three hemoplasmas. Mosquito pools were also assessed in a PCR assay that amplifies cytochrome B; a positive result indicated the mosquitoes had fed on mammalian blood.
Results- The overall prevalence of hemoplasma DNA in blood of the feral cats was 7.4% (M. hemofelis, Candidatus M. haemominutum, or both) but none of the mosquito pools were positive for hemoplasma DNA or cytochrome B.
Conclusions. The prevalence of hemoplasma DNA in feline blood was similar to previous studies and demonstrates that the organisms are common in the region. Collected mosquitoes did not contain fresh mammalian blood and there was no evidence that they had ingested hemoplasma DNA during previous blood meals. Future field studies need to include alternate methods to capture freshly fed mosquitoes and laboratory studies are needed to determine if mosquitoes, first, can acquire hemoplasmas from infected cats and, second, transmit hemoplasmas during a subsequent blood meal.

Evaluation of Gastric Transit Time, Motility, and pH During Sevoflurane Anesthesia in Dogs

SC Cochran, DC Twedt, P Boscan

Purpose: The effects of inhaled anesthetics on gastric function have not been described. The purpose of this study was to measure gastric transit time, motility, and pH during anesthesia with sevoflurane.
Methods: Eight healthy dogs were anesthetized with sevoflurane and anesthesia was maintained for 7.9 ± 1.6 hours. Gastric contraction characteristics and pH were measured using a wireless sensor capsule (SmartPill®) when awake and during anesthesia. The dogs ingested a SmartPill® capsule at least 30 minutes before anesthesia. Gastric transit time was calculated as the time between when the capsule entered and left the stomach. Motility index was quantified by calculating the area under the curve divided by time.
Results: All values are mean ± SD and are compared using the Mann Whitney test. Sevoflurane anesthesia decreased gastric motility. Luminal maximum pressure decreased from 50.4 ± 29.0 when awake to 10.8 ± 11.2 mmHg when anesthetized ($p<0.01$), mean peak amplitude of contractions decreased from 11.8 ± 5.8 to 3.5 ± 1.9 mmHg ($p<0.01$) and motility index decreased from 0.69 ± 0.56 to 0.02 ± 0.06 ($p=0.01$). Frequency of contractions of 3.3 ± 1.0 and 2.5 ± 1.5 contractions/minute showed no change ($p>0.1$). Luminal gastric pH did not change during anesthesia. The pH was 1.97 ± 0.72 while awake and 2.28 ± 0.68 during anesthesia ($p>0.1$). Decreased gastric motility during anesthesia did not return to normal until 12-15 hours after recovery from anesthesia. Decreased motility delayed gastric emptying as the capsule did not exit the stomach until 43.1 ± 18.6 hours post administration, a significant delay from the normal gastric emptying time in dogs of 14.0 ± 9.0 hours ($p<0.01$).
Conclusion: General anesthesia with sevoflurane reduced gastric motility by decreasing the amplitude of contractions. Reduced gastric motility continued for 12-15 hours following anesthesia. Therefore, gastric transit time or emptying time can be delayed for up to 30 hours following anesthesia.

Detection of Bartonella henselae IgM in serum of naturally exposed cats

J Ficociello, C Bradbury, A Morris, MR Lappin

Purpose. Bartonella henselae has been associated with illness in cats and people. However, results of blood culture, PCR on blood, or B. henselae IgG in serum have not correlated to the presence or absence of clinical disease. In addition, results of B. henselae IgG in serum are poorly predictive of bacteremia. We recently validated a Bartonella spp. IgM ELISA using serum collected sequentially from cats experimentally infected by exposure to B. henselae infected Ctenocephalides felis. The purpose of this study was to determine whether B. henselae IgM results can be used to predict clinical bartonellosis or bacteremia.
Materials/Methods. Samples from cats with and without fever (n = 183), with and without stomatitis (n = 121), and healthy shelter cats (n = 51) that had been used in previous studies of Bartonella IgG antibodies and Bartonella spp. PCR had been stored at – 80C. The samples were thawed and assessed in the Bartonella spp. IgM assay. The distribution of the paired sample results was evaluated by logistic regression to determine odds ratios and 95% confidence intervals. Wilcoxon's rank sum test was used to determine whether Bartonella IgM ELISA titer magnitude was associated with presence of fever or stomatitis. Significance was defined as $p < 0.05$. Results were used to calculate PPV and NPV for presence of disease and for presence of bacteremia as defined as a positive PCR assay result.
Results. Results from analysis revealed no statistical difference association between IgM or IgM titer magnitude and the presence of fever or stomatitis. The PPV and NPV of Bartonella IgM and disease were approximately 40% in all analyses. The PPV and NPV of Bartonella spp. IgM for prediction of bacteremia were approximately 15% and 85%, respectively.
Conclusions. Bartonella spp. IgM antibodies do not correlate with the presence of fever or stomatitis and are not accurate for the prediction of the Bartonella spp. bacteremia.

Liposomal clodronate for depletion of myeloid suppressor cells and treatment of cancer in dogs

SD Hafeman, AM Guth, JL Sottnik, SW Dow

Purpose: Tumor-associated macrophages and myeloid suppressor cells help promote tumor growth and increased numbers of these cells have been associated with a poor prognosis in many tumors. Liposomal clodronate (LC; dichloromethylene bisphosphonate encapsulated in phosphatidylcholine liposomes) has been effectively used for phagocytic cell depletion in the treatment of IMHA in dogs, and in anti-tumor studies. Liposomal clodronate may therefore be a safe and effective treatment of multiple tumor types in dogs. Materials/Methods: Liposomes were prepared containing clodronate or phosphate buffered saline. The effects of the different liposomes on macrophage killing were assessed using murine macrophage cells lines and canine malignant histiocytosis cell lines via the MTT assay to assess cell viability. The effectiveness of in vivo depletion of phagocytic cells was also assessed by flow cytometry after i.v. administration of different LC types. Tumor bearing mice were treated with weekly LC i.v., and tumor growth and cell populations were measured. Canine patients with spontaneous tumors were treated with bi-weekly LC i.v. as single agent therapy and evaluated for tumor response and toxicity. Results: Liposomal clodronate demonstrates significant killing of macrophages (> 90% killing of DH82 cells) in vitro and phagocytic cells (2 fold reduction) in vivo. Treatment with liposomal clodronate has significant anti-tumor activity in mice (growth curve $p < 0.05$ compared to untreated control). Nearly 50% of canine patients treated with LC as single agent therapy showed tumor responses with minimal toxicity. Conclusions: Liposomal clodronate demonstrates significant anti-tumor activity through depletion of myeloid suppressor cells. This phenomenon is applicable to canine patients with spontaneous tumors. Combination therapy with standard chemotherapy may work synergistically with LC to provide enhanced anti-tumor activity.

Flow cytometric determination of anti-erythrocyte antibody-mediated anemia in cats

ML Hart, S Dow, CB Webb

Purpose: Determine if a flow cytometry protocol could identify feline anti-erythrocyte antibodies attached to red blood cells (RBC) of cats with a presumptive diagnosis of immune-mediated hemolytic anemia (IMHA).
Material/methods: EDTA-preserved whole blood from 3 anemic cats with a presumptive diagnosis of IMHA was processed concurrently with samples from non-anemic cats. Ten microliters of whole blood was washed 3 times and the RBCs incubated in fluorescence activated cell sorting buffer alone or with goat anti-feline IgM or IgG FITC-conjugated antibodies (Serotec). The cells were washed and analyzed using a Cyan flow cytometer. Untreated cells were used to set the threshold for non-specific fluorescence.
Results: Three cats (2, 5, and 16 years of age) with a regenerative anemia and a presumptive diagnosis of IMHA were positive for anti-erythrocyte antibodies using the described protocol. The packed-cell volume of the anemic cats ranged from 6% to 17%. One cat was FeLV+, tested Coombs positive, and showed 2+ saline agglutination. The second cat also showed marked RBC saline agglutination and icteric serum. A third cat was both anemic and thrombocytopenic. All anemic cats were negative by PCR for M. haemofelis and M. hemominutum. All 3 anemic cats had RBCs that were positive by flow cytometry for IgM (mean 8.0% +/- 3.3 std dev) and 2 that were positive for IgG (mean 3.4% +/- 1.8) anti-erythrocyte antibodies. The non-anemic cats' RBCs stained a mean of 0.5% +/- 0.4 with anti-feline IgM and 0.3% +/- 0.3 with anti-feline IgG antibodies.
Conclusions: Flow cytometry can be used to detect feline anti-erythrocyte antibodies in clinical cases of anemia in cats. This should be an important tool in future studies of the prevalence and pathophysiology behind immune-mediated hemolytic anemia in the feline species.

Susceptible Genotypes to Brucellosis in American Bison (Bison bison): Preliminary Assessment

JA Herman, ND Halbert, AJ Piaggio, JC Rhyan, MD Salman

Purpose: The aim of this presentation is to investigate the role of a single nucleotide polymorphism within the bison prion protein gene in natural resistance to brucellosis samples collected from Yellowstone National Park.
Materials/methods: Bison used are part of a feasibility study between state and national agencies to explore alternative methods to managing bison infected with brucellosis. Animals are quarantined on state mandated pastures until serology results are released. Serological tests were conducted on all samples by National Veterinary Services Laboratory (Ames, IA). DNA was extracted from 177 bison tissue samples. Prion protein gene sequence was obtained using PCR. Associations between seroreactor status of the animal, genotype, and sex were analyzed.
Results: No significant associations were found between the prion protein genotype and seroreactor results to brucellosis. There were no significant associations between sexes in this species either.
Conclusions: From this study we can conclude that the prion protein gene sequence should not be used as a management option for brucellosis susceptibility until further research is completed.

Detection of Eubacterial DNA in Blood of Dogs by Real Time PCR Assay

LA Ireland,T Hackett, J Veir, MR Lappin

Purpose: Sepsis is a common complication of critical illness. In one study 49% of critically ill dogs and cats that had blood cultures performed had bacteremia and animals with severe disease were 10 times more likely to die if they had concurrent bacteremia. Isolating bacteria can be problematic and it can take up to 5 days for the results of blood culture. Delays in treatment may result and may contribute to higher mortality. We hypothesize that detection of bacteremia in dogs by real time PCR is more sensitive than blood culture by comparing eubacterial rDNA PCR to blood culture among critically ill canine patients and control dogs undergoing elective OHE.
Materials/Methods: Polymerase chain reaction (PCR) has been used to identify the presence of microbial DNA in clinical specimens. As blood of healthy dogs is not always sterile, the result of a conventional PCR assay may not correlate to the presence of illness. In a pilot study, samples taken from critically ill dogs and healthy dogs (ovariohysterectomy and dentistry patients) were evaluated using the assay. The method that was most suitable for gram-negative sepsis was then applied to additional bacterial species. Septic patients were included in the study if they had an abnormal temperature, high or low white blood cell counts, and had a suspected bacterial infection based on clinical signs, surgical findings and cytologic analysis.
Results: From this study, we had 10 PCR positive healthy dogs and 3 PCR positive septic dogs. The mean CFU/ml of healthy dogs and septic dogs were 117.6 and 98.6, respectively. The standard deviation of CFU/ml of healthy dogs and septic dogs were 37.2 and 16.6, respectively. Thus, the level of CFU/ml may not correlate with sepsis.
Conclusions: Samples obtained during the current study will be used to determine if PCR results correlate to blood culture results as well as determining if PCR is more sensitive than blood culture at detecting bacteremia.

Efficacy of Adeno-Associated Gene Therapy in Equine Bone Marrow Derived Mesenchymal Stem Cells

A Mandel, LR Goodrich, VW Choi, J Kisiday, CW McIlwraith, RJ Samulski

Purpose: Adeno-associated virus (AAV) is a vector with seven different serotypes that has potential for genetically modifying bone marrow derived mesenchymal stem cells (BMDMSC) to efficiently express therapeutic genes for long periods of time with a low incidence of immune reactions and cell toxicity for future gene therapy applications in joint diseases.

Materials and Methods: BMDMSC were harvested from bone marrow of the ileum in horses between the ages of 3 and 5 years. Three days after seeding, AAV vector serotypes 1-6, and 8, carrying a GFP gene expression cassette were used to transduce cells using 6000 viral particles per cell. A quantitative analysis of fluorescence intensity and the percentage of transduced cells was measured using flow cytometry at weeks 0, 2, 4, and 6. RNA was harvested from cells and vector toxicity was measured by quantitative PCR and relative gene expression of MMP's and Aggrecanase. The viability of the cells was also measured using ethidium bromide.

Results: AAV with GFP serotypes 1-6 and 8 in BMDMSC's revealed serotypes 2 and 3 as the most efficient vectors for transduction. Flow cytometry for BMDMSCs on day 5 following transduction revealed a transduction efficiency of 90% for serotype 2, 53% for serotype 3, 45% for serotype 5, and 34% for serotype 6 at initial week 0. The transduction efficiency was greatest for serotypes 2 and 3. mRNA of cytokines will be analyzed in an attempt to determine any inflammatory effects.

Conclusion: A difference exists in transduction efficiency between all serotypes and this study highlights those differences. In BMDMSCs, serotypes 2 and 3 appear to be the most. This information will be a foundation for future in vivo studies using AAV as a gene therapy vector for joint diseases. The AAV vectors may be able to transduce joint tissue or extracellular cartilage matrix, in vivo, which is important when determining which serotype of AAV to utilize in the clinical arena.

Methicillin-resistant Staphylococcus spp. contamination of stethoscopes in a small animal veterinary teaching hospital

K McCord, K Stanton, D Bolte, D Hyatt, K Lunn

Purpose: Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant S. pseudointermedius (MRSI) are considered emerging pathogens in companion animals. MRSA is associated with nosocomial infections, and the organism can be transmitted between humans and animals by fomites. Human studies have shown staphylococcal contamination rates of stethoscopes of 66% to 100%. We hypothesized that at least 60% of stethoscopes at the CSU-VTH are contaminated with greater than 20 colony-forming units (CFU) of Staphylococcus spp. per square centimeter, with a small percentage of these being MRSA and MRSI. The purpose of this study was to count the number of CFU of staphylococci present on the diaphragms of 100 stethoscopes and determine the percentage of organisms that are MRSA or MRSI. Material/methods: Blood agar was used as the culture substrate. Only large diaphragms were used to help increase the surface area for isolation. An MRSA enrichment broth-soaked swab was rubbed across the surface area of the diaphragm and cultured in broth. Results: 22% of all stethoscopes cultured positive for S. aureus (7%) or S. intermedius (16%). One stethoscope produced both species. MRSA was cultured from 2%, and MRSI was cultured from 5%, of all stethoscopes. Staphylococci of any species were found on 93% of stethoscopes. 6% of stethoscopes were contaminated with >20 CFU of any Staphylococcus species. Only 3% of stethoscopes were contaminated with greater than 20 CFU of S. aureus and S. intermedius. Only one stethoscope was contaminated with >20 CFU of MRSA and one was contaminated with >20 CFU of MRSI. Conclusions: Staphylococci were present on most stethoscopes, but only a small percentage were contaminated with methicillin-resistant organisms, as hypothesized. These results indicate that stethoscopes are potential fomites in the spread of nosocomial infections, and regular cleaning and disinfection of this important piece of equipment should be employed.

Determination of eubacterial DNA in the peripheral blood of healthy neonatal foals from birth to 72 hours of age

NA McGreevey, ES Hackett, JK Veir, MR Lappin, DP Lunn, PM McCue

Purpose: The purpose of this study was to optimize a 16S rDNA qPCR using a region common to all eubacteria for use with equine blood. Once optimized, the assays were applied to total DNA extracted from blood samples of healthy foals to assess whether eubacterial DNA was amplified transiently in foals during the first 72 hours of life. Materials/methods: Optimized qPCR assays were evaluated in silico for suitability for amplification of DNA from commonly detected organisms in neonatal foals via BLAST analysis. Serial dilutions of representative isolates of Eschericia coli, Streptococcus equi subsp. zooepidemicus, and Enterococcus faecalis were added to equine whole blood for evaluation in vitro. Sensitivity of the qPCR assays for the three organisms was 0.7 cfu/mL equivalent of DNA, 429 x 10-15 g, and 318 x 10-15 g of DNA respectively. Blood was collected from seven healthy foals at birth, 1, 2, 3, 4, 8, 12, 24, 48, and 72 hours of age and blood samples were evaluated by both blood culture and qPCR analysis. Results: In silico analysis results suggest the assay is capable of detecting most pathogens associated with neonatal foal sepsis. In vitro analysis amplicons were consistent with published sequences and did not suggest the presence of PCR inhibitors in equine blood. Bacteria were cultured from 9 of 70 samples (12.9%). The positive samples were from 4 of 7 foals that remained healthy throughout and subsequent to the study. All positive blood cultures were from blood samples obtained at 12 hours of age or earlier. All samples were negative in the qPCR assay. Conclusions: The qPCR assay appeared to have a high analytic sensitivity in titration experiments. The lack of concordance between assay results suggests that the qPCR assay is less sensitive than culture or that the cultures were falsely positive. Positive foal blood cultures may also suggest that healthy foals develop transient bacteremia early in the post partum period.

Prevalence of Bartonella spp. DNA in conjunctival cells collected from shelter cats with and without conjunctivitis

C McInnis

Purpose: Conjunctivitis is a common, painful condition of cats with potential to induce pathologic corneal changes and compromise vision. Bartonella spp. have been implicated as a cause of feline conjunctivitis but studies confirming the association are lacking. The objective of the study is to determine the Bartonella spp. DNA prevalence in conjunctival cells of from cats with and without conjunctivitis housed in Oregon and Florida shelters and so likely had been exposed to fleas. Materials and Methods: Participating shelter veterinarians were asked to collect blood (1 ml in EDTA) and conjunctival swabs under topical anesthesia from cats with clinical evidence of conjunctivitis prior to administration of antibiotics. For each cat with conjunctivitis, samples from a healthy cat without conjunctivitis were collected. The samples were stored at 4C until shipped to CSU for assessment in a conventional PCR assay that amplifies DNA of 7 common Bartonella spp. The DNA prevalence rates were compared between groups of cats using Fisher's exact test. Results: Of the 34 cats with conjunctivitis assessed to date, 10 (29%) had Bartonella DNA amplified from blood but none (0%) had Bartonella DNA isolated from the conjunctival samples. Of the 27 control cats, 10 (37%) had Bartonella spp. DNA isolated from whole blood samples and one (4%) had Bartonella DNA isolated from the conjunctival sample. There were no significant differences in the prevalence of Bartonella spp. DNA isolated from conjunctival samples or from blood samples between cats with and without conjunctivitis ($p=0.44$ and $p=0.58$, respectively). Conclusions: While Bartonella spp. infection was common based on the blood results, there was a low prevalence of Bartonella DNA in conjunctival cells from cats with and without conjunctivitis. These results failed to confirm an association between Bartonella spp. and feline conjunctivitis, suggesting that Bartonella may not be an etiologic agent of this condition.

Metronomic dosing of cyclophosphamide reduces the ratio of T regulatory/TH17 cells and prevents growth of fibrosarcoma in mice

LA Mitchell, DL Gustafson, BJ Biller

Purpose: Continuous administration of low doses of chemotherapy drugs is referred to as metronomic chemotherapy (MC). MC is currently being evaluated because of its ease of oral administration, lower toxicity profile, and promising efficacy data. The goals of this study were to evaluate the effects of MC on the balance between T regulatory (Treg) and TH17 cells and on tumor growth in mice with fibrosarcoma. We hypothesized that metronomic dosing of cyclophosphamide (CYC) would reduce the number of immunosuppressive Treg cells while promoting the expression of inflammatory TH17 cells, therefore favoring the development of effective antitumor immunity. Materials/methods: Tumors were established by injecting 250,000 MCA 205 fibrosarcoma cells subcutaneously in the flank. Treated mice received 20mg/kg/day of CYC, delivered in drinking water, beginning on day 3 after tumor injection. Serial evaluation of the number of circulating Treg and Th17 cells was performed on days 6, 13 and 20 as well as in the spleen at the time of euthanasia. Results: Metronomic dosing of CYC significantly reduced tumor growth throughout the 24 day study period. In both the spleen and the blood, mice receiving metronomic CYC demonstrated statistically increased percentages of TH17 cells. This corresponded to a decrease in the percentage of Treg, suggesting that metronomic dosing of CYC results in polarization between these two T cell subsets. Conclusions: Metronomic dosing of CYC is effective in reducing tumor burden in mice. This delivery method and dosing schedule promotes the expression of pro-inflammatory TH17 cells, while reducing the number of immunosuppressive Treg cells. Future work will focus on the mechanism by which metronomic dosing of this drug stimulates the production of TH17 cells and how this inflammatory cell can be exploited to make chemotherapy drugs more effective, especially when used in combination with immunotherapy.

Outcome of Radioactive Iodine Therapy in Cats Receiving Prior Methimazole Therapy

RE Oman, KF Lunn

Purpose: Radioactive iodine (I-131) is the preferred treatment for cats with hyperthyroidism. Prior to I-131 treatment, many cats receive methimazole to lower thyroxine (T4) levels. There is concern that methimazole can enhance thyroidal I-131 uptake causing post-treatment hypothyroidism. While some veterinary hospitals require that methimazole be discontinued 7-10 days prior to I-131 treatment, the Veterinary Teaching Hospital (VTH) at CSU does not. The purpose of this study was to evaluate the results of I-131 treatment in hyperthyroid cats in which methimazole treatment was stopped at different time intervals prior to receiving I-131. Materials/methods: A retrospective medical records search was performed to identify hyperthyroid cats receiving I-131 treatment at CSU. The time interval from cessation of methimazole therapy to injection of I-131, and the lowest recorded post-therapy T4 level, were documented. Cats were divided into two groups; those receiving I-131 treatment within 2 days of stopping methimazole and those receiving I-131 treatment three or more days after stopping methimazole. Hypothyroidism was defined as a T4 below the normal reference range (< 1 ug/dl). Results: Of the 50 cats assessed, 33 received I-131 within 2 days of stopping methimazole. Of those 33, 17 (52%) had a T4 < 1 ug/dl, and the remaining 16 (48%) had a normal or elevated T4 after I-131. 17 cats received I-131 3 or more days after stopping methimazole. Of these 17, 10 (59%) had a T4 < 0.05.

Results. To date, 50 cats with azotemia and 30 normal cats have been evaluated. Results to date do not indicate significant differences in antibody prevalence rates or antibody levels between azotemic and non-azotemic cats.

Conclusions. Results suggest that development of antibodies to these antigens is not associated with azotemia in cats. Alternately, the results may be affected by the small number of cases entered into the study to date. Additional samples are being collected and further data analysis controlling for the number and types of vaccines administered will be performed upon completion of the study.

The Pharmacokinetics of Mirtazapine in Cats with Chronic Kidney Disease

JM Quimby, DL Gustafson, KF Lunn

Purpose: This study was designed to determine the pharmacokinetics of mirtazapine, an appetite stimulant, in cats with chronic kidney disease (CKD) and age-matched controls (AMC) after administration of a single oral 1.88 mg dose. Materials/Methods: Two cats with stable CKD from each IRIS (Internation Renal Interest Society) Stage (II, III and IV) were utilized. Six age-matched cats with normal CBC, chemistry and urinalysis served as controls. Blood samples were collected prior to and 0.5, 1, 1.5, 2, 4, 8, 24, and 48 hours after oral administration of 1.88 mg of mirtazapine. Serum was collected, frozen and mirtazapine concentrations were measured by LC/MS/MS. Non-compartmental pharmacokinetic modeling was performed. Results: Increased vocalization and affection were the only side effects noted. Mean half-life was 16.4 +/- 5.1 hours (CKD) and 12.3 +/- 1.8 hours (AMC). Mean peak serum concentration was 110.6 +/- 30.8 ng/ml (CKD) and 79.6 +/- 21.7 ng/ml (AMC). Mean area under the curve (AUC) was 770.7 +/- 225.5 ng/ml□hr (CKD) and 555.5 +/- 175.4 ng/ml□hr (AMC). Mean clearance was 0.61 +/- 0.1 L/hr/kg (CKD) and 0.79 +/- 0.16 L/hr/kg (AMC). The Mann-Whitney test was used to compare groups and a statistically significant difference in AUC and clearance was found ($p = 0.03$ for both). A significant negative correlation between creatinine and clearance was identified using a Spearman rank test ($p = 0.002$). Conclusion: CKD delays the clearance of mirtazapine. A single low dose of mirtazapine was well tolerated and resulted in a half-life that is compatible with 48 hour dosing intervals in cats with CKD.

The Effects of Curcumin on Feline Vaccine-Associated-Sarcoma Cells

BA Qurollo, DH Thamm

Feline vaccine-associated sarcoma (VAS) is a soft tissue sarcoma arising from sites where vaccinations have been administered. It is suggested that inflammation associated with vaccine components causes proliferation of fibroblasts and myofibroblasts that undergo neoplastic change in genetically predisposed cats. Novel therapies are needed. Curcumin, a derivative from the spice turmeric, has been reported to act against several cancer-associated molecular targets including platelet-derived growth factor receptor (PDGFR) and matrix metalloproteinases (MMP), two targets implicated in VAS tumorogenesis and tumor progression. In humans, mice and rats, curcumin is metabolized primarily through glucuronidation, and studies have shown poor bioavailability reducing curcumin's efficacy in vivo. Because cats lack glucuronyl transferase and have a deficient glucuronidation pathway, it is conceivable that curcumin could have increased bioavailability in this species. We evaluated the antiproliferative effects of curcumin against feline VAS cell lines using a bioreductive fluorometric assay. At 25 uM, curcumin dramatically inhibited the viability of PDGF-BB-stimulated VAS and FSA cell lines. VAS cells exposed to 5 uM of curcumin showed decreased invasion through a Matrigel basement membrane suggesting a negative effect on MMPs. Alterations in the PDGFR signaling pathway was demonstrated with Western blots showing a decrease in phosphorylated AKT, a kinase activated. downstream from PDGFR, in cell lysates exposed to 20 uM of curcumin for 1 hour. In conclusion, curcumin appears to be able to modulate processes in VAS cells associated with the malignant phenotype at concentrations of 5-25 uM. It remains to be determined whether these concentrations are achievable in cats.

Prevalence of Selected Zoonotic and Vector-Borne Agents in Dogs and Cats on the Pine Ridge Reservation

AV Scorza and MR Lappin

Purpose. To estimate the prevalence of intestinal parasites and vector borne agents of dogs and cats in the Pine Ridge Reservation, South Dakota.

Materials/Methods. Fecal samples were examined for parasites by microscopic examination after fecal flotation and for Giardia and Cryptosporidium by IFA. Giardia and Cryptosporidium IFA positive samples were genotyped. Cat sera were tested for antibodies against Bartonella spp., Toxoplasma gondii, and FIV antibody, as well as antigens of FeLV and Dirofilaria immitis. Dog serum was tested for antibodies against T. gondii, Borrelia burgdorferi, Ehrlichia canis, and Anaplasma hagocytophilum as well as D. immitis antigen. Total DNA extracted from blood was assessed in PCR assays for DNA of Rickettsia spp., Bartonella spp., Ehrlichia spp., Anaplasma spp., haemoplasmas, and Babesia spp. (dogs only).

Results. In the 84 dog fecal samples, Giardia spp. (32%) was most common followed by Taenia spp. (17.8%), Toxascaris leonina (9.5%), Toxocara canis (7.1%), Cryptosporidium spp. (7.1%), hookworms (3.5%), and Cystoisospora canis (2.3%). All nine feline fecal samples were negative. Nine Giardia spp. isolates were sequenced and were dog genotypes. Four Cryptosporidium spp. isolates were sequenced and were Cryptosporidium canis. Antibodies against T. gondii were detected in 15% of the 82 dog sera but all sera were negative for other antibodies as well as D. immitis antigen. Of the 32 cat sera, 37.5% had antibodies against Bartonella spp. and 6% had antibodies against T. gondii. FeLV antigen was detected in 10% of the cats but all were negative for D. immitis antigen and FIV antibodies. None of the 92 dog blood samples and 39 cat blood samples was PCR positive for DNA of the target agents.

Conclusions. Enteric parasites in dogs and Bartonella spp. antibodies in cats were common on this reservation. This information could be used to help develop a preventive medicine plan for these animals and their owners.

Treatment of Canine Lymphocytes with the immunotoxin, HA22

A Scott, J Perry, P Kiser, A Avery

Treatment of Canine Lymphocytes with the immunotoxin, HA22. Scott, A, J Perry, P Kiser, A Avery. Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO. Purpose: To determine the effect of the anti-human-CD22 immunotoxin, HA22, on rate of cell death in primary canine lymphocytes in vitro. Materials/methods: First, the affinity of HA22 for canine CD22 was determined using a competitive assay with PE conjugated anti-canine CD22 on neoplastic CD22+ lymphocytes. This was compared to a similar competitive assay using unconjugated anti-canine CD22. Second, Lymphocytes, from normal canine peripheral blood and lymph nodes as well from lymphoma bearing dogs, were isolated and cultured with or without HA22. The cells were then stained with propidium iodide (PI) or Annexin-V-FITC and results were obtained using flow cytometry. Culture with doxorubicin was used as a positive control. Results: The affinity study showed similar inhibition of binding of the PE conjugated anti-CD22 Ab by HA22 and the unconjugated anti-CD22 Ab. In normal canine lymphocytes, the rate of cell death in culture exceeded that of the negative control, but was less than the positive control (doxorubicin) at time points of 1, 24, 48 and 72 hours. Primary neoplastic cells appeared to be too unstable to culture for a significant amount of time outside of the body and no significant data was obtained from them as a result. Conclusions: HA22 showed affinity for the binding to canine CD22. In culture, the rate of cell death of the normal canine lymphocytes treated with HA22 fell in a range between the negative and positive controls. This would be expected if the CD22+ cells were being selectively killed by HA22 while having no effect on the CD22- cells in the culture. Further studies should be done to specifically identify the population of CD22+ cells and HA22's effect on them as well as determining the in vivo effects of HA22 on CD22+ canine lymphomas.

Regional analgesia of the pinna, ear canal, and soft tissues of the lateral face in mesocephalic canids

SL Shaver, PM Mich, LR Whalen

Purpose: To determine anatomic landmarks for local anesthetic blockade of the great auricular, auriculotemporal, and internal auricular (caudal, middle, lateral) nerves.

Materials and Methods: Mesocephalic canine cadavers weighing 20-40kg were dissected. Perineural blockade was simulated by injection of new methylene blue dye (0.2-0.5ml) using a 22ga 1" needle. Blockade was deemed successful with epineurium staining.

Results: Auriculotemporal nerve (75% accuracy): The needle was placed rostral to the horizontal external ear canal, caudal to the temporomandibular joint, 1/3 distance between the caudo-dorsal aspect of the zygomatic arch and the mandibular angular process, angled 15 degrees rostrad and inserted to a depth of 2cm. Great auricular nerve (67% accuracy): The width of the wing of the atlas was measured. From the ventro-caudal aspect of the wing, the needle was positioned ventrad 1/2 and caudad 1/4 of the measured width, along a line parallel to the jugular furrow and inserted through the subcutaneous tissue and m. platysma (approx. 1cm in a lean animal). The needle was angled approximately 45 degrees dorsad, fanning from dorsal to ventral as dye was injected. Internal auricular branches (80% accuracy): The horizontal external ear canal was palpated as proximally as possible. The needle was placed caudal to the canal, dorsal to a line drawn between the mandibular angular process and the paracondylar process of the occipital bone, inserted to the temporal bone, and withdrawn slightly prior to injection.

Conclusions and Clinical Relevance: Described anatomic landmarks allowed for reliable perineural delivery of dye. Cases such as total ear canal ablation and deep ear cleaning may benefit from these techniques. Pain associated with these procedures is difficult to manage with systemic analgesics and general anesthesia. Further studies are needed to assess accuracy of placement by individuals other than the study investigators.

Detection of calprotectin and its correlation to apoptosis within the equine gastrointestinal tract from horses with black walnut extract-induced laminitis

ML Shoemaker, L Chiavaccini, J Belknap, B Charles, EJ Ehrhart, DM Hassel

Purpose: Equine laminitis is a debilitating and life-threatening condition whose precise pathogenesis remains unresolved, however gastrointestinal disturbances are the most common disease entities associated with laminitis. An investigation into the events occurring within the gastrointestinal tract of horses exposed to black walnut extract will provide further insight into the pathogenesis of this devastating disease.

Materials and Methods: Colon tissue from horses exposed to black walnut extract (BWE), a proven inducer of laminitis, were collected during the developmental (3hr) and late (10-12 hr) stages of induced laminitis and were examined for inflammatory and apoptotic events within the gastrointestinal wall in comparison to untreated control horses. Formalin-fixed, paraffin-embedded colon tissue was sectioned and standard immunohistochemical techniques were used to detect expression of calprotectin as an indicator of inflammation, and caspase-3 active, as an indicator of apoptosis. The level of calprotectin expression and neutrophil migration was scored on a scale of 0 (no inflammation) to 5 (severe inflammation with many extravascular neutrophils).

Results: Colon from horses exposed to BWE at both 3 and 10-12 hours post exposure scored consistently higher than controls indicating the presence of an inflammatory response to BWE. Percentage of caspase-3 positive cells was not different between BWE and control tissue.
Conclusions: The identification of calprotectin expression and the characterization of inflammation within the intestinal wall of horses exposed to BWE may provide further insight into the pathogenesis of laminitis and identification of novel methods for the prevention and treatment of laminitis secondary to gastrointestinal tract disease.

Annexin A2 and alpha-enolase antibodies in cats with and without azotemia

C Sonius, JR Hawley, M Lappin

Purpose. Renal disease is a leading cause of morbidity and mortality in cats, yet our current understanding of the causes in feline patients is limited. Recent research at Colorado State University showed that inoculation of cats with vaccines grown on the Crandell-Rees Feline Kidney (CRFK) cell line leads to the generation of antibodies that cross-react with feline renal cell lysates. Recently, two of the immunodominant antigens to which antibodies are formed have been determined to be alpha-enolase and annexin A2, both of which are linked to autoimmunity and renal disease in humans. The purpose of this study was to investigate the relationship between vaccination, azotemia, and antibodies to alpha-enolase and annexin A2 in cats with and without azotemia.

Materials/Methods. Sera from azotemic and non-azotemic cats with a minimum of 5 years of vaccine history were collected. Sera were assayed in optimized Indirect ELISAs to detect antibodies to alpha-enolase and annexin A2. Percentages of cats in each group that were positive for the two antibodies were compared by Fisher's exact test. Wilcoxon's rank sum test was used to determine whether annexin A2 or alpha enolase titer magnitude was associated with presence of fever or stomatitis. Significance was defined as $p < 0.05$.

Results. To date, 50 cats with azotemia and 30 normal cats have been evaluated. Results to date do not indicate significant differences in antibody prevalence rates or antibody levels between azotemic and non-azotemic cats.

Conclusions. Results suggest that development of antibodies to these antigens is not associated with azotemia in cats. Alternately, the results may be affected by the small number of cases entered into the study to date. Additional samples are being collected and further data analysis controlling for the number and types of vaccines administered will be performed upon completion of the

Correlation Between Geometric Parameters Identified on Computed Tomographic Images and Radiographic Images of Horses

EE Strobel, CE Kawcak

In a previous study, horses with third metacarpal condylar fracture had significant differences in third metacarpal bone geometry when compared to the opposite limb and horses that had no fracture. Geometry was determined by evaluation of computed tomographic scans. A simpler means of assessing geometry, such as radiographic evaluation could be used more commonly by equine practitioners as a means of identifying “at-risk horses”. The purpose of this study is to determine if abnormalities on computed tomographic scans can be correlated to radiographic abnormalities, and serve as a screening tool for horses with abnormal third metacarpal geometry that might be predisposed to fractures. Computed tomographic scans of third metacarpal condyles (n=16) were analyzed for geometrical shape using customized computer modeling software (OsteoApp, Colorado State University). Radiographic images from the same horses were analyzed for geometric shape using a software package (EFilm, Merge Technology, Milwaukie, WI). The ratio of lateral-to-medial condylar width and dorsal-to-palmar curvature were analyzed for each horse. A spearman correlation coefficient was used to evaluate correlation between techniques. When evaluating the 30 degrees dorsal measurement, which is the projection that should be closest to the radiographs, the r^2 value was 0.50, demonstrating no significant correlation. There was also no correlation for the 0 degrees measurement, with a r^2 value of 0.10. The curvature measurements had an r^2 value of 0.13 and therefore had no significant correlation. The results of this study show that radiographic assessment of joint surface geometry is unreliable.

Correlation between right atrial and jugular pressures in lateral recumbent horses under anesthesia

K Tam, M Rezende, P Boscan

Purpose: Right atrium pressure is considered the true central venous pressure (CVP) and while it provides relevant information for patient management under anesthesia, the technique is invasive and cumbersome, and therefore it is not routinely used in clinical cases. The goal of the study is to compare and correlate jugular pressure and right atrium pressure in lateral recumbent horses under anesthesia. Materials/methods: Nine horses were anesthetized using a standard protocol (sedation with xylazine, induction with ketamine and diazepam, and maintenance with sevoflurane). All horses were positioned in left lateral recumbency. Fluid administration was at 5 ml/kg/hr and heart rate, ECG, arterial blood pressure, temperature and blood gases were used for patient monitoring. For the study, a 14 ga 28F catheter introducer was inserted into the right jugular vein to measure jugular pressure. A 7F 110cm catheter was placed into the right atrium, via the catheter introducer, to measure right atrial pressure. Both jugular and right atrium pressures were measured simultaneously with an aneroid manometer (cmH2O). Results: The spearman correlation coefficient indicates that the right atrium and jugular pressures correlate with an R-Squared = 0.70. The data suggests that jugular pressure can be used in lateral recumbent horses to estimate the CVP with moderate confidence. Conclusions: Jugular pressure in horses is easy to measure. In the present study the jugular pressures showed moderate correlation with right atrial pressures. While jugular pressure cannot replace right atrium pressure for CVP measurements, it can be used to estimate right atrium pressure and CVP in lateral recumbent horses.

The effects of administering oral powder electrolytes on the incidence of colic in horses participating in a week long 100 mile ride

WT Walker, K Tisher, AE Hill, RJ Callan

The effects of administering oral powder electrolytes on the incidence of colic in horses participating in a week long 100 mile ride WT Walker1, K Tisher2, AE Hill1, RJ Callan1. 1. Colorado State University College of Veterinary Medicine, Fort Collins, CO. 2. Littleton Equine Medical Center, Littleton, CO. Purpose: Horses on long distance endurance rides may colic, potentially due to dehydration, fatigue, or electrolyte imbalances. This study evaluated the effect of oral powder electrolytes on incidence of colic in horses participating in a 100 mile horseback ride. Materials/methods: Forty horses in control (n=18) and treatment (n=22) groups were rested on days 0 and 4 and ridden 20 miles on days 1,2,3,5 and 6, were offered ad libitum mix hay and water when not being ridden, and were offered the same amount of water on the trail. The treatment group received oral electrolytes in grain twice per day while the control group received grain only. Blood samples were collected each evening and later analyzed for PCV and plasma sodium, chloride, potassium, calcium, magnesium, total protein, albumin, BUN, creatinine, glucose, and bicarbonate concentrations. Results: None (0.0%) of the control horses and 5 treated horses (22.7%, P=0.053) experienced colic. All colics occurred after a rest day (3 episodes on day 1; 2 on day 4). Horses that colicked had other risk factors for colic including; first time on the ride, long distance travelled to the ride, young age, and lack of conditioning before the ride. Serum analyses are pending. Conclusions: The administration of oral powder electrolytes was associated with an increased incidence of colic (P=0.053), which only occurred after a rest day. The relationship of colic with serum electrolyte and metabolite concentrations needs to be further evaluated. Other risk factors may have contributed to the occurrence of colic.

Prevalence of enteric parasites in veterinary student owned dogs that frequent dog parks

A Wang, R Ruch-Gallie, P Lin, AV Scorza, MR Lappin

Purpose: In a previous study of feces from dogs in Colorado, 16.2% were positive for agents with zoonotic potential; Giardia spp. and Cryptosporidium spp. were the most prevalent and are both infectious immediately when passed in feces. Dog parks are very popular in urban areas, but there are no current studies attempted to correlate visits to dog parks and risk of colonization by enteric parasites. The purpose of this study is to determine whether dog park visitation is associated with an increased prevalence of enteric parasites in dogs owned by PVM students at CSU.

Materials/Methods: Feces were submitted with a completed survey form detailing dog park attendance rates, fecal character scores, and other clinical information. Feces were examined for parasites by microscopic examination and for Giardia and Cryptosporidium by a commercial IFA. Positive samples were genotyped using the glutamate dehydrogenase, b-giardin and triose phosphate isomerase genes for Giardia spp. and the heat shock protein-70 gene for Cryptosporidium spp.

Results: Feces from 52 dogs attending dog parks (9.6% positive) and 42 dogs not attending dog parks (0% positive) have been analyzed to date. Of the five Giardia positive dogs, two were coinfected with Cryptosporidium, each of the dogs attended dog parks, and one had diarrhea. Sequence analysis was available from four Giardia isolates (two assemblage D, one assemblage C, one assemblage C and D mixed infection) and one Cryptosporidium isolate (C. canis). The Giardia prevalence rates between the two populations were not statistically different (Fisher's exact test; $p = 0.062$).

Conclusions: The findings suggest that dogs that frequent dog parks are more likely to colonized by Giardia spp. but presence of Giardia in feces does not correlate with the presence of diarrhea. Based on power calculations, an additional 101 dogs will be entered into the study to determine if the trends are statistically significant.

Storage of Bovine Sperm for 20 h between Semen Collection and Sexing

JL Anema, JK Graham, RW Lenz, GE Seidel, Jr

Purpose: This study was designed to optimize bovine sperm storage for 20 h between semen collection and sex sorting followed by cryopreservation. Materials/Methods: Two successive ejaculates were obtained from each of 5 Holstein and 3 Jersey bulls. Nothing except antibiotics was added to the control samples until staining with Hoechst 33342 for sorting. For Treatment 1, semen was diluted 9:1 to result in 24 mM MOPS. Treatment 2 was Treatment 1 + 2% egg yolk. For each group, a subsample was sorted by flow cytometry shortly after collection; sperm then were frozen following standard procedures. The other subsample was stored at 15-18 degrees C and sorted 20 h after collection followed by cryopreservation. Samples were evaluated post-thaw for subjective progressive and total motility, by computer-assisted sperm analysis (CASA), and by flow cytometry for sperm viability using propidium iodide and SYBR-14. Results: Results of Treatment 1 exceeded the control, while results for Treatment 2 did not. Second ejaculates were superior to first ejaculates. Addition of MOPS maintained pH about 0.2 units higher than the control, but pH declined similarly over time in all groups. While responses for the 20 h sort were numerically lower than the 0 h sort (P>0.1), the majority of 20 h responses were acceptable for most, but not all bulls. Conclusions: Storing sperm in 24 mM MOPS was beneficial. Surprisingly, 2% egg yolk negated beneficial effect of MOPS, possibly due to increasing osmolarity by ~15 mOsM/kg due to pH adjustment.

Endocrine action of interferon-tau on the corpus luteum in sheep: Implication for antiluteolytic and luteotrophic mechanisms

AQ Antoniazzi, LE Henkes, RC Bott, RL Ashley, JE Bruemmer, GD Niswender, G Moss, B Alexander, JFC Oliveira, TE Spencer, FW Bazer, TR Hansen

Interferon-tau (IFNT), a major secretory protein of ruminant conceptuses during early pregnancy prevents regression (luteolysis) of the corpus luteum (CL). It acts in a paracrine manner to silence transcription of estrogen receptor alpha (ESR1) and oxytocin receptor (OXTR), preventing release of prostaglandin F2 alpha (PGF) from the endometrium. Greater concentrations of IFNT in uterine vein blood from pregnant compared to non-pregnant ewes and induction of extra uterine interferon-stimulated genes (ISGs) in pregnant ewes provoked the hypothesis that IFNT acts through endocrine action on the CL to modulate major genes involved in luteolysis. ESR1, OXTR, prostaglandin transporter (SLCO2A1), PGF receptor (PTGFR) and prostaglandin E2 receptor subtypes 2 (PTGER2), 3 (PTGER3) and 4 (PTGER4) mRNAs were examined in ovine CL on Days 12 to 15 of the estrous cycle or pregnancy. These mRNAs also were examined in CL after 24h infusion of 200 µg BSA or IFNT (2 x 107 antiviral units) into the uterine vein using osmotic pumps on Day 10 of the estrous cycle. At 12h after surgical installation of pumps, half of the ewes from BSA or IFNT groups received a single 5 mg injection of PGF. PTGFR mRNA decreased (P<0.05; SAS-GLM) between Days 12 and 15. PTGER3 mRNA declined (P<0.005; day x pregnancy status) by Day 14 in cyclic, but not pregnant ewes. Following a 24h infusion into the uterine vein, SLCO2A1 mRNA decreased (P<0.001) in BSAPGF, IFNT and IFNTPGF compared to BSA-infused ewes. PTGFR mRNA was down regulated (P<0.001) in IFNT and IFNTPGF compared to BSA and BSAPGF-infused ewes. PTGER4 mRNA was greater (P<0.05) in IFNT and IFNTPGF compared to BSA-infused ewes. In conclusion, IFNT has acute (24h) effects on the CL during maternal recognition of pregnancy in ewes by suppressing PTGFR (antiluteolytic) and increasing PTGER3 (luteotrophic) mRNAs. Also, diminished SLCO2A1 in response to IFNT would impair release of PGF by large luteal cells, and thereby, intraluteal-lytic action of PGF.

The expression profile of the chemokine receptor CXCR4 and specific T-cell markers in peripheral blood and endometrium during early pregnancy in cows

RL Ashley, NP Smirnova, and TR Hansen

Chemokines and their receptors are pivotal factors in implantation and vascularization of the placenta. Activation of the chemokine receptor 4 (CXCR4) by its ligand, CXCL12, causes recruitment of leukocytes into the uterus of pregnant females and stimulates trophoblast proliferation and invasion. Using semi-quantitative RT-PCR (qRT-PCR), we recently demonstrated that CXCR4 mRNA in peripheral blood was present on d 75, increased from d 75-90, and then plateaued through d 190 of gestation. Based on the ample data providing roles for CXCR4 during early pregnancy in other species and the high degree of sequence homology between species, we hypothesized that expression of CXCR4 mRNA in peripheral blood and endometrium would increase during the time of implantation in cows. To test this hypothesis, qRT-PCR was performed on blood samples from pregnant cows at days 0, 11, 20, 32, and 75 of gestation. Blood cell CXCR4 mRNA was significantly ($P<0.05$; Newman-Keuls Multiple Comparison Test) up-regulated from d 20-32 of pregnancy with an even greater ($P<0.05$) increase by d 75, when compared to all other days. Analysis through qRT-PCR for different surface markers of T-cells revealed that CD8, T-cell receptor-beta (TCR-B), and TCR-gamma (TCR-G) transcripts were expressed in a pattern similar to CXCR4. CD8 mRNA at d 75 of gestation was significantly ($P<0.05$) greater compared to days 11, 20, and 32 of pregnancy. Likewise, both TCR-B and TCR-G mRNAs were significantly ($P<0.05$) up-regulated at d 75 compared to all other days evaluated. qRT-PCR was also performed on endometrial samples from pregnant cows on days 14, 19, 23, and 50. CD8 and TCR-B mRNA was significantly ($P<0.05$) greater on d 19 compared to d 23 of gestation, while expression of TCR-G mRNA was significantly ($P<0.05$) up-regulated on d 19 and 23 compared to d 14 of pregnancy. These data are interpreted to mean the CXCL12/CXCR4 pathway and associated immune cells are activated during development of placental-maternal exchange in cows.

Micro RNA regulation of genes in bovine oocytes and embryos

JP Barfield, GJ Bouma, GE Seidel Jr

Purpose: The objective was to study expression of miRNAs in bovine oocytes and preimplantation embryos.

Materials/methods: In experiment 1, in vivo-matured oocytes were collected by transvaginal aspiration of 7 superstimulated cows and compared to in vitro-matured oocytes aspirated from abattoir ovaries and matured in vitro for 23 h. After vortexing, maturation of oocytes was confirmed by visualization of the 1st polar body. In experiment 2, in vitro-matured oocytes were generated as described. Subsets were fertilized in vitro or activated parthenogenetically by incubation in 5μM ionomycin for 5 min followed by 10 μg/mL cycloheximide + 5 μg/mL cytochalasin B for 5 h. After 18 h and 12 h, respectively, fertilized and activated oocytes were centrifuged to enable visualization of pronuclei. Zygotes with 2 polar bodies and 2 pronuclei and parthenotes with 2 pronuclei were selected. Total RNA was extracted from 30 pooled oocytes for each replicate (n=90/treatment) and reverse transcribed. MiRNA expression was evaluated by RT-PCR using primers for 384 miRNAs. Relative expression levels were analyzed with a t-test of normalized values.

Results: In experiment 1, 8 miRNAs were expressed only in in vivo matured oocytes, 5 only in in vitro matured oocytes, and 7 were expressed in both. In experiment 2, 6 miRNAs were expressed only in zygotes, 1 miRNA, only in parthenotes, and 13 miRNAs, in both, with significant upregulation of miR-574-5p and miR-125a-5p in zygotes ($p<0.05$). In vitro matured oocytes and zygotes had 14 miRNAs in common, with miR-375 significantly upregulated in zygotes ($p<0.05$). Major pathways potentially targeted by these miRNAs include electron transport chain, pentose phosphate pathway and cell cycle regulation.

Conclusions: Several of these candidate miRNAs may be important for regulation of bovine oocyte maturation and embryo development.

LIN28 is a Regulator of Endocycles in Trophoblast Stem Cell Differentiation

VA Enriquez, J Guttormsen, B Fromme, GJ Bouma, QA Winger

The placenta provides the maternal-fetal exchange of nutrients and is therefore necessary for fetal survival. Upon fibroblast growth factor 4 (FGF4) deprivation in vitro, proliferative trophoblast stem (TS) cells differentiate into polyploid, nonproliferating trophoblast giant (TG) cells and undergo endoreduplication. Endoreduplication is a rare event that circumvents the mitotic cycle generating an endocycle; genomic replication without cell division. The regulatory process mediating the switch from mitosis to endoreduplication and differentiation of TS to TG cells is still largely unknown. Cyclin dependent kinase 1 (CDK1) regulates the transition between G2/M phases, and the initiation of cell replication after genomic replication. p57, p27, and p21 act to inhibit CDKs, effectively blocking cell cycle transition. When CDK1 is inhibited using RO3066, TS cells differentiate into TG cells and undergo endoreduplication. Blocking CDK1 activity in other cell types results in apoptosis, suggesting that most cells cannot be forced into an endocycle. In this study, we employed Tcfap2c KO (KO) and WT TS cell lines to identify regulators of endoreduplication. KO TS cells fail to differentiate and begin endoreduplication upon removal of FGF4. LIN28, a postulated regulator of endoreduplication, expression decreases as TS cells differentiate into TG cells. However, KO cells maintain Lin28 mRNA levels throughout differentiation. LIN28 binds to transcripts encoding cyclins A and B that activate CDK1 to allow G2/M transition in mitotic cell populations. LIN28 is localized to the cytoplasm of proliferating WT TS cells and cycles between the cytoplasm and nucleus of TG cells. However, LIN28 is located in the cytoplasm and nucleus of KO TS cells with or without FGF4. Treatment of mutant TS cells with the CDK1 inhibitor RO3066 results in cell death. These results indicate that LIN28 is active in regulating the endocycle of TG cells by inhibiting CDK1.

The role of proline-rich 15 in trophoblast cell migration and invasion

KC Gates, JD Cantlon, RV Anthony

Purpose: Proline-rich 15 (PRR15) is a nuclear protein expressed by trophoblast cells during early conceptus development, coinciding with the period of rapid conceptus elongation in ruminants. In sheep, PRR15 mRNA is undetectable on day (d) 11, is detectable on d13, with peak expression on d15 and 16, followed by diminished expression on d17, 21 and 30. Previously, we infected d8 blastocysts with a lentiviral vector expressing a short-hairpin (sh) RNA which targeted degradation of PRR15 mRNA. Conceptuses expressing PRR15-specific shRNA failed to elongate by d15, and most failed to survive in utero. In contrast, conceptus development was not impacted by control lentiviral vectors, inferring that PRR15 is required for conceptus development and survival. The purpose of our study is to investigate the role of PRR15 in trophoblast cell migration and invasion. Materials: Ovine conceptuses were collected at d15 of gestation and cells were dispersed in culture to develop trophoblast cell lines. The resulting cell lines were plated on plastic or Matrigel (6.4 mg/ml: 1 mm thick) for 12, 24, 36 or 48 hrs. Quantitative reverse transcription PCR was used to measure changes in PRR15 mRNA concentrations. Results: Primary trophoblast cells grown on Matrigel underwent phenotypic changes indicative of invasion into the matrix. These changes correlated with increasing concentrations of PRR15 mRNA, peaking (»20 fold increase) at 36 hrs. Conclusions: PRR15 expression increases in ovine trophoblast cells when grown on a matrix that induces differentiation into a migratory and invasive phenotype. Infection of these cells with the PRR15 shRNA lentivirus, prior to plating on Matrigel, will reveal its impact on the trophoblast cell transcriptome, as assessed by microarray analysis. This approach will also be used to determine if PRR15 is required for trophoblast cell migration and invasion. These studies will provide insight into the regulation of ruminant conceptus development.

Evaluating the Efficacy of Metronomic Cyclophosphamide using Regulatory T cell Populations and Markers of Angiogenesis in Canine Soft Tissue Sarcomas

JA Harper, D Rice, L Strange, B Biller

Objective: The uninterrupted administration of low, oral doses of chemotherapy drugs (also known as metronomic chemotherapy) is thought to selectively target tumor angiogenesis as well as regulatory T cells (Treg), both of which can facilitate tumor growth. This study was performed to determine whether evaluation of tumor growth, tumor angiogenesis and determination of Treg numbers can be used to assess the efficacy of metronomic dosing of oral cyclophosphamide (CYC) in dogs with soft tissue sarcomas.

Procedure: Peripheral blood and tumor biopsies were obtained from five client-owned dogs with soft tissue sarcomas pre-treatment, and following 14 and 28 days of CYC therapy. Tumor size was measured in two dimensions using calipers at each sample collection. Patients then received local anesthesia and a core tumor sample was taken. These tissue biopsies were stained for endothelial cells using immunohistochemistry (IHC) and tumor microvessel density (MVD) was evaluated. Immunostaining and flow cytometry were performed on all blood samples to enumerate Treg populations.

Results: Tumor size remained stable during the study period. Although tumor MVD and Treg numbers tended to decrease, there were no significant differences in these parameters between pre-treatment and post-treatment time points.

Conclusions: Our results suggest that the doses of CYC utilized in this study were too low to significantly affect tumor growth over a 28 day period. Interestingly, there was a notable decrease in tumor MVD and Treg cells between pre-treatment and day 14, both of which then increased by day 28. These trends suggest that metronomic CYC may be initially beneficial but its effects are not complete and are reversible.

Role of nitric oxide in the expression of gonadotrophin releasing and gonadotrophin inhibiting (RFamide related peptide) hormone in mouse development

P Kumar, K Tsutsui, Huang, SA Tobet

Nitric oxide (NO) has been implicated in various physiological functions, including brain development, cardiovascular regulation, and reproductive functions. Immunoreactive neuronal nitric oxide synthase (nNOS) has been demonstrated in neurons surrounding gonadotropin releasing hormone (GnRH) neurons and NO has also been suggested as a regulator of GnRH release. A role for NO in the regulation of RFamide-related peptide [RFRP; the mammalian homolog of gonadotrophin inhibiting hormone (GnIH)] in mammals is undetermined. The present study tested whether genetic disruption of nNOS affects the detection of immunoreactive GnRH and RFRP neurons in development. Paraformaldehyde fixed brains from postnatal day 0 WT and KO were sectioned coronally and processed for immunoreactive GnRH and RFRP in adjacent sections. The number of immunoreactive GnRH neurons in KO mice (171+22.67 in one third of the total number of sections) was significantly greater ($p=0.05$) compared to the WT (106+16.0). The difference was mostly attributable to neurons in the region of the organum vasculosum of lamina terminalis (OVLT). Immunoreactive RFRP neurons were counted from the plane of the paraventricular nuclei to mammillary nuclei. The majority of cells were located in the dorsomedial nuclei and again there were significantly more ($p<0.001$) immunoreactive RFRP in KO (569.75+23.7) than in WT (350.25+32.8). The RFRP immunoreactive fibres projected toward GnRH neurons in the region of OVLT in both WT and KO even at this early age (P0). It is possible that NO alters the expression of both GnRH and RFRP early in development. The physiological role of this regulation and the mechanism of its generation awaits discovery.

Mouse retinal expression of the Mu-opioid receptor and its preferential endogenous agonist ß-endorphin

J Mower, J Anglen, SK Gallagher, J Vigh

The endogenous opioid peptide, ß-endorphin, is one of many diverse cleavage products of the large precursor protein proopiomelanocortin (POMC). The mu-opioid receptor (MOR) is a G protein-coupled receptor (GPCR) through which ß-endorphin regulates many physiological functions, and is the target for most analgesic drugs. Expression of opioid peptides and their receptors in the mammalian retina has not been studied in details and the existing sparse data is controversial. Methods: Using transgenic mouse models and immunohistochemical techniques we sought to characterize MOR and ß-endorphin expression in the mouse retina through cell morphology, size, distribution, and colabeling studies with known retinal cell markers. Results: ß-endorphin was shown to be expressed by a subpopulation of cholinergic (starburst) amacrine cells, of both orthotopic and displaced subtypes. Antibody directed against MORs stains neuronal somas and processes in the inner retina. MOR staining does not colocalize with POMC+ cholinergic amacrine cells. Instead, immunolabeling shows at least two distinct MOR+ cell-types in the inner nuclear layer (INL) and ganglion cell layer (GCL). Some MOR+ somas appear to be GABAergic, colocalize with the glutamic acid decarboxylases isoform GAD-67. No colocalization of MOR and the molecular marker for glycinergic amacrine cells (Gly-T1) was observed. Conclusion: Our findings point to a diverse population of putative amacrine cells and retinal ganglion cells that express the mu-opioid receptor and, through yet unidentified neuromodulatory pathways, may respond to the endogenous opioid peptide ß-endorphin.

Paraventricular Nucleus of the Hypothalamus: Novel Vascular Development and Relation to Adjacent Cell Types

J Schow, JG Knoll, Q Zhang, SA Tobet

MThe region of the paraventricular nucleus of the hypothalamus (PVN) is relatively unique in the central nervous system (CNS) for its extensive vascular supply that is easily distinguished from surrounding vessels by its dense “wing-shaped” pattern of vessels flanking the third ventricle. There is little known about the development, potential sex differences, or functional relevance of the vasculature of this region. The PVN itself is a heterogeneous nucleus with multiple neuronal cell types responsible for modulating different aspects of physiology. Two important populations of neurons are those that synthesize vasopressin (antidiuretic hormone), which functions to regulate blood pressure, and those that synthesize corticotrophin-releasing hormone (CRH), which plays a major role in stress responses. We are investigating the development of the PVN vasculature in relation to the surrounding cells and endocrine hormones produced in this region. We have observed the pattern formation in mice from embryonic day (E)17 to adulthood using several techniques. From these observations, it is apparent that multiple changes to the vasculature occur during development and that the PVN pattern does not fully develop until weaning (around postnatal day 19). Currently, we are using computer assisted image analysis based on brightfield microscopy with immunoreactive platelet endothelial cell adhesion molecule (PECAM) to measure the area of blood vessels in 50µm sections. From this measurement, we have identified a possible sex difference showing that the PVN area in females may be greater than in males. We are also using dual-fluorescence confocal microscopy in thicker sections (250µm) to measure vascular volume and proximity of other cell types to blood vessels. In the longer term we plan to visualize the relationship between cells and vessels and their relative placement to each other in three dimensions to better understand the development of this uniquely vascularized region.

MICRORNAS IN EQUINE OVARIAN FOLLICULAR FLUID

JC Silveira, GJ Bouma, EM Carnevale

Reproductive aging in women coincides with a decline in oocyte quality. The horse is a valuable animal model to study reproductive aging, as follicular waves and hormone profiles are very similar between women and mares. Our overall goal is to identify factors controlling oocyte quality and competence during reproductive aging. Studies have reported microRNAs (miRNAs) presence in blood serum, and miRNA profiles appear to correlate with certain disease states. MiRNAs are non-coding RNAs, and participate in gene regulation by cleaving target mRNAs or repressing translation. Recent studies demonstrate miRNAs play a role in female reproductive function and fertility. We postulate that presence of distinct miRNAs correlates with oocyte competency. To test the importance of miRNAs in oocyte competence, we determined the presence of miRNAs in ovarian follicular fluid of aging mares. Follicular fluid (FF) provides an important microenvironment for proper development of oocytes. FF from pre-ovulatory follicles (36h after hCG) was collected from 3 young mares (8, 10, and 13 yr) and 3 old mares (25, 25, and 29 yr) and screened for presence of miRNAs. FF samples were centrifuged to remove cells. Total RNA (including the miRNA fraction) was isolated from the supernatant using TRI-Reagent BD (Molecular Research Center, Inc.). Real time PCR analysis was conduced using miRNA profiling plates (384 Human miRNome MicroRNA Profiling Kit, System Biosciences). The initial screen identified 38 miRNAs with at least a 2 fold differential expression between FF isolated from young and old mares. Four miRNAs ($P<0.05$; Student t-test) were selected to confirm their differential expression in a larger number of FF samples from young (n=7, mean 9 yr) and old (n=8, mean 23 yr) mares. We conclude that miRNAs are present in FF, and exhibit an age-dependent difference in expression. Further studies are needed to determine where miRNAs are being produced and their function.

GABA and the Developing Paraventricular Nucleus of the Hypothalamus

M Stratton, K McClellan, S Tobet

The paraventricular nucleus (PVN) of the hypothalamus is a central player in the regulation of many physiologic functions that include food intake, stress responses and cardiovascular function. It is a master controller of the hypothalamic-pituitary-adrenal (HPA) axis. Major depressive disorder (MDD) has been associated with a dysregulation of HPA axis function. Interestingly, there is evidence for increased likelihood of major depressive disorder in individuals with mutations in genes encoding GABA receptors. Gamma-aminobutyric acid (GABA) is a neurotransmitter that during development acts as a secreted factor that regulates neuronal migration, proliferation, and differentiation. We hypothesize that some of the increased predisposition to MDD may be mediated by altered formation of the PVN during development due to alterations in GABA signaling. To test this hypothesis we are investigating the role of GABA in fetal PVN development in transgenic and knockout mouse models. Mice lacking the R1 subunit of the GABAB receptor at embryonic day 17 (E17) have altered distribution of cell containing immunoreactive estrogen receptor-alpha in and around the PVN as well as decreased immunoreactive BDNF within the PVN. We have also identified a decrease in the number of neurons containing immunoreactive estrogen receptor-alpha in and around the PVN in response to fetal exposure to the GABAA receptor antagonist bicuculline (also at E17). Additional preliminary data from in vitro video microscopy indicates altered movement characteristics of cells in the developing PVN in response to the GABAB receptor antagonist saclofen. By examining the location and number of cells characteristic to the PVN, along with live cellular responses both by video microscopy and pharmacological indices of signaling we are determining key factors responsible for the formation of the PVN early in development.

Inducible photoreceptor degenerative model in goldfish

D Varland, C Suh, J Vigh

Retinal degenerative diseases characterized by photoreceptor cell loss are one of the leading causes of permanent blindness among humans. In order to study the process and provide new ideas for rescuing or reconstructing the retinal tissue, genetic and non-genetic animal models have been used. A single intraperitoneal injection of N-methyl-N-nitrosourea (MNU) has been shown to induce selective and permanent photoreceptor cell loss in various mammalian models, making MNU a prime candidate for use in models of induced photoreceptor degeneration. Our overall purpose is to establish an inducible photoreceptor degeneration model in fish, where, as opposed to mammals, the retina is capable of regeneration following damage. MNU (4 ul of 14 w/v % in of DMSO) was applied via direct, intraocular injections into the posterior chamber of the left eye, along side a sham injection (4 ul of DMSO) into the contralateral eye, under MS-222 anesthesia. After one week, fish were euthanized and the retinal tissue was harvested. MNU effects were studied with standard histological techniques: immunohistochemical and nuclear labeling. Anti-PKCa immunolabeling of Mb type bipolar cells was used to identify retinal layers. The nuclear dye, ToPro3, was used to quantify the somas in the nuclear layers of the retina in MNU-treated versus control eyes. The number of photoreceptor somas in the outer nuclear layer of the MNU-treated retina decreased significantly compared to the control, contralateral eyes in every fish tested. Similar soma count comparisons in the inner nuclear layer showed that MNU did not decrease the numbers of bipolar, horizontal, and amacrine cells. These results demonstrate that direct intraocular injections of MNU in the goldfish lead to selective and inducible photoreceptor degeneration similar to previous observations. Thus, MNU may be used in modeling long term effects of photoreceptor degeneration such as retinal regeneration and/or rewiring.

New approaches for the identification of biochemical markers of infection and nerve damage in leprosy

R Al-Mubarak, PJ Brennan, VD Vissa

Mycobacterium leprae, an obligate intracellular pathogen, survives by invading the host peripheral nervous system (Schwann cells and macrophages) and causes nerve damage and deformity, the main manifestations of leprosy. However, the mechanisms of nerve damage in leprosy are poorly understood. Since M. leprae is characterized by massive genomic decay, it is highly dependant on host genes for growth. M. leprae can also control host genes expression. Like other diseases and infections these activities will be associated with changes in the lipid profile of the host tissues. In addition, other studies have been conducted in order to understand the interaction between M. leprae and Schwann cells in vitro, and many mechanisms have been proposed for Schwann cells demyelination in leprosy, such as hypophosphorylation of the axonal proteins, and autoimmunity against the host's own antigens. In this study we used the nine-banded armadillo (Dasypus novemcintus) as a model to understand the host- M. leprae interaction at the molecular level. We examined the entire spectrum of host lipids and proteins in armadillo nerves before and after M. leprae infection. Lipid samples in triplicate were subjected to Electrospray ionization/mass spectrometry (ESI-MS/Q/TOF). Also, using mass spectrometry approaches we analyzed changes in the protein expression profile and modification (e.g. phosphorylation) of the nerve fractions separated by one or two-dimensional gel electrophoresis (2DE) and compared infected to the naïve animal nerves. The preliminary data show increased levels of certain lipid groups mainly neutral lipids in the infected tissues compare to the naïve ones. The protein profile showed no obvious differences between the infected and naive nerves. However, there were increased amounts of immunoglobulins IgG and IgM in the infected nerve. This new lipidomic, and proteomic approach is designed to lead to markers of infection and nerve damage in leprosy.

Oral Bioavailability of a Novel Francisella tularensis Chemotherapeutic

L. Best, B. Cranmer, S. Knudson, J. Cummings, K. England, P. Tonge, R. Slayden

Francisella tularensis is a gram-negative bacterium that causes the disease Tularemia in mammals. F. tularensis is considered a potential biological weapon by the United States government due to its low infectious dose, potential for aerosol transmission, and high virulence. A lead-based alkyl-substituted diphenyl ether, SBPT04, has shown promise as an antimicrobial against F. tularensis. A survival study explored the efficacy of three different formulations of PT04 delivered via oral gavage against a Shu4 infection of F. tularensis in a murine model. Listed in ascending order of treatment success the formulations were: 5% ethanol, 10% ethanol 6% cremaphore 1% citric acid, and 10% ethanol 30% PEG 400 6% EtPGS. A second study aimed to identify the effect of different formulations on SBPT04 bioavailability and tissue distribution in C57/BL6 mice. Plasma, lung, liver, and spleen samples were analyzed using LC/MS for concentrations of SBPT04 and the glucuronidated conjugate (SBPT04g). 10% ethanol 30% PEG 400 6% EtPGS formulation was least effective in the survival study but achieved the greatest amount of SBPT04 tissue perfusion.

Comprehensive evaluation of the effects of chemotherapy in the guinea pig model of tuberculosis

ML Caraway, DJ Ordway, CA Shanley, EA Orme, DS Bucy, L Haskell-Dove, M Henao-Tamayo, MR Harton, S Shang, SL Kraft, AJ Lenaerts, RJ Basaraba, IM Orme

Pulmonary tuberculosis in the guinea pig is an extremely useful model for chemotherapy testing due to the fact that its disease pathology is similar to what is seen in humans. A comprehensive analysis of the impact of chemotherapy on Mycobacterium tuberculosis disease progression, pathology, and immunity in this model has been lacking in the field. We clearly demonstrate using a realistic low dose aerosol model of delivery of a M. tuberculosis strain of high virulence that treatment for 24 weeks with isoniazid, rifampicin, and pyrazinamide clears the animal of detectable bacilli. However, the lung pathology reveals that after chemotherapy residual persistent bacilli remain within necrotic and calcified granulomatous lesions. Tracking the immune response by flow cytometry and PCR we found that our highly virulent strain of M. tuberculosis induced Foxp3+T regulatory cells which was associated with a decline in Th1 immunity and eventual death of the control animals. During chemotherapy either by immune modulation and/or reducing the bacterial burden led to Foxp3 T regulatory cell elimination and protective immunity to be re-established. This study was supported by a generous grant from the Bill and Melinda Gates Foundation

Sindbis virus usurps the cellular HuR protein to stabilize its transcripts and promote productive infections in mammalian and mosquito cells

EL Chaskey, KJ Sokoloski, AM Dickson, CJ Wilusz, J Wilusz

The mechanisms utilized by viruses to protect their transcripts from the cellular RNA decay machinery, as well as the biological relevance of this protection, are largely unknown. We demonstrate that Sindbis virus and four other alphaviruses use U-rich 3'UTR sequences in their RNAs to recruit the cellular HuR protein during infections of both human and mosquito cells. HuR binds the viral RNAs with high specificity and affinity. Furthermore, Sindbis virus induces the selective movement of HuR protein out of the nucleus of mammalian cells during infection thereby increasing the cytoplasmic pool of the protein available to the virus. Finally, knockdown of HuR protein in both human and mosquito cells results in a significant increase in the rate of decay of Sindbis virus RNAs and diminishes viral yields. Collectively these data indicate that Sindbis virus, and likely other alphaviruses, usurp the HuR protein to avoid the cellular mRNA decay machinery and maintain a highly productive infection.

Liposome-Nucleic Acid Adjuvants Elicit Effective Mucosal Immunity

AJ Duffy, KL Propst, R Kedl, SW Dow

Development of effective mucosal vaccine adjuvants has become an increasingly important goal with the recent emergence of a new pandemic flu virus and the potential for emergence and rapid spread of other viral or bacterial pathogens. We previously reported that cationic liposome, non-coding plasmid DNA complexes (CLDC) were effective vaccine adjuvants for inducing cell-mediated and humoral immune responses against protein or peptide antigens following parenteral immunization. Therefore, in the current study we investigated the ability of CLDC to function as mucosal vaccine adjuvants. To address this question, humoral and T cell responses were assessed in mice following intranasal (i.n.) immunization with CLDC-adjuvanted vaccines, and immune responses were compared to those elicited by cholera toxin B and CpG ODN adjuvants. In addition, the effects of CLDC adjuvant on antigen uptake and trafficking by antigen presenting cells in the airways and draining lymph nodes was evaluated. We found that i.n. immunization with CLDC-adjuvanted vaccines effectively induced local and systemic antibody responses. Moreover, vaccines delivered with CLDC were particularly effective in generating antigen specific T cell responses in the lungs. The addition of CLDC enhanced antigen uptake by monocytes and DC in the airways and by DC in draining lymph nodes. Vaccine responses elicited by CLDC were found to be dependent on MyD88 signaling. Finally, immunization with CLDC and heat-killed bacteria generated significant protection against lethal pulmonary challenge with Burkholderia pseudomallei. Therefore, we concluded that CLDC can function as effective mucosal vaccine adjuvants for eliciting balanced humoral and cell-mediated immunity.

Epidemiological aspects of Neospora caninum in Alaskan canids

SA Elmore, TM O'Hara, LR Ballweber

Purpose: Little is known about the sylvatic cycle of Neospora caninum in arctic and subarctic ecosystems. Serosurveys have shown exposure to the organism in a variety of arctic and subarctic mammals, including caribou (Rangifer tarandus), musk-oxen (Ovibos moschatus), and gray wolves (Canis lupus), but the definitive hosts for N. caninum in these ecosystems have not yet been identified. Domestic dogs and coyotes (Canis latrans) are the only confirmed definitive hosts for N. caninum, although oocysts genetically compatible with N. caninum have been found in red fox (Vulpes vulpes). Gray wolves and arctic fox (Vulpes lagopus) may also be definitive hosts, given the close taxonomic relationship with dogs, coyotes, and red foxes. Methods: In this study, feces from domestic sled dogs (n=15), grey wolves (n=157), red foxes (n=6), arctic foxes (n=95), and coyotes (n=5) were opportunistically collected and frozen at -80C until processing. Feces were analyzed using a nested polymerase chain reaction (PCR) protocol with the previously described primers Np6+/Np21+ and Np6/Np7, which target the Nc5 region. Amplicon sequencing followed the PCR, and resulting sequences were compared with existing data on GenBank. Results: Neospora caninum DNA was detected in feces from domestic sled dogs, arctic fox, and grey wolves. Conclusions: The detection of N. caninum in the feces of Alaskan canids suggests that these animals are most likely exposed to N. caninum through diet. Further research will address the intermediate host status of arctic canid prey species and continue surveys of canid feces for evidence of natural infections.

Evidence of reduced gene flow among bobcats due to habitat fragmentation in southern California

JS Lee, EE Boydston, LM Lyren, R Alonso, EW Ruell, SN Bevins, ML MacMillan, KR Crooks, S VandeWoude

Human activities, such as urbanization, can result in the fragmentation of once contiguous natural habitat, resulting in patchy habitat interspersed within and around a growing urban landscape. Animals living among fragmented landscapes often have reduced movement between habitat patches when the urban matrix separating patches is hostile. Mammalian carnivores with large home ranges, such as bobcats, may be particularly affected by habitat fragmentation. Restricted movement between habitat patches can lead to reduced gene flow, inbreeding, and a loss of genetic diversity through genetic drift. The current study seeks to determine if human development -- including two major highways -- is reducing gene flow between habitat patches in metropolitan areas south of Los Angeles. Seventeen microsatellite loci were amplified to allow genotypic analysis of 106 bobcats from three core habitat patches separated by large freeways. Population genetic analyses indicate that population substructure does exist in association with landscapes fragmented by urban development, and that 2 distinct subpopulations exist within our study location. This finding suggests that urbanization and a large highway (Interstate –5) are barriers to gene flow, preventing admixture of the two subpopulations. Substructure was not detected between habitats separated by another highway (SR 91) which may be due to population mixing in these areas. Further analysis will be conducted on genotypic structure of a retrovirus (Feline Immunodeficiency Virus) infecting approximately 20% of the genotyped animals to evaluate the utility of tracking barriers to gene flow using a genetic marker with higher mutation rates than the host genome.

Identification of new Mycobacterium leprae variable number tandem repeat loci for strain typing

W Li, RM Sakamuri, M Price, B Hall, NY Robbins, RW Truman, T Gillis, V Vissa

To increase the repertoire of genomic markers suitable for Mycobacterium leprae genotyping in different applications spanning phylogenetics and transmission studies, we have re-examined the M. leprae genome for short tandem repeat (STR) loci and sampled clinical isolates from a diverse range of geographic locales.
Thirty three loci were amplified from a global collection of 66 M. leprae isolates by multiplex PCR. Another set of 58 Indian isolates were screened for 20 of these 33 loci, using single or multiplex PCR assays for detecting variable number of tandem repeat (VNTR) polymorphisms.
Polymorphisms were found in eight loci (ML1, ML8, ML10, ML11, ML15, ML18, ML29 and ML55) in our study.
Among eight new polymorphic VNTR loci, two markers (ML1 and ML8) showed potential for strain typing purposes as 2 or 3 variants were seen in multiple samples in different countries. . ML1 locus is made of 52 bp tandem repeats. ML1 VNTR allele in these global strains also correlates with a previously described system of subtyping into four groups based on three single nucleotide polymorphisms. One, two and three copy alleles can be seen. ML8 locus is made of 37 bps tandem repeats. One and two alleles can be seen. One copy of ML8 has high frequency in Asian countries. The other six loci for strain typing will depend on further testing in additional isolates. These markers, when added to the existing panel of polymorphic VNTRs can aid in monitoring the pathogen responsible for continued incidence of leprosy in the world even after years of wide scale implementation of multidrug therapy.

Intestinal macroparasites and mercury (Hg): the chemical ecology within the alimentary tract of a piscivore host

A Linton, TM O'Hara, KB Beckmen, M Salman, LR Ballweber

In recent years, parasites have been receiving recognition for their role in evaluating ecosystem-health, and for their potential use as indicators of marine pollution. Cestodes and acanthocephalans have been shown to accumulate heavy metals at much higher concentrations than detected in host tissues. The primary objective of this project is to study toxicant-parasite interactions in a piscivore host, specifically, by assessing the role of gastrointestinal macroparasites in mercury distribution and biotransformation (ecotoxicoparasitology). We hypothesize that parasites alter the dynamics of mercury absorption (bioavailability), and that these toxicant-parasite interactions affect the overall health of the host. Intestinal tracts from wild canids, which will serve as a reference population for this study, were processed; parasites were removed, weighed, counted, and identified to species. Luminal contents and various tissue samples were also collected. Samples were analyzed for mercury at the Wildlife Toxicology Laboratory, University of Alaska—Fairbanks (UAF). Determination of trophic levels of intestinal fauna will be instrumental in delineating ecological relationships within the host intestine, and will be accomplished by stable isotope analysis in the coming months. To date, total mercury (THg) analysis has been carried out on 83 wolves. Hepatic THg concentrations ranged from 5.7 ppb wet weight (ww) to 7,300 ppb ww. Renal THg concentrations ranged from 11.2 ppb ww to 4,600 ppb ww. Total mercury concentrations measured in the livers of "coastal" wolves (n=19) were significantly higher than those in the livers of wolves from interior Alaska (n=64) ($Z = 5.23$, 1 d.f., $p = <0.0001$), with mean concentrations of 2,216.89 and 128.17 ppb ww, respectively. This newly recognized difference in THg concentrations between land-locked and coastal wolves identifies a geographical "hot spot" for Hg and will provide important insight into the feeding ecology of these animals.

Walleye Dermal Sarcoma Virus (WDSV) Orf C: a possible oncolytic therapy

E Magden, C Brewster, J Rovnak, S Quackenbush

Walleye dermal sarcoma virus (WDSV) is a complex retrovirus that causes the growth of cutaneous tumors in walleye fish throughout North America. The tumors that form with this viral infection have been shown to spontaneously regress on a seasonal basis. WDSV encodes three accessory proteins (rv-cyclin, Orf B, Orf C) that are necessary for regulation of virus expression, tumor formation and tumor regression. Orf C has previously been shown to target the cell mitochondria and induce apoptosis, likely contributing to the observed seasonal tumor regression. To further define the mechanism(s) of apoptosis, we generated a recombinant lentivirus that expresses WDSV Orf C (Lenti-Orf C). Transduction of cells with Lenti-Orf C resulted in expression of Orf C in the nucleus and mitochondria consistent with previous findings. With this recombinant Orf C-expressing virus we intend to identify cellular proteins that interact with Orf C to define the mechanisms of apoptosis induction. A mouse mammary tumor cell line will be used to assess the in vivo oncolytic capabilities of this recombinant virus.

Strain-Specific Neuropathology of Feline Immunodeficiency Virus

C Miller, M MacMillan, S Huitrón-Resendiz, SJ Henriksen, J Elder, H Bielefeldt-Ohmann, S Vandewoude

Feline immunodeficiency virus (FIV) is a naturally-occuring lentivirus of domestic cats, and is the causative agent of feline AIDS. Similar to human immunodeficiency virus (HIV), the pathogenesis of FIV involves infection of lymphocytes and macrophages, and results in chronic progressive immune system collapse and death. Clinical presentation ranges from subclinical viremia to the development of secondary, opportunistic infections of the respiratory, gastrointestinal, and urinary tract. Neuropathologic correlates of FIV infection, however, have not yet been elucidated, and may be relevant to understanding HIV-associated neurologic disease (NeuroAIDS). Cats were exposed to two well-characterized FIV strains with divergent clinical phenotypes and a chimeric strain as follows: PPR (relatively apathogenic), C36 (immunopathogenic), and PCenv (a chimeric virus consisting of a PPR backbone with substituted C36 env region). A sham inoculum control group was also included. Peripheral nerve conduction velocity, CNS imaging studies, viral loads and hematologic analysis were performed over a 12 month period. At termination of the study (350 days post-inocculation), brain sections were obtained from six anatomic locations known to be involved in human and primate lentiviral neuroAIDS. Histological and immunohistochemical evaluation with seven markers of inflammation revealed that PCenv infection resulted in mild inflammation of the CNS, microglial activation, neuronal degeneration and apoptosis. The C36 strain had similar effects, although to a lesser degree, while the PPR strain induced minimal abnormalities. Conduction velocity aberrations were noted peripherally in all three groups at 50 weeks post-infection. This study illustrates the potential for FIV to provide valuable information about neuroAIDS pathogenesis and therapeutic intervention.

Studies of Burkholderia pseudomallei beta-lactam resistance mechanisms

DA Rholl, HP Schweizer

Burkholderia pseudomallei is the etiological agent of melioidosis. Due to its high levels of intrinsic antibiotic resistance and potential use in bioterrorism, it is designated a CDC category B select agent. Late generation beta-lactams, specifically ceftazidime, have more than halved the mortality rate of melioidosis, but resistance to the drugs has emerged. The penA gene codes for a putative twin arginine transport (TAT)-secreted beta-lactamase in B. pseudomallei, but its role in resistance has not been well studied. We investigated the effects of penA deletion, mutation, and up-regulation, as well as TAT deletion. We also examined the role of two LysR-family regulators flanking penA, which were suspected to control beta-lactamase production. Deletion of penA reduced the minimum inhibitory concentration (MIC) between 4- and 64-fold for 3 of 8 beta-lactams tested, with other drugs showing only minor changes. A single amino acid substitution in the TAT signal sequence of PenA or TAT deletion had the same effects as penA deletion, confirming the association between the two. Over-expression of penA of ~33-fold, achieved through single-copy chromosomal insertion of the gene under an inducible promoter, increased the resistance levels for all beta-lactams tested (2- to 8-fold). Interestingly, increased production of PenA affected piperacillin and ceftazidime more than other drugs (4- and 8-fold, respectively) despite the fact that deletion of the penA gene provided only a 2- to 4-fold decrease in MIC. Using qRT-PCR we showed that deletion of the two LysR-family regulators had no effect on penA regulation. In conclusion, although its regulation remains unknown, the TAT-dependent PenA beta-lactamase plays a significant role in the beta-lactam resistance profile of B. pseudomallei.

A novel role of nucleophosmin in mRNA export and quality control

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

Processing of the 3' end of most mRNAs in mammalian cells involves cleavage of pre-mRNAs and the subsequent addition of 150-200 adenylate residues. Previously we have shown the 32 kDa nucleophosmin (NPM) protein is deposited on the 3' UTR of mRNAs as a direct result of 3'end processing. Therefore NPM effectively 'marks' mRNAs that have undergone successful 3' end processing. NPM is over-expressed in many cancers and has been implicated as a major cause of oncogenic transformation in non-translocation lymphomas. We wish to determine the functional consequences of NPM deposition on mRNAs. To address this, we have used shRNA technology to create HeLa NPM knockdown cells. In these NPM-depleted cells, we find that the length of the poly(A) tail on multiple mRNAs is significantly extended. Furthermore, Fluorescence In Situ Hybridization (FISH) assays demonstrate that polyadenylated RNAs accumulate in nucleus of NPM knockdown cells. These data strongly suggest that NPM deposition plays a role in RNA quality control and/or the export of mRNAs out of the nucleus. Importantly, we have successfully recapitulated the mRNA hyperadenylation phenotype in polyadenylation assays using nuclear extracts prepared from these cells, thus providing us with a powerful tool to study mechanistic aspects of NPM regulation of poly(A) tail length. Finally, UV cross-linking assays have allowed us to visualize at least one other factor deposited on mRNAs during polyadenylation suggesting that NPM may be only one member of a protein complex comprising the polyadenylation 'mark'. We are currently purifying additional components and anticipate that these will provide new insights into the role of NPM and the polyadenylation mark complex in hyperadenylation, mRNA export, and nuclear mRNA surveillance.

MOLECULAR EPIDEMIOLOGY OF LEPROSY IN CEBU, PHILLIPPINES

RM Sakamuri, M Kimura, W Li, HC Kim, WC Black 4th, M Balagon, Paul Saunderson, R Gelber, SN Cho, PJ Brennan, and V Vissa

Leprosy is caused by Mycobacterium leprae. A quarter million of new leprosy cases were detected annually throughout the world. M. leprae is not cultivable under in vitro conditions, and has long incubation time before the disease is diagnosed. The causes for continued prevalence in endemic countries are still unknown. In this regard, molecular tools for differentiation of isolates of M. leprae are needed to track and control transmission of leprosy. Genome sequencing of M. leprae facilitated the identification of genetic markers such as variable number of tandem repeat (VNTR) and single nucleotide polymorphic (SNP) loci. We standardized methods for DNA extraction from the clinical samples and also developed rapid and high throughput methods like multiplex-PCR and fragment length analysis (FLA) for VNTR analysis and PCR-RFLP for SNPs to facilitate molecular strain typing. We have applied these methods on the samples obtained from leprosy patients in Cebu, Philippines, an island province where nearly 400 cases are detected annually. Thus far, we have compiled a database containing molecular and epidemiological information from over 300 leprosy patients since 2004. VNTR alleles were found to be highly discriminatory when assessed at 16 loci, yielding a population structure comprising of five clades. On the other hand, SNP subtyping based on three markers showed 85% of Cebu, Philippine isolates are of type 1 and the remaining of type 3. Typical VNTR signatures also distinguish SNP type 1 from type 3 isolates. Within multiple cases families, patients had highly similar M. leprae VNTR profiles, indicating a possible common source of transmission, stability of VNTR markers during short range transmission, and therefore suitability of VNTR typing for tracing transmission clusters within contacts. Ongoing investigations are directed towards longitudinal molecular typing combined with geographical and epidemiological linkage identifications within the endemic communities.

Temporal association of large granular lymphocytosis, neutropenia, proviral load, and FasL mRNA in cats with acute feline immunodeficiency virus infection

WS Sprague, JA Terwee, S. VandeWoude

Neutrophils comprise the majority of white blood cells in circulation and play an important role in innate immunity, particularly during acute infection. Neutropenia during acute human immunodeficiency virus (HIV-1) infection has been reported, but there is a paucity of information describing the pathogenesis of this condition. Neutropenia with concurrent large granular lymphocyte (LGL) lymphocytosis has been demonstrated in both LGL leukemia and common variable immunodeficiency syndrome in people, and in both syndromes an increase in soluble Fas ligand (sFasL) has been associated with neutrophil apoptosis leading to neutropenia. During acute FIV-C-PG infection, we observed that cats develop LGL lymphocytosis concurrent with a marked neutropenia that is temporally associated with the rise and fall of feline immunodeficiency virus (FIV) proviral loads. LGLs are thought to be natural killer cells, which express either CD56 or CD8/CD57. Flow cytometric analysis demonstrated increases in CD56 and CD8 peripheral blood cell surface expression during acute FIV-C-PG infection; however, only CD56 temporally increased and decreased with FIV proviral loads. Expression of FAS-L mRNA was increased at the same time points as these peripheral hematologic abnormalities, and also decreased as FIV proviral load reached set point. We describe an interesting temporal association between innate immune responses and viral load during acute FIV-C-PG infection that has similarities with HIV and other non-viral-associated immunological dyscrasias of people. Ongoing studies are designed to investigate the mechanism of neutrophil apoptosis during acute FIV infection as a means to increase our understanding innate correlates of immunity during lentiviral disease. These studies will be the basis for development of novel therapies for immunodeficiency virus infection aimed at inhibiting the Fas-FasL apoptosis pathway.

Chemokine Receptor 4 (CXCR4) is Up-Regulated in Bovine Peripheral Blood Mononuclear Cells following Infection with Bovine Viral Diarrhea Virus in vitro

CM Weiner, NP Smirnova, S Vandewoude, H Van Campen, TR Hansen

Purpose: Bovine viral diarrhea virus (BVDV) infection leads to monetary losses in cattle operations due to morbidity and impaired growth. One reason is the difficulty in identifying pregnant cattle carrying BVDV persistently infected (PI) fetuses. We previously described a type-I interferon (IFN-I) response to acute BVDV infection in PI fetuses in vivo and biomarkers to identify heifers carrying a PI fetus, one of which is chemokine-receptor 4 (CXCR4). We hypothesized that: (i) infection of peripheral blood mononuclear cells (PBMC) with ncp BVDV in vitro would upregulate IFN stimulated genes (ISG) and CXCR4; (ii) blocking CXCR4 with CXCR4 antagonist AMD3100 or binding with its ligand stromal-derived factor 1(SDF-1a) will change CXCR4 expression and prevent viral entry. Materials/methods: Peripheral blood mononuclear cells (PBMCs), isolated from BVDV-naïve steer blood, were infected with ncp BVDV2 in vitro at moi of 1. PBMCs were treated with AMD3100 and/or SDF-1a prior to and during infection. Cells were collected and processed for RT-PCR and flow cytometry staining post infection. Results: At 32 h post infection (hpi), IFN-1 pathway (IFN-ß, ISG-15), antiviral molecules (retinoic acid inducible gene I, RIG-I), CXCR4 and CD8 mRNAs increased ($P < 0.05$). Treatment of PBMC with AMD3100 prior to BVDV infection did not affect BVDV RNA replication, but abrogated CXCR4 mRNA concentraton. SDF-1a treatment increased the expression of BVDV, CXCR4, IFN- ß, ISG15, RIG-I, CD4, and CD8 mRNA. Conclusions: 1. Up-regulation of IFN-1 pathway genes, RIG-I, CXCR4, and CD8 in BVDV infected PBMC at 32 hpi may indicate that CXCR4 expression is induced during BVDV in vitro infection by IFN-1 in bovine PBMC. 2. Blocking CXCR4 with AMD3100 and/or SDF-1a did not prevent viral entry and/or replication, but abrogated a BVDV-induced increase in CXCR4 expression. SDF-1a, as with other viral agents, modulated infection. Funded by the USDA-NIFA (2008-35204).

Development & optimization of a multiplex microsphere based feline cytokine assay

BA Wood, KP O'Halloran, S VandeWoude

Cytokines play an important role in host innate immune response against an infection. Traditionally, enzyme-linked immunosorbent assays (ELISAs) have been used to determine cytokines levels; however, because of the relatively large serum volume needed to test for an individual cytokine (e.g., up to 100 uL), the number of cytokines that can be tested in serum collected at a single time-point from laboratory animals is limited. Recently, an alternate technology has been developed in which multiple cytokines can be detected in as little as 50 uL using microsphere based immunoassays. Cytokine detection kits are available for laboratory animals, such as mice and non-human primates; however, kits are currently not available for domestic felids. To develop feline microsphere based immunoassays, antibody pairs from commercially available feline ELISA cytokine kits (IFN-g, IL-10 and IL-12; R&D Systems) were combined with magnetic microspheres (Luminex Corporation). The assay was optimized by testing a single factor at a time, including capture antibody concentration, detection antibody concentration, standard curve detection range, cross-reactivity between analytes, multiplex standard curves, streptavidin-PE concentration, and the number of microspheres used per well. The standard curve detection range in the multiplex assay was found to be 15.6-2000 pg/mL for IFN-g, 31.25-4000 pg/mL for IL-10 and 9.7-1250 pg/mL for IL-12. When assay validation is completed, this multiplex assay will be used to examine the cytokine response of domestic cats infected with host-adapted and non-host adapted (i.e., cross species) feline immunodeficiency virus strains.

FIV transcription levels in tissues from single and co-infected cats

X Zheng, J TerWee, S VandeWoude

Feline immunodeficiency virus (FIV) infects domestic cats and ultimately causes fatal immune dysfunction. Puma lentivirus (PLV) is phylogenetically related to FIV and occurs naturally in pumas (Puma concolor). Our laboratory developed a feline model of cross-species lentiviral transmission that demonstrates that PLV infects domestic cats but results in aborted avirulent infection. Further, PLV infection partially protects against virulent FIV clinical disease. To study causes for differential pathogenicity and factors restricting viral coinfection, we evaluated FIV proviral load, RNA transcripts and cytokine IFNg, IL10, IL12 levels from 14 tissues harvest at 159 days post-infection. PLV provirus and RNA was found in very low levels in intestinal tissues and were virtually undetectable in bone marrow and thymus. FIV provirus and RNA were highest in bone marrow, thymus and spleen. FIV provirus tended to be higher in co-infected animals, whereas FIV RNA tended to be higher in singly-infected animals. No obvious discernable differences in cytokine levels were detected. This study demonstrates that regional differences in FIV infection at the tissue level might be related to differential pathogenicity during co-infection with PLV, but analyses of earlier timepoints post-infection are necessary to further study this phenomenon.

Neoamphimedine, a new Topoisomerase II alpha inhibitor, in Metnase-mediated DNA decatenation

CL Amerin, AK Ashley, DV LaBarbera, JA Nickoloff

Following replication of DNA, chromosomes become topologically intertwined or catenated. Successful resolution (decatenation) of chromosomes is essential for cellular division, and if decatenation fails, cell death, cell arrest or gross chromosomal aberrations can result. Decatenation is mediated by topoisomerase IIα (TopoIIα), thus this enzyme has been exploited as a chemotherapeutic target, though cancer cells can develop resistance to TopoIIα inhibitors. Metnase is a human protein that interacts with TopoIIα and promotes decatentation and resistance to TopoIIα inhibitors, thus, potentiating carcinogenesis. Novel TopoIIα inhibitors may prove useful in preventing or delaying the development of resistance. Neoamphimedine (NeoA), a marine pyridoacridine, blocks TopoIIα-dependent decatenation of DNA in vitro. While the mechanism of action remains elusive, NeoA does not promote stabilization of cleavable complexes. NeoA also delays formation of human epidermoid-nasopharyngeal and colon tumors in nude mice similarly to the TopoIIα poison etoposide. Our hypothesis is that NeoA will block Metnase-potentiated decatenation. To test this hypothesis, we have optimized an *in vitro* decatenation assay using using kinetoplast DNA (highly interlocked DNA circles). Further, mitotic cells have been identified via immunocytochemistry (ICC) against FOXM1 in human fibrosarcoma cells to determine mitotic index. FOXM1 ICC allows visualization of mitotic arrest following inhibition of decatenation, and can demonstrate *in vivo* cellular ability to bypass the decatenation checkpoint, indicative of resistance to top2a inhibitor(s). NeoA may prove useful in custom chemotherapy including combined treatments with traditional TopoIIα inhibitors, and/or with to-be-developed Metnase inhibitors in tumors that over-express Metnase.

Evaluating an Air-Liquid Interface Culture of Normal Human Bronchial Epithelial Cells

M Bushey, B Hawley, J Volckens

Current techniques to model inhaled aerosol exposures in vitro are limited in their physiological relevance. For example, many in vitro models rely on suspending an aerosol in an aqueous solution before dispersing this mixture onto submerged cells. However, this technique does not account for the fact that human lung cells typically exist at an air-liquid interface. Furthermore, particles suspended in solution prior to the exposure can undergo both physical and chemical reactions that alter their toxic properties. To address these issues, we have developed a novel air-liquid interface exposure system to study the effects of aerosols on lung cells. As part of this research, we also sought to demonstrate that normal human bronchial epithelial cells cultured at an air-liquid interface display signs of cellular differentiation matching that of bronchial epithelia in vivo. Normal human bronchial epithelial cells were cultured at an air-liquid interface on collagen-coated membranes over a period of four weeks. Selected wells were then: (1) paraffin embedded at days 1, 10, 24 and 28; (2) sectioned on a microtome; (3) stained with eoisin and hematoxylin; and (4) imaged using transmitted light microscopy. Imaged cells showed progressive signs of cellular differentiation, including formation of a pseudo-stratified columnar epithelium. Additionally, the emergence of ciliated, mucin-producing, and basal-like cells was apparent.

Evaluating the inflammatory response of human lung cells to woodsmoke exposure

B Hawley, J Volckens, M DeFoort

Approximately ½ the world's population uses biofuels for cooking and heating, with wood being the most common. Human exposure to biofuel combustion emissions (i.e., woodsmoke) has been associated with pulmonary disease, eyesight degradation, cancer, and adverse pregnancy outcomes. Consequently, woodsmoke exposure is an important global health concern. Recent effort has been given to the design and dissemination of 'improved cookstoves,' with the expectation that these technologies will reduce emissions and improve health. Although research has been conducted on the effect of traditional cookstove emissions (i.e., the 'three-stone fire'), there is a lack of information on the health effects associated with exposure to improved cookstove emissions. This research seeks to investigate inflammation and stress in human bronchial epithelial cells exposed to emissions from improved and traditional cookstoves. We hypothesize that exposure to improved cookstove emissions will reduce levels of inflammation in normal human bronchial epithelial (NHBE) cells compared to emissions from a traditional three-stone fire.

Woodsmoke was generated from three different cookstoves. Particulate matter and gaseous emissions were monitored while the stoves were operated at the CSU Engines and Energy Conversion Laboratory. NHBE cells were exposed in vitro using a novel exposure system. Expression of cyclooxgenase-2, heme oxygenase-1, and interleukin-8 were characterized at 1 and 24 hours using real-time RT PCR.

Emissions from each cookstove were substantially different, with the three stone fire having the highest emissions. Expression levels of inflammatory genes were also significantly higher than control cells, with the 3-stone fire having the greatest effect. These results provide preliminary evidence in support of the improved cookstove hypothesis.

Defining Metnase Function in Plasmid DNA Integration

J Nie, JA Nickoloff

Integration of foreign DNA into genomic DNA is important for retroviral infection, human gene therapy, and construction of transgenic cell lines and animals. Integration can also mediates gene transfering from apoptotic cells to nearby cells, potentially transferring oncogenes from a dying malignant cell to normal cells. Metnase is a protein uniquely expressed in higher order primates, including humans. The function and importance of this enzyme has not been fully understood. Metnase contains a SET (Lysine methylase) domain and a *Mariner* transposase (nuclease) domain, as well as a phosphorylation domain. Previous research demonstrated that integration of plasmid and viral DNA is proportional to Metnase expression level. Metnase also promotes NHEJ (Non-Homologous End-Joining) of transfected linear plasmid DNA. It has been shown that Metnase requires both the SET domain and the nuclease domain to function in NHEJ. The recruitment of Metnase to DNA double strand breaks can be blocked by mutation of the S495 phosphorylation domain. We hypothesize that Metnase relies, at least partially, on its NHEJ activity to promote DNA integration. In this project, we aim to determine if the SET, nuclease and/or S495 phosphorylation domains, which are required for efficient NHEJ, are also required to promote DNA integration, as well as a NHEJ protein Ligase IV. This will be tested by cotransfecting puromycin expressing plasmid and wild-type or mutant Metnase expressing plasmids into the Metnase-null HEK-293T cells. This project will provide better understanding in how Metnase promotes DNA integration and its role in maintaining genomic stability.

Role of Rad51 ATPase in Double-Strand Break Repair by Homologous Recombination

JD Wischhusen, J Nie, JA Nickoloff

The Rad51 protein is known for its role in initiating homologous recombination (HR) during the repair of DNA double-strand breaks (DSBs). Rad51, which contains an ATPase domain, forms a nucleoprotein filament at broken ends of DNA to initiate HR. In a study conducted by the Symington lab, several mechanisms were elucidated regarding the binding of Rad51 protein. First, Rad51 requires ATP in order to bind to DNA. Second, binding of the protein is independent of ATP hydrolysis. Lastly, inhibition of hydrolysis provides stabilization of the nucleoprotein filament, otherwise the filament is dissembled. We are investigating the role of the ATPase domain in HR by measuring HR efficiency and HR outcomes in three different yeast strains: (1) wild-type; (2) mutated ATPase domain on Rad51; and (3) mutated ATPase domain on both Rad51 and paralogs Rad55 and Rad57. HR efficiency and HR outcomes are determined by measuring gene conversion tract lengths, tract directionality, and tract continuity in the three yeast strains. This project will generate a rich data revealing a clearer picture of the mechanism of Rad51 mediated strand invasion in the early stages of homologous recombination.

Effects of Survivin and Survivin Inhibition in Canine Osteosarcoma

JK Shoeneman, DH Thamm, JB Charles, EJ Ehrhart

Canine osteosarcoma (OSA) is a disease with an extremely high mortality rate. Current therapies for OSA merely slow the progression of metastatic disease. New approaches to therapy are needed. Survivin is a protein with both anti-apoptotic and pro-proliferative activity, commonly elevated in human cancer. Survivin expression is a negative prognostic factor in dogs with lymphoma, and previous work has demonstrated that both canine lymphoma and OSA cell lines contain high levels of survivin. We hypothesized that inhibition of survivin in canine OSA cells will inhibit cell cycle progression, and cause an increase in apoptosis and chemotherapy sensitivity. To determine the effects of survivin inhibition in canine OSA, we knocked down survivin via siRNA in two canine OSA cell lines. The knockdown was confirmed by western blot analysis. Total and live cell number was assessed by manual cell counting with trypan blue. Caspase-3/7 Assay analysis was used to determine levels of apoptosis in cells with survivin inhibition compared to control cells. Cells were then treated with antineoplastic drugs carboplatin (CPT) or doxorubicin (DOX) +/- survivin knockdown and caspase assays repeated. Finally, propidium iodide staining and flow cytometry analysis was used to determine the effect of survivin knockdown on cell cycle progression. Survivin knockdown resulted in approximately 85% inhibition of survivin protein expression. It also decreased total and viable cell number, and increased apoptosis when compared to controls. Survivin knockdown enhanced OSA sensitivity to CPT and DOX. Cell-cycle analysis demonstrated both mitotic arrest and apoptosis. We have successfully shown that survivin inhibition increases apoptosis and chemosensitivity, and inhibits cell cycle progression. Thus, survivin may be a viable therapeutic target for the treatment of both canine and human OSA. Future studies will evaluate the role of survivin expression in outcome following treatment in canine OSA.