Scientific Proceedings

15th Annual Research Day

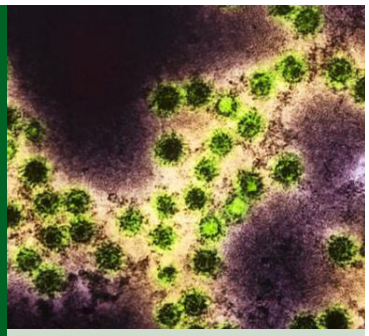
January 25, 2014 • Hilton Fort Collins

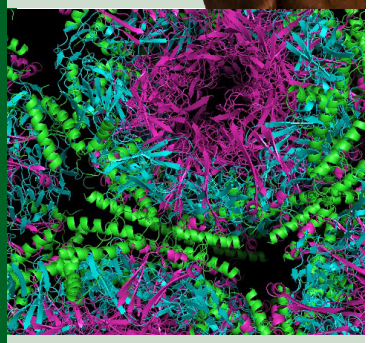
Our 15th Annual Research Day showcases the academic work of 145 aspiring scientists in Colorado State University's College of Veterinary Medicine and Biomedical Sciences. The day gives our rising stars vital experience presenting their research findings to a scientific audience through poster displays and talks. The day also provides young researchers with an avenue for feedback to help them develop ideas that, in many cases, will become lifelong scientific pursuits. In a sign of significance, the research projects on display are sponsored by two dozen well-respected companies, foundations, and institutions concerned with improving human, animal, and environmental well-being. Thank you for supporting and engaging with our presenters – undergraduate students, graduate students, veterinary residents, and post-doctoral fellows – as they pursue research that will help animals, people, and the planet!

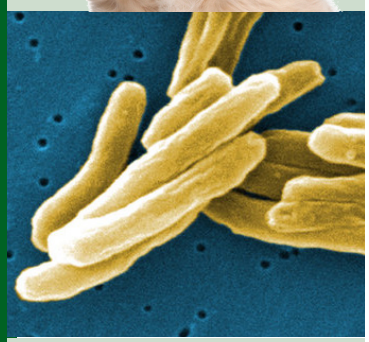
Colorado State University

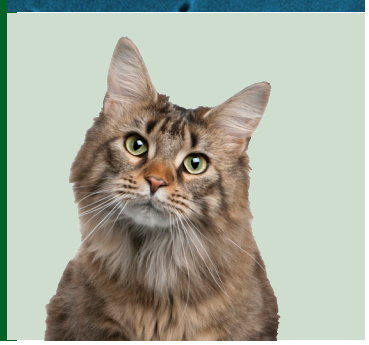
College of Veterinary Medicine and Biomedical Sciences

Biomedical Sciences
Clinical Sciences
Environmental and Radiological Health Sciences
Microbiology, Immunology, and Pathology









Zoetis Research Award Winner

Dr. Greg Amberg seeks subcellular insights into hypertension

Dr. Greg Amberg received the 2013 Zoetis Award for Veterinary Research Excellence, an honor that confers the role of keynote speaker at the College of Veterinary Medicine and Biomedical Sciences Research Day. His lecture is titled, "Mitochondria link calcium and redox signaling in the vasculature."

Amberg, an associate professor in the Department of Biomedical Sciences, received his Doctor of Pharmacy from Idaho State University, followed by his doctorate in cell and molecular physiology and pharmacology from the University of Nevada. He received postdoctoral training in cardiovascular physiology in the laboratory of Dr. Fernando Santana at the University of Washington, and then joined the Colorado State University faculty in 2007.

The Amberg Lab investigates the poorly understood mechanisms controlling calcium channels in arterial smooth muscle. This research investigates a novel regulatory mechanism where reactive oxygen species (ROS) increase calcium channel activity in arterial smooth muscle cells. Increased calcium channel opening elevates smooth muscle calcium and causes arterial contraction. Importantly, elevations in calcium entry and reactive oxygen species formation are thought to be related to the development of cardiovascular diseases including hypertension, atherosclerosis, and stroke.

Specifically, the Amberg Lab investigates the temporal and spatial interface functionally linking redox and calcium microdomains at the subcellular level and relates this to arterial dysfunction and the development of hypertension. This work uses a combinatorial approach that includes total internal reflection fluorescence (TIRF) microscopy, voltage-clamp electrophysiology, fluorescent calcium imaging and video microscopy of intact arteries, and molecular techniques. This work is designed to provide mechanistic insights into events underlying altered redox and calcium signaling during hypertension and may lead to the development of new rational therapies for managing and preventing disease.

Dr. Amberg was named a Pew Scholar in the Biomedical Sciences in 2010. His work is supported by the National Institutes of Health – National Heart, Lung, and Blood Institute.

Schedule Of Events

		Room
11:30-12:00	Poster set up	Salon III, IV
12:00	Opening remarks – Dr. Susan VandeWoude, associate dean for research and graduate education	Salon II
12:05	Zoetis Research Award Winner – Dr. Gregory Amberg *"Mitochondria link calcium and redox signaling in the vasculature "*	Salon II
12:45	Break	
1:00-5:00	Oral Presentation I: Clinical Sciences	Salon I
1:00-5:00	Oral Presentation II: Basic Sciences	Salon V
1:00-5:00	Oral Presentation III : Clinical/Basic Sciences	Salon II
1:00-3:00	Poster Session I Judging: Odd-numbered Posters	Salon III, IV
3:15-5:00	Poster Session II Judging: Even-numbered Posters	Salon III, IV
5:00-6:00	Social Hour	Salon III, IV
6:00	Awards	Salon III, IV

SESSION 1: CLINICAL SCIENCE

1:00-5:00PM

Salon I

1:00	Ledesma-Feliciano	Use of transabdominal ultrasonography to assess fetal characteristics in Reeve's muntjac deer (Muntiacus reevesi)	MIP
1:15	Barnard	Phase I study evaluating the combination of autophagy inhibition by hydroxychloroquine and doxorubicin treatment in canine non-Hodgkin's lymphoma	CS
1:30	Burden	Ovarian function following prostaglandin administration to mares in diestrus: a retrospective study	CS
1:45	Contreras-Menakis	Post-amputation orthopedic disease in canine amputees: preliminary results of an online survey	CS
2:00	Curran	CHOP versus LAP for treatment of CHOP-relapsed canine lymphoma.	CS
2:15	Doddman	Geographic disparities in clinical characteristics of duodenitis/proximal jejunitis in horses in the United States	CS
2:30	Fagre	Intake procedures in Colorado animal shelters	CS
2:45	BREAK		
3:00	Fink	Field comparison of Seresto® (10% imidacloprid/4.5% flumethrin) collar and a placebo collar placed on cats.	CS
3:15	Fitzpatrick	Pharmacokinetics of subcutaneous ondansetron in healthy geriatric cats, cats with chronic kidney disease and cats with liver disease.	CS
3:30	Fujishiro	Administration of an intranasal Bordetella bronchiseptica vaccine and a subcutaneous FVRCP vaccine is superior to the subcutaneous FVRCP vaccine alone in a FHV-1 challenge model	CS
3:45	Hamil	An analysis of dose distribution following the administration of electronic brachytherapy to the canine nasal cavity	ERHS
4:00	Jones	The development of an indirect computed tomography lymphography protocol for sentinel lymph node detection in head and neck cancer	ERHS
4:15	Koch	Innate immune responses of primary equine respiratory epithelial cells to infection with a modified live influenza virus vaccine.	CS
4:30	Adrian	Sedated computed tomographic angiography: a novel method for improving the diagnosis of canine pancreatitis	ERHS
4:45	Linke	A novel avian influenza antiviral technology targeting respiratory epithelium: proof-of-principle in an avian model	CS

SESSION 2: BASIC SCIENCE

1:00-5:00PM

Salon V

1:00	Bender	Pharmacokinetics of a PrPC siRNA therapeutic that can cross the blood brain barrier	MIP
1:15	Birkenheuer	RV-cyclin and its role in tumor development	MIP
1:30	Cartwright	The Role of Various DNA Repair Pathways in the Formation of Chromosomal Inversions	ERHS
1:45	Casali	Amoebal Models of Non-tuberculosis Mycobacteria (NTM) Infection	MIP
2:00	Davenport	Assessing prion species barriers and the new host effect with RT-QuIC methodology	MIP
2:15	Elder	Determining the presence of blood-borne prions at various time points throughout infection	MIP
2:30	Grover	Profiling early lung immune responses in the guinea pig model of tuberculosis (TB)	MIP
2:45	BREAK		
3:00	Grubaugh	West Nile Virus Population Dynamics in Wild-caught Birds	MIP
3:15	Gustafson	Hedgehog pathway expression in canine transitional cell carcinoma and normal canine bladder	CS
3:30	Hazenfield	Comparison of first throw knot security of six different friction knots	CS
3:45	Higgins	Are marine fish a source of Brucella infection?	MIP
4:00	Hoover	Monitoring RML Scrapie Prion-Seeding Activity in Neuroblastoma Cells with RT-QuIC	MIP
4:15	Johnson	Activated mesenchymal stem cells amplify antibiotic activity against chronic Staphylococcus aureus infection.	MIP
4:30	Lee	The one assay to find them all: targeted genome capture and next-generation sequencing to simultaneously detect all feline viruses and bacteria	MIP
4:45	Li	ICV infusion of (pro)renin receptor antagonist (mPRO20) attenuates prorenin and DOCA-salt induced hypertension	BMS

SESSION 3: CLINICAL/BASIC SCIENCE

1:00-5:00PM

Salon II

1:00	Marshall	Short-course antibiotic administration in dogs with pneumonia: 72 cases (2002-2013)	CS
1:15	Martin	Evaluation of two dry therapeutic diets for dogs with acute diarrhea	CS
1:30	Nakamoto	Prevalence of zoonotic parasites in shelter dogs in Veracruz, Mexico	CS
1:45	Noyes	Associations between isolation of Mannheimia haemolytica, antimicrobial resistance and use, and morbidity and mortality in feedlot cattle	CS
2:00	Parkinson	Establishment of a Reference interval for Fibrinogen in Healthy Ornate Box Turtles (Terrepene ornata ornata)	CS
2:15	Ruple-Czerniak	Risk factors for the development of canine lymphoma in North American dogs: 18,826 cases (1990-2009)	CS
2:30	Wennogle	Effects of subcutaneous or intranasal vaccine administration on clinical signs in FHV-1 infected cats without previous vaccination	CS
2:45	BREAK		
3:00	McMillan	DNA damage produced by 64Cu-ATSM high LET Auger electrons	ERHS
3:15	Myrick	Evaluation of the accuracy of a commonly used dynamometric wire tensioner	CS
3:30	Prasad	Small RNA response of Culex quinquefasciatus to West Nile virus infection: relationship to vector competence.	MIP
3:45	Regan	Amplification of tumor vaccine immunity by co-administration of ondansetron	MIP
4:00	Rico	Liposome-antigen-nucleic acid complexes protect mice from lethal challenge with Western and eastern equine encephalitis viruses	MIP
4:15	Rosenberg	Optimized Methodology for Obtaining and Analyzing Limited Quantities of Soil Contaminated with Radionuclides	ERHS
4:30	Samson	Evaluation of aerosol shedding in growing pigs following administration of porcine reproductive and respiratory syndrome virus vaccine	Other
4:45	Smith	Dynamic HLA-linked changes in insulin-binding B cells in pre-diabetic and new onset diabetic patients as well as their first-degree relatives.	MIP

Departmental Abbreviations

BMS: Biomedical Sciences
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
MIP: Microbiology, Immunology, and Pathology

POSTER PRESENTATIONS

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

#1	Adney	Experimental infection of goats with MERS-CoV	MIP
#2	Akin	Nav1.6 somato-dendritic localization in hippocampal neurons is via an ankyrinG-independent mechanism	BMS
#3	Alout	Effect of mass drug administration of ivermectin on malaria infection in Burkina Faso, West Africa	MIP
#4	Anna	Development of two tabletop exercises of zoonotic disease outbreaks to train senior veterinary students at The Ohio State University	Other
#5	Ball	Genetic modification of stem cells with scAAV-equine-BMP-2 and protein expression before and after cryopreservation	CS
#6	Bascuñán	Use of laser-guidance for canine limb circumference measurement	CS
#7	Beale	Development of immune mediated polyarthritis after vaccination	CS
#8	Becker	Effects of iron chelation on monocyte metabolism; changes in CD-36 and GLUT-1 expression	MIP
#9	Bell	Low Dose Radiation exposure causing time related saturation of Gamma-H2AX in Human Fibroblast cells despite differences in absorbed dose	ERHS
#10	Benson	Quantitative comparison of advanced glycation end-products in the serum of diabetic to non-diabetic cats	CS
#11	Brock	Cerenia® for the management of vomiting and inappetence associated with chronic kidney disease in cats.	CS
#12	Bromberek	Does exercise-induced pulmonary hemorrhage affect career longevity and performance among South African Thoroughbred racehorses?	CS
#13	Brown	Pathogenesis of Francisella tularensis infection in cottontail rabbits	MIP
#14	Burgess	Factors associated with large animal inpatient shedding of Salmonella enterica in a veterinary teaching hospital	CS
#15	Burton	Investigating systemic endectocides as a novel strategy for West Nile virus control in Northern Colorado	MIP
#16	Cadmus	Salmonella prevalence in baby poultry at feed stores	MIP
#17	Cameron	Characterization of exosomes and their role in FIV infection- a pilot study	MIP
#18	Charley	Interactions between segmented RNA viruses and the RNA decay machinery	MIP
#19	Chotiwan	The role of fatty acid synthase during dengue virus replication in mammalian cells	MIP
#20	Cleys	Prenatal Androgenization Decreases Ovine Placental gDNA Methylation and Alters Placental Gene Expression	BMS

#21	Colbath	Comparison of the immunosuppressive properties of allogeneic and autologous equine mesenchymal stem cells	CS
#22	Coleman	mRNA decay is altered in myotonic dystrophy patient cells	MIP
#23	Crouch	LIN28 in Exosomes Secreted by Human Placental Cells and Ovarian Cancer Cells	BMS
#24	Curtis	Micro-CT assessment of bone healing and strength after stereotactic radiation therapy for local control of osteosarcoma	MIP
#26	Dailey	MicroRNA expression changes associated with chemo-sensitivity in canine cancer cell lines	CS
#25	Dang	Actin cytoskeleton modulates local L-type calcium channel signaling and ERK activation in gonadotropes.	BMS
#27	Daniel	Laparoscopic ovariohysterectomy in goats	CS
#28	Denkers	Urinary Shedding of Prions in Chronic Wasting Disease Infected White-tailed Deer	MIP
#29	Doran	Hypophosphatemia in hyperventilating dogs	MIP
#30	Dozier	Could the inhibition of the cellular decay machinery contribute to the pathogenicity of bovine viral diarrhea virus infections?	MIP
#31	Edmondson	Pathologic and cardiovascular characterization of pheochromocytoma associated catecholamine-induced cardiomyopathy in dogs	MIP
#32	Engen	A comparison of dose-dependent outcomes in induction of cytogeno-toxic responses by novel glucosyl flavonoids	ERHS
#33	Enriquez	Ovarian cancer cell-secreted exosomes contain LIN28 and unique RNA signatures capable of inducing invasion and migration	BMS
#34	Farrell	Validation of a smartphone-based point-of-care hemoglobin assay for use in dogs	CS
#35	Felgenhauer	Mesenchymal Stem Cell Immune Modulation of Canine Lymphocyte Responses	CS
#36	Ferguson	Effect of estriol on urodynamic findings 24 hours after dosing in female spayed reseach beagles	CS
#37	Forster	Modulation of Canine Gut Hormones with Bean Consumption and Weight Loss	ERHS
#38	Fowles	Canine COXEN: cross-species genomic applications for predicting chemosensitivity in dogs	CS
#39	Fox	Endoplasmic reticulum/plasma membrane junctions function as mem-brane protein trafficking hubs	BMS
#40	Freund	Quantum dot labeling of canine mesenchymal stromal cells for longi-tudinal visualization	BMS
#41	Garbino	Rapid in-vitro assay to detect CWD prions in deer saliva	MIP
#42	George	IGHV usage and somatic hypermutation analysis in canine B cell chronic lymphocytic leukemia.	MIP
#43	Gibas	High variability in the risk estimates of zoonotic tuberculosis	CS
#44	Good	Commuting and air pollution: A multi-pollutant exposure study	ERHS
#45	Harbison	Effects of dietary rice bran or navy bean on human plasma cytokine levels and leukocyte telomere length	ERHS
#46	Henderson	Whole Body Analysis of CWD Prion Peripheralization	MIP

#47	Herrington	Effects of mRNA decay on transcription: maintenance of steady state mRNA levels through buffering	MIP
#48	Hill	Measuring cytokine profiles longitudinally during prion infection	MIP
#49	Hoaglund	Robotically improving accuracy of the cervid prion cell assay (CPCA) method	MIP
#50	Hohnbaum	Presence of toxigenic Pasteurella multocida ssp. Multocida evaluated in the oral cavity of cats with feline chronic gingivostomatitis.	CS
#51	Hong	In Vitro susceptibility of human influenza A and B viruses to nitazoxanide and tizoxanide	CS
#52	Hornig	Evaluation of a point-of-care glucose and beta-hydroxybutyrate meter operated in various environmental conditions in prepartum and postpartum sheep	CS
#53	Hoxmeier	Transmission and maintenance of Mycobacterium ulcerans by Anopheles gambiae	MIP
#54	Hyatt	Of mice, men, and elephants continued: the relationship between articular cartilage zonal thicknesses and body mass	Other
#55	Jalkanen	Zinc finger protein mRNAs are regulated post-transcriptionally in stem cells: A tale of fingers and (poly(A)) tails.	MIP
#56	Johnson	Degenerative and infectious change in heart valves from Northern Sea Otters	MIP
#57	Kane	Molecular interaction analysis of prions and potential peripheral receptors	MIP
#58	Kirkley	Looking Beyond the Neuron: Neuroinflammation in California Sea Lions Exposed to Domoic Acid	ERHS
#59	Kumor	Assaying the role of Platelet endothelial cell adhesion molecule-1 (PECAM-1) in vitro	MIP
#60	Lake	Building a virtual cat: a physiologic-based pharmacokinetic (PBPK) model for investigating drug dosing in cats	CS
#61	Lear	Evaluation of a model demonstrating mitigation of nociceptive response to oxytetracycline injection site inflammation by flunixin meglumine in dairy cows	CS
#62	Lee	Quantitative measurement of bacterial 16s rRNA genes in plasma of FIV infected cats	MIP
#63	Loughridge	Deaths related to musculoskeletal injury peak mid racing season in Colorado racehorses	CS
#64	Lyon	Attenuated Activity of Clofazimine in a Mouse Model Exhibiting Caseous Necrosis	MIP
#65	Maeda	Homologous recombination repair is required for G2-phase potentially lethal damage repair	ERHS
#66	Martinez	The effects of pathogen reduction technology on malaria (Plasmodium falciparum) in whole blood units	MIP
#67	Matthews	Evidence for endothelial to mesenchymal transition in canine degenerative mitral valve disease	CS
#68	Miller	Characterization of the Salivary Antibody Response in FIV-infected Domestic Cats	MIP

#69	Moon	Pathogenic consequences of flavivirus-mediated suppression of the cellular RNA decay machinery	MIP
#70	Morges	Phase II evaluation of VDC-1101 in canine cutaneous T cell lymphoma	CS
#71	Mosovsky	Interferon-gamma enhancement of antibiotic activity against Burkholderia is mediated by induction of reactive oxygen species	MIP
#72	Neisler	Evaluation of the clinical utility of a Lig based ELISA for early and inexpensive diagnosis of leptospirosis in dogs	CS
#73	Newett	Evaluation of Live Bp82 Vaccination Efficacy in Goats	MIP
#74	Ngai	Use of filter paper to quantify polychlorinated biphenyl (PCB) in bottlenose dolphins whole blood	Other
#75	Olsen	Prion seeding activity in peripheral tissues of primary passage and host-adapted murine chronic wasting disease	MIP
#76	Ortega	Prions in plants: potential assay for detection of PrP^{res} in grasses from Rocky Mountain National Park	MIP
#77	Pabilonia	Using Conservation Genetics to Improve ex-situ Management of the Critically Endangered Buffon Macaw (Ara ambiguus guayaquilensis)	MIP
#78	Pauls	Development of a harness to facilitate a novel method of canine gait analysis utilizing inertial motion sensors.	BMS
#79	Penilla	Detecting SNPs by Deep Sequencing in the Insecticide Resistance Genes of Aedes aegypti	MIP
#80	Potter	Evaluation of coliphage dynamics in bighorn sheep, domestic sheep and cattle: implications for bacteriophage therapy	MIP
#81	Richardson	Effect of 2-aminoimidazole Compounds on Advanced Glycation End Products	MIP
#82	Romero	Paracrine and Endocrine action of Conceptus-derived Interferon-Tau during Early Pregnancy in Ewes	BMS
#83	Saklou	Comparison of accelerated hydrogen peroxide and per oxygen disinfectants as misting applications	CS
#84	Salmon	Dietary fatty acids do not predict insulin resistance in the presence of similar body weight and visceral adiposity	BMS
#85	Sampaio	A yeast colony morphology phenotypic transition associated with loss-of-heterozygosity	ERHS
#86	Selariu	Mechanism of vertical transmission of Chronic wasting disease (CWD) in animal models	MIP
#87	Sharif	Copy number variation mediated by dispersed repeats in yeast	ERHS
#88	Shields	The Role of the C_2A Domain of Synaptotagmin in Asynchronous Release	BMS
#89	Shoeneman	Survivin inhibition via EZN-3042 in canine lymphoma and osteosarcoma	CS
#90	Shropshire	Evaluation for associations of Bartonella species with azotemia and hematuria in cats.	CS
#91	Steel	Induction of Oxidative Stress during Flavivirus Infection Enhances RNA Replication.	MIP
#92	Stutzman-Rodriguez	Novel gammaherpesviruses in mountain lions and domestic cats: variations in prevalence and predictor variables	MIP

#93	Tangtrongsup	Effect of dexamethasone concentration on chondrogenic differentiation of equine bone marrow-derived mesenchymal stem cells	CS
#94	Tuttle	Early detection of clinical disease in guinea pigs experimentally infected with Mycobacterium tuberculosis.	MIP
#95	Walsh	β-Amyloid- and proinflammatory cytokine-induced cofilin-actin rod formation requires prion-dependent activation of NADPH oxidase.	Other
#96	Weishaar	Expression and function of polo-like kinase in canine cancer	CS
#97	Whitaker	Comparing the effect of docosahexaenoic acid (DHA) supplementation of western and low-fat diets on myocardial fatty acid composition	BMS
#98	Willingham	Assessing mother to offspring transmission of chronic wasting disease using transgenic mouse models	MIP
#99	Yoo	Modulation of coagulation and fibrinolysis by carbon monoxide and nitric oxide in dogs: a thromboelastographic analysis	MIP
#100	Zhang	Regulation of H19 lncRNA by RNA-binding proteins in muscle cells.	MIP

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Congratulations Again to 2013 CVMBS Research Day Winners

Oral Presentations

First Basic **Michelle N. Sullivan** "Endogenously-generated lipid peroxidation products dilate rat cerebral arteries by activating TRPA1 channels in the endothelium"

Second Basic **Kimberly Yore** "Determination of the Flea Species Infesting Dogs in Florida and Bartonella spp. Prevalence Rates"

First Clinical **Laura E. Selmic** "Oncologic outcome and prognostic factors in 1134 dogs with appendicular osteosarcoma treated at a single institution"

Second Clinical **Christine K. Ellis** "Differentiation between Healthy Cattle and Cattle Infected with Mycobacterium bovis using the Volatile Organic Compound Profiles Present in Breath"

Poster Presentations

First **Christy Wyckoff** "Bioassay detection of chronic wasting disease prions in soil"

Second **Audrey Ruple-Czerniak** "Risk factors for the development of malignant histiocytosis in Bernese Mountain Dogs"

Third **Ashley Neff** "Differential regulation of mRNA stability in human induced pluripotent stem cells"

Golden Pipette Award – Department of Biomedical Sciences

2014 CVMBS Research Day Organizing Committee

Brad Borlee – Faculty Chair – Microbiology, Immunology, and Pathology

Dawn Duval – Assistant Faculty Chair – Clinical Sciences

Phil Fox – Biomedical Sciences

An Dang – Biomedical Sciences

Dan Regan – Clinical Sciences

Shannon McLeland – Clinical Sciences

Brock Sishc - Environmental and Radiological Health Sciences

Cory Sicard - Environmental and Radiological Health Sciences

Claire Birkenheuer - Microbiology, Immunology, and Pathology

Brendan Podell - Microbiology, Immunology, and Pathology

Sue VandeWoude - CVMBS Associate Dean of Research

Aimee Oke – Committee Coordinator- CVMBS College Office

Veterinary Summer Scholars Program

College of Veterinary Medicine and Biomedical Sciences

The Veterinary Summer Scholars program was initially established through support from Merck-Merial to provide an opportunity for veterinary schools to expose students in their first and second years of veterinary medical school to biomedical research. With continued support from Merial, several other organizations, CVMBS and faculty mentors have contributed funds to provide summer stipends for program participants. The current Veterinary Student Scholars program gives veterinary students hands-on exposure to veterinary medical research to introduce them to potential research careers. CSU CVMBS recently received funds from the National Institutes of Health and will be able to further expand the very successful program next year.

Twenty-nine veterinary students from CSU and abroad participated in the 2013 CSU Veterinary Summer Scholar program. Students spent the summer working in research labs, attending weekly research seminars and field trips to CSU, federal and state research facilities. Many of the projects conducted by CSU students last summer are being presented today at the CVMBS Research Day.

2013 Summer Scholars Sponsors

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Morris Animal Foundation

American Humane Association

American Society of Lab Animal Practitioners

University of Alaska, Fairbanks

Royal Dick School of Veterinary Science, Scotland

CSU College of Veterinary Medicine

To view the research of students funded in 2013 or to apply for the summer 2014 program, please visit the website at: http://csu-cvmbs.colostate.edu/dvm-program/Pages/Veterinary-Scholars-Program.aspx

PVM Student Grant Program

Center for Companion Animal Studies
Department of Clinical Sciences

In 2006, the HESKA Corporation made a $20,000 donation to support research that involved PVM students. That year, the monies were used to support 5 excellent projects chosen from 9 that were submitted. With continued collaboration from the HESKA Corporation, the Young Investigator Grant Program was opened to other corporate and non-corporate donors. The amount of funding has continued to grow yearly. In 2013, $68,500 was raised and distributed to 24 different projects all of which involved a PVM student as a scientist. Many of those projects are being presented today at the CVMBS Research Day. Colorado State University offers thanks to all sponsors of this program and is looking forward to advancing the veterinary sciences with our partners in the years to come while concurrently involving PVM students in clinical research.

2013 PVM Student Grant Program Sponsors

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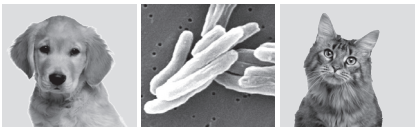
To view the grants funded in 2013 or to make a donation, please visit the Center for Companion Animal Studies website at: www.csuvets.colostate.edu/companion



ORAL PRESENTATIONS

CLINICAL SCIENCE

Session I – Salon I

1:00-5:00PM

Use of transabdominal ultrasonography to assess fetal characteristics in Reeve's muntjac deer (Muntiacus reevesi)

Carmen Ledesma-Feliciano, Kelly Walton, Erin McNulty, Amy Nalls, Kelly Anderson, Jeanette Hayes-Klug, Candace Mathiason

Reeve's muntjac deer (Muntiacus reevesi) is a small South Asian deer species used as a prion transmission and pathogenesis model. Mother-to-offspring transmission is a specific area of interest for researchers and requires insight into maternal reproductive efficacy and fetal characteristics during infection. Assessing gestation and fetal characteristics requires use of ultrasonography to collect information on fetal growth and development. Here we describe the use of transabdominal ultrasonography to assess fetal growth characteristics and viability in muntjac deer. Estrus was synchronized with 2 PGF2 αinjections, given 11 days apart, and verified through vaginal cytology that displayed sperm and increased superficial epithelial cells. Evaluations to date have demonstrated pregnancy as early as 35 d after the second PGF2 injection, with embryos measuring between 0.5 to 1 cm in length and displaying fetal heart movement. Fetal mineralization was apparent at approximately 60 d with a fetal crown-rump distance of 3 to 4 cm. At 90 d, the crown-rump distance was 8 to 9 cm. Ultrasonographic examination proved to be beneficial in evaluating fetal development in muntjac deer. Current and future goals of this study include reliably detecting pregnancy in muntjac does, continued assessment of fetal development throughout gestation, assessment of fetal variables to estimate gestational time, and identification of any differences in fetal characteristics or viability in various control and experimental protocols of prion infection. These parameters can not only benefit a variety of experimental protocols but also provide a basis for successful reproductive management of muntjac deer colonies.

Phase I study evaluating the combination of autophagy inhibition by hydroxychloroquine and doxorubicin treatment in canine non-Hodgkin's lymphoma

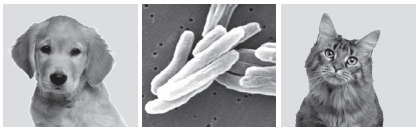
Rebecca A. Barnard, Luke A. Wittenburg, Ravi K. Amaravadi, Daniel L. Gustafson, Andrew Thorburn, and Douglas H. Thamm

Autophagy is a lysosomal degradation process that allows for the recycling of cellular material, potentially contributing to resistance and survival for a number of different cancers. Thus pharmacologic autophagy inhibition is currently being explored in human clinical trials; however, it has not been investigated for use in canine cancers. Canine non-Hodgkin's lymphoma (NHL) is one of the most prevalent tumor types and though most patients initially respond to CHOP therapy, relapse occurs within 6-11 months. Multi-agent therapy can also be cost and time prohibitive for owners so single agent doxorubicin (DOX) may be used as an alternative, yet the response rate is substantially lower. Therefore, a Phase I, single arm, dose escalation trial was conducted in dogs to determine a maximum dose of HCQ that can be combined with DOX. HCQ was administered daily by mouth throughout the trial, beginning 72 hours prior to DOX, which was given intravenously on a 21-day cycle. Peripheral blood mononuclear cells and biopsies were collected before and 3 days after HCQ treatment and assessed for autophagy inhibition and HCQ concentration. A total of 30 patients were enrolled in the trial. HCQ alone was well tolerated with only mild lethargy and gastrointestinal-related adverse events. The overall response rate (ORR) was 100%, stable disease or better, with median progression free interval (PFI) of 5 months. Pharmacokinetic analysis revealed a 100 fold increase in HCQ in tumors compared to plasma, but no correlation was observed between the two. While autophagy biomarkers did demonstrate inhibition in some patients, but there was no correlation between plasma and tumor response indicating that plasma measurements cannot be used in lieu of tumor measurements. In conclusion, the superior ORR and comparable PFI to single agent DOX provide strong support for further evaluation via randomized, placebo-controlled trials in canine NHL.

Ovarian function following prostaglandin administration to mares in diestrus: a retrospective study

Chelsie A. Burden, Ryan A. Ferris, Patrick M. McCue

Prostaglandins (PGF) are routinely used in the reproductive management of the mare to induce luteolysis and provide an opportunity for an early return to estrus. The objectives of this study were to 1) determine the average interval from prostaglandin administration to the subsequent spontaneous ovulation, 2) evaluate the effect of follicle size, mare age, and month of treatment on ovarian follicular response, 3) describe the fate of large follicles (≥35mm) at the time of PGF administration, and 4) determine incidence of hemorrhagic anovulatory follicles (HAF) formation following PGF. Reproductive records of 275 mares managed over a total of 529 estrous cycles were reviewed. All mares were administered prostaglandins in mid-diestrus. Mares were subsequently examined by transrectal ultrasonography to monitor follicular development and determine the day of the next spontaneous ovulation. The average interval from prostaglandin administration to ovulation was 8.4 ± 2.5 days and was correlated with the size of the largest follicle at time of prostaglandin administration, with a longer interval to subsequent ovulation occurring if the follicle was small at the time of PGF administration. Large follicles (≥35mm) had one of three outcomes following PGF treatment including: ovulation within 48 hours (14.5%), ovulation post 48 hours (75.4%), or regression (10.1%) followed by emergence and subsequent ovulation of a new follicle. Incidence of HAF formation occurred in 12/529 cycles (2.3%). No significant differences in mare age or season on outcome following PGF administration were noted. This study gives insight to proper timing of subsequent reproductive examination as well as the proportion follicular regression and incidence of hemorrhagic anovulatory follicle formation following PGF administration in the mare.



Post-amputation orthopedic disease in canine amputees: preliminary results of an online survey

Elena Contreras-Menakis, Felix Duerr

Canine amputees may suffer from orthopedic disease in one or more of their remaining limbs. In the veterinary literature, there are only two case reports and one case series describing surgical management of orthopedic disorders in canine amputees and four studies describing post-amputation changes in gait. In order to further investigate occurrence of post-amputation orthopedic disorders and surgical and/or medical treatment selection, an online survey of canine amputee owners was conducted. Frequencies of occurrence of post-amputation orthopedic disorder, timeframe, treatment category chosen, and surgery performed, were recorded. There were a total of 126 survey respondents. Forty-one percent (n=49/120 respondents) indicated that their canine amputee had a subsequent orthopedic disorder, which occurred at an average of 18 months (17.5 +/- 30 months) post-amputation; 32 were thoracic limb amputees, and 17 were pelvic limb amputees. Fifteen out of these 17 pelvic limb amputees had orthopedic disease in the contralateral pelvic limb, and two of the 15 also had orthopedic disease in a thoracic limb. Of the 32 thoracic limb amputees with orthopedic disease, 22 had disease in at least one of their pelvic limbs. Eight pelvic and 12 thoracic limb amputees selected surgical treatment, all for pelvic limb disorders, consisting of 11 cranial cruciate ligament ruptures, treated by tibial plateau leveling osteotomy (n=8/11), tibial tuberosity advancement (n=1/11) and extracapsular repair (n=2/11) surgeries, four patellar luxation surgeries, three hip luxations, and two fractures. Twenty-nine amputees did not undergo surgery, due to various factors including concurrent neoplastic process, geriatric age, or non-surgical orthopedic conditions. In this survey population, 41% (n=49/120) of canine amputees had subsequent orthopedic disease which occurred at an average of 1.5 years post-amputation, more often in a pelvic limb. Surgery was selected as treatment for subsequent pelvic limb disorders by 41% (n=20/49) of amputees with orthopedic disease.

CHOP versus LAP for treatment of CHOP-relapsed canine lymphoma.

Kaitlin M. Curran, Janet C. Lori, and Douglas H. Thamm

Introduction: Canine lymphoma is responsive to initial chemotherapy; however, it then becomes resistant to drugs in the initial protocol and second remission durations are shorter. The objective of this study was to evaluate whether choice of first rescue protocol (re-induction with CHOP or switching to lomustine, L-asparaginase, and prednisone [LAP]) affected outcome in dogs that had relapsed following an initial CHOP protocol Methods: Data were collected retrospectively from 49 dogs from one institution over a 10-year period. Signalment, clinical presentation, initial treatment specifics, relapse and rescue specifics, adverse events, best response, progression-free survival (PFS) and overall survival (OS) time were evaluated. PFS and OS estimates were calculated using the Kaplan-Meier method and differences between groups compared using logrank analysis. Multivariate analysis was performed using forward and reverse stepwise Cox regression. Results: All dogs were in remission for at least 45 days following CHOP discontinuation. 30 dogs were re-treated with CHOP and 19 were treated with LAP as their first rescue protocol. There was no difference in overall response rate or percent complete response between groups. Univariate variables predictive of PFS included rescue choice, first remission duration (FRD) and adverse effects necessitating delay/reduction. Variables predictive of OS included FRD and institution of CHOP retreatment at some point during rescue therapy. On multivariate analysis, delay/reduction remained significant for PFS. FRD and CHOP re-treatment were significant for OS.Conclusions: Dogs undergoing CHOP re-treatment at some time following relapse had a superior outcome to those that never received CHOP re-treatment.

Geographic disparities in clinical characteristics of duodenitis/proximal jejunitis in horses in the United States

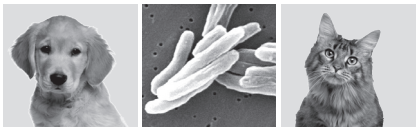
Courtney Doddman, Amy L Steiler, Elizabeth J Elzer, Ann Hess, Louise Southwood, Brett Tennant-Brown, and Diana M Hassel

Duodenitis-proximal jejunitis (DPJ) is an idiopathic disease of horses characterized by abdominal pain, dysfunction of the proximal small intestine and subsequent development of profuse nasogastric reflux. Anecdotal evidence notes that prevalence of DPJ is highest in the southeastern United States and that clinical features of DPJ differ among regions through the USA, but no study has investigated the role of geographical location and associated causative factors. Case records were compared from veterinary referral hospitals in 3 different geographic locations from the years 1997 through 2011 to determine if significant differences in clinical, clinicopathologic and prognostic characteristics exist among horses with DPJ. Data was reported as means and standard errors for each variable and hospital. One-way ANOVA F-tests were performed for each variable and pairwise comparisons of means were performed for each variable to compare pairs of hospitals. The three hospitals compared were Colorado State University (West), University of Georgia (SE), and University of Pennsylvania (NE). Significant differences between hospitals were observed in heart rate at presentation, maximum body temperature, mucous membrane color, character of reflux, peripheral blood total protein, peritoneal fluid total protein, neutrophil count, days of hospitalization, albumin, sodium, chloride, GGT, AST, and creatinine. This study confirmed anecdotal reports of geographical differences in clinical presentation of DPJ. Horses from the University of Georgia and University of Pennsylvania had more severe clinical signs and more severe biochemical abnormalities than horses presenting with DPJ at Colorado State University, with a trend toward the most severe abnormalities from University of Georgia horses. The mechanisms by which these differences occur have yet to be elucidated but may be related to differences in bacterial pathogens.

Intake procedures in Colorado animal shelters

Anna C Fagre and Rebecca Ruch-Gallie

Purpose: To determine intake procedures, with a focus on infectious disease testing, in Colorado animal shelters. Methods: A survey was designed and administered to shelter supervisors across the state of Colorado via SurveyMonkey or mailed hard copy. Information collected concerned general shelter characteristics and intake procedures performed in various circumstances. Descriptive statistics were reported for overall intake procedures. Chi-square analysis will be utilized to compare differences in intake procedures between shelter types and animal source. Results: Only 77.3% (34/44) of shelters report vaccinating all animals upon intake, with young age (65.8%; 25/38), pregnancy (55.3%; 21/38), and mild existing illness (39.5%; 15/38) being cited as the top reasons for not administering core vaccines. While respondents perceive heartworm disease as a risk to the canine (66.7%) and feline (31.4%) populations in Colorado, only 43.2% (16/37) report testing dogs and 5% (2/40) report testing cats on intake. When respondents were asked if they perceive endoparasitic disease as a risk to the canine and feline populations in Colorado, 61.8% (21/34) and 51.5% (17/33) did, respectively. However, many shelters responding to disease screening questions commented that they only screen in the presence of clinical signs or other suspicion of infection. Conclusions: Few shelters test dogs and cats for infectious diseases and those that do utilize tests for diagnostic purposes rather than routine screening. Additionally, vaccination protocols in several shelters are not consistent with Association of Shelter Veterinarian (ASV) guidelines.



Field comparison of Seresto® (10% imidacloprid/4.5% flumethrin) collar and a placebo collar placed on cats.

Heidi K Fink, Sara Wennogle, Wendell L Davis, Cristiano Von Simson, and Michael R Lappin.

Seresto® collar (Bayer Animal Health) has been shown to control flea and tick infestations for eight months and to be effective in preventing transmission of Bartonella henselae and Cytauxzoon felis among cats. While collars avoid some of the compliance issues associated with topical products, some cats object to wearing collars. The purpose of this study was to evaluate tolerance of client-owned cats for the Seresto® collar or a placebo collar. A total of 96 client-owned cats, greater than 10 weeks of age, were enrolled in the study. Cats systemically ill, hairless breeds, or cats declawed in all four limbs were excluded. Cats were randomized by household to wear a placebo collar for 14 days followed by Seresto® for 14 days or a Seresto® collar for 28 days. Examinations by a veterinarian were performed on Days 0, 14, and 28. Owners recorded daily systemic and local health observations. All but two cats, including one that entrapped it's mandible in the collar and one that developed local pyodermatitis (Seresto®), completed the 28 day study. Local lesions and licking occurred in the first 14 days and licking was more common in Seresto® cats. No local lesions were reported for placebo cats after switching to Seresto® and only one Seresto® cat had reports of licking after Day 14. Housing status, single or multiple cat household, and whether a collar had been worn previously were not associated with side-effects. Adverse events detected for cats wearing Seresto® were similar to those for cats wearing placebo collars and to cats wearing identification collars reported in a previous study. Although cats were more likely to lick the collar area in the first 14 days of placement while wearing Seresto® collars, the data suggests that cats originally intolerant of collars become receptive over time.

Pharmacokinetics of subcutaneous ondansetron in healthy geriatric cats, cats with chronic kidney disease and cats with liver disease.

Rikki L Fitzpatrick, Luke A Wittenburg, Ryan J Hansen, Paul L Lunghofer, Dan L Gustafson, and Jessica M Quimby.

Ondansetron is a 5-HT3 receptor antagonist and an effective anti-emetic in cats. The purpose of this study was to compare the pharmacokinetics of subcutaneous ondansetron in geriatric cats to cats with chronic kidney disease (CKD) and liver disease using a limited sampling strategy. 12 geriatric cats, 16 CKD cats and 8 liver disease cats were enrolled. Based on limited sample modeling, blood was drawn 30 minutes and 2 hours following a 2 mg subcutaneous injection of ondansetron. Ondansetron concentrations were measured by liquid chromatography coupled to tandem mass spectrometry. Drug exposure (AUC) was predicted using a limited sampling approach based on multiple linear regression analysis of previous full sampling studies and clearance estimated using non-compartmental methods. The AUC was 344.8 ± 101.6 ng/mL • hr in the geriatric cats, 414.1 ± 142.0 ng/mL • hr in the CKD cats and 592.4 ± 314.4 ng/mL • hr in the liver cats. The calculated clearance of ondansetron was determined to be 1.08 ± 0.27 L/hr/kg in the geriatric cats, 0.94 ± 0.25 L/hr/kg in the CKD cats and 0.83 ± 0.47 L/hr/kg in the liver cats. A one-way ANOVA demonstrated no statistically significant difference between groups, although a subjective decrease was seen in clearance for liver cats. A subset of cats (10 CKD, 5 liver) was age-matched to geriatric cats and this did not affect results. There was no significant difference in the clearance of subcutaneous ondansetron in CKD or liver disease cats when compared to geriatric cats. Additional cats with liver disease should be assessed for increased statistical power.

Administration of an intranasal Bordetella bronchiseptica vaccine and a subcutaneous FVRCP vaccine is superior to the subcutaneous FVRCP vaccine alone in a FHV-1 challenge model

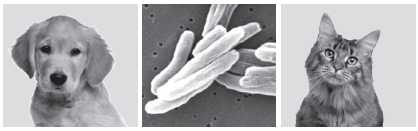
Madeline A Fujishiro, Krystle L Reagan, and Michael R Lappin

Concurrent administration of an intranasal (IN) feline herpesvirus 1 (FHV-1) and feline calicivirus vaccine (FCV) with a subcutaneous (SQ) FHV-1, FCV and panleukopenia (FVRCP) vaccine was shown to give superior protection to use of the SQ vaccine alone in a previously reported FHV-1 challenge study. The effect was believed to be from local non-specific immune stimulation induced by the IN vaccine. Bordetella bronchiseptica is another important respiratory pathogen of cats. The objective of this study was to compare clinical signs and FHV-1 shedding rates after FHV-1 inoculation of kittens vaccinated with both an IN B. bronchiseptica vaccine (Nobivac Feline-Bb; Merck Animal Health) and a SQ FVRCP vaccine (Nobivac 1-HCP; Merck Animal Health) versus kittens vaccinated with the SQ FVRCP vaccine alone. A total of 16 specific pathogen free 8 week-old kittens were randomized into 2 groups. At 10 weeks of age, one group was concurrently administered the IN B. bronchiseptica vaccine and the SQ FVRCP vaccine and the other group was administered the SQ FVRCP vaccine alone. The USDA challenge strain of FHV-1 was administered by IN inoculation 7 days after vaccination. Ocular scores and respiratory scores were determined daily by trained, masked individuals. Pharyngeal swabs were collected for performance of a quantitative PCR assay for FHV-1 DNA and GAPDH with results reported as the FHV-1/GAPDH ratio. There were no statistical differences in total clinical scores between groups of cats prior to vaccination, infection, or one-week post-infection. However, during week 2 and 3 post-infection, cats administered both vaccines had significantly lower total clinical scores than cats administered the FVRCP vaccine alone. There were no differences in FHV-1 shedding. The results support the use of the 2 vaccines concurrently in cats with high risk of exposure to FHV-1 shortly after primary immunization. Funded by a grant from Merck Animal Health.

An analysis of dose distribution following the administration of electronic brachytherapy to the canine nasal cavity

Lauren E Hamil, David Zhang, James T Custis

Electronic brachytherapy (EB) is a type of radiation that utilizes low energy x-rays to treat neoplastic lesions. X-rays in this kilovoltage range are preferentially absorbed by bone, unlike megavoltage external beam radiation. Thus, EB has been reserved as a treatment for superficial lesions away from bone. While external beam radiation therapy remains the gold standard treatment modality for canine nasal tumors, the recent availability of an affordable, mobile, veterinary specific EB system (Axxent) requiring minimal radiation shielding has resulted in the reported use of EB in the treatment of canine nasal tumors. Currently, there is no report of absorbed dose to bone in these patients and post-treatment times remain insufficient to determine late effects or toxicity. BrachyVision software (BVS) has been designed for use with EB to model dosimetry to the tumor and surrounding normal tissues. This software models x-ray attenuation based on distance from the source regardless of density and thus treats all tissues as being equivalent to water. In five canine cadaver heads, a unilateral nasal tumor was created with soft-tissue equivalent material. Using BVS, dose was calculated and prescribed to each of three interstitial dwell positions within the nasal tumor. Axxent was used to deliver the prescribed dose. Concurrently, an ion chamber measured absorbed dose at pre-determined positions perpendicular to the source. Paired t-tests were used to compare calculated and measured doses. The mean differences between estimated and measured dose for the nasal septum, hard palate, and maxilla were -20%, 72% and 65%. For all points along both the hard palate and maxilla, measured dose was significantly lower than the calculated dose ($p<0.001$). Possible causes for this difference are underestimation of x-ray attenuation and increased absorption by bone. The potential to overdose bone and underdose soft-tissue structures distal to bone should be considered when using EB.



The development of an indirect computed tomography lymphography protocol for sentinel lymph node detection in head and neck cancer

Matthew D Jones, Elissa K Randall, Lisel K B Ruterbories, Susan L Kraft

Purpose: Identifying lymph node metastases in patients with head and neck cancer provides important staging and prognostic information for making treatment decisions. Indirect computed tomography (CT) lymphography could potentially be a more sensitive and accurate, widely available, cost effective, and safe method for identifying sentinel lymph nodes for metastasis, with the goal of improving cancer patient management. Methods: Canine and feline patients with biopsy proven tumors of the head and neck underwent routine staging CT. 0.3 – 0.5 ml of iohexol 350 mgI/ml was slowly injected into the center of the tumor. Images were analyzed for tumor and lymph node characteristics, and for intensity and pattern of contrast enhancement of lymphatic vessels or lymph nodes. Cytology or histopathology was performed on identified sentinel lymph nodes. Results: Sixteen patients were enrolled. Tumor types included squamous cell carcinoma (SCC), fibrosarcoma, melanoma, osteosarcoma, myxosarcoma, and thyroid carcinoma. Contrast was seen in the lymphatic vessels in 7/16 patients and visualized in lymph nodes in 5 of those 7 patients as follows: 1. Ear base mass – ipsilateral superficial cervical lymph node. 2. Mandibular SCC – medial of 2 ipsilateral mandibular lymph nodes. 3. Maxillary melanoma – lateral of 2 ipsilateral mandibular lymph nodes. 4. Rostral mid mandible fibrosarcoma – right and left medial mandibular lymph nodes. 5. Maxillary mass (granulomatous cellulitis) – ipsilateral parotid and superficial cervical lymph nodes. Conclusions: The sentinel lymph node as determined by CT lymphography was sometimes a distant or an unexpected lymph node. Further development and refinement of the protocol is needed to increase the rate of lymphatic system visualization before this becomes a viable procedure.

Innate immune responses of primary equine respiratory epithelial cells to infection with a modified live influenza virus vaccine.

Drew W Koch, Heidi L Pecoraro, Lori Bentsen, Gisela Soboll-Hussey, Gabriele A Landolt

Equine influenza virus (EIV) is an important equine respiratory pathogen that has serious health and economic impacts. To design effective EIV control strategies, it is crucial to understand how to invoke long-term protective immunity. While systemic antibody titers have long been used as a correlate for protection from EIV, systemic antibody titers are not always accurate predictors of clinical protection. The cold-adapted, modified live virus (MLV) vaccine FluAvert is thought to mimic natural infection more closely as it uses similar pathways to process and present antigen, yet, it induces only weak systemic antibody responses. Despite this, the vaccine has been shown to provide clinical protection for up to 12 months after a single vaccine administration. This suggests that mechanisms other than humoral responses are critically important in EIV immunity. While the adaptive immunity to EIV has been well characterized, there is a lack of information regarding the innate immune responses during EIV infection. Understanding early mucosal immune responses may help to elucidate the apparent lack of correlation between antibody titers and clinical protection observed with use of the MLV vaccine. The objective of this study was to compare the innate immune responses of primary equine respiratory epithelial cell (EREC) cultures following inoculation with either a closely related wild-type EIV strain or the MLV strain. Our hypothesis was that the MLV vaccine elicits similar innate immune responses in equine airway cells as the wild-type virus. Protein and mRNA expression of IFN-alpha, IFN-gamma, IL-4, IL-6, IL-10, IL-17, TNF-alpha, and TLR3 & 9 were determined in virus inoculated ERECs. Our results indicate that ERECs support infection and replication of EIV. Moreover, EIV infection results in the upregulation of mRNA and cytokine expression and their expression levels are likely associated with viral replication efficiency.

Sedated computed tomographic angiography: a novel method for improving the diagnosis of canine pancreatitis

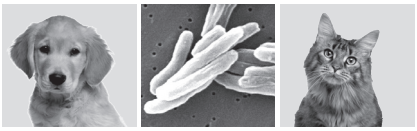
Anna M Adrian, David C Twedt, Susan L Kraft, Angela J Marolf

Computed tomography (CT) is considered accurate for diagnosing human pancreatitis. A combination of clinical signs, bloodwork and ultrasound (US) are commonly used to diagnose canine pancreatitis. Although advanced disease can be detected with US, there are inherent limitations. After clinical and US diagnosis of pancreatitis, a sedated 3-phase angiographic CT was performed, followed by US guided aspirates and a cPLi assay. Images were evaluated for portion of visible pancreas, pancreatic size, evaluation of the pancreatic attenuation, presence of peri-pancreatic changes and contrast enhancement pattern. On CT 9/9 pancreata were visualized in their entirety. One of those dogs had a previous body and left limb pancreatectomy. In 9/9 dogs the right limb and body and in 4 dogs additionally the left limb of the pancreas were affected and enlarged. The parenchyma was homogenous in all left limbs, 7/8 bodies and 8/9 right limbs. 3/8 left limbs, 6/8 bodies and 6/9 right limbs had ill-defined borders. The mesentery was hyperattenuating surrounding 3/8 left limbs and bodies and 4/9 right limbs. Surrounding free fluid was seen in 2/9 cases. The contrast enhancement pattern in all 3 phases was homogenous in 6/8 left limbs and bodies and in 8/9 right limbs. In patients with heterogenous contrast enhancement the delayed phase was best to identify ring-like and patchy enhancement. 5/9 dogs had a positive cPLi test, 1/9 was questionable and were 3/9 normal. Fine needle aspirates showed inflammation in 3/9 cases, 3/9 were non-diagnostic and 3/9 were normal. CT identified thrombi in the portal vein in 2/9 dogs and a cholelith in 2 dogs. Inflammatory changes of the pancreas can lead to enlargement, visible attenuation differences, ill-defined borders and altered contrast enhancement. The apperance of the surrounding mesentery can vary. Careful evaluation of the portal vein is recommended to evaluate the presence of a thrombus.

A novel avian influenza antiviral technology targeting respiratory epithelium: proof-of-principle in an avian model

Lyndsey Linke, Kristy Pabilonia, Jeffrey Wilusz, Johannes Fruehauf, Gabriele Landolt, Roberta Magnuson, Francisco Olea-Popelka, Sushan Han, Mo Salman.

Avian influenza virus (AIV) is often a consequence, economically relevant disease of poultry. The lack of robust prophylactics underlines the urgency to develop more effective control measures in poultry, as a means of controlling AIV transmission and reducing the impact outbreaks have on poultry operations. This work provides proof-of-concept for an innovative viral-targeted intervention strategy for preventing AIV in poultry. The objective was to investigate the inhibition of AIV in chickens using the RNA interference (RNAi) prevention strategy, Transkingdom RNAi (tkRNAi). TkRNAi uses nonpathogenic bacteria to generate and deliver silencing RNAs to mucosal epithelial tissues. Using tkRNAi, we have developed a novel RNAi antiviral capable of generating and delivering small interfering RNAs (siRNAs) targeting 2 key AIV genes required for viral replication. These novel RNAi vectors (termed anti-AIV vectors) were delivered in vitro and in vivo and their protective efficacy against AIV challenge was assessed. We first evaluated AIV (H6N2 and H8N4) suppression after treating chicken epithelial cells with these anti-AIV vectors. Viral shedding, assessed by TCID50, indicates these anti-AIV vectors significantly reduced viral titers compared to untreated controls, corresponding with up to 4.2 logs reduction in infectious virus titer. Subsequently, a series of pilot studies using these anti-AIV vectors were conducted in chickens. Results indicate this novel RNAi antiviral does inhibit AIV shedding in chickens, without concomitant side effects to the host. This proof-of-concept work could represent a new antiviral technology with the potential to prevent AIV infection and transmission in chickens. Demonstrating the value of this novel approach could translate into a cost-effective technology that limits outbreaks in poultry, and could represent a transformative approach with great potential to have a sustained and significant impact on other susceptible species, including humans.



ORAL PRESENTATIONS

BASIC SCIENCE

Session II – Salon V

1:00-5:00PM

Pharmacokinetics of a PrPC siRNA therapeutic that can cross the blood brain barrier

Heather Bender and Mark Zabel

The emergence of prion diseases in wildlife populations and the increasing impact of prion diseases on human health has led to an increase in the study of antiprion compounds. Recent studies have found antiprion compounds that can inhibit the infectious prion isomer (PrPRes) or down regulate the normal cellular prion protein (PrPC). These compounds are often found through the screening of drug or chemical compound libraries. However, most of these chemicals cannot cross the blood brain barrier to effectively inhibit PrPRes formation in brain tissue or to specifically target neuronal PrPC. Also, these compounds tend to have multiple off target effects, and are often too toxic to use in animal or human subjects. Therefore, we have proposed using siRNA that is targeted towards PrPC, and complexed to the RVG-9r peptide, which will target the siRNA to nicotinic acetylcholine receptors within the CNS. Our siRNA therapeutic has proven effective in eliminating prion disease from several neuronal cell lines. We are now testing the PrPC siRNA in vivo through an intravascular route, and evaluating the pharmacokinetics using live imaging and flow cytometry. To avoid serum degradation and facilitate passage through the blood brain barrier in vivo, we have complexed our PrPC siRNA - RVG-9r peptide to liposomes. Wild type mice treated with the siRNA therapeutic through an intravascular route have detectable siRNA and peptide signals in the brain using the IVIS live imaging system within 15 minutes after injection. There is minimal to no peripheral detection of siRNA and peptide using live imaging. Using flow cytometry, we can detect a 25-90% decrease in neuronal PrPC and a 30-80% decrease of PrPC within the kidneys 24 hours after treatment. We are now trying to optimize the PrPC siRNA therapeutic for the maximum decrease in neuronal PrPC expression.

RV-cyclin and its role in tumor development

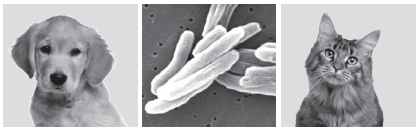
Claire H Birkenheuer, Sandra L Quackenbush, and Joel Rovnak

Walleye dermal sarcoma virus, a complex retrovirus, is the cause of seasonal dermal sarcoma in walleye fish. This virus requires tumor development for replication, and encodes two proteins that are expressed before genomic replication of the virus. These two proteins are thought to be responsible for oncogenesis. Retroviral cyclin (RV-cyclin) is one of these proteins. RV-cyclin interacts with host cyclin dependent kinase 8 (CDK8). CDK8 is a highly conserved protein across species, and has oncogenic-like properties in human cancers such as colon cancer and melanoma. It is responsible for efficient RNA polymerase II transcription elongation of another set of oncogenes, the immediate-early genes (IEGs), which include FOS, EGR1, and JUN. We hypothesized that RV-cyclin's interaction with CDK8 would enhance RNA polymerase II transcription elongation of the immediate early genes leading to tumor development. To test this hypothesis, RV-cyclin was expressed in human cell lines, and shown to increase transcript levels of the IEGs using reverse-transcription quantitative PCR (RT-qPCR). This occured in HeLa cells transiently transfected with RV-cyclin, as well as in serum-stimulated HCT116 cells which stably expressed RV-cyclin. Chromatin immunoprecipitation experiments and nuclear run-on experiments demonstrated that RV-cyclin's interaction with CDK8 enhanced RNA polymerase II transcription elongation of EGR1, as RV-cyclin expression increased RNA Pol II occupancy across the EGR1 gene locus and gave rise to a greater number of nascent-biotinylated EGR1 transcripts. Additionally RV-cyclin had no effect on phosphorylation events in the MAPK pathway as measured by SDS/PAGE and westernblot analysis, or on mRNA decay of the immediate early genes, measured using an actinomycing D transcriptional shut-off with RT-qPCR analysis. In conclusion, these data point to a role for RV-cyclin in walleye dermal sarcoma development, where RV-cyclin enhances transcription of the IEGs leading to tumor formation and growth.

The Role of Various DNA Repair Pathways in the Formation of Chromosomal Inversions

Ian M Cartwright, Matthew D Genet, Kaitlin Hannenburg, Takamitsu A Kato

Chromosomal inversions are considered to be stable chromosomal aberrations. Inversions cause a rearrangement of the chromosome, but the do not cause any loss of genomic information. Tradiationally, rBanding or gBanding techniques have been used to identify chromosomal inversions. The newest technique being used to look at inversions is mBanding, this fluorescence technique limits the study to a single chromosome and can only identify chromosomal inversion larger then 20 megabases. In this study we have used a single-cycle EdU staining technique that uses an Alexa Fluor Azide fluorescent probe to identify a single strand of DNA. Our protocol allows us to identify chromosomal inversions on the order of 0.6 megabases. In this study we have evaluated the role of various DNA repair pathways on the formation of chromosomal inversions. Homologous recombination, nonhomologous endjoing, and fanconi anemia CHO mutants were exposed to 0, 1, or 2 Gy of gamma irradiation and analyzed for inversions. To exclude the possibility of false inversion attributed to two sister chromatid exchanges occurring on a single chromatid we only counted inversions smaller then a width of a single chromatid, roughly 15 megabases in size, which we classified as micro inversion. Using previous research, we confirmed that the observed inversions were true inversions by comparing the number of observed inversions to the number of observed rings at each dose. We used a Poisson distribution to calculate the expected "false" inversions, 2 sister chromatid exchange (SCE) on one chromosome. The values for total false inversions and 2 SCE events within 15 megabases were similar to the observed numbers. We have shown that homologous recombination repair is the primary repair pathway associated with inversion formation. The inversions frequency in the mutant cells returns to the frequency observed in the control CHO10B2 when the mutation is corrected.



Amoebal Models of Non-tuberculosis Mycobacteria (NTM) Infection

Amy L Casali, William H Wheat, and Mary Jackson

Free-living amoebae (FLA) are widespread in the environment and have been shown to host a variety of potentially pathogenic microbes, including mycobacteria species. With the increased development and use of immune-modulating drugs, opportunistic infection with organisms that were previously considered non- or sub-pathogenic is increasing. Non-tuberculosis mycobacteria (NTM) cause infrequent but serious disease, particularly in the immune-compromised, and have been responsible for outbreaks of nosocomial infections in many areas of the world. We postulate that FLA hosting NTM may act as an effective "Trojan horse" allowing NTM unfettered access to potential hosts, and may shield the bacilli from antibiotics and disinfectants. In order to determine whether NTM within amoebae are rendered more resistant to broad-spectrum antibiotics, three species of Acanthamoeba, and two strains of Hartmanella species were infected with three strains of Mycobacterium massiliense and two strains of Mycobacterium chelonae known to be sources of opportunistic infections. Infected amoebae were treated with high doses of ciprofloxacin and clarithromycin (5 mg/mL) and at specified time points post-infection, an aliquot of these cultures were extracted, the amoebae lysed, and the surviving bacterial contents plated to test for NTM growth. The infected amoebae were also examined microscopically to visualize the NTM within the amoebae. Finally, the infected amoebae are allowed to encyst, then lysed and plated to determine NTM survivability in the amoebal cyst. These data may provide important information to help detect sources of nosocomial infections that can be resistant to conventional antibiotic therapy and disinfectant treatment, and will likely provide a means to investigate an alternative path of infection for these NTM.

Assessing prion species barriers and the new host effect with RT-QuIC methodology

Kristen A. Davenport, Davin M. Henderson, Candace K. Mathiason, Edward A. Hoover

The structural characteristics of the donor and recipient PrP are understood to play a major role in the propensity for trans-species prion transmission. Most studies of the effects of primary or tertiary prion protein structures on trans-species prion transmission have relied upon animal bioassays, making the influence of prion protein structure vs. host co-factors (e.g. cellular constituents, trafficking, and innate immune interactions) difficult to dissect. As an alternative strategy, we are using real-time quaking-induced conversion (RT-QuIC) to investigate the propensity for and the kinetics of trans-species prion conversion. RT-QuIC provides better-defined and easily mutable conditions of seeded conversion to study the specific role of native PrP:PrPRES interactions as a component of the species barrier. Specifically, we will compare chronic wasting disease (CWD) and bovine spongiform encephalopathy (BSE) prions by seeding each prion into its native host recPrP (full-length bovine recPrP, or full-length white tail deer recPrP) and into the opposite species. Upon establishing the characteristics of intra-species and inter-species prion seeding for CWD and BSE prions, we will evaluate the seeding kinetics and cross-species seeding efficiencies of BSE and CWD passaged through felines, a permissive host for both CWD and BSE. We hypothesize that both BSE prions and CWD prions passaged through felines will seed human recPrP more efficiently than BSE or CWD from the original hosts. In other words, the new host effect dampens the species barrier between humans and BSE or CWD. This is particularly relevant as we investigate potential means of transmission of CWD to other hosts.

Determining the presence of blood-borne prions at various time points throughout infection

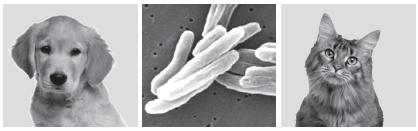
Alan M Elder, Davin M Henderson, Amy V Nalls, Edward A Hoover, Anthony E Kincaid, Jason C Bartz, and Candace K Mathiason

Human and animal transmissible spongiform encephalopathies (TSEs) are efficiently transmitted during both clinical and subclinical stages of disease. To date, four of the 227 variant Creutzfeldt-Jakob disease (vCJD) patients acquired their infection via blood transfusion from subclinical donors. Furthermore, it is currently thought that as many as one in 1,250 individuals may be asymptomatic carriers of vCJD. The development of a highly sensitive and specific antemortem diagnostic tool is important to detect carriers of the disease and to better understand the biological significance of prionemia. We have previously demonstrated blood-borne prions in subclinical and clinical TSE-infected hosts using the in vitro whole-blood-optimized real-time quaking-induced conversion assay (wboRT-QuIC) (100% specificity and >92% sensitivity). Here we investigated hematogenous prions collected from longitudinal TSE studies in hamsters, white-tailed deer, and muntjac deer inoculated by various routes (intravenous (IV); intracranial (IC); oral (PO); intraperitoneal (IP); and aerosol) using the wboRT-QuIC assay. Initial detection of circulating hematogenous prions occurred very early in subclinical disease and was present through the clinical stage of disease, until death of the animal. These studies will expand our understanding of the temporal status and biological significance of blood-borne prions.

Profiling early lung immune responses in the guinea pig model of tuberculosis (TB)

Ajay Grover, Brian Kalet, Crystal Shanley, Randall Basaraba, Ian Orme and Diane Ordway

The guinea pig model of tuberculosis (TB) has been used extensively to study the immune responses and has aided in identifying TB vaccine candidates that are currently in clinical trials. The immune responses have been studied earlier in guinea pig after day 30 post-infection but the status of early immune responses is not known. We used custom Taqman Real-time PCR arrays to study the levels of early immune response and propose that guinea pig, being an outbred animal model can be categorized into high responders and low responders like humans. High responders show higher inflammatory immune response than low responders as early as day 19 post-infection that correlates with pathology of the lungs. There was no significant difference between the early immune responses of BCG vaccinated guinea pigs and the control guinea pigs. TB antigen-specific IFN-γ could be detected in the blood as early as day 5 post-infection. We conclude that early stage inflammatory immune responses can be correlated with the lung pathology.



West Nile Virus Population Dynamics in Wild-caught Birds

Nathan D. Grubaugh, Darci R. Smith, Angela M. Bosco-Lauth, Doug E. Brackney, Corey Rosenberg, Todd Felix, Scott Sieke, Aaron C. Brault, and Gregory D. Ebel

Wild birds are the most important vertebrates in the West Nile virus (WNV) transmission cycle. Several studies have demonstrated that they generally impose purifying selection on the virus, and have suggested that they may select for novel WNV variants. However, the extent to which different important avian species influence WNV at the population level is poorly understood. Therefore, we evaluated whether different wild birds have distinct impacts on WNV populations. Specifically, we serially passed WNV in three important wild birds that are competent hosts for WNV and experience varying levels of mortality: American crows (AMCR; Corvus brachyrhynchos), house sparrows (HOSP; Passer domesticus), and American robins (AMRO; Turdus migratorius). After five sequential triplicate passages in each bird species, we will define changes to WNV population diversity and consensus sequence, replication and pathogenesis, and competitive fitness. Preliminary results indicate that AMCRs infected with the serially passaged viruses developed higher levels of viremia and experienced earlier mortality compared to birds infected with the unpassaged virus. The HOSPs developed an earlier peak viremia with the passaged virus, however the mortality and viremia differences compared to the unpassaged virus were insignificant. Passage in birds resulted in the generation of viruses with increased fitness gains in the same species and chicks compared to the unpassaged virus. Ongoing studies in AMROs will determine if other wild bird species exert similar evolutionary pressures on WNV. Additionally, fitness studies in mosquitoes will determine whether passage in birds also leads to increased fitness in mosquitoes, which was previously observed in our laboratory. Sequencing results will determine if the fitness differences are due to genetic changes in WNV following sequential passage. Collectively, these results will lend insight into the role of wild birds in the selection of novel WNV genotypes.

Hedgehog pathway expression in canine transitional cell carcinoma and normal canine bladder

Tanya Gustafson, Barbara Biller and Barbara E Kitchell

Transitional cell carcinoma (TCC) is the most commonly diagnosed tumor of the canine urinary system. Overall survival with traditional chemotherapy in this disease has not improved for many years, and so additional research into new therapeutic targets is needed. Hedgehog signaling, which regulates normal embryonic development, represents one such target. When activated in adult cells, Hedgehog signaling promotes oncogenesis and has been found to play a central role in human bladder cancer. Expression of Hedgehog signaling in canine TCC has not been previously evaluated. Therefore, in this study, Hedgehog signaling was investigated in five TCC cell lines. Hedgehog ligands (Sonic, Indian, and Desert Hedgehog), Patched 1 (PTCH1), Smoothened (SMO) and the signaling effectors Glioma-associated oncogene 1, 2 and 3 (GLI1, GLI2, GLI3) expression were evaluated through reverse transcriptase PCR. Protein expression was confirmed by Western blot. Interestingly all five canine TCC cell lines expressed Indian Hedgehog (IHH) rather than Sonic Hedgehog (SHH) as in the human disease. The Hedgehog receptor, PTCH1, was uniformly expressed at the RNA level across all five cell lines. The intermediate signaling molecule SMO was highly expressed in four out of five lines. Evaluation of the effector GLI genes revealed GLI2 expression at the RNA and protein level in all 5 cell lines. GLI1 was detectable at the RNA level but not at the protein level in all lines. GLI3 was expressed at the RNA level in 2 out of 5 cell lines. This study suggests that Hedgehog signaling does have a role in canine TCC. Further studies are needed to evaluate expression of these factors in canine TCC tumor samples, and to investigate the effect of inhibition of this pathway on tumor growth and invasion.

Comparison of first throw knot security of six different friction knots

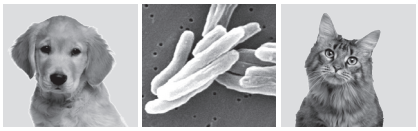
Kurtis M. Hazenfield and Daniel D. Smeak

Maintenance of first throw knot security on an encircling suture ligature placed around a vascular pedicle is vitally important for surgical hemostasis. First throw knot security is of paramount importance during ligation of any vessel as it prevents slippage or unwanted loosening until subsequent locking throws are placed. The purpose of this study was to compare first throw knot security of six different friction knots. The surgeon's throw (ST), Miller's knot (MK), Ashley modification of the Miller's knot (AMK), modified Miller's hand-tie (MHT), constrictor knot (CK) and strangle knot (SK) were evaluated. Each knot was constructed with 2-0 polyglyconate around each of two balloon dilation catheters utilized as small and large diameter vascular pedicle models and tested to failure ten times. Leak pressure measurements were normally distributed for all groups and were summarized using the mean and 95% confidence intervals. Leak pressure in all groups was compared using one-way ANOVA and Student's t-test was used to compare between groups. A least square means differences Student's t-test was used to compare identical knots between groups. $P<0.05$ was considered significant. All knots outperformed the ST in both the large and small diameter pedicle models. The MK, CK, and SK withstood pressures of >360mmHg regardless of model diameter. The ST and AMK leaked at pressures below physiologic arterial pressure in both models. The SK, CK, and MK displayed excellent global first throw knot security, consistently withstanding supra-physiologic pressures. During in-vitro testing, the SK, CK, and MK were far superior to all other friction knots, suggesting that they may be preferred for first throw use in vascular pedicle ligation and should be further evaluated for clinical use. The ST consistently leaked at sub-physiologic pressures, suggesting that it should be avoided for use as a first throw in vascular pedicle ligation.

Are marine fish a source of Brucella infection?

Jennifer Higgins, Suzin Webb, Allison Tuttle, and Richard Bowen

Purpose: The aim of this project was to determine whether marine fish are naturally infected with Brucella spp. Brucella was first isolated from marine mammals in 1994, and has since been classified as two novel species, B. ceti and B. pinnipedialis. Infection is endemic in many populations of marine mammals; however, routes of disease transmission are poorly defined. In addition, there have been three cases of zoonotic infection with marine Brucella spp. with the source of infection unknown. We hypothesize that fish are naturally infected with Brucella in the marine environment and are a potential source of disease for marine mammals and humans. Materials/methods: A polymerase chain reaction (PCR) approach was used to investigate whether Brucella infection can be detected in a population of Atlantic herring (Clupea harengus) harvested from the coast of southern New England. Samples of liver, spleen, and kidney were collected from all fish. Sample size (n=60) was calculated based on that required to detect infection in the population given an assumed 5% prevalence rate and a 95% confidence interval. Initial analysis focused on liver tissue, and extracted DNA was subjected to PCR analysis. A real-time multiplex PCR assay that targets the Brucella genus-specific gene bcsp31 was utilized in this study.Results: All liver (n=60) and spleen (n=12) samples were found to be PCR negative. Conclusions: Brucella was not detected in the herring sampled. Therefore, prevalence of infection in this population of herring is less than 5%. If marine fish are naturally infected with Brucella, prevalence of infection may be higher in fish sharing habitat with a resident population of Brucella-positive marine mammals. These fish populations will be targeted for sampling in future studies.



Monitoring RML Scrapie Prion-Seeding Activity in Neuroblastoma Cells with RT-QuIC

Clare E Hoover, Davin M Henderson, Mark D Zabel, Edward A. Hoover

Detection and monitoring of prion infection in cell cultures can be problematic due to low target protein levels in cell lysates and extended time required for detection by standard assays. We adapted the real time quaking induced conversion (RT-QuIC) assay to detect mouse-adapted scrapie (RML) by using a recombinant normal Syrian hamster prion protein (SHrPrP) (truncated, AA90-231) (Wilham et al., PLoS Pathog, 2010) as substrate and cell lysates as seeds. The sensitivity of this assay system in detecting RML prion-seeding activity in infected mouse brain homogenate was ≥10-7. For in vitro cell infection studies, murine neuroblastoma (N2A) cells were exposed in suspension to a 0.1% RML brain homogenate for 30 minutes. The cells were then seeded in 12-well plates, allowed to grow to confluency, and passaged up to six times with media changes and washes over 4 weeks. At each passage, 1 x 106 cells were removed and lysed in PBS/EDTA/Triton-x. Samples were diluted to 1 x 10^-3 in a 0.1% SDS/PBS buffer and used as seeds in RT-QuIC with appropriate controls. RML prion-seeding activity was detected in all passages of inoculated cells but no uninfected N2A control cells maintained and assayed in parallel. Our results validate the use of RT-QuIC for rapid detection and monitoring of RML scrapie prion infection using the SHrPrP substrate in N2A cell lysates. RT-QuIC has great utility in higher throughput cell culture studies in which prion protein levels are too low to be examined by assays such as western blotting or immunostaining.

Activated mesenchymal stem cells amplify antibiotic activity against chronic Staphylococcus aureus infection.

Valerie A Johnson, Tracy Webb, Stephen Dow

Background/Rationale: Antimicrobial resistance is one of the greatest challenges facing the medical community today and new interventions are needed. Recent studies have shown that mesenchymal stem cells (MSC) exhibit antimicrobial activity when activated by Toll-like receptor (TLR) ligands in vitro. We hypothesized that activated MSC could enhance the activity of conventional antibiotics in common wound infections. Methods: MSC derived from adipose tissue of mice were expanded in vitro and activated with TLR ligands to assess the effects on antimicrobial activity and production of antimicrobial peptides (AMP). The effects of MSC treatment in vivo were assessed using a mouse model of chronic S. aureus biofilm infection. Mice were treated with a series of 4 MSC i.v. injections, with or without antibiotic treatment, and bacterial load assessed over 14 days via IVIS imaging and bacterial culture of the wound site. Results: Activation of MSC with TLR ligands significantly increased production of the AMP CXCL10 and inhibited bacterial growth in vitro. The most potent TLR agonist in vitro was the TLR3 agonist polyI:C. In the chronic S. aureus infection model, activation of MSC with polyI:C resulted in decreased bacterial counts. Conclusions: Treatment with activated MSC induced AMP production and enhanced antibiotic therapy in vivo. These results suggest that stem cell therapy using activated MSC may be an effective means to enhance antibiotic therapy for chronic infections

The one assay to find them all: targeted genome capture and next-generation sequencing to simultaneously detect all feline viruses and bacteria

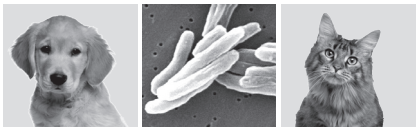
Justin S Lee, Sue VandeWoude

The development of new strategies for the detection of existing and novel pathogens would greatly benefit infectious disease diagnoses and research. Targeted genome capture is a newly developed technology whereby rare nucleic acids within complex samples (i.e. blood) can be enriched using oligonucleotide capture probes. The result is amplification of target RNA or DNA, originally present as a minute fraction of the total nucleic acids from a sample. One of the most informative applications of targeted genome capture is the subsequent use of enriched DNA for next-generation sequencing, which can provide robust genetic sequence data for targeted nucleic acids while minimizing non-target sequences. These technologies have many potential uses, but their application in animal health research and clinical settings remains relatively unexplored. Many infectious agents cause chronic, latent infections with low replication rates, rendering them undetectable by current assays despite remaining pathogenic or transmissible. We hypothesized that the combined use of these technologies would enable the simultaneous detection and characterization of multiple feline viral and bacterial pathogens. Sixteen samples representing domestic cats, bobcats, and pumas with known and unknown pathogen status were screened using a capture probe library designed to enrich diverse pathogen sequences. All 16 post-enrichment samples were pooled and sequenced on a MiSeq platform (Illumina Technologies, Inc.). We detected most of the targeted pathogens known to be present in the samples, as well as several previously undetected pathogens. The on-target genetic sequence data was sufficient to definitively characterize all pathogens detected. However, the capture assay had low specificity, and therefore, non-target genomic DNA sequences were abundant in most samples. This promising pilot study is the basis for our current project to expand the number of targeted pathogens, evaluate the sequencing of both RNA and DNA, and validate the use of this methodology with clinical samples from domestic cats.

ICV infusion of (pro)renin receptor antagonist (mPRO20) attenuates prorenin and DOCA-salt induced hypertension

Wencheng Li and Yumei Feng

We previously reported that the binding of prorenin to (pro)renin receptor (PRR) plays a major role in brain Ang II formation and development of deoxycorticosterone acetate (DOCA)-salt hypertension. In this study, we designed and developed an antagonistic peptide (mPRO20) to block the binding of prorenin to PRR. Fluorescence-labeled mPRO20 bound to both mouse and human brain tissue, and the dissociation constant was 14.5nM and 0.9nM respectively. The binding was blocked by co-incubation with prorenin, indicating the specificity of mPRO20 to PRR. To test the in vivo effect of mPRO20, C57BL/6 mice were implanted with telemetric probes and intracerebroventricular (ICV) cannula. ICV infusion of mouse prorenin (300 ng in 3μl for 10 minutes) increased BP (ΔMAP: 28±3.4 mmHg); and this effect was attenuated by mPRO20 in a dose-dependent manner (maximum effect, ΔMAP: 7±2.9 mmHg). Chronic ICV infusion of mPRO20 (40μg/kg/day, 21 days) attenuated the development of hypertension (113±3.3 vs. 134±3.6 mmHg, $P<0.05$), and the increase in brain Ang II levels (904±47 vs. 1371±88 pg/g) induced by DOCA-salt. In addition, ICV infusion of mPRO20 improved autonomic function and spontaneous baroreflex sensitivity in mice treated with DOCA-salt. In summary, mPRO20 binds to both mouse and human PRR, decreases Ang II formation and hypertension induced by either prorenin or DOCA-salt. We conclude that mPRO20 may be a novel PRR antagonist for the treatment of hypertension.



ORAL PRESENTATIONS

CLINICAL/BASIC SCIENCE

Session III – Salon II

1:00-5:00PM

Short-course antibiotic administration in dogs with pneumonia: 72 cases (2002-2013)

Hannah R Marshall, Raegan J Wells, and Michael R Lappin

The recommended duration of antibiotic administration for bacterial pneumonia in dogs is two weeks beyond radiographic resolution. This guideline can lead to months of therapy in some patients. Prolonged antibiotic administration may be unnecessary and increases the risk for development of resistant bacteria. Guidelines for humans with community acquired or ventilator associated pneumonia suggest shorter durations of therapy, with courses as short as 3 to 5 days. A retrospective evaluation of dogs treated for pneumonia at the CSU VTH over an 11 year period was performed. Medical records of 416 dogs diagnosed with pneumonia between 2002 and 2013 were evaluated. Of 416, 72 dogs received antibiotics for 35 days or less, with radiographic improvement, radiographic resolution, or significant clinical improvement following treatment. Dogs were excluded (344) due to treatment beyond 35 days (14.4%), no follow up (13%), concurrent significant disease (8.4%), or euthanasia (46.8%). Criteria tabulated included cultured organism, duration and type of antibiotic administration, pneumonia classification, and clinical or radiographic response to treatment. There were 56 (77%) dogs with bronchopneumonia, 11 (15.2%) had aspiration pneumonia, 2 (2.7%) with interstitial pneumonia, and 3 (4.1%) classified as other. The time categories for treatment were under 10 days (8.3%), 10-14 days (19.4%), 14-28 days (50%), and 28-35 days (12.5%). Twenty five dogs (34.7%) had continuation of the antibiotic prescription at an average of 14 days after initiation due to incomplete radiographic resolution, adding 7-14 days of antibiotic treatment. The remainder of the dogs did not receive continuation of the prescription (47, 65.3%). This was based upon either radiographic (35, 48.6%) or clinical (12, 16.6%) improvement. These data suggest that a shorter antibiotic course may be a safe alternative to prolonged administration in some patients. They also provide historical evidence of shorter courses to support the safety of future prospective studies.

Evaluation of two dry therapeutic diets for dogs with acute diarrhea

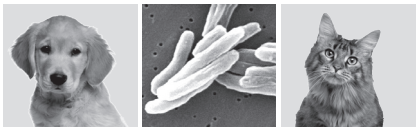
Laura ER Martin, Sara A Wennogle, H Xu, C Jean-Phillip, Michael R Lappin

Acute diarrhea is a common cause of morbidity in dogs, and in shelter dogs may delay time to adoption and strain shelter resources. The purpose of this study was to compare the use of two diets, Purina EN® and Hill's Science Diet i/d®, in a population of young, otherwise healthy shelter dogs with acute diarrhea of at least 2 days duration. Diarrhea was characterized as ≥ 4 on the Nestle Purina Fecal Scoring system. All dogs were evaluated for gastrointestinal parasites using fecal flotation and fecal immunofluorescence assay for Giardia and Cryptosporidium spp. (Meridian Diagnostics) on study entry, and all were administered Drontal Plus® (Bayer Animal Health) for 3 days. Dogs were randomly assigned to be fed EN® or i/d®, with feces and appetite scored daily for 14 days. To date, 13 dogs fed EN® and 9 dogs fed i/d® met the entry criteria and completed the study through at least Day 11. Dogs fed EN® had a mean fecal score of < 3 by Day 2 and dogs fed i/d® had a mean fecal score < 3 by Day 5, but these findings were not statistically significant. Of the dogs fed i/d®, 44.4% had a recurrence of a fecal score > 3 after Day 7 versus 15.4% of the dogs fed EN® ($p = 0.18$). Dogs fed EN® had a significantly greater ($p = 0.02$) proportion of normal stools between Days 1 – 7 (81.7%) than dogs fed i/d® (63.2%). Parasites were detected in the feces of 38.4% of dogs fed EN® and 55.4% of dogs fed i/d® but these results were not significantly different. Both diets were well tolerated and apparently effective in this study design, with dogs fed Purina EN® having a greater proportion of normal stools than those fed Hills Science Diet i/d®.

Prevalence of zoonotic parasites in shelter dogs in Veracruz, Mexico

Meagan Y Nakamoto, Andrea V Scorza, Mariel A Domingez, Dora R Salas, and Michael R Lappin

This study aimed to determine the prevalence of enteric parasites in shelter dogs in Veracruz, Mexico and to genetically characterize the Giardia duodenalis and Cryptosporidium spp. isolates. Fecal samples were submitted to the University of Veracruz, Mexico and parasites were microscopically identified after fecal flotation (FF). Samples were then shipped to Colorado State University for Giardia and Cryptosporidium immunofluorescence assay (FA) and molecular analysis. FA positive samples had total DNA extracted. PCR amplification of the SSU RNA and the heat shock protein 70 (hsp70) genes were performed on Cryptosporidium FA positive samples while PCR amplification of the triose phosphate isomerase (TPI), glutamate dehydrogenase (GDH), and beta-giardin (BG) genes were performed on Giardia FA positive samples. Of the 222 dogs examined from the La Roca shelter in Veracruz, Mexico, 97% were positive for at least one enteric parasite and over 60% tested were positive for multiple parasites. Ancyclostoma caninum, Strongyloides stercoralis, Uncinaria stenocephala, and Trichuris vulpis were found in 184 (82.9%), 65 (29.3%), 78 (35.1%), and 32 (14.4%) of the 222 samples respectively. Five Cryptosporidium spp. and two Cryptosporidium canis isolates were amplified by the 18S rRNA and by the HSP-70 assays respectively. Of the 30 Giardia spp. isolates, 16, five, and one samples were amplified by the GDH, TPI, and BG genes, respectively. Fourteen of the 19 (73.7%) PCR positive isolates typed as assemblage D by any of the three genes. Assemblages C, F, and A were amplified from two, one, and three samples, respectively. One isolate typed as assemblage D by GDH and assemblage A by TPI. The prevalence of enteric parasites of stray dogs in the La Roca shelter in Veracruz is high. The majority of Giardia assemblages were dog host adapted, but two were the human adapted assemblage A, suggesting the dogs were exposed to human feces.



Associations between isolation of Mannheimia haemolytica, antimicrobial resistance and use, and morbidity and mortality in feedlot cattle

Noelle R Noyes, Katharine M Benedict, Sheryl P Gow, Calvin W Booker, Sherry J Hannon, Tim A McAllister, and Paul S Morley

Purpose: Mannheimia haemolytica has been implicated as one of the most significant etiological agents associated with bovine respiratory disease in cattle. Objectives of this study were to explore risk factors associated with isolation of susceptible and resistant M. haemolytica in a commercial feedlot setting, and to explore associations between colonization and health outcomes. Materials/Methods: Cattle (n=5,498) from 4 feedlots located in Alberta, Canada were randomly enrolled and sampled on arrival and then later in the feeding period. Nasopharyngeal samples were cultured for M. haemolytica and tested for resistance to 21 antimicrobials. Records of antimicrobial use (AMU) and health events were collected. Associations between AMU history and isolation of M. haemolytica, and colonization and health outcomes, were modeled using generalized estimating equations (GEE). Results: Models showed a negative association between parenteral AMU in enrolled cattle and isolation of M. haemolytica (OR 0.2, 95%CI 0.02 – 1.2, P=0.006) and a positive association between parenteral AMU in penmates of enrolled cattle and isolation (OR 1.5, 95%CI 1.05 – 2.2, P=0.02). AMU was not associated with isolation of single-resistance M. hemolytica of any phenotype. However, parenteral AMU in penmates of enrolled cattle was associated with greatly increased odds of recovering multi-drug resistant M. haemolytica in sampled cattle (OR 23.9, 95%CI 8.4 – 68.3, P<0.0001). In addition, cattle from which M. haemolytica was recovered on arrival had a higher likelihood of being diagnosed with fever within 10 days of arrival (OR 1.7, 95%CI 1.1 – 2.4, P=0.07). Conclusions: These findings generally support AMU protocols that target high-risk and clinically ill cattle, particularly at arrival in the feedlot. However, the link between AMU and MDR M. haemolytica warrants further investigation to determine the effects of such MDR on treatment efficacy.

Establishment of a Reference interval for Fibrinogen in Healthy Ornate Box Turtles (Terrepene ornata ornata)

Lily A Parkinson and Matthew Johnston

The number of turtle-owning households in the United States is on the rise. Providing veterinary care for this growing population of pet turtles can be a challenge, as many diagnostic tools available in other domestic species are not well researched in chelonians. Even turtle blood chemistry panels require a great deal of further study, making the interpretation of any results from a blood draw challenging for veterinarians. To begin to glean useful information from blood draws in pet chelonians, reference values for blood parameters in pet turtle species must be established. It has been suggested that fibrinogen may be a useful blood component to quantify in turtles with infections. This study sought to establish a reference interval for fibrinogen in ornate box turtles (Terrepene ornata ornata). A total of 42 healthy turtles and five turtles deemed to be ill were enrolled in the study. Cohort sorting occurred via complete blood counts and a blood chemistry panel. All healthy turtles were also strategically dewormed prior to fibrinogen quantitation to further assure their health. A D'Agostino and Pearson omnibus normality test indicate that the healthy turtle fibrinogen values form a Gaussian distribution while inclusion of the unhealthy cohort results in a non-Gaussian distribution (p<0.0001). Results of this study indicate that fibrinogen may assist veterinarians caring for turtle patients. Fibrinogen could be a useful blood test to determine whether a turtle has an underlying infectious of inflammatory process.

Risk factors for the development of canine lymphoma in North American dogs: 18,826 cases (1990-2009)

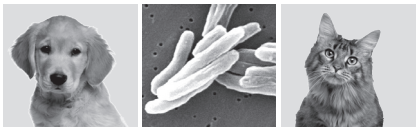
Audrey Ruple-Czerniak and Paul S Morley

Purpose: Lymphoma is one of the most common life-threatening cancers of dogs, accounting for up to 25% of all diagnosed malignancies. Despite the frequency with which lymphoma is diagnosed in dogs, few risk factors for the development of the disease have been identified. Materials and methods: A retrospective case-control study design was utilized to examine associations between exposure variables and the outcome of canine lymphoma. The Veterinary Medical Database, the most comprehensive source of veterinary medical information available in the United States, was used to identify dogs diagnosed with canine lymphoma at veterinary teaching hospitals in the United States and Canada. A comparison group was selected from the same population of dogs and was matched to the case population in a 1 to 4 ratio based upon admitting institution, year of hospital admission, and age of the dog. The association between risk factors and the diagnosis of canine lymphoma was estimated using logistic regression models. Results: Our study population consisted of 67,712 dogs admitted to one of 26 veterinary teaching hospitals in North America. Results of the multivariable logistic regression analysis showed more than 25 breeds at increased risk of developing lymphoma and more than 35 breeds at decreased risk of developing disease as compared to dogs of mixed breed. Dog breeds classified by the American Kennel Club as belonging to the Sporting, Working, or Terrier groups were found to have increased risk of developing lymphoma and those breeds belonging to the Hound and Toy groups were found to have decreased risk of developing lymphoma as compared to dogs of mixed breed. In addition, male dogs were found to be at higher risk of developing lymphoma as compared to females. Conclusions: These data suggest dogs of different breeds and genders have different levels of risk associated with development of lymphoma.

Effects of subcutaneous or intranasal vaccine administration on clinical signs in FHV-1 infected cats without previous vaccination

Sara A Wennogle, Michael R Lappin

While a transient state of immunosuppression after SQ vaccination has been reported in the dog, similar information is not available for cats. The objective of this study was to determine the effects of one dose of IN or one dose of SQ modified live virus containing vaccine on the clinical signs of FHV-1 in cats with chronic infection. Mixed sex, 7 month-old, vaccine-naïve kittens (n = 12) with mild FHV-1 associated illness were randomly divided into 2 groups of 6 kittens (3 males, 3 females) and housed and handled separately to avoid cross-contamination between groups. Total clinical scores, ocular scores, respiratory scores, and FHV-1 DNA shedding were determined before (Days 0 – 6) and after (Days 7 – 20) vaccination with either a FHV-1, FCV, and FPV containing SQ vaccine or a FHV-1 and FCV containing IN vaccine. Ocular scores in kittens vaccinated SQ were significantly greater Days 7 - 20 when compared to Days 0 - 6 ($p= 0.041$); this finding was not apparent in the kittens vaccinated IN. Total clinical scores, total respiratory scores, and viral DNA shedding were not different between the groups within the time periods. Although mild, the findings suggest that kittens infected with FHV-1 prior to vaccination may have clinical signs exacerbated transiently after administration of SQ modified live vaccines. The mechanism for this finding is not currently known. Use of IN modified live FHV-1 and FCV containing vaccines should be considered in kittens with known or suspected FHV-1 infections. The project was funded by Pfizer Animal Health.



DNA damage produced by 64Cu-ATSM high LET Auger electrons

Dayton D. McMillan, Junko Maeda, Justin Bell, Takamitsu A. Kato

The oxygen status of tumors is an important clinical diagnostic factor. In radiotherapy applications, hypoxic tumors display resistance to traditional external beam radiotherapy, instigating interest in finding alternative, more effective methods to treat these tumors. 64Cu-diacetyl-bis(N4-methylthiosemicarbazone) (64Cu-ATSM) has shown clinical usefulness in imaging and experimental radiotherapy of solid state tumors due to its ability to concentrate in hypoxic tissue regions and emit multiple forms and energies of radiation. Intrinsic to the use of 64Cu-ATSM for radiotherapy is the decay mechanism of 64Cu which emits high linear energy transfer (LET) auger electrons. Presently the biological significance for cell killing and DNA damage due to high LET electrons released in the decay of 64Cu is unknown. To evaluate how high LET auger electrons play a role in cell death and tumor control, Chinese hamster ovary (CHO) DNA double strand break (DSB) proficient and deficient cell lines were treated with 64Cu-ATSM. Initial results show similar cell survival at constant doses of 64Cu-ATSM, indicating lethality due to high LET radiation. Survival of CHO cells exposed to 64Cu-ATSM is compared with other known high and low LET forms of radiation. These findings indicate 64Cu-ATSM may have potential clinical advantages over low LET radiations for more effectively treating hypoxic tumors.

Evaluation of the accuracy of a commonly used dynamometric wire tensioner

Shyla E Myrick, Christopher M Gauthier, and Ross H Palmer

Circular external skeletal fixators (CESF) are used in human and veterinary medicine for fracture and arthrodesis stabilization, skeletal deformation correction, and bone transport. They utilize fine tensioned wires that engage bone segments and secure them to a modular frame. The wire tension has significant effects on overall fixator stiffness, which can greatly affect healing. Significant variation has been shown in the accuracy of wire tensioning devices in human orthopedics, but this has not yet been evaluated in veterinary wire tensioners. The purpose of this study was to evaluate the accuracy of the most commonly used US veterinary dynamometric wire tensioner. Five new IMEX wire tensioners were tested by tensioning a Kirshner wire affixed to the load cell of a servohydraulic materials testing machine via a custom testing fixture. The wire was loaded to each of the 3 preset manufacturer settings on the tensioner, which correlated with the appropriate wire tension for three different fixator ring sizes (66, 84, and 118 mm). The tensioners were each tested 6 times at each ring size while actual wire tension was measured. Percentage error of the tensioners at each setting was calculated by dividing the actual wire tensions by the expected values and subtracting 100. The mean measured and standard deviation tension values for 66, 84, and 118 mm rings were 50.4±3.5, 78.6±2.4, and 105.4±2.6 kg, respectively. The percentage error was -11.5%, -2.9%, and 11% for the 66, 84, and 118 mm rings, respectively. The tensioners tested tended to under-tension the wire in small ring configurations and over-tension the wire in large ring configurations compared to the tension value given by the device. Caution should be used when tensioning wires in a 66 mm ring fixator when large loads are expected.

Small RNA response of Culex quinquefasciatus to West Nile virus infection: relationship to vector competence.

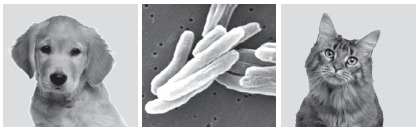
Abhishek N Prasad, Doug E Brackney, Benjamin J Dodd, Darci R Smith, Thomas D Harrison, Corey L Campbell, Jennifer E Beane, and Gregory D Ebel

Arthropod-borne viruses (arboviruses) are a taxonomically diverse group of viruses that perpetuate in transmission cycles between insects and vertebrate hosts. Culex mosquitoes are a major vector of arboviruses worldwide, including West Nile virus (WNV). Vector competence is the ability of an arthropod host to transmit a pathogen, and is influenced by intrinsic and extrinsic factors. However, the molecular determinants within the host that influence vector competence remain poorly understood. Variation in vector competence has long been recognized between individuals, populations within species, and between species. When exposed to the same virus-containing bloodmeal, some mosquitoes fail to become infected, others become infected but limit virus replication and dissemination, while others develop disseminated infection and ultimately transmit virus. RNA interference (RNAi) is the primary antiviral immune response in mosquitoes to arbovirus infection, however, the role it plays in shaping vector competence in mosquitoes is unknown. We sought to characterize the mosquito RNAi response to WNV infection and determine its influence on the vector competence using colonized Culex quinquefasciatus mosquitoes. Mosquitoes were exposed to WNV in an artificial bloodmeal and held for various periods of EI. Small RNA (sRNA) responses were then profiled using deep sequencing. To characterize the early sRNA responses to WNV, midguts were removed from mosquitoes 12, 24, and 72 hours after feeding and sRNAs mapped to the WNV genome. To assess the relationship between sRNA responses and virus dissemination from the midgut, midguts and legs were removed from mosquitoes at 7 and 14 days post feeding. sRNA responses from mosquitoes that permitted WNV dissemination from the midgut into peripheral tissues were compared to those that limited WNV to the midgut. The goal of this project is to characterize the sRNA responses of mosquitoes to WNV infection, and determine the extent to which RNAi influences vector competence in this system.

Amplification of tumor vaccine immunity by co-administration of ondansetron

Daniel Regan, Amanda Guth, Michelina Petri, Steven Dow

We have recently discovered that inflammatory monocytes recruited to vaccine-draining lymph nodes can function as potent suppressors of humoral and cell-mediated immune responses to vaccination. Recruitment of these inflammatory monocytes is mediated primarily by local production of CCL2 secondary to vaccine adjuvant-induced inflammation. We also found that monocyte migration blockade by means of small molecule antagonists of the CCL2 receptor, CCR2, could amplify vaccine immunity in mice. Therefore, we sought to identify already approved and cost-effective drugs that could be repurposed for use as monocyte migration inhibitors and novel vaccine enhancing agents (VEA) in humans and companion animals. In silico modeling was performed to identify potential CCR2 antagonists from a library of existing, FDA-approved, and safe drugs. Ondansetron was identified as a strong, predicted CCR2 antagonist. Monocyte migration assays and Ca2+ flux signaling assays confirmed the CCR2 antagonist activity of ondansetron in vitro, while an inflammation model confirmed monocyte migration inhibition in vivo in mice. Therefore, the VEA activity of ondansetron was assessed in a murine lymphoma vaccine model. We found that co-administration of ondansetron significantly enhanced the anti-tumor activity of the lymphoma vaccine in a therapeutic tumor vaccine setting, resulting in significant inhibition of tumor growth compared to mice receiving the tumor vaccine alone or ondansetron alone. Co-administration of ondansetron at the time of vaccination resulted in significant amplification of tumor-specific T cell immunity, including both CD4 and CD8 T cell responses. Mice treated with ondansetron also had significant reductions in macrophage infiltration into tumor tissues, along with significant reductions in tumor angiogenesis. The vaccine enhancing activity of ondansetron appeared to be mediated through enhancement of antigen-specific T cell responses, a reduction in tumor-macrophage accumulation, and decreased tumor angiogenesis. We conclude therefore that ondansetron may be effectively repurposed as an effective VEA for tumor vaccines.



Liposome-antigen-nucleic acid complexes protect mice from lethal challenge with Western and eastern equine encephalitis viruses

Amber B Rico, Aaron T Phillips, Tony Schountz, Ann M Toth, Don L Jarvis, Ann M Powers, Kenneth E Olson

Alphaviruses are mosquito-borne viruses that cause significant disease in animals and humans. Western and eastern equine encephalitis virus (WEEV and EEEV), two New World alphaviruses, can cause fatal encephalitis and EEEV is a select agent of concern in biodefense. However, we have no antiviral therapies against alphaviral disease and current vaccine strategies target only a single alphavirus species. In an effort to develop new tools for a broader response to outbreaks, we designed and tested a novel alphavirus vaccine comprised of cationic lipid nucleic acid complexes (CLNCs) and the ectodomain of WEEV E1 protein (E1ecto). Interestingly, we found that the CLNC component, alone, had therapeutic efficacy, as it increased survival of CD-1 mice following lethal WEEV infection. Immunization with the CLNC-WEEV E1ecto mixture (lipid-antigen-nucleic acid complexes; LANACs) using a prime/boost regimen provided 100% protection in mice challenged with WEEV subcutaneously, intranasally, or via mosquito. Mice immunized with LANAC mounted a strong humoral immune response, but did not produce neutralizing antibodies. Passive transfer of serum from LANAC E1-ecto immunized mice to non-immune CD-1 mice conferred protection to WEEV challenge, indicating that antibody is sufficient for protection. In addition, the LANAC E1-ecto immunization protocol significantly increased survival of mice following intranasal or subcutaneous challenge with EEEV. In summary, our LANAC formulation has therapeutic potential and is an effective vaccine strategy that offers protection against two distinct species of alphavirus irrespective of the route of infection.

Optimized Methodology for Obtaining and Analyzing Limited Quantities of Soil Contaminated with Radionuclides

Brett L Rosenberg, Georg Steinhauser, and Thomas E Johnson

Following major nuclear accidents, radionuclides diffuse through soils differently based on soil composition and the chemical properties of the nuclides. It is necessary to be able to identify small quantities of various nuclides, especially several years after their deposition, through radiometric methods, such as gamma spectroscopy, LSC, NAA, and proportional counting; and non-radiometric methods, such as mass spectrometry. In this study, the top 15 cm of soil was obtained from different sites in the Fukushima prefecture, from the gate of the NPP to 40 km from the NPP, using a soil core sampler. Each sample was cut into six 2.5 cm sections to analyze trends in nuclide diffusion. Since only limited quantities of soil were available, we sought to optimize the analysis of the material. 134Cs and 137Cs activity was measured using nondestructive gamma spectroscopy prior to any other step. Further analyses will be done after cutting the 2.5 cm samples into quarters to detect levels of 36Cl, 3H, 90Sr, and other nonvolatile radionuclides, including 239Pu, 240Pu, and 241Pu. 36Cl activity will be analyzed in the first quarter after separation from other nuclides through volatilization of chloride in the soil using sulfuric acid and the reaction of hydrochloric acid vapors with silver nitrate. The second quarter will be heated to collect 3H in the form of tritiated water. The third quarter will be used for the chemical dissolution and chromatographic separation of 90Sr and other nonvolatile radionuclides. The fourth quarter will be saved for future studies. Preliminary results show that 134Cs and 137Cs activity relative to soil depth remained linear on a log scale and maintained a constant ratio for certain soils. Some soils have shown no or minimal Cs activity. Additional analysis is still ongoing.

Evaluation of aerosol shedding in growing pigs following administration of porcine reproductive and respiratory syndrome virus vaccine

Anna Samson, Scott Dee, Joel Nerem, Brett O'Brien

Porcine reproductive and respiratory syndrome (PRRS) costs the United States swine industry one billion dollars annually. 60% of weaned pigs are PRRSv negative, yet 58% percent will become PRRSv positive by market age. This is a driving factor for producers to vaccinate their growing pigs. PRRSv has been demonstrated to travel through the air 9.1 km. Transmission of a vaccine virus to negative herds or herds vaccinated with a different strain is a concern. This characteristic should be evaluated on individual strains because aerosol transmission of PRRSv is isolate dependent. The objective of this study was to monitor aerosol shedding of the Fostera PRRS vaccine virus in growing pigs. One thousand and four weaned pigs were vaccinated with Fostera PRRS vaccine in a commercial nursery. The pigs were confirmed PRRSv negative by PCR and ELISA. Oral fluids were collected in the nursery on days 3, 7, 14, and 21 post-vaccination to monitor PRRS viremia by PCR. Four air samples were collected daily with a liquid cyclonic collector for 21 days. Air samples were tested for PRRSv RNA by PCR. Sequencing, virus isolation and titration were performed on all positive air samples. A positive control was conducted to determine the concentration at which the air collectors used could detect this vaccine strain. This was an observational study (n=1) and descriptive statistics were used to present the results. Four of 42 (9.5%) indoor air samples and 1 of 42 (2%) outdoor samples were positive, however, were unable to be sequenced due to inadequate quantity. A TCID50 assay conducted on all positive air samples was zero, and attempted growth on PAM cells was unsuccessful. Oral fluids confirmed shedding of Fostera PRRSv at all the time points post-vaccination. These data demonstrated minimal risk of transmission of the Fostera vaccine strain.

Dynamic HLA-linked changes in insulin-binding B cells in pre-diabetic and new onset diabetic patients as well as their first-degree relatives.

Mia J. Smith, Shannon K. O'Neill, Thomas A. Packard, Lisa Fitzgerald-Miller, Daniel Stowell, Rochelle Hinman, Peter A. Gottlieb, and John C. Cambier

Given the efficacy of B cell depleting therapies in type 1 diabetes (T1D), as well as the observed necessity for anti-insulin B cells for disease development in NOD mice, we hypothesize that insulin-binding B cells (IBCs) play a pathogenic role in development of T1D in humans. Moreover, we believe pathogenic IBCs are normally silenced by anergy, a type of B cell tolerance wherein autoreactive cells occupy peripheral lymphoid organs but are antigen unresponsive. Thus, we hypothesize there is a loss of anergic IBCs prior to development of T1D that contributes to disease. Using a magnetic particle enrichment scheme to enrich for IBCs, we explored the frequency and surface phenotype of IBCs in the peripheral blood of subjects along the continuum of T1D development. We found a decrease in anergic IBCs in the peripheral blood of pre-diabetic and new onset patients that returns to normal levels in diabetics 1 year post diagnosis. Interestingly, in first-degree relatives, we found that some individuals exhibit a normal anergic B cell phenotype, while others display a decrease of anergic B cells similar to the pre-diabetic and new onset patients. Stratifying FDRs based on their known HLA status, we found the FDRs with the fewest anergic B cells tend to have the high-risk HLA alleles linked to development of T1D. Moreover, when we examined the total B cell population, we found a similar decrease in the anergic population, suggesting loss of anergy, regardless of autoantigen specificity, may predispose individuals to development of autoimmunity in general, the precise nature of which is determined by other genetic risk factors such as HLA. Hence, our results suggest a role for perturbation of B cell anergy in development of T1D, and perhaps other autoimmune diseases, and shed light on the role of autoreactive B cells in the pathogenesis of T1D.

POSTER PRESENTATIONS

Experimental infection of goats with MERS-CoV

Danielle R Adney, Vienna R Brown, Helle Bielefeldt-Ohmann, and Richard A Bowen

The Middle East respiratory syndrome coronavirus (MERS-CoV) was recently discovered in Saudi Arabia and adjoining countries. Clinical signs associated with human infection mimick those resulting from infection with severe acute respiratory syndrome (SARS) virus, and MERS-CoV poses a substantial threat to global public health. While the virus reservoir has not fully been elucidated, field studies support the hypothesis that bats and dromedary camels may contribute to human infection. Case studies also suggest that goats may play an important role in MERS epidemiology, although their specific role is currently unknown. Furthermore, species tropism has limited available animal models to Rhesus macaques, making development of other models essential to understand transmission, pathogenesis, and to evaluate therapeutics. We experimentally inoculated goats of differing ages with MERS-CoV to gain appreciation of their potential to serve as a reservoir host or alternative animal model. Goats inoculated with the virus were co-housed with non-inoculated goats, and were monitored for clinical disease, viral shedding, and seroconversion. Animals were necropsied at sequential time points after infection, and a battery of tissues was harvested for characterization of pathology and virus load. Initial results suggest that goats produce antibodies in response to MERS-CoV exposure and, as in humans, infection is associated with a range of disease severity. If we find that goats shed significant quantities of virus, that would indicate that goats may plan an epidemiologically important role as competent hosts for MERS-CoV, and that goats may be a valuable animal model for this emerging pathogen.

Nav1.6 somato-dendritic localization in hippocampal neurons is via an ankyrinG-independent mechanism

Elizabeth J Akin, Kristen Brown, Sanaz Sadegh, Aubrey V Weigel, Jean-Baptiste Masson, Diego Krapf, Michael Tamkun

Voltage-gated sodium (Nav) channels are responsible for the initiation of the neuronal action potential. This function is innately tied to its subcellular polarized distribution in neurons, with a high concentration of Nav protein within the axon initial segment (AIS). This localization, and thus function, is lost after traumatic brain injury. Furthermore, the number and isoform of Nav channels is altered in some forms of epilepsy. Despite the importance of this protein and its localization, little is known about the real-time dynamics of this channel on the neuronal surface. To address Nav localization and dynamics, we created a Nav1.6 construct tagged with the photoswitchable fluorophore, Dendra2, which was expressed in cultured rat hippocampal neurons. We combined single-particle tracking with photoactivated localization microscopy (spt-PALM) such that we could follow a small subset of Nav1.6-Dendra2 molecules. A steady-state density of active fluorophores was maintained via a low-power activation laser and the trajectory of each molecule was determined using an automated detection and tracking algorithm. Consistent with previous observations, this method showed that AIS Nav1.6 channels are stably anchored, presumably to AnkyrinG. In contrast, somato-dendritic Nav1.6 channels showed both diffusive behavior and periods of transient confinement within specific membrane regions, thus creating small membrane clusters. To determine if this transient confinement is due to interactions with ankyrinG, we removed the ankyrin binding motif (ABM) from Nav1.6. Despite the loss of axonal localization, this mutant channel still trafficked effectively to the surface. Surprisingly, single-particle tracking of both the full-length and mutant channels demonstrated that both channels have similar behaviors of transient confinement. This implies that the Nav1.6 somatic localization is by a novel, ankyrinG independent method.

Effect of mass drug administration of ivermectin on malaria infection in Burkina Faso, West Africa

Alout Haoues, Seaman Jonhatan, Burton Timothee, Nowak Wojtek, Krajacich Benjamin, Meyers Jacob, Yerbanga Serge, Cohuet Anna, Bougouma W Roland, Dabiré K Roch, Foy D Brian

Mass drug administration of ivermectin to control filariasis and onchocerciasis diseases has been shown to reduce the mosquito survivorship, particularly in Anopheles gambiae the main vector of malaria in Africa. The treatment also reduced the proportion of mosquitoes that are able to transmit the malaria parasite, Plasmodium falciparum. Thus ivermectin has been proposed as a novel drug to control malaria transmission. To determine the underlying mechanism, we tested in field situation whether ivermectin inhibits the development of Plasmodium parasites in the Anopheles mosquito vector or whether ivermectin kills preferentially malaria-infected mosquitoes. We first performed indoor collection of blood fed mosquitoes in two villages in Burkina Faso, one received ivermectin treatment the other not (control). Mosquitoes were held in field insectary during six days before dissection and malaria parasite counting. Prevalence and intensity of infection were compared between the treated and the control villages. We also infected recently-collected mosquitoes with Plasmodium field isolates using the standard membrane feeding assay protocol in presence or absence of a low dose of ivermectin. Survivorship after blood feeding was analyzed. Parasites were counted in midguts at day 7 and analyzed using a generalized linear mixed model to account for pseudo-replication between the different blood donors. From field data, oocyst prevalence dropped only during the first week after ivermectin treatment but the number of parasite does not decrease. From the lab experiment, the low dose of ivermectin does not inhibit the parasite development and does not affect the survival of infected mosquitoes during the first week after blood feeding. It suggests that ivermectin protect from infection but does not affect the parasite development. Further work is ongoing to reproduce the field conditions where the drug last longer with parasites in the human bloodstream and where the fluctuation of various environmental factors impacts vector-pathogen interactions.

Development of two tabletop exercises of zoonotic disease outbreaks to train senior veterinary students at The Ohio State University

Madeline M Anna, Jeanette M O'Quin, Armando E Hoet, and John M Crawford

This Master's level integrative writing project developed two tabletop exercises (TTX) centering on zoonotic disease outbreaks of veterinary public health importance to train veterinary medical students at The Ohio State University (OSU). Veterinarians are increasingly taking on important roles in the field of public health. This creates a clear need to train senior veterinary students to handle public health issues, especially collaborating with local and state partners to combat zoonotic disease concerns. While TTX are commonly used for disaster response preparedness, they haven't been utilized in veterinary training suggesting that this project will add new material to the existing knowledge base of TTX. Two TTX were developed with the goals of increasing veterinary student knowledge of public health infrastructure and the veterinarian's role in zoonotic disease response. The two zoonotic diseases chosen to incorporate into TTX were Chlamydophila psittaci and Salmonella Newport. Learning objectives were developed in order to measure outcomes. The exercises were expertly reviewed by 3 veterinarians employed at the CDC and state and local health departments, and piloted with two separate groups of senior veterinary students in the OSU preventive medicine rotation. Results showed positive feedback from the participants. The success of these TTX with veterinary students led to inclusion of the Salmonella TTX in a continuing education session for practicing veterinarians at the Ohio Veterinary Medical Association's 2013 Midwest Veterinary Conference. The initial success of this interactive method in training medical students and professionals suggests development of more TTX and wider use in veterinary medical colleges.

Genetic modification of stem cells with scAAV-equine-BMP-2 and protein expression before and after cryopreservation

Alyssa N Ball, Laurie R Goodrich

Fracture treatment in horses is fraught with difficulties. During the repair of long-bone fractures, equine surgeons work at the mechanical limits of implants available for bone and the regions where implants are applied often have a paucity of soft tissue coverage. Catastrophic fractures and the plight of recovery have been spotlighted in the horse most recently by the racing careers of Eight Belles and Barbaro, respectively. Bone marrow derived mesenchymal stem cells (MSCs) have shown efficacy in their ability to accelerate healing of a variety of connective tissue injuries, including bone, using various growth factors to direct their differentiation in vitro. Furthermore, many studies have shown osteo-induction of MSCs in response to genetic modification with bone morphogenic protein-2 (BMP-2) using a gene therapy vector, self-complementary Adenoassociated Viral Vector (scAAV). Our hypothesis was that genetic modification of MSCs overexpressing BMP-2 will induce osteogenesis in cell culture monolayer and protein expression will not be reduced if cells are cryopreserved. Our specific aims were to determine the most appropriate dose of scAAVequine-BMP-2 to induce osteogenesis of MSCs in cell culture monolayer and to compare BMP-2 expression in MSCs before and after cryopreservation. Our preliminary data suggests MSCs should be transduced with 12,000 viral particles per cell (vpc) as they appeared more osteogenic and stained more positively for calcium than cells transduced with doses of 4,000 or 8,000 vpc. Our data also suggests that cells should be maintained for 7 days post-transduction before cryopreservation. Cells maintained for 4 days post-transduction scored and stained less osteogenic, although their BMP-2 protein levels were elevated. Data is pending following cryopreservation. In the future, cells should be placed in a fibrin glue to determine if changes in protein expression occur. If not, a delivery method for fracture repair in the horse can be perfected.

Use of laser-guidance for canine limb circumference measurement

Ana Bascuñán, Sasha Foster, Sangeeta Rao, Penny Regier, Felix Duerr

Purpose: Assessment of muscle mass in canine patients is used as an outcome measure of orthopedic disease for clinical and research purposes. Definitive measurement of muscle mass involves advanced imaging and is not practical in most cases, thus limb circumference is used to estimate changes in muscle mass over time. Previous studies have attempted to describe an objective method for measurement of limb circumference, but have shown poor inter- and intra-tester reliability. In humans, several devices have been created that improve reliability of measurement by controlling height and angle of measurement along the limb. However, these devices are difficult to apply to the canine patient due to differences in anatomy. To address this shortcoming, the authors have developed a device that projects a laser line across the canine limb to guide placement of the measuring tape. We hypothesized that this device would decrease inter-tester variability in measurement. Materials and Methods: Ten observers measured eight canine thighs as previously described (CONV group) and utilizing the novel device (LASER group). The greater trochanter and lateral femoral condyle were used as palpable landmarks. One measurement was taken by estimating the midpoint between these landmarks (CONV group) and a second measurement of the same thigh was made using the laser-guidance device (LASER group). Measurements were taken separately and in a random order by each observer. The intraclass correlation coefficient (ICC) was calculated for each method of measurement. Results: The ICC for the LASER group (0.885, 95% CI 0.77-0.999) was higher with a tighter confidence interval when compared to the CONV group (0.858, 95% CI 0.71-1.005). Conclusions: The laser-guidance device may decrease variability in measurement of canine limb circumference. Further evaluation of the device with a larger sample size is indicated.

Development of immune mediated polyarthritis after vaccination

Victoria J Beale, Julia K Veir

Canine immune-mediated polyarthritis (IMPA) is described as inflammation in multiple joints that can lead to systemic effects as well joint pain and lameness. A causal relationship between vaccination and IMPA in dogs has been suggested, however, there is a lack of research to verify this association.The purpose of this retrospective case controlled study was to investigate if there is a correlation between vaccination and the development of IMPA compared to a control population. Medical records from 18 client-owned dogs with IMPA were obtained between 2009 and 2013. Dogs were diagnosed with IMPA based on clinical signs, joint fluid analysis with microscopic evidence of sterile inflammation and a negative serology for tick borne disease. Frequency of recent vaccination was compared between the IMPA group and an age and temporally (presentation to the same clinical service +/- 14 days) matched control group. Breed and sex were determined to be variables that would be unlikely to influence vaccination status. There was no statistical difference between the two groups in age (median 6 years, range 1-11). In the IMPA group 12 (66%) were spayed females, 5 (28%) were castrated males, and 1 (6%) was an intact male. In the control group 1 (6%) was an intact female, 10 (55%) were spayed females, 6 (33%) were castrated males and 1 (6%) was an intact male. One of 18 dogs (6%) in the IMPA group had been vaccinated within 30 days of onset of clinical signs and presentation to CSU-VTH. Of the control group, 1 of 18 dogs (6%) was vaccinated within 30 days of presentation to CSU-VTH. There was no statistical difference between these two groups. There was no correlation found between recent vaccination and onset of IMPA in this group of dogs.

Effects of iron chelation on monocyte metabolism; changes in CD-36 and GLUT-1 expression

Kirsten Becker, Natalie Lakey, David Ackart, Brendan Podell, and Randall Basaraba

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), infects nearly one third of the world's population. A loss of metabolic homeostasis during Mtb infection in humans and guinea pigs may contribute to TB pathogenesis by altering immune cell function and providing a readily available source of host-derived nutrients. Iron is a critical cofactor for numerous enzyme systems in both Mtb and host cells. Mtb effectively outcompetes host-derived iron through the production of iron siderophores known as mycobactin and exochelin in the intracellular microenvironment. The chelation of host iron leads to downstream changes in gene expression and cellular metabolism, which we hypothesize contributes to the increased availability of host-derived lipids and carbohydrates to intracellular bacilli. We hypothesized that treatment of monocytes with the iron chelators mycobactin or deferoxamine mesylate (DFO), increases expression of GLUT-1 and CD36, membrane transporters for glucose and long chain fatty acids, respectively. The human monocyte cell line, THP-1, was treated with DFO and mycobactin, followed by analysis of relative gene expression of GLUT-1 and CD36 by qRT-PCR. Expression of these proteins was also analyzed by western blot. It is anticipated that enhanced expression of both membrane transporters will increase cellular uptake of glucose and fatty acids, as measured by incorporation of C13 heavy glucose and palmitic acid isotopes in both iron chelator-treated cells and cells infected with M. bovis BCG. Correcting or preventing the alterations in cellular metabolism by Mtb infected macrophages may represent a therapeutic target that can be used as an adjunct to conventional antimicrobial drug treatment of TB.

Low Dose Radiation exposure causing time related saturation of Gamma-H2AX in Human Fibroblast cells despite differences in absorbed dose

Justin J Bell, Carissa M Burke, Ian M Cartwright, Takamitsu A Kato

Studies have been conducted to predict the mortality and carcinogenic effects of low dose radiation (LDR) exposures. LDR is generally defined between 0 to 100mSv or about 0.1Gy. Commonly, LDR exposures to the public come from environmental exposures caused by high background levels often based on and individual's geographic location or occupation. Generally most of the LDR research that has taken place uses epidemiologic studies in order to project the risk associated with certain radiation exposures and the likelihood of genetic mutations or prediction of cancer formation. At the cellular level, radiation exposures are known to cause breaks in the cell's DNA. Innate DNA damage repair mechanisms in cells are responsible for mending said damaged DNA. These DNA repair pathways however have the potential of causing miss repairs that can alter the natural sequence of nucleic acids thus creating genetic mutations that can be extrapolated to carcinogenesis or the initiation of apoptosis. This study focuses on the cellular response of four human fibroblast cell lines, AG1522 and GM2149 GM38 and GM8399, to prolonged LDR exposures. Cells were initially synchronized in G0 by contact inhibition as a means to prevent further cell cycling before being exposed to two different LDR Cs-137 sources. Differences in Radioactive sources allowed for sample exposures to vary in dose rate from 1cGy/hr to 5.5 cGy/hr. A γ-H2AX assay was performed as a means to quantify the DNA damage the cells had occurred, as well as study the response and prevalence of γ-H2AX over time with constant LDR exposure. Immunocytochemistry followed by florescence microscopy was utilized to detect and quantify the number of γ-H2AX foci present. Preliminary data suggests that there is a time dependent saturation of γ-H2AX activation that appears to be similar between the variance of LDR exposure yet relative to each cell line.

Quantitative comparison of advanced glycation end-products in the serum of diabetic to non-diabetic cats

Jeret M Benson, Rebecca M Timmons, and Craig B Webb

In human diabetics oxidative stress is known to increase production of advanced glycation end-products (AGE). Increased production of AGE contributes to the adverse clinical consequences associated with Type 2 diabetes. The hypothesis of this study is that oxidative stress increases AGE production in the serum of diabetic (DM) cats. Feline-specific assays for AGE quantification were developed and applied to serum samples from cats with and without DM. The methylglyoxal (AGE precursor) reaction with diaminobenzene to form 2-methylquinoxaline is the basis for the methylglyoxal diaminobenzene assay (Diamino MEG). The methylglyoxal reaction with 2,4-dinitrophenylhydrazine to form bis (2,4-dinitrophenylhydrazone is the basis for the alternative methylglyoxal 2,4-dinitropheylhydrazine (DNPH MEG) assay. Both protocols were adapted to run as spectrophotometric microplate assays for free serum feline methylglyoxal. D-sorbitol was measured indirectly using a modified R-biopharm® (Boehringer Mannheim) kit to quantify formazan. Serum from 21 DM and 26 non-DM cats were compared using these assays. Sample BCA total protein was not significantly different between groups. Mean serum sorbitol concentration tended to be higher in DM cats, but this was not a statistically significant difference between DM cats, 95.0 mM (±12.7) and non-DM cats, 91.8 mM (±19.2). Using the Diamino MEG assay, methylglyoxal appeared minimally increased in the non-DM group, mean of 0.134 mM (±0.13), but this difference was also not statistically significant compared to the DM-group mean 0.12 mM (±0.07). This difference appeared even smaller using the DNPH MEG methodology. Although no statistical differences were seen between DM and non-DM groups for antioxidant capacity or AGE and AGE precursors, the DM cats in this study were deemed clinically well controlled and stable. Future work will compare well controlled to poorly controlled or newly diagnosed diabetic cats over time to determine if insulin therapy decreases AGE production in this population of Type 2 diabetic cats

Cerenia® for the management of vomiting and inappetence associated with chronic kidney disease in cats.

WilliamT Brock, K Moses, D Bolotin, K Patricelli, and Jessica M Quimby

Cerenia® is commonly used for acute vomiting. A recent pharmacokinetic and toxicity study in cats indicated that longer-term usage appears safe. The aim of this study was to assess the efficacy of Cerenia® for management of chronic vomiting and inappetence associated with feline chronic kidney disease (CKD). Forty-one cats with stable IRIS Stage II or III CKD, no known concurrent illness and a complaint of chronic vomiting and inappetence attributed to CKD were enrolled in a placebo-controlled, blinded clinical study. A CBC, serum biochemistry, urinalysis, urine culture, T4 and blood pressure were required for entry. Cerenia® was administered at a dose of 4 mg orally (median 1.1 mg/kg, range 0.6-2.9 mg/kg) daily for 2 weeks. Owners kept daily logs of vomiting incidence, appetite and activity scores. Physical exam, weight, body condition score, and serum biochemistry were performed before and after the trial period. Mann Whitney statistics were used to compare treatment groups. Thirty-three cats successfully completed the trial: 21 cats received drug (9 Stage II cats, 12 Stage III cats) and 12 cats received placebo (7 Stage II cats, 5 Stage III cats). There was a statistically significant decrease in vomiting in CKD cats that received Cerenia® ($p = 0.002$). There was no statistically significant difference in appetite scores, activity scores, weight or serum creatinine between groups. Cerenia® was demonstrated to palliate vomiting associated with chronic kidney disease, however did not appear to significantly improve appetite or result in weight gain in cats with Stage II and III CKD.

Does exercise-induced pulmonary hemorrhage affect career longevity and performance among South African Thoroughbred racehorses?

Julia L Bromberek, Paul S Morley, Kenneth W Hinchcliff, and Alan J Guthrie

Purpose: Exercise induced pulmonary hemorrhage (EIPH) has a high incidence among Thoroughbred racehorses, causing it to be a major concern in the racing industry. Efforts have been focused to determine how to minimize EIPH occurrence and how EIPH affects racehorse welfare and performance. Many studies have assessed the association of EIPH and concurrent racing performance, but none have investigated long-term impacts of EIPH on career performance. Therefore, we sought to determine whether EIPH is associated with career performance (longevity, races, wins, places, and earnings) among a population of South African Thoroughbred racehorses not treated with furosemide or nasal dilator strips. Materials/Methods: Two populations of South African Thoroughbred racehorses were combined: a randomized, placebo-controlled, crossover field trial and a cross-sectional study evaluating EIPH at five racetracks. Both populations were followed until retirement. Combined, 1,034 horses underwent post-race tracheobronchoscopic examination, independently evaluated as severity grade 0-4 by three investigators blinded to the horses' identity and race performance. Racing records for all horses were obtained from a commercial database after retirement from competitive racing and were summarized into career performance variables. Results: Overall, 70% of horses showed evidence of EIPH. Controlling for age, sex, and original study population, horses with EIPH started in approximately 4 more races than horses without (95% CI 1.99 to 6.18). Lifetime earnings were significantly, but not meaningfully, higher among horses with EIPH grade > 1 compared with those with EIPH grade 0 (adjusted average change in earnings 1.69 Rand, 95% CI 1.08 to 2.64). EIPH was not significantly associated with career longevity (adjusted HR 0.88, 95% CI 0.77 to 1.01), lifetime wins, or lifetime places. Conclusions: EIPH does not appear to be associated with career performance.

Pathogenesis of Francisella tularensis infection in cottontail rabbits

Vienna R. Brown, Danielle R. Adney, Helle Bielefeldt-Ohmann, Richard A. Bowen

Francisella tularensis is a highly virulent, zoonotic bacterium that causes significant natural disease and is also of concern as an organism for bioterrorism. Serologic testing of wildlife is frequently used to monitor spatial patterns of infection and quantify exposure. Cottontail rabbits are a natural reservoir for this bacterium in the U.S., although very little work has been done experimentally to determine how these animals respond to infection; thus, information gathered from field samples has provided an incomplete picture. The objective of this study was to provide an initial characterization of clinical disease, bacteremia, pathology, and antibody kinetics of Eastern cottontail rabbits (Sylvilagus floridanus) experimentally infected with five strains of F. tularensis. We infected rabbits with four field strains, including MA00-2987 (A1b strain), WY96-3418 (type A2), KY99-3387 and OR96-0246 (type B strains) and SchuS4 (type A1a strain), a widely used, virulent laboratory strain. The results clearly indicate that infection with different clades of the bacterium resulted in significantly varied patterns of disease as well as gross and histopathology. Based on this new information, we plan to evaluate the less virulent strains for a longer duration to monitor long term immune responses and the ability to clear infection, both of which could be important determinants of environmental maintenance.

Factors associated with large animal inpatient shedding of Salmonella enterica in a veterinary teaching hospital

Brandy A Burgess, Paul S Morley

During nosocomial Salmonella outbreaks in veterinary hospitals there tends to be widespread environmental contamination. Previous work indicates patient isolates can have the same phenotype as environmental isolates, suggesting animals to be a likely source. Factors for animal shedding have been identified however many of these studies focus on a subset of inpatients with results being minimally generalizable to the general hospital population. The objective of this study was to determine factors associated with fecal shedding of S. enterica in the general inpatient population at a large animal veterinary hospital. Inpatients included in this case-control study had fecal samples collected and cultured, using standard techniques, from March 2002 through December 2012, as part of ongoing infection control efforts. Factors related to patient stress and defense mechanisms were evaluated. Data on factors of interest were collected retrospectively from electronic medical records. Multivariable logistic regression was used to evaluate associations between animal factors and fecal shedding of S. enterica. During the study period, there were approximately 11,061 inpatients of which 6.3% (n=695) were fecal culture positive for S. enterica. The majority of culture positive inpatients were bovine (72%) and equine (22%) with the remaining being New World camelid, small ruminant, and porcine. The findings of this study will provide a better understanding of factors associated with fecal shedding in the general large animal inpatient population, allowing for the implementation of evidence based preventive measures. This information will be integral to risk management related to periods of epidemic as well as endemic disease.

Investigating systemic endectocides as a novel strategy for West Nile virus control in Northern Colorado

Timothy A Burton, Amy Robinson, and Brian D Foy

West Nile virus (WNV), the leading cause of arboviral encephalitis in the United States, is primarily transmitted by members of the Culex spp. genus of mosquitoes. Control of WNV in the U.S. is limited to widespread insecticide applications in an effort to control Culex populations, an often expensive, poorly targeted approach with limited efficacy. We seek to investigate a novel strategy for controlling Culex populations that addresses these issues. Through testing of systemic endectocides - drugs that have activity against parasitic invertebrates – it was determined that Culex mosquitoes are sensitive to ivermectin applications at an LC(50) dose of about 50 ng/mL. After coating bird seed with an ivermectin solution and allowing chickens to ingest this formulated feed for a number of days, Culex tarsalis mosquitoes are allowed to take a blood meal from the treated birds. Differing mortality occurred depending on the drug concentration; 95% of mosquitoes died within five days after taking a bloodmeal from a chicken fed on a 200mg ivermectin/kg birdfeed diet for seven days, while a diet of 50mg/kg caused no significant mosquito mortality. The C. tarsalis and WNV control strategy being investigated involves introducing medicated birdfeed stations into areas of high WNV transmission. Wild birds ingesting feed from these stations will take ivermectin into their system, and after a short period of time the drug begins to circulate in the bloodstream. As a result, mosquitoes feeding on these ivermectin-medicated birds might see heightened levels of mortality, interrupting the reservoir-vector life cycle of the virus and reducing transmission in the treated area.

Salmonella prevalence in baby poultry at feed stores

Kyran J Cadmus, Sarah M Millonig, Matthew Erdman, and Kristy L Pabilonia

Humans can be exposed to Salmonella through direct or indirect contact with live chicks and ducklings (baby poultry). Salmonella outbreaks traced to contact with baby poultry purchased at agricultural feed stores or directly from mail-order hatcheries occur yearly in the United States. This study assessed the prevalence of Salmonella in baby poultry enclosures at feed stores in Colorado. Surveys characterized chick sourcing information, housing and cleaning habits, and display or distribution of Salmonella educational information. Salmonella was detected in over half (63%) of the stores. In total, Salmonella was detected in 69/171 samples and thirteen different serotypes were identified. Serotype Typhimurium (14%) was most commonly isolated, followed by serotype Senftenberg (8%). Salmonella Typhimurium and Salmonella Infantis serotypes isolated matched strains causing multistate outbreaks in humans in 2013. Multiple serotypes were isolated from 23% of stores. Stores sold a median of 100 birds per week (range 10-1000). Ten of 27 stores (37%) that sold multiple species commingled the species in the same enclosure. Of 13 stores that purchased birds from multiple hatcheries, seven (54%) commingled birds from different hatcheries in the same enclosure. Chicks were sourced from 10 different hatcheries, with 43% using more than one hatchery. Most (66%) stores cleaned cages at least daily, ranging in frequency from twice daily to every other week. Salmonella educational material was posted or distributed by 50% of stores. As backyard poultry becomes more popular across the US, many people who have no previous poultry knowledge or experience are purchasing chicks. Salmonella is very common in feed store poultry and store practices may contribute to dissemination of the organism. Practices to minimize Salmonella in baby poultry should be evaluated, in order to prevent human exposure to Salmonella in backyard flock settings.

Characterization of exosomes and their role in FIV infection- a pilot study

Ashley D Cameron, Sue VandeWoude, Ryan M Troyer, Christine S Olver

Exosomes, 10-50 nm endosomally-derived microvessicles, contain proteins, viral antigens, and mRNA and appear to have a role in immunomodulation by regulation of antigen presentation or mRNA transfer to recipient cells. Feline immunodeficiency virus and human immunodeficiency virus are lentiviruses with similar disease pathogenesis and host cell tropism. The role of exosomes in HIV infection in vitro has been discussed but results are conflicting regarding up- or down-regulation of the immune response. Characterization of exosomes in cats is limited and their role in FIV infection both in vivo and in vitro remains unexplored. In this pilot study, Western blot and electron microscopy were used to identify the presence of exosomes from infected and non-infected cat serum and in vitro feline kidney cells (CRFK) culture. Electron microscopy confirmed the presence of exosomes in blood. Additionally, Western blot analysis identified an exosome protein marker Heat shock protein 90 (Hsp90) determining the presence of exosomes from blood using ultracentrifugation. Virus particles (80-100nm) and exosomes are similar is shape (spherical) and size. For co-infection of CRFK cells and exosomes with the intention of identifying consequences of exosomes and FIV infection in vitro, we determined the preferred protocol for exosomes isolation excluding virus particles. Exoquick TC and Optiprep gradient protocols were used to identify exosomes and virus particles from infected CRFK supernatant probing with Hsp90 for the presence of exosomes and for viral protein p24. Hsp90 distributed throughout the Optiprep gradients while the virus concentrated to the heavier fractions of the gradient. Exoquick TC samples were inconclusive. Our data demonstrates that the Optiprep protocol will be the best method in determining exosome effects on in vitro FIV infection of CRFK cells. Future studies aim to determine the effect of exosomes on FIV infection.

Interactions between segmented RNA viruses and the RNA decay machinery

Phillida A Charley, Eric Sefing, Brian Gowen, and Jeff Wilusz

In order to successful infected a host, RNA viruses have to outsmart the host cellular RNA decay machinery. Previous work in our laboratory has focused on how positive sense RNA viruses bypass the cellular RNA decay machinery to ensure the stability of their transcript. Little is known about how segmented RNA viruses, like Junin virus (JUNV) (an arenavirus) or Rift Valley Fever virus (RVFV) (a bunyavirus), interface with the cellular RNA decay machinery. Since many elements that regulate RNA stability can be found in the 3` untranslated regions (UTR) of transcripts, we hypothesize that the 3`untranslated region (UTR) of segmented RNA viruses is pivotal to how the transcript avoids the cellular RNA decay machinery. To test this hypothesis, so far we have cloned the 3`UTR of all of the mRNA from various segments of JUN and RVFV into an expression vector and assessing how RNAs with these 3' UTR regions act as substrates for the major enzymatic steps in mRNA decay using deadenylation, exonuclease and decapping assays. In addition, we have also assessed the interaction of cellular proteins with these viral 3' UTR regions using a UV crosslinking approach. Interestingly, we have found that many of the RNAs that contain JUNV 3` UTR segments interact strongly with a ~55 KDa cellular protein. We are currently characterizing this protein and its relationship to JUNV biology.

The role of fatty acid synthase during dengue virus replication in mammalian cells

Nunya Chotiwan, Sudip Khadka, Doug LaCount, Richard J. Kuhn and Rushika M Perera

Dengue virus (DENV) modulates host lipids to facilitate its infection in both mammalian and insect cells. Specific host membrane rearrangement as well as a unique lipid repertoire is induced during virus replication. We are interested in fatty acid synthase (FAS), a key enzyme of the lipid biosynthesis pathway, which is expressed at high levels in several diseases including obesity and cancers. During DENV infection, FAS is activated by the viral nonstructural protein 3 (NS3) and localizes with double stranded RNA at sites of viral RNA replication. Moreover, the inhibition of this enzyme by commercially available compounds, C75 and Cerulenin, disrupted DENV replication in both human and mosquito host cells. FAS is a dimeric enzyme composed of 7 domains. Yeast two hybrid assays have revealed the interaction between the protease domain of NS3 and the dehydratase (DH) domain of FAS. We are currently investigating if other domains of FAS interact with DENV proteins using a split luciferase assay and trying to identify the mechanism of FAS recruitment by NS3. Additionally, we are pursuing the potential of the DH domain to act as an inhibitor that disrupts DENV replication by either interfering with the interaction between FAS and NS3, or by stimulating a cellular environment that is refractory to viral infection. FAS is a high profile therapeutic target. Understanding the mechanism of cellular utilization of FAS using a virus that perturbs FAS will help us understand the mechanism of this enzyme in several diseases.

Prenatal Androgenization Decreases Ovine Placental gDNA Methylation and Alters Placental Gene Expression

Ellane R Cleys, Jennifer L Halleran, Vanessa A Enriquez, Juliano da Silveira, Quinton A Winger, Jason E Bruemmer, Colin M Clay, Gerrit J Bouma

Sex steroid hormones estradiol and testosterone regulate epigenetic programing in many tissues, including programing gene expression in the placenta and fetus. While estradiol is known to regulate early placenta development, little research has investigated the role of testosterone in placentation despite its rise in maternal circulation during pregnancy and placenta-induced pregnancy disorders. Using androgenized pregnant ewes, we investigated the role of testosterone in placental cell gene expression and DNA methylation. Placental androgenization decreased global DNA methylation in gestational day 90 (GD90) placentomes. In vivo androgenization also changed expression of genes involved in placenta epigenetic gene expression, angiogenesis, and growth factors, including increased H19, VEGF, and IGF2 and decreased IGFBP1 and IGFBP2. As androgen receptor (AR) complexes with histone demethylases in human prostate to regulate AR target genes, we investigated if the same could be occurring in the sheep placenta. Western blot analysis and immunohistochemistry showed trophoblast expression of histone demethylase KDM1A and uterine epithelium expression of histone demethylase KDM4D. Prenatal androgenization increased AR and KDM4D protein in GD90 placentomes. Additionally, increased ESR1 and DNMT1 protein was found in placentomes from androgenized ewes. Coimmunoprecipitation showed that AR formed complexes with KDM1A in sheep placentomes and that AR-KDM1A complexes are recruited to ARE half-sites in promoter regions of genes regulating placenta development and vascularization, including VEGFA. In conclusion, placental androgen signaling is capable of altering DNA methylation and gene expression, likely through AR-KDM1A complexes. Additionally, androgens appear to be an important regulator of trophoblast epigenetic programing for placental development and function, and aberrant androgen signaling may contribute to the development of placental disorders.

Comparison of the immunosuppressive properties of allogeneic and autologous equine mesenchymal stem cells

Aimée Colbath, Jennifer Phillips, Wayne McIlwraith, Steven Dow and Laurie Goodrich

The purpose of this study was to investigate the immunosuppressive properties of equine allogeneic and autologous mesenchymal stem cells (MSC), with the ultimate goal of determining whether allogeneic MSC would be suitable for treatment of musculoskeletal inflammatory conditions. The hypothesis of the study was that both allogeneic and autogenous equine MSC would be immune suppressive in vitro, demonstrated by a decrease in T lymphocyte proliferation. The immune suppressive properties of MSC were determined using lymphocyte suppression assays. Equine autologous and allogeneic MSC were cultured with in vitro activated equine T lymphocytes at ratios of 1:10, 1:100 and 1:1000 (MSC:lymphocyte). Flow cytometry was used to assess MSC effects on lymphocyte proliferation, by the CFSE dye dilution method. Cytokine production by T cells was assessed by intracellular IFN-g staining and flow cytometric analysis. We observed a dose-dependent suppression of lymphocyte proliferation by MSC. Addition of autologous MSC (at a 1:10 ratio) suppressed proliferation by 22%, while addition of allogeneic MSC suppressed proliferation by 29%. A student T-test revealed no difference between proliferation suppression by autologous versus allogeneic MSC ($p= 0.11$).We also observed a dose-dependent suppression of INF-g production by T cells when autologous or allogeneic MSC were added to the activated cultures. A greater suppression of IFN-g was noted by the allogeneic MSC when compared to the autogenous MSC ($p= 0.0014$). These results indicate that both autologous and allogeneic equine MSC are immune suppressive for T cells. Further research will focus on the immune mechanisms by which MSC suppress T cell responses, and whether autologous and allogeneic MSC exert equivalent anti-inflammatory activity when injected into inflamed joints. Finally, the authors will utilize an in vivo model with single and repeated injections of allogeneic equine MSC to sequentially measure indicators of joint inflammation using synovial fluid analysis, physical examination parameters, and lameness scores.

mRNA decay is altered in myotonic dystrophy patient cells

Stephen J Coleman, Ashton C Herrington, Hend Ibrahim, Alexa Dickson, Jeffrey Wilusz, and Carol J Wilusz

Type 1 Myotonic dystrophy (DM1) is a chronic and progressive autosomal dominant neuromuscular disease resulting from a trinucleotide repeat expansion in the 3'UTR of the dystrophia myotonica protein kinase (DMPK) gene. Pathogenesis is linked to the accumulation of the toxic repeat-containing RNA in nuclear foci and sequestration of RNA-binding proteins, such as Muscleblind (MBNL1). MBNL1 and another RNA binding protein whose function is affected in DM1, CELF1, have both been implicated as regulators of mRNA decay leading to the hypothesis that the stability of cellular mRNAs may be altered in DM1 and contribute to pathogenesis. Preliminary data demonstrate that mRNAs from three transcripts that were previously shown to be bound by CELF1; SOX9, TUT1, and ZNF37A, are stabilized in DM1 patient myoblasts compared to control cells. Moreover, knockdown of mutant DMPK mRNA in DM1 patient cells results in restoration of decay to rates similar to those in myoblasts from normal individuals. These data support the hypothesis that mRNA stability is affected in myotonic dystrophy. Sequencing of mRNA (RNA-seq) will be used to perform a global analysis in these samples to evaluate changes in mRNA decay, splicing, and polyadenylation that result from expression of the toxic repeat-containing DMPK RNA.

LIN28 in Exosomes Secreted by Human Placental Cells and Ovarian Cancer Cells

Amanda R Crouch, Juliano C da Silveira, Vanessa A Enriquez, Ellie Cleys, Gerrit J Bouma, Quinton A Winger

The RNA binding protein LIN28 is present in less differentiated cells, including stem cells and cancer cells, and is involved in cellular metabolism and proliferation pathways. Recent studies revealed that LIN28 is also present in tumor cells that are generally associated with a poor prognosis, and our studies indicate it is possibly involved in ovarian cancer cell migration and invasion. Placental trophoblast cells exhibit similar characteristics (migration, invasion) to cancer cells during early placental development. Both cancer and trophoblast cells are known to secrete small vesicles called exosomes. They are released into the extracellular fluid and can be taken up by other cells and can regulate cell function. The overall goal of this project is to demonstrate a role for LIN28 in exosome release by placental cell lines (ACH3P scramble and knock down) and ovarian cancer cell lines (IGROV-1 and OV420). We hypothesize that LIN28 is present in the exosomes and regulates their secretion. The presence of LIN28 in exosomes would represent a potential mechanism of transport of LIN28 between cells. Exosomes secreted from each of these cell lines will be isolated and tested for the presence of LIN28A. In addition, we plan to demonstrate that knock down of LIN28A in cells causes a decrease in exosome secretion in culture media. These data will provide critical insight into the regulation of exosome release by trophoblast and ovarian cancer cells, and may lead to development of novel diagnostic tools to study placental disease and ovarian cancer development.

Micro-CT assessment of bone healing and strength after stereotactic radiation therapy for local control of osteosarcoma

Ryan Curtis, Jamie Custis, Nicole Ehrhart, Sara Gookin, and Seth Donahue

Canine patients receiving definitive-intent stereotactic radiation therapy (SRT) for the local treatment of canine osteosarcoma at Colorado State University's Flint Animal Cancer Center have achieved similar median survival times compared to the current standard of care (amputation and chemotherapy). Despite the ability of SRT to achieve local tumor control, there is a high risk of pathologic fracture following treatment. Reasons for fracture include the degree of existing osteolysis as well as the inability of bone to remodel naturally following radiation. Zoledronic acid (ZA), a potent bisphosphonate and bone resorption inhibitor, and parathyroid hormone (PTH), a potent anabolic bone agent, are therapeutic candidates for decreasing this fracture risk post-irradiation. We hypothesized that combined ZA/PTH treatment would increase bone volume and strength more than either treatment alone. Using an orthotopic model of canine osteosarcoma in athymic rats, we evaluated bone healing following clinically-relevant doses of SRT (total dose of 36 Gy in 3 once daily fractions). Groups included 36 Gy SRT only, 36 Gy SRT plus ZA , 36 Gy SRT plus ZA and PTH, and 36 Gy SRT plus PTH. Micro-CT analysis of affected femurs was used for the assessment and comparison of bone mineral density, bone volume, and polar moments of inertia between treatment groups. Significant increases in bone volume were shown in both the ZA and the combined ZA/PTH treated groups as compared to SRT treatment alone. The polar moment of inertia, used as a surrogate measure of bone strength, was also increased in these groups. This work provides further evidence for expanding the potential indications for ZA and PTH therapy, including post-irradiated bone disease due to canine osteosarcoma.

MicroRNA expression changes associated with chemo-sensitivity in canine cancer cell lines

Deanna D Dailey, Ann M Hess, Kenneth L Jones, Joe Brown, Jared S Fowles, Gerrit J Bouma and Dawn L Duval

MicroRNAs (miRNA) are small non-coding RNA molecules involved in post-transcriptional gene regulation. Deregulation of miRNA expression occurs in cancer and miRNA expression profiles have been associated with drug sensitivity in human cancer cells and tumors. Algorithms have been developed that utilize gene expression profiles from NCI60 human cancer cells to predict response to chemotherapy drugs in cancer cells and patient tumors, including canine samples. Modification of these algorithms to incorporate miRNA expression profiles is desirable, as miRNAs have increased stability in patient samples such as blood or serum. The purpose of this study was to characterize miRNA levels in 16 canine cancer cells lines and identify miRNAs commonly associated with drug sensitivity. Total RNA, including small RNA, was extracted from 16 canine cancer cell lines and next generation sequencing was performed on an Illumina platform at the Genomics and Microarray Core (UC Denver). Differential miRNA expression analysis was conducted based on the five most and least sensitive cell lines for each of four chemotherapy drugs (doxorubicin, carboplatin, cisplatin and paclitaxel) utilizing voom in the limma R package. Differentially expressed miRNAs were confirmed via RT-qPCR on the Roche LightCycler480 PCR platform using 384 well plates. Next generation sequencing data identified miRNAs from 4 miRNA families (let-7, mir-146, mir-148/152 and mir-21) commonly associated with drug sensitivity for these drugs. Drug associated changes in the relative level of four miRNAs were confirmed by RT-qPCR: let-7i (FC=-1.98, p=0.042) in cisplatin resistant cells, mir-146a (FC=-7.74, p=0.007) in carboplatin resistant cells, and mir-21 (FC=3.89, p=0.006) and mir-148a (FC=3.63, p=0.007) in doxorubicin resistant cells. Our findings confirm that miRNA expression changes in canine cancer cells are associated with drug sensitivity. This represents an important first step toward identifying miRNA signatures predictive of individual tumor responses to chemotherapy for canine and human patients.

Actin cytoskeleton modulates local L-type calcium channel signaling and ERK activation in gonadotropes.

An K. Dang, Dilyara Murtazina, Christianne Magee, Amy M. Navratil, Colin M. Clay, and Gregory C. Amberg.

The binding of gonadotropin-releasing hormone (GnRH) to its receptor on gonadotrope cells in the anterior pituitary initiates signaling cascades that result in enhanced luteinizing hormone (LH) biosynthesis and secretion. This process is essential for follicular maturation and ovulation. Previous research suggests that the extracellular signal regulated kinase (ERK) is activated by Ca2+ influx through L-type Ca2+ channels to increase LH synthesis. What is missing, however, is direct demonstration of localized Ca2+ influx at the plasma membrane and how the spatiotemporal dynamics of these channels affect downstream signaling. We hypothesize that disrupting actin cytoskeleton, which is important for membrane organization and cell remodeling, will affect localized GnRH-induced Ca2+ influx and ERK activation. To test this hypothesis, we used a combination of TIRF microscopy and electrophysiology to image subplasmalemmal Ca2+ influx in the gonadotrope cell line αT3-1. Using this approach we visualized discrete sites of Ca2+ influx ("Ca2+ sparklets") which produced microdomains of elevated Ca2+ on the cell surface. Pretreatment with Jasplakinolide (Jas; 100nM), a pharmacological disruptor of actin cytoskeleton, decreased the Ca2+ influx at GnRH (3nM) induced Ca2+ sparklets sites, but did not change the number of sites on the plasma membrane. Jas did not affect Ca2+ sparklet activity or density induced by PKC agonist phorbol 12, 13-dibutyrate (PDBu; 50 nM) or L-type Ca2+ channel agonist FPL64176 (500 nM). Therefore, actin disruption with Jas interrupts GnRH signaling and ERK activation by decreasing the Ca2+ influx through L-type Ca2+ channels, but does not affect Ca2+ activity or ERK activation downstream of GnRH when activating PKC or L-type Ca2+ channels directly. In summary, these data indicate that GnRH engages the actin cytoskeleton for localized Ca2+ influx through L-type Ca2+ channels and ERK phosphorylation, and demonstrates the specificity in spatial and temporal organization to propagate extracellular signals into intracellular signals for LH biosynthesis.

Laparoscopic ovariohysterectomy in goats

Alexander J. Daniel, Jennifer Hartman, Katlin J. Hornig, Tim Holt, Robert J. Callan, Stacey R. Byers, and Eileen S. Hackett

Uterine and ovarian tumors are reported in pet goat populations and often result in unfavorable outcomes. In addition to clinical signs associated with reproductive cancers, intact females can display unwanted behavior during estrus including vulvar hemorrhage and uterine fluid expulsion. High surgical complications are observed with ovariohysterectomy via open laparotomy in goats, including hemorrhage, incomplete removal, incisional infection and abdominal adhesion formation. There is a need to offer minimally invasive ovariohysterectomy to pet goat owners, with minimal associated post-operative morbidity. The purpose of the present study is to prospectively describe surgical technique, anesthetic technique, surgical times, complications and outcomes in 12 intact juvenile female goats. We hypothesize that laparoscopic ovariohysterectomy will be well tolerated and result in minimal post-operative morbidity in juvenile goats. Cases were enrolled with informed client consent in this descriptive IACUC approved clinical study. Follow-up information was obtained by owner interview. Four goats have thus far been enrolled and have undergone laparoscopic ovariohysterectomy. Surgical ovariohysterectomy was facilitated through use of a tissue sealing device (Ligasure, Covidien, Mansfield, MA). Mean surgical time was 44 minutes, with a standard deviation 12 minutes, for completion of the laparoscopic procedure. Surgical procedures were well tolerated by all goats. Direct surgical complications including hemorrhage, incomplete removal, incisional infection or other morbidities were not observed. All goats were healthy at the time of follow-up and owners had a high customer satisfaction with the procedure. Though preliminary work is promising, further study of this laparoscopic ovariohysterectomy technique in goats is warranted to investigate more fully the effects and expected outcomes of this procedure.

Urinary Shedding of Prions in Chronic Wasting Disease Infected White-tailed Deer

Nathaniel D Denkers, Davin M Henderson, Candace K Mathiason, and Edward A Hoover

Purpose: Chronic wasting disease (CWD) is unique among prion diseases in its efficient lateral transmission in nature, yet the dynamics and magnitude of shedding and its immediate and long term consequences remain unknown. The present study was designed to determine the frequency and time span in which CWD prions are shed in urine from infected white-tailed deer using adapted real-time quaking-induced conversion (RT-QuIC) methodology. Methods: Longitudinal urine samples were collected by free catch or catheterization over a 2-year period (every 3 months for the first 15 months, then every month until study termination) from aerosol-route-infected white-tailed deer [CWD+ (n=6); CWD- (n=3)]. High speed centrifugation pelleted material from 200µl of urine was harvested, re-suspended in buffer, and used as seed in RT-QuIC assays employing recombinant Syrian hamster prion PrP substrate. Prion seeding activity was recorded as thioflavin T binding fluorescence (480 nm emission) using a fluorimeter-shaker. Results: In our initial studies, prion seeding activity has been demonstrated in urine collected at 9 months post-inoculation in 2 of 2 deer, and intermittently at later time points in 5 of 6 CWD+ inoculated deer. Urine from all sham-inoculated control deer remained negative for prion seeding activity in RT-QuIC. Conclusion: CWD prions (as inferred by prion seeding activity by RT-QuIC) are shed in urine of infected deer as early as 9 months post inoculation and intermittently or at varying levels throughout the disease course. Further studies are in progress examining the time frame, sample variables, correlates, and magnitude of urinary prion shedding in deer.

Hypophosphatemia in hyperventilating dogs

Hannah Doran, Seung Yoo, Christine S Olver

Purpose: Anecdotally, hypophosphatemia is seen in nervous dogs that have prolonged panting during visits to the clinic. While the veterinary literature asserts that respiratory alkalosis causes hypophosphatemia, we are not aware of recent veterinary studies that have proven the relationship between respiratory alkalosis and hypophosphatemia. Objectives: The aim of our study was to test the hypothesis that respiratory alkalosis causes hypophosphatemia in anesthetized dogs. Methods: Five sedated dogs were anesthetized (Propofol 5 mg/kg IV, 10 mg/mL), intubated, and manually hyperventilated to reach and maintain an EtCO2 of 15-20 mmHg. Blood collection was as follows: baseline time 0 with spontaneous ventilation, after 5 minutes of hyperventilation, after 10 minutes of hyperventilation, and at 20 minutes after a 10 minute period of spontaneous ventilation. A blood gas analysis and a plasma biochemistry panel were performed on each sample within 20 minutes. Results: Mean venous pH was significantly increased and mean PvCO2 was significantly decreased at 5 and 10 minutes compared to baseline but not at 20 minutes. Mean phosphorus was significantly decreased at 5, 10, and 20 minutes compared to baseline. One dog did not reach an adequate level of respiratory alkalosis so was excluded from analysis. Phosphorus did not return to baseline levels after our 10 minute recovery time. Conclusion: We conclude that hyperventilation in dogs causes hypophosphatemia which we hypothesize is due to shifting to the intracellular compartment. Additionally, we hypothesize that this is the cause of hypophosphatemia in nervous and panting dogs. This is an important consideration for clinicians attending to nervous dogs so that further clinical work up of hypophosphatemia is not performed.

Could the inhibition of the cellular decay machinery contribute to the pathogenicity of bovine viral diarrhea virus infections?

M Kaitlin Dozier, Stephanie Moon, John Anderson, Jeffrey Wilusz

Although bovine viral diarrhea virus (BVDV) is a widespread concern in the livestock industry, numerous questions remain regarding its mechanisms of pathogenesis as well as how it persists in the bovine cell. An understanding of these processes may prove pivotal in the development of novel prophylactic therapies for BVDV. Based on research performed in our laboratory with other members of the Flaviviridae, we hypothesize that BVDV inhibits the action of Xrn1, an exoribonuclease that plays a major role in regulating cellular gene expression and normally destroys mRNAs that are not useful for cellular actions (which would include BVDV mRNAs). This hypothesis will be tested by determining the half-lives of certain bovine cell mRNAs as well as the accumulation of uncapped mRNAs with and without BVDV infection in order to ascertain if Xrn1 activity is compromised. We will also test directly in Xrn1-inhibition assays using recombinant protein if the highly structured 5' untranslated region of BVDV RNA contains an element that stalls Xrn1 and represses its activity. If BVDV does indeed target Xrn1 for repression during infection, future experiments will determine the effect of maintaining Xrn1 activity on BVDV replication, persistence and pathogenesis to assess the value of this novel virus-host interaction as a potential therapeutic target. Work performed using recombinant Xrn1 protein and BVDV RNAs may also uncover a very sensitive screening assay for small molecule inhibitors that would be amenable to high throughput applications. Therapeutics that decrease the prevalence of BVDV would have significant value in the cattle industry.

Pathologic and cardiovascular characterization of pheochromocytoma associated catecholamine-induced cardiomyopathy in dogs

Elijah F. Edmondson, Jan Bright, Chuck Halsey, EJ Ehrhart

Pheochromocytoma associated catecholamine-induced cardiomyopathy is a well-recognized entity in man and has been described in mice and nonhuman primates but has not yet been identified or described in dogs. In this retrospective study, nine dogs were identified with histologically confirmed pheochromocytomas and concurrent cardiovascular pathology observed histologically (n = 6), echocardiographically (n = 4), and/or electrocardiographically (n = 5). Histopathology included multifocal cardiomyocyte necrosis with contraction bands, cardiomyocyte degeneration, myocardial hemorrhage, lymphohistiocytic myocarditis, and interstitial fibrosis. Clinical procedures including electrocardiographic and echocardiographic examinations, Doppler blood pressure measurement, and auscultation were available for five dogs and consistently revealed concentric or mixed (eccentric and concentric) ventricular hypertrophy. Additional changes observed included arrhythmias, systemic hypertension, and heart murmurs. The myocardial lesions observed in this series of dogs are similar to those observed in humans with pheochromocytoma associated catecholamine-induced cardiomyopathy. Catecholamine-induced cardiac disease is reversible with medical treatment; therefore, recognition of this cardiomyopathy has the potential to reduce morbidity and mortality in dogs with pheochromocytomas.

A comparison of dose-dependent outcomes in induction of cytogenotoxic responses by novel glucosyl flavonoids

Anya Engen, Junko Maeda, David E. Wozniak, Colleen A. Brents, Justin J. Bell, Mitsuru Uesaka, Yasushi Aizawa, and Takamitsu A. Kato

Quercetin (Q) and its glucosyl derivative rutin (R) are naturally occurring flavonoids ubiquitous in plants, fruits and vegetables. Both compounds are known inducers of sister chromatid exchange (SCE), micronuclei (MN) and inhibitors of poly (ADP ribose) polymerase (PARP). Q is a polyphenolic aglycone, and R is nearly identical chemically distinguished by two glucosyl attachments. Monoglucosyl rutin (MO) and maltooligosyl rutin (MA) are novel derivatives of R manufactured with additional glucosyl modifications to improve R's water solubility, and presently do not have toxicity data. We hypothesize that MO and MA induce similar cytogenotoxic responses at the same concentrations as those of Q and R. The current study uses an in vitro system of Chinese hamster ovary (CHO) cells to examine differences in SCE and MN frequencies, cell viability, and PARP inhibition as genotoxic, cytotoxic, and bioactivity endpoints, respectively. SCE was determined by incorporation of 5'-bromodeoxyuridine (BrdU) in treated cells for two mitotic cycles and differential staining. MN frequencies were quantified using the cytokinesis-block micronucleus (CBMN) technique. Cell viability was assessed using colony formation and growth inhibition assays. PARP activity was measured with ELISA. As anticipated, Q exhibited the most potency, inducing a mean SCE frequency of 27% at 0.1 PPM ($p<0.05$) and MN response of 8% at 10 PPM ($p<0.05$). Q significantly inhibited cell growth and PARP by 50% at 10 PPM. MA is shown to be a stronger SCE inducer and PARP inhibitor compared to MO at 1,000 PPM, though responses are not statistically significant. While similar MN frequencies of 2.5% and viability responses (50% growth inhibition) were observed for both novel glucosyls at 1,000 PPM. All four flavonoid compounds induced genotoxic, cytotoxic, and bioactivity responses. However, the novel glucosyl flavonoids require substantially higher treatment doses to induce the same responses as Q and R despite sharing similar chemical makeups.

Ovarian cancer cell-secreted exosomes contain LIN28 and unique RNA signatures capable of inducing invasion and migration

Vanessa A. Enriquez, , Juliano C. da Silveria, Monique A. Spillman, Quinton A. Winger, and Gerrit J. Bouma

Ovarian cancer is the 5th most deadly cancer among women in the United States and the most lethal gynecological malignancy in the world. Recent studies reveal that human tumor cells release cell-secreted vesicles called exosomes. Exosomes are endosome-derived vesicles containing bioactive materials, including RNAs and miRNAs that can be detected in body fluids. Importantly, stem cell factor LIN28, a regulator of let-7 miRNAs, is present in ovarian cancer cells. Our preliminary data revealed high LIN28 and low let-7 miRNAs levels in aggressive human ovarian cancer cells compared to low LIN28 and high let-7 miRNAs in less aggressive human ovarian cancer cells. Moreover, this RNA signature is also present in their secreted exosomes. We hypothesized that ovarian cancer cell-secreted exosomes are taken up by target cells and induce change in gene expression and cell behavior. Our data revealed that IGROV1 (high LIN28) secreted exosomes are taken up by HEK293 cells which lead to increased levels of LIN28 and increased invasion and migration in HEK293 cells. In addition, various genes involved in epithelial-mesenchymal transition, including TIMP1 (25-fold higher), FOXC and NOTCH1 (11-fold-higher), CDH1 (6-fold higher), MMP2 (5-fold higher), MMP9 (4-fold higher), and ZEB1 (3-fold higher) were up-regulated in HEK293 cells following uptake of IGROV1-secreted LIN28 positive exosomes. We postulate that ovarian cancer cell-secreted exosomes contain a distinct RNA signature capable of inducing phenotypic changes in non-cancer cells, as well as distinguishing aggressive, advance staged ovarian tumors from early stage tumors. Therefore, we performed RNAseq transcriptome analysis on IGROV1 and OV420 (low LIN28) secreted exosomes. These results yielded 195 differentially expressed RNAs. Data from this study are important for elucidating the role ovarian cancer cell-secreted exosomes have on early metastasis and tumor progression, an area in ovarian cancer biology in critical need of advancement.

Validation of a smartphone-based point-of-care hemoglobin assay for use in dogs

Kate Farrell, Lauren Sullivan, and Joel Ehrenkranz

Purpose: To prospectively investigate whether a smartphone-based spectrophotometric assay for bedside measurement of hemoglobin concentration has sufficient accuracy and reproducibility to measure the hemoglobin concentration in canine blood samples. Materials/Methods: Blood samples were obtained using EDTA tubes submitted to the clinical pathology laboratory for a complete blood count (CBC). Each sample was run on the smartphone device in duplicate using two strips, to obtain a total of four readings per dog. Variance component estimates were made to evaluate smartphone reading variability within and between dogs. The readings were averaged to obtain one smartphone hemoglobin concentration and compared to the CBC reference hemoglobin. A standard linear calibration was used to compare the methods. Bland-Altman analysis was used to compare the hemoglobin value obtained by the smartphone device with the reference method and to determine if the difference between the methods was a function of hemoglobin concentration. Results: The hemoglobin concentrations measured by smartphone were compared to the reference hemoglobin value for 73 dogs. Correlation (r) between the two devices was 0.83 ($p< 0.001$). The mean error for the smartphone hemoglobin concentration was positive [0.73 g/dL, 95% CI (0.39, 1.06), $p< 0.001$]. The variance between the smartphone and reference hemoglobin values was not due to hemoglobin concentration. Observed variability within the smartphone assay was largely due to differences between dogs (92%); the remaining variability (8%) was due to multiple readings from an individual blood sample. Conclusions: A point-of-care smartphone-based photometric method for measuring hemoglobin is a suitable alternative to the laboratory analyzer for determining hemoglobin concentration in dogs. There was excellent intra-sample reproducibility on the smartphone device, indicating good repeatability. Smartphone hemoglobin measurement averaged 0.73 g/dL higher than reference values. These data demonstrate that bedside hemoglobin determination using a smartphone-based device represents a clinically useful point-of-care alternative to a laboratory hemoglobin measurement.

Mesenchymal Stem Cell Immune Modulation of Canine Lymphocyte Responses

Jessica Felgenhauer, Allison Bradley, Jordan Dunham, Tracy Webb, Amanda Guth, and Steven Dow

Mesenchymal stem cells (MSCs) are attractive for therapy of inflammatory diseases because of their potent immune modulatory properties in mouse and human studies. However, little is known about their immune modulatory properties or the mechanisms of these effects in other species such as dogs, despite the fact that MSC therapy is now being utilized widely in companion animals. Therefore, we hypothesized that canine MSC would exert immune suppressive properties in vitro and that this immune suppression would be mediated by factors secreted by the MSC. Previous studies in rodent and human MSC systems have suggested that MSC immune modulatory properties could be mediated by release of PGE2, reactive oxygen or nitrogen, production of indoleamine 2,3-dioxygenase (IDO), or by secretion of immune suppressive cytokines such as TGF-b and IL-10. To determine whether canine MSC were immune suppressive, we established an MSC:T cell co-culture system to assess the effects of MSC on suppression of lymphocyte proliferation. Specific pathways involved in this T cell inhibitory effect were also assessed using specific pathway inhibitors in vitro. T cell proliferation was assessed using CFSE-labeled T cells, and IFN-g release was quantitated by ELISA. Specific pathway inhibitors evaluated included indomethacin for PGE2, NAC, BSO and catalase for ROS, aminoguanidine and LNMMA for iNOS, anti-TGF-B for TGF-B, and 1-MT for IDO inhibition, and neutralizing antibodies for TGF-b or IL-10 mediated pathways. We found that canine MSC significantly inhibit T cell proliferation, suggesting that canine MSC are also immune suppressive. By identifying the specific pathways utilized for these inhibitory effects, it may be possible to engineer canine MSC to become even more immune modulatory for use in therapy of inflammatory and autoimmune diseases in clinical studies in dogs.

Effect of estriol on urodynamic findings 24 hours after dosing in female spayed reseach beagles

Leah E Ferguson, Laura Martin, and Michael R Lappin

Estriol is a short acting estrogen used for the management of urinary incontinence that is available as a commercial product in many countries (Incurin®, Merck Animal Health). While safe when administered daily, some dogs maintain continence on Incurin® administered every other day. The objective of this study was to describe select urodynamic findings in dogs 24 hours after administration of Incurin®. Research beagles (n = 5) that had been used in a previous vaccine study had an ovariohysterectomy performed 10 months before this study. The maximum urethral closure pressure (MCUP) and functional profile length (FPL) were determined 3 times over 2 weeks prior to starting Incurin® administration. Incurin was dosed at 2 mg, PO, daily in the morning for 14 days. On the morning of the 15th day, each dog received xylazine 1 mg/kg and atropine 0.02 mg/kg subcutaneously. A triple lumen urinary catheter was placed with the guidance of a speculum and a urethral pressure profile (UPP) was obtained using standard operating procedures at the Veterinary Teaching Hospital. The percentage increase in MUCP and FPL were determined by comparing the measurements obtained 24 hours after the 15th dose of Incurin® compared to the last baseline measurements before Incurin® therapy was begun. Twenty four hours after the 15th daily dose of Incurin®, the MUCP and FPL were numerically increased compared to baseline in 4 of 5 dogs. The MUCP increases ranged from 0 to 222.2% (Mean = 83.8%; Median = 17.6%) The FPL increases ranged from 0 to 40% (Mean = 13.6%; Median = 12.7%). A urinalysis collected at the time of data collection was normal for all dogs. The results support the clinical observations that Incurin® induces urodynamic changes that would promote continence for greater than 24 hours in many dogs.

Modulation of Canine Gut Hormones with Bean Consumption and Weight Loss

Genevieve M Forster, John E Bauer, and Elizabeth P Ryan

Obesity is a major disorder associated with lifestyle factors in U.S. dogs and an overweight/obese condition has been shown to exhibit alterations in inflammation, metabolism, and gut hormone expression that together create a favorable environment for tumorigenesis. Emerging evidence suggests that dry bean consumption can promote health in humans by reducing inflammation, improving metabolism, beneficially altering gut hormone expression, and reversing dyslipidemia and obesity; resulting in reduced risk for cancer development. Given the increasing neoplasia rates for canines and the potential for dry beans to modulate gut hormone expression, we evaluated a subset of dogs for changes in gut hormones. Thirty overweight/obese, but otherwise clinically healthy, adult male and female, client owned dogs were randomized to one of three isocaloric, macro and micronutrient matched diet groups: control (0% bean), black bean (25% w/w), or navy bean (25% w/w). Dogs were calorically restricted for 4 weeks to achieve weight loss, and plasma samples were taken throughout the study for clinical and experimental analysis. Within each group, 6 dogs that showed the greatest decrease in cholesterol over baseline measures were selected for serum gut hormone analysis using a multiplex microsphere-based assay. Dogs undergoing weight loss without beans showed a median decrease of 15% decrease in glucagon and dogs consuming a bean diet showed a median decrease of 31-70% in glucose-dependent insulinotropic peptide, a 27-35% increase in insulin, and a 10% increase in adiponectin. These preliminary results provide insight into the mechanisms by which dietary bean intake can reduce canine obesity that may be related to a reduced risk for developing neoplasia.

Canine COXEN: cross-species genomic applications for predicting chemosensitivity in dogs

Jared S Fowles, Ann M Hess, Dawn L Duval, Douglas H Thamm, and Daniel L Gustafson

Genomic strategies for personalized cancer treatment are in its infancy for veterinary oncology. The co-expression extrapolation (COXEN) method has successfully predicted chemosensitivity in vitro and in vivo in human cancer. Implementing genomic strategies across species allows dogs to take advantage of the wealth of available human data, and humans to take advantage from veterinary clinical trials. Our purpose is to explore COXEN's utility in a cross-species extrapolation of gene expression models (GEMs) to predict chemosensitivity in canine cancer from a human reference set. Gene expression and drug sensitivity data for 6 chemotherapeutics were publicly available for the human NCI60 panel. Microarray analysis was performed or obtained for 30 canine cell lines (ACC30) and 49 canine osteosarcoma tumors (COS49). Drug sensitivity was calculated for the ACC30 via proliferation assays. Significance Analysis of Microarrays (SAM) was performed on sensitive and resistant NCI60 lines to obtain gene signatures. Co-expressed genes for GEM development were selected after correlation of human and canine gene expression data. COXEN analysis generated NCI60-based GEMs that averaged 71% accuracy in predicting ACC30 sensitivity for 6 drugs, 5 being significant by binomial test (p values <= 0.032). GEMs for paclitaxel, and lomustine resulted in COXEN scores that significantly correlated with actual sensitivity data (P =0.0012, 0.0439, Spearman). For doxorubicin, a model built only on the NCI60 was 70% accurate in predicting clinical response in the COS49 (p = 0.0577 and 0.0649, binomial and Log Rank). A doxorubicin model with NCI60-derived genes but built on a subset of COS49 (COS16) was 85% accurate in the remaining samples (COS33) (p = 0.0193 and 0.0062, binomial and Log Rank). Results are encouraging for COXEN in interspecies applications. Further study may show this method beneficial in veterinary clinical trials, which could strongly support this treatment strategy in both human and canine cancer.

Endoplasmic reticulum/plasma membrane junctions function as membrane protein trafficking hubs

Philip D. Fox, Christopher J Haberkorn, Aubrey V Wiegel, Elizabeth J Akin, Mathew J Kennedy, Diego Krapf and Michael M Tamkun.

Endoplasmic reticulum/plasma membrane (ER/PM) junctions are well known for their role in store-operated Ca2+ influx via the Stim/Orai complex. We provide evidence for a novel role of ER/PM junctions as trafficking hubs for insertion and removal of plasma membrane proteins in HEK cells and neurons. By simultaneously visualizing ER/PM junctions and various transmembrane protein cargoes with total internal reflectance (TIRF) microscopy, we demonstrate that the vast majority of exocytotic delivery events for a recycled membrane protein, or for a membrane protein being delivered to the PM for the first time, occur at ER/PM junctions. Likewise, we observed stable clathrin clusters and functional endocytosis of PM proteins preferentially at EM/PM junctions. Thus, ER/PM junctions serve to organize the molecular machinery for both insertion and removal of cell surface proteins, highlighting a novel role for these unique cellular microdomains in neuronal secretory trafficking.

Quantum dot labeling of canine mesenchymal stromal cells for longitudinal visualization

Kristin Freund, Megan Aanstoos-Ewen, Laura Chubb, Ruth Rose, Tracy Webb, Nicole Ehrhart

Introduction: Determination of mesenchymal stromal cell (MSC) fate in vivo is critical for evaluating MSC efficacy; yet in vivo tracking of MSCs has been notoriously difficult. Quantum dots (QDs) are a novel labeling alternative to traditional fluorophores. Their advantages over traditional fluorophores, such as green fluorescent protein, make QDs attractive for MSC labeling and tracking in a complex in vivo environment. However, there have been no published data regarding the labeling of canine MSCs with QDs. The study objectives were: 1) develop a method for QD labeling of canine MSCs; 2) determine the duration of fluorescence in vitro; and 3) seed QD-labeled MSCs onto a demineralized bone matrix (DBM), determine percentage cell adherence, and confirm proliferation. We hypothesized that QDs could be visualized up to 14 days in vitro and that QD-labeled MSCs would adhere and proliferate when seeded onto DBM. Materials and Methods: Canine adipose-derived MSCs were labeled using QDs and imaged daily using fluorescent microscopy for 14 days. Labeled cells were seeded onto 0.25cc of canine DBM and cultured for 7 days. Results: QDs were passed on to daughter cells; however, the distribution between cells was not even. Fluorescence of QD-labeled MSCs was successfully observed for 14 days. Thirty-one percent of labeled cells adhered to DBM. Proliferation of QD-labeled MSCs on DBM was confirmed for up to 7 days. Discussion/Conclusion: Canine MSCs can be successfully labeled with QDs; these can be visualized using fluorescent microscopy for 14 days. Successful adherence to and proliferation on DBM was achieved. These data can be utilized to design in-vivo studies to determine the bone-regeneration potential and cell fate of an MSC/DBM tissue product.

Rapid in-vitro assay to detect CWD prions in deer saliva

Nina Garbino, Davin M. Henderson, Nathaniel Denkers, Amy V. Nalls, Candace Mathiason and Edward A. Hoover.

Chronic Wasting Disease (CWD) is an emergent efficiently transmitted prion disease (or transmissible spongiform encephalopathy) of cervids (deer, elk and moose) that results in a progressive uniformly fatal neurodegenerative disease. Prion diseases are characterized by the templated conversion of normal cellular prion protein into an abnormal conformation that is usually relatively insoluble and protease-resistant. Currently, CWD has been identified in 22 States, 2 Canadian provinces and South Korea and has been detected in two new states within the last year. Bioassay studies in deer and cervidized transgenic mice have indicated that shedding of infectious prions in excreta including saliva, urine and feces is likely an important factor in CWD transmission. Understanding the dynamics of prion shedding in saliva will be useful for antemortem diagnosis and surveillance of CWD in both captive and wild populations. The aim of this study, therefore, was to apply a new rapid in-vitro assay [real-time quaking-induced conversion (RT-QuIC)] to determine the time of onset, length and pattern of shedding of prions in deer saliva following infection. Thus far 142 blinded, longitudinally collected then randomized saliva samples from 17 CWD-infected and 3 uninfected white-tailed deer have been tested. Preliminary data demonstrate that shedding in saliva occurs as early as 6 months after infection, thus at least one year before deer exhibit clinical symptoms of CWD.

IGHV usage and somatic hypermutation analysis in canine B cell chronic lymphocytic leukemia.

Stacey George, Serena Mancha, Robert Burnett, and Anne Avery

Somatic hypermutation of the immunoglobulin heavy chain variable region (IGHV) gene is prognostic in human B-cell chronic lymphocytic leukemia (B-CLL). B-CLL clones that have undergone somatic hypermutation ("mutated") are associated with an indolent course, whereas "unmutated" clones yield a poorer prognosis. Additionally, a bias toward usage of certain IGHV genes in human B-CLL implies recognition of a common antigen, suggesting antigenic stimulation as a possible etiology. This study aimed to determine whether canine B-CLL has similar characteristics such that it could become a model for the human disease. DNA was extracted from convenience blood samples from 63 dogs with B-CLL. Immunoglobulin genes were amplified with PCR. PCR amplicons were sequenced directly as well as cloned and sequenced. Acquired sequences were compared to germline IGHV using NCBI BLAST to determine IGHV usage. In accordance with human studies, sequences were considered mutated if they differed more than 2% from the published germline sequence. In 15 cases we could not detect the neoplastic clone. In the remaining 48, we could unequivocally identify the clone and determine IGHV usage. Of 85 possible IGHV genes in the dog, 22.9% (11/48) of B-CLLs utilized VH41. VH41 was found in 75% (6/8) of boxers, a breed thought to be predisposed to the disease. VH41 does not appear to be common in dogs without B-CLL. A subset of clones (21/48, 43.8%) featured unmutated IGHV. All of the VH41 clones identified were either unmutated or highly similar to the germline sequence. Canine B-CLL shares some features with human B-CLL, including biased IGHV gene usage and populations of mutated and unmutated B-CLLs. Usage of VH41 predominates in B-CLL and does not appear to be common in dogs without B-CLL. Biased VH41 usage supports an antigenic-stimulation model for oncogenesis; thus naturally-occurring canine B-CLL has potential as a model for human B-CLL.

High variability in the risk estimates of zoonotic tuberculosis

Meghan E Gibas, Francisco Olea-Popelka

Recently, the World Health Organization classified zoonotic tuberculosis (TB) as a "neglected zoonosis". Conditions in the developing world including living in direct contact with animals infected with Mycobacterium bovis and the consumption of unpasteurized milk and dairy products, significantly increases the risk of this infection for humans. Current literature on zoonotic TB is inconclusive, incomplete, conflicting, and poorly communicated among health professionals. This has resulted in a serious misconception that only a small proportion (1.4%) of human TB patients suffer from zoonotic TB globally. This study is a systematic review of all currently available data on zoonotic TB evaluating the design, inclusion and exclusion criteria, laboratory protocols used, region, temporal components, and demographic characteristics of the study population (risk factors) in each study that estimated the risk of zoonotic TB. The preliminary findings indicate that the proportion of human TB patients suffering from zoonotic TB ranges from 0.4% to 45% depending on the region, epidemiological scenario, and methods and protocols used to isolate and differentiate M. bovis. This analysis will provide insight into the current variation in the estimation of the risk of zoonotic TB and will identify regions with a higher risk for this infection among humans, livestock, and wildlife. Thus, valuable information will be generated to better understand the risk of zoonotic TB worldwide, which will help to elucidate important epidemiological aspects of this neglected zoonosis. This new information will be used to design strategies to more effectively diagnose, prevent and control zoonotic TB.

Commuting and air pollution: A multi-pollutant exposure study

Nicholas Good, Taylor Carpenter, Anna Molter, Jennifer Peel, and John Volckens

The Fort Collins Commuter Study is a five-year project that will assess personal air pollution exposure and indicators of acute subclinical health responses to airborne pollution. The study examines exposures to multiple air pollutants during 30-hour periods of continuous personal monitoring, enabling the relative contributions of multiple activities to be assessed. Given that road traffic is a major source of air pollution, exposure during commuting may make up a substantial fraction of a person's daily exposure. The first aim of the Fort Collins Commuter Study is to examine how the choice of commuting route and transport mode impacts exposure. Fifty participants are being recruited into the study. Each commuter is completing eight full days of exposure assessment and is randomized to travel by car and by bicycle from their home to their workplace and follow pre-arranged nominally higher and lower traffic routes. The result is a total of 400 commute-days of exposure data. Each commuter carries a backpack containing monitoring equipment and is fitted with a personal heart rate and activity monitor. Exposure to black carbon, particulate matter, carbon monoxide, particle number concentration, nitrogen dioxide, volatile organic compounds and noise are being measured. The location of the commuter is recorded using a GPS receiver. The results presented will examine how route and transport mode impacts commuter exposure to multiple pollutants. The relative contribution of exposure during commuting will be assessed in the context of the total daily exposure. Initial results suggest commuting can often be a substantial contributor to daily total personal exposure, but other activities can also be dominant factors.

Effects of dietary rice bran or navy bean on human plasma cytokine levels and leukocyte telomere length

Gregory Harbison, Erica Borresen, Genevieve Forster, Susan Bailey and Elizabeth Ryan

Emerging studies suggest increased rice bran (RB) and navy bean (NB) consumption is associated with prevention of cancer and other aging-related chronic diseases. This study examined the relationship between dietary RB and NB consumption by healthy adults and colon cancer survivors on plasma inflammatory cytokine levels and leukocyte telomere length (TL). Blood samples used in this study were collected from individuals enrolled in the Beans/Bran Enriching Nutritional Eating For Intestinal Health Trial (BENEFIT, NCT01929122). The primary goal of this study was to determine relationships between cytokine levels (TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, sCD40L, VEGF), telomere length, and effects of RB or NB dietary intake for 2 and 4 weeks. We currently have samples from 24 colorectal cancer survivors and 11 healthy volunteers with each participant randomized by BMI and sex to a control, RB, or NB diet group. Peripheral blood mononuclear cells (PBMCs) and plasma were isolated for TL measurement and cytokine level quantification, respectively. PBMC relative TL was measured by real-time PCR. Cytokine levels were measured using Human Cytokine/Chemokine Immunoassay (Milliplex® MAP). Interim data analysis suggests a possible relationship between IFN- γ levels and TL independent of dietary study groups. TNF-α and IL-8 were also observed to correlate with TL in some participants. These trends were not statistically significant and merit further evaluation in a larger sample set. Similarly, no statistically significant differences were observed during interim analysis for cytokine levels or telomere length across dietary groups compared to control and baseline. Findings will be reported on an individual and group basis, and the identification of participants that did respond across diet groups for both cytokine levels and telomere will be presented. This suggests the possibility of observing significant changes in a longer dietary intervention study. This work is supported by NIH NCI R21CA161472.

Whole Body Analysis of CWD Prion Peripheralization

Davin M. Henderson, Nathaniel D. Denkers, Amy V. Nalls, Candace K. Mathiason and Edward A. Hoover.

Chronic wasting disease (CWD) is transmitted with unique efficiency among free ranging cervids in North America (now involving 22 U.S. states, 2 Canadian provinces and South Korea). Likewise, the level of CWD prion dissemination outside the central nervous system and consequent shedding via secreta and excreta, e.g. saliva, urine, feces, nasal fluids appears greatest among the prion diseases, likely responsible for the florid horizontal spread of CWD. Determining how and when CWD prions disseminate throughout the body during disease course is key to understanding how prions may efficiently spread throughout populations. A robust characterization of prion distribution in an entire host organism has not been possible and remains unknown. Whether prion trafficking varies by route of infection is likewise unknown. We hypothesize that CWD prion distribution is more robust in hosts independent of species infected and that route of introduction influences trafficking and tissue distribution of CWD. To explore these hypotheses and gain more comprehensive and robust insight into CWD pathogenesis, we are employing real-time quaking induced conversion (RT-QuIC) in an expanded micro-well format to permit increased throughput and a more global whole body view of prion distribution in infected white-tailed deer. Data to be presented from initial studies suggest that intra-cerebrally infected deer develop a more limited disseminated prion distribution body-wide vs. that in deer infected by the aerosol route.

Effects of mRNA decay on transcription: maintenance of steady state mRNA levels through buffering

Ashton Herrington, Jerome Lee, Ju Youn Lee, Ashley Neff, Bin Tian, Jeffrey Wilusz and Carol J Wilusz

There is considerable evidence that transcription influences mRNA decay and several models for how this can occur have been put forward. Recent studies suggest the cell may also have mechanisms by which mRNA decay rates can also feedback and influence transcription. We examined the differences in mRNA decay between human foreskin fibroblasts (HFF) and genetically identical induced pluripotent stem (iPS) cells that were derived from these HFFs. When we assessed changes in mRNA abundance between the two cell types we found that there was a negative correlation between decay and abundance. Transcripts that were more stable in iPS cells were frequently of lower abundance than in HFFs. Conversely, destabilized transcripts tended to be more abundant. We undertook a global analysis of mRNA decay rates in C2C12 myoblasts following depletion of the deadenylase PARN. We determined that several mRNAs were significantly stabilized in a PARN knockdown cell line. Intriguingly, as before, we found that the abundance of these stabilized transcripts either was not altered, or was slightly reduced. The opposite was true for the transcripts that were destabilized in PARN KD cells – their abundance tended to increase. We validated the changes in mRNA decay and abundance and also measured newly transcribed pre-mRNAs as an indicator of transcription rate. In each case, despite clear decreases in mRNA decay rates, the mRNA abundance and pre-mRNA abundance were reduced instead of increased as accepted models would predict. These results imply that the cell is able to buffer mRNA levels by down-regulating transcription to compensate for reduced mRNA decay. In yeast, the XRN1 exoribonuclease modulates both decay and transcription (Haimovich et al Cell, 2013). We currently are investigating whether this is also the case in mammalian cells and investigating the roles of various decay enzymes such as XRN1, CCR4, and DCP2 in regulating transcription.

Measuring cytokine profiles longitudinally during prion infection

Dana C. Hill, Breanna Smith, Mark D. Zabel*

Many studies have been performed analyzing the effects of inflammation during acute and chronic transmissible spongiform encephalopathies. Both peripheral and central nervous system immune responses likely play a role in prion-associated neurodegenerative disease due to persistent release of inflammatory cytokines from microglial cells, astrocytes and other immune cells. Identifying cytokines present throughout prion disease will help determine if they mediate immunopathology that contributes to neuropathology and wasting disease. The purpose of this study is to measure cytokine profiles longitudinally in transgenic mice infected with prions as compared to control animals inoculated with normal brain homogenate. In a preliminary study, serum cytokine levels were measured in cervidPrP-expressing mice infected with CWD prions, and control mice inoculated with normal brain homogenate using the BioPlex suspension array system. We have analyzed IL-1β, TNF-α, IFN-γ, GM-CSF, IL-2, IL-6, IL-10, IL-4, and IL-5 at baseline levels, day one post inoculation, and at two-week intervals through terminal disease. At the time of sacrifice, mouse serum and brain homogenate cytokine levels were also analyzed. As a distinct continuation of this study, TgA20 mice infected with RML mouse-adapted prions will be analyzed in a similar longitudinal fashion, with additional sacrifices at 40, 60 and 80 days post inoculation for extended comparison of central cytokine profiles. Age and sex related cytokine variations will be taken into account and in addition to the cytokines listed above, IL-12p70, IL-13, and IL-18 will also be analyzed. This study represents the first longitudinal experiment analyzing systemic and neuro-inflammatory responses in the same prion-infected animals throughout their entire disease course.

Robotically improving accuracy of the cervid prion cell assay (CPCA) method

Elizabeth Hoaglund, Jifeng Bian, Carla Calvi, and Glenn Telling

Chronic Wasting Disease (CWD) is a naturally occurring prion disease of cervids resulting in degenerative signs similar to other transmissible spongiform encephalopathies (TSEs). Historically, CWD has been characterized by mouse bioassay, which is time consuming and expensive. Recently, there has been a push to quantify prion infectivity by the newly developed cervid prion cell assay (CPCA), which is faster and more economical. Further refining the CPCA will develop a high throughput method to measure infectivity in CWD. This study aimed to see if automation of the CPCA could improve accuracy of reported results. It was demonstrated that performing critical steps, such as making the single cell suspension before transfer to filter plates, can be improved for accuracy through the use of a robot. Continuing to refine this assay to a high throughput design will allow for more experimentation to be done comparing CWD strains from different infected animals, or even from animal to animal within a given study population. Greater use of this assay will not only save time and costs, but will further serve to reduce and replace animal bioassays in the future for determining prion levels in animals suffering from CWD. Future directions include investigating whether infection efficiency is different between different cervid species.

Presence of toxigenic Pasteurella multocida ssp. Multocida evaluated in the oral cavity of cats with feline chronic gingivostomatitis.

April C Hohnbaum and Roxane MacLellan

Feline Chronic Gingivostomatitis (FCGS) is an oral disease recognized in domestic cats. Currently there is no known etiology of this disease and thus treatment options are limited. This study aims to investigate possible infectious pathogens present in the affected cats' oral cavities that may contribute to the pathogenesis of FCGS. The agents that will be investigated are Pasteurella multocida ssp. Multocida, Feline Herpes Virus 1, and Calicivirus. Their presence in feline oral cavities will be evaluated by 1) swabbing the caudal oral vestibule and culturing the swabs for presence of P. multocida ssp. Multocida , 2) determining presence of all three organisms by PCR analysis, and 3) analyzing biopsy samples of affected cats' caudal mucosa by histopathology. In addition to these goals, if P. multocida ssp. Multocida is present, this study also seeks to specifically investigate the presence serotypes A and D, and the presence of the toxA gene encoding for the Pasteurella Multocida Toxin (PMT). The serotype and presence of the toxA gene will be identified by PCR. Two populations of cats will be compared. The control population will be cats without evidence of oral or dental disease. The test population will be client owned cats diagnosed with FCGS. If one or more of the above agents are found in higher numbers in the oral cavities of the test population than in the control population, or if P. multocida ssp. Multocida serotypes A or D, or the toxA gene is found in higher levels in the test population, it would indicate that more research needs to be conducted to find out what role, if any, these agents have in contributing to the development of FCGS.

In Vitro susceptibility of human influenza A and B viruses to nitazoxanide and tizoxanide

Cindy Hong, Drew Koch, Lori Bentsen, and Gabriele Landolt.

Human influenza epidemics can affect all age groups yearly, most notably causing risks of serious complications among children younger than age two, adults age 65 or older, and people with preexisting medical conditions. While antiviral drugs are available for prevention and treatment of influenza, antiviral resistance development, mediated through mutational adaptation, is a considerable problem and can limit the effectiveness of treatment. Thiazolides, such as nitazoxanide (NTZ) and its active metabolite tizoxanide (TIZ), have been found to have broad antibacterial, antiprotozoal, and antiviral activity. While the mechanism of action of NTZ is not fully understood, its antimicrobial effect is thought to be mediated by a cell-specific rather than a virus-specific mechanism. This suggests a low potential for the development of resistance to thiazolides by viral mutational adaptation. Consequently, NTZ and its derivatives may serve as valuable alternatives to conventional antiviral therapy provided that they demonstrate inhibitory activity against human-lineage influenza A and B viruses. In this study, we examined the in vitro antiviral activity of NTZ and TIZ against four human-lineage influenza A virus isolates, including two oseltamivir resistant strains, as well as one influenza B virus.. Both NTZ and TIZ inhibited replication of all viruses tested with 50% effective concentrations (EC50) ranging from 0.06 to 0.25 mM and from 0.05 to 0.13 mM, respectively.. These results suggest that NTZ and TIZ exhibit antiviral activity against human influenza A and B virus and may be useful for treatment. Further investigation is currently being conducted to determine whether NTZ and TIZ work synergistically with other classes of influenza antiviral drugs. . Additionally, clinical trials are underway to determine the efficacy of NTZ and TIZ in human patients.

Evaluation of a point-of-care glucose and beta-hydroxybutyrate meter operated in various environmental conditions in prepartum and postpartum sheep

Katlin J Hornig, Stacey R Byers, Robert J Callan, Timothy Holt, Megan Field, Hyungchul Han

The objective of this study was to compare beta-hydroxybutyrate (BHB) and glucose concentrations measured with a dual-purpose point-of-care (POC) meter designed for use in humans and a laboratory biochemical analyzer (LBA) to determine whether the POC meter would be reliable for on-farm measurement of blood glucose and BHB concentrations in sheep in various environmental conditions and nutritional states. Thirty-six pregnant mixed-breed ewes involved in a maternal feed restriction study were utilized for this project. Blood samples were collected from each sheep at multiple points throughout gestation and lactation to allow for tracking of gradually increasing metabolic hardship. Whole blood glucose and BHB concentrations were measured with the POC meter and compared with serum results obtained with an LBA. A total of 464 samples were collected. Whole blood BHB concentrations measured with the POC meter compared well with LBA results, and error grid analysis showed the POC values were acceptable. Whole blood glucose concentrations measured with the POC meter had more variation, compared with LBA values, over the glucose ranges evaluated. Results of error grid analysis of POC-measured glucose concentrations were not acceptable, indicating errors likely to result in needless treatment with glucose or other supplemental energy sources in normoglycemic sheep. The POC meter was user-friendly and performed well across a wide range of conditions. The meter was adequate for detection of pregnancy toxemia in sheep via whole blood BHB concentration. Results should be interpreted with caution when the POC meter is used to measure blood glucose concentrations.

Transmission and maintenance of Mycobacterium ulcerans by Anopheles gambiae

J. Charles Hoxmeier, Brice D. Thompson, Brian Foy, Karen Dobos

Buruli Ulcer disease is a severe, ulcerative disease of the skin resulting from infection with Mycobacterium ulcerans. Considered a neglected tropical disease, Buruli Ulcer disease is increasing in incidence and prevalence while basic questions remain regarding its route of transmission and environmental maintenance. This study evaluated the interaction between Anopheles gambiae mosquitoes and M. ulcerans bacilli to determine if this species could play a role as a vector or as a reservoir for this disease. Mosquitoes were raised in water inoculated with live, virulent M. ulcerans, gamma-irradiated M. ulcerans (whole, dead), or no supplemental bacteria, over a period of 8-10 days at 28C and 70% humidity. The subsequent development of the mosquitoes was monitored on a daily basis. A Kaplan-Meier survival curve was generated and indicated a significant difference ($p \leq 0.001$) in survival to adulthood between mosquitoes raised with live M. ulcerans compared to irradiated M. ulcerans. PCR was used for detection of M. ulcerans DNA, and immunofluorescence (IF) imaging to localize and visualize M. ulcerans on the mosquito. Mosquitoes from both the live and dead M. ulcerans-fed groups demonstrated positive PCR signals. IF analysis demonstrated different M. ulcerans contamination patterns on the external tissues of the proboscis between treatment groups. Finally, we initiated metabolomics profiling and bacterial culture of adult mosquitoes from all treatment groups, in an effort to begin to understand the differences observed in developmental delay due to the impact of water and insect contamination with live M. ulcerans. Many mosquito species, including A. gambiae, are common in regions in which Buruli Ulcer disease is endemic, and the most prominent risk factor for development of disease is an association with standing water. A relationship between mosquitoes and M. ulcerans may play a role in the transmission and environmental maintenance of Buruli Ulcer disease.

Of mice, men, and elephants continued: the relationship between articular cartilage zonal thicknesses and body mass

Linda Hyatt, Jos Malda, Mattie van Rijen, and Mark van Turnhout

Articular cartilage is able to function in allowing efficient joint movement due to the highly organized and functionally specific matrix network of collagen fibrils dividing it into three zones. The Department of Orthopaedics at the Utrecht University Medical Center conducted a study of 58 mammalian species, demonstrating that the thickness of the articular cartilage has a negative allometric relationship to body mass; thus animals with a larger body mass have relatively thinner articular cartilage than those with small body masses. The purpose of this particular study, was to build on the aforementioned study and determine how the zonal organization of articular cartilage varies with body mass. The prediction is that the widths of the zones of articular cartilage will always maintain the same proportion to the total width of articular cartilage, despite a change in body mass. To determine the zonal organization across species with different body masses, 33 samples from the previous study were used, representing 10 species of mammals. Ostochondral core samples in paraffin were sectioned and either left unstained, stained with hematoxylin and eosin, or picrosirius red for different methods of image analysis in order to determine the cell density of the layers, width of the layers, and light retardance patterns within the layers. The results may have implications in the field of tissue engineering for joints damaged by trauma or aging.

Zinc finger protein mRNAs are regulated post-transcriptionally in stem cells: A tale of fingers and (poly(A)) tails.

Aimee L Jalkanen, Ashley T Neff, Ju Youn Lee, Bin Tian, Jeffrey Wilusz, and Carol J Wilusz

The C2H2 zinc finger proteins (ZNFs) are highly conserved transcription factors important for development, differentiation and tumor suppression. Global analysis of mRNA decay rates in human induced pluripotent stem (iPS) cells and genetically matched human foreskin fibroblasts (HFF) revealed that mRNAs encoding C2H2 ZNFs were significantly more stable in iPS cells than in fully differentiated fibroblasts but surprisingly had reduced abundance. Given the large number of C2H2 ZNF mRNAs affected (> 100), coordinated changes in their expression potentially have wide-ranging impacts on pluripotency and differentiation. Our goal is to characterize the mechanisms, sequences and factors involved in modulating decay of C2H2 ZNF mRNAs. Decay of most mRNAs initiates with removal of the poly(A) tail and can be regulated through association of trans-acting factors with the 3'UTR. We find that ZNF mRNAs have unusually short poly(A) tails in iPS and HeLa cells. In HFFs, the poly(A) tails appear even shorter which might contribute to reduced ZNF mRNA stability in these cells. In HeLa cells, a luciferase reporter plasmid bearing the ZNF12 mRNA 3'UTR produces a reporter transcript with the same short poly(A) tail as the endogenous ZNF12 mRNA suggesting that the sequences responsible for restricting the poly(A) tail are contained within the 3'UTR. The ZNF12 3'UTR reporter also showed decreased luciferase activity compared to controls. This may be linked with the observation that ZNF mRNAs are predominantly restricted to the nucleus in HeLa cells where they would not be able to access the translation machinery. In conclusion, the 3'UTR of ZNF mRNAs appears to play a significant role in multiple aspects of post-transcriptional regulation including control of poly(A) tail length and translation repression. We are currently testing whether the 3'UTR is also sufficient to allow differential regulation of decay in iPS and HFFs and to narrow down the sequence elements involved.

Degenerative and infectious change in heart valves from Northern Sea Otters

Sam Johnson, Verena Gill, Kathy Huntington, E.J. Ehrhart, Brad Charles, E. Christopher Orton, Colleen Duncan

Northern sea otters (Enhydra lutris kenyoni) have a high prevalence of mitral and aortic valvular endocarditis (VE), characterized by proliferative nodules containing fibrin, necrotic debris, inflammatory cells and often, identifiable bacteria. Coxiella burnetii can manifest as VE in chronic human infections and given their shared habitat with infected pinnipeds (Callorhinus ursinus and Eumetopias jubatus), it is hypothesized that sea otters may also have been exposed to this organism. Human patients with degenerative heart valve disease are predisposed to infection by Coxiella leading to VE. The prevalence of heart valve lesions in sea otters suggests that they may have a form of degenerative valve disease similar to that of humans. In canines and humans, a characteristic sign of myxomatous mitral valve disease (MMVD) is endothelial-mesenchymal transdifferentiation (EMT), a mechanism marked by myofibroblast expression of a-smooth muscle actin (a-SMA). The objective of this study was to determine the presence of C. burnetii in 10 sea otter valves with VE lesions and 16 valves without lesions, and further, to identify whether or not these valves undergo degenerative changes consistent with canine and human EMT. All of the sea otter heart valves were PCR negative for C. burnetii, indicating an absence of the bacterium within the valves. a-SMA immunohistochemical staining of the sea otter heart valves showed increased intensity and altered distribution consistent with canine degenerative change. EMT within sea otter heart valves, diseased or not, suggests a similar pathogenesis to canines and humans and raises further questions about sea otter degenerative valve disease and its relationship to bacterial infection and VE.

Molecular interaction analysis of prions and potential peripheral receptors

Sarah J Kane and Mark D Zabel

Mouse knockout studies have revealed a critical role for the innate immune system, specifically Complement, in peripheral prion propagation. The work in this study aims to elucidate whether Complement proteins bind prions in order to identify the molecular players directly involved in binding prions prior to propagation in lymphoid tissues and subsequent neuroinvasion and neurodegeneration. Surface Plasmon Resonance (SPR) technology was used to observe whether Complement proteins C3b, C3d, Complement Receptors CD21/35, C1q, and Factor H bind high density PrPC thought to mimic prion amyloid, as well as bona fide infectious prions enriched from an elk with Chronic Wasting Disease (CWD). Our studies reveal CD21/35 and C1q bind both high density PrPC and prions from infected brain sturdily, whereas C3b, C3d, and Factor H bind high density PrPC minimally and brain prions negligibly. These data support in vivo data revealing CD21/35 deficiencies as more protective than deficiencies in their ligands C3b and C3d, and may provide therapeutic target(s) for preventing disease spreading. Future directions include elucidating the role of Factor H in peripheral prion propagation in a mouse adapted Scrapie model. Colorado State University Prion Research Center, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences.

Looking Beyond the Neuron: Neuroinflammation in California Sea Lions Exposed to Domoic Acid

Kelly S Kirkley, James E Madl, Colleen Duncan, Frances M Gulland, and Ronald B Tjalkens

California sea lions (CSLs) exposed to the marine biotoxin domoic acid (DA) develop an acute or chronic toxicosis marked by neurological dysfunction and seizures that is often refractory to current treatments. Experimental seizure studies have shown glial activation and the resultant oxidative and inflammatory mediator release to play a significant role in generation of seizures through perturbing glutamate-glutamine cycling, but the involvement of these pathways in DA toxicosis is unknown. Sections from archived hippocampi from 7 control and 13 CSLs diagnosed with DA toxicosis were immunofluorescently stained for markers of gliosis and oxidative/nitrative stress and changes in glutamine synthetase (GS) and compared to pathological changes observed in hematoxylin and eosin stained sections. Quantitative counts revealed increasing loss of microtubule associated protein-2 positive neurons with elevations in 4-hydroxynonenal based on chronicity of exposure while the number of activated glia expressing nitric oxide synthase 2 and tumor necrosis factor-α followed a pattern based on pathological severity. There were no significant changes in the amount of GS positive cells, but there was substantial upregulation of 3-nitrotyrosine in GS expressing cells and in neurons especially in chronic DA animals. These changes were most consistently seen in the dentate gyrus and in the cornu ammonis (CA) sectors CA3, CA4, and CA1; however, CA2 and subiculum were also often affected. The results of this study indicate that gliosis and resultant changes in GS are possibly important mechanisms in DA induced seizure and combinatorial therapy aimed at limiting these changes could be effective in treating animals

Assaying the role of Platelet endothelial cell adhesion molecule-1 (PECAM-1) in vitro

Alexandra M Kumor, Amir N Ahmed, Conner L Jackson, Alan R Schenkel

Prior studies have shown that PECAM deficient mice spontaneously develop micro-bleeds to an unknown stimulus. This results in fibrosis that resembles human Idiopathic Pulmonary Fibrosis (IPF). Particulate Matter 2.5 (PM2.5) has been shown to cause lung hemorrhage at high doses, and may be a trigger of IPF. We hypothesize that particulate matter 2.5 has adverse effects on endothelial cell PECAM-dependent function other than cell viability. To examine the effect of PM 2.5 on lung endothelial cell function we are purifying lung endothelial cells and culturing them with PM 2.5. In initial test doses we expected to see cell death, none was observed, although contaminating macrophages may have ingested most of the PM 2.5. Next we will test cell barrier function with retro viral vectors to inhibit PECAM-1 expression.

Building a virtual cat: a physiologic-based pharmacokinetic (PBPK) model for investigating drug dosing in cats

Renee C. Lake, Ryan J. Hansen, Paul J. Lunghofer, Daniel L. Gustafson

Background: Cats have known genetic abnormalities in UGT1A6 and ABCG2, leading to alterations in glucuronidation and drug transport that have often resulted in severe drug toxicities. Predicting drug disposition in cats via extrapolation from canine-based pharmacokinetic models for drugs metabolized via such pathways is thus rarely appropriate, especially for drugs lacking an extensive clinical database. The purpose of this research is to develop a physiologic-based pharmacokinetic (PBPK) model of the cat to better simulate drug disposition by virtue of modeling differences with regard to metabolic pathway efficiencies. Doxorubicin (DOX) is selected as the initial drug for modeling given its frequent use in feline cancers. Methods: Weights and volumes of relevant organs and tissues were collected from necropsies conducted on 28 adult cats (>1 yr. old, 14 male/14 female). Biochemical parameters for DOX metabolism and tissue disposition were measured from microsomal and cytosolic subcellular fractions isolated from freshly harvested feline organs. Enzyme specific activity was determined for NADPH:cytochrome C reductase and carbonyl reductase (CBR1) due to their known importance in DOX metabolism. Initial DOX metabolism activity was validated via incubation of DOX with feline liver microsomes over a 3 hr. time period. Results: Physiologic weights showed mean values of 15.82 g (heart), 109.63 g (liver), 33.95 g (kidney), 24.44 g (stomach), 63.15g (small intestine). NADPH:cytochrome C reductase showed average activities (units/mg) of 0.1056 (liver), 0.0190 (kidney), 0.0019 (heart), and 0.0570 (small intestine), and CBR1 activities (units/mg) were 0.0151 (liver), 0.0533 (kidney), 0.0104 (heart), and 0.0187 (small intestine). DOX metabolism showed expected decreases in DOX and associated increases in the primary metabolite Doxorubicinol. Conclusions: Experimental protocols for the characterization of feline metabolism and physiology parameters were developed, with successful demonstration of enzyme specific activities and DOX metabolism, representing a first step toward development of a PBPK model for drug dosing in cats.

Evaluation of a model demonstrating mitigation of nociceptive response to oxytetracycline injection site inflammation by flunixin meglumine in dairy cows

Andrea S Lear, Alex Yager, Stacey R Byers, Jason Ahola, and Robert J Callan

The purpose of this study was to determine if flunixin meglumine administration could mitigate nociceptive pain associated with oxytetracycline injection site inflammation as measured objectively by an algometer. Oxytetracycline (10 mg/kg) was administered intramuscularly at a single site in the semimembranosus/semitendinosus muscle group of the hind leg in 5 non-lactating, mature cull dairy cows. The opposite rear limb was used as the control site which received a sham injection. Cows were randomized receiving either flunixin meglumine (2.2 mg/kg) or equivalent volume of 0.9% saline IV at 24-hour intervals for 5 days. An algometer was used to measure the amount of pressure applied directly at the injection site required to elicit a nociceptive response at 1, 6 and 24 hours after treatments, daily. Statistical analysis was performed using standard ANOVA and pairwise comparisons. The oxytetracycline injection site demonstrated a more sensitive nociceptive response compared to site that received the sham injection. Cows treated with flunixin meglumine resulted in higher algometer pressure necessary to elicit a nociceptive response from 78-120 hours after oxytetracycline injection compared to control animals. In conclusion, flunixin meglumine appeared to mitigate nociceptive response at the oxytetracycline injection site after 78 hours of treatment. Additional studies are needed to further refine this model for assessing the mitigation of inflammatory mediated pain by nonsteroidal anti-inflammatory drugs. This study was funded through private donations.

Quantitative measurement of bacterial 16s rRNA genes in plasma of FIV infected cats

Jonathan Lee, Alora LaVoy, Lin Zhang, Rita D. Simoes, Gregg A. Dean

Natural or experimental infection of cats with feline immunodeficiency virus (FIV) causes an immunodeficiency syndrome similar to HIV infection of people and SIV infection of macaques. All three viruses are associated with chronic immune activation, even under conditions where viral replication is well controlled. Because the gastrointestinal tract is a primary target organ of FIV infection, it is possible that translocation of microbial products across a damaged mucosal barrier could be responsible for driving systemic immune activation. Previous research has shown that FIV infection targets CD4+ T cells in the lamina propria of the intestine, subsequently leading to a damaged GI mucosal barrier. Evidence of bacterial translocation concomitant with systemic immune activation, however, has not been demonstrated in FIV infected cats. Hypothesis: FIV infection causes disruption of the intestinal mucosa resulting in microbial translocation and subsequent systemic immune activation. To address the hypothesis, a longitudinal study of FIV infected cats was performed. Expression of the proliferation marker Ki67 in peripheral blood lymphocytes was used as an indicator of systemic immune activation. Soluble CD14 (sCD14) was measured by ELISA and used as a surrogate marker for circulating bacterial lipopolysaccharide. However, a direct measure of a bacterial product was needed to definitively address this aspect of the hypothesis. To accomplish this, a quantitative, real-time PCR assay for bacterial 16S rRNA genes was developed and utilized. The present study sets the stage for further investigation using the FIV/cat model to explore therapeutic interventions aimed at reducing microbial translocation and thereby mitigating the negative sequelae of chronic immune stimulation.

Deaths related to musculoskeletal injury peak mid racing season in Colorado racehorses

Alice B Loughridge and Chris E Kawcak

Surveillance of the diseases and injuries that result in the death of racehorses is of paramount importance in order to identify the most common causes and risk factors for fatality. Since 2000, the Colorado Racehorse Postmortem Evaluation Program has ensured that every racehorse that dies while at a Colorado racetrack is necropsied and the cause of death and any pathological findings are documented. The goal of this project was to survey the causes of death in racehorses at Arapahoe Park Racetrack. A total of n=111 racehorses died or were euthanized from 2000-2012. The most common cause of death was due to musculoskeletal injury, followed by gastrointestinal, and respiratory conditions. Of the musculoskeletal-related deaths, proximal sesamoid bone fractures were the most common cause of fatality in both Thoroughbreds and Quarter Horses, followed by carpus injuries. Interestingly, a sharp increase in the number of deaths related to musculoskeletal injury was observed at week 7 of the racing season and this continued until week 13. The exact reason for this pattern of injury is unclear, but it is possible that at the height of the summer, the track becomes dryer and firmer. It is also possible that as the racing season progresses, minor injuries progressively worsen with the accumulation of high-speed exercise. Knowledge about the most common fatal injuries and characteristics of those injuries can be used to update racetrack veterinarians at Arapahoe Park so they may be more likely to detect a minor abnormality before it becomes a catastrophic injury.

Attenuated Activity of Clofazimine in a Mouse Model Exhibiting Caseous Necrosis

Edward R Lyon, Veronica Gruppo, Elizabeth Brooks, Scott Irwin, Christopher Schrupp, Brendan Prideaux, Véronique Dartois, and Anne J Lenaerts

It is estimated that approximately one third of the world is infected with M. tuberculosis. The emergence multiple drug resistant and extensively drug resistant tuberculosis threatens to undermine our efforts to control the epidemic spread of this pathogen. Novel drugs and combination drug regimens are desperately needed to reduce the global TB burden. Clofazimine (CLF) has recently gained renewed interest as a drug that could potentially shorten the duration of therapy or be used in combination to treat drug resistant infections. As such, we wished to examine the efficacy of this drug in the 'Kramnik' mouse model of infection which exhibits caseous necrotic granulomas that more closely resemble pulmonary disease in humans. When compared to Balb/c mice which do not develop highly organized caseous necrotic granulomas, we observed attenuated activity in the lungs of Kramnik mice. In the spleens of Kramnik mice which do not present with caseous necrosis, CLF activity was equivalent to that observed in Balb/c mice. When CLF was administered prior to the formation of necrotic granulomas, activity was restored, implicating a central role for the pathological process in the diminished activity. As CLF is currently being considered for treatment of TB in humans, it will be important to understand the mechanism responsible for the attenuated activity.

Homologous recombination repair is required for G2-phase potentially lethal damage repair

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We have carried out the first study aimed at examining the DNA repair of potentially lethal damage (PLD) during the G2-phase of the cell cycle. A novel cyclin-dependent kinase 1 inhibitor, RO-3306, was used to arrest cells in G2. We have found that while arrested in G2, wild type Chinese hamster ovary (CHO) cells, display a significant downward modulation of radiation-induced cell-kill in comparison to cells released from G2 immediately after a genotoxic insult such as radiation. Quantitatively, both the extent of the genotoxic damage, scored as the number of chromosomal aberrations, and the severity of interference with normal cell proliferation, was measured by the length of the lag time preceding the recovery of delayed entry into mitosis were reduced by holding of the cells in the G2 phase of the cell cycle. This arrest is thereby allowed cells to recover from damage and increase survival. This G2 PLD repair capability was moreover observed in non-homologous end joining (NHEJ) mutants, and not in homologous recombination (HR) mutant cell lines. Based on our cell survival assays for cells whose DNA was damaged in G2, NHEJ-mutant cell lines were found to be very sensitive to gamma-ray exposure when compared to G2-HR mutant. Our findings suggest that, following exposure to ionizing radiation during the G2 phase of the cell cycle, the NHEJ DNA repair pathway is responsible for the majority of the non-PLD DNA damage repair, and conversely, that the HR DNA damage repair pathway is responsible for the repair of PLD DNA damage during this phase of the cell cycle

The effects of pathogen reduction technology on malaria (Plasmodium falciparum) in whole blood units

Caitlyn Martinez, Shawn Keil, Christine Olver

Plasmodium falciparum (one of several species of malaria that infect humans) is a deadly blood borne parasite transmitted by mosquitos that can also be spread through infected blood units. Malaria causes over half a million deaths each year. It is difficult to screen for and detect in blood units increasing the likelihood of transmission of the organism. Pathogen Reduction Technology (PRT) (adding riboflavin to blood products and exposing them to UV light) has previously been shown to significantly reduce pathogen viability, greatly reducing the transmission risk of many types of harmful organisms. Our hypothesis was that treating whole blood units using PRT would reduce malaria viability to a level that decreases the risk of transfusion transmission of the parasite. We cultured serially-diluted blood from untreated and PRT-treated infected blood units for parasite growth. We scored these either positive or negative for parasite using thick smear slide preparations. The log reduction produced by the treatment was calculated based on the thick smear results. We showed that there is a considerable reduction in viable parasite load of treated blood units. This indicates the likely future value of this treatment for clinical implementation, especially in regions where Malaria is endemic and blood transfusions are necessary. Future studies may include the efficacy of this treatment on different species of malaria (P. vivax, P. ovale, P. malariae, P. knowlesi), and to determine if efficacy is dependent on parasitemia or parasite stage.

Evidence for endothelial to mesenchymal transition in canine degenerative mitral valve disease

Derek Matthews, Christopher Orton

Degenerative mitral valve disease is an important cause of heart failure and death in dogs and humans. The disease shows remarkable pathologic similarity in both species, including deposition of glycosaminoglycan, net catabolism of the fibular ECM, and focal areas of high-density myofibroblasts that are thought to mediate ECM pathology. The origin and mechanism of increased myofibroblast density is unknown. We hypothesized that regions of high density myofibroblasts in degenerative mitral valves might be the result of endothelial to mesenchymal transition (EndMT). To address this hypothesis, we determined presence of CD-31, a marker of endothelial cells, and α smooth muscle actin (αSMA), a marker of myofibroblasts, on canine normal and degenerative mitral valves. We reasoned that if EndMT is active that cells expressing both CD-31 and αSMA would be present within the regions of high density myofibroblast. We further reasoned that TGF-β1, a known initiator of EndMT, would co-localize to myofibroblast region. The anterior leaflet of the mitral valve was collected from six dogs that died for reasons unrelated to heart failure. The leaflets were scored using the Whitney scale and leaflets scoring higher than type 1 were deemed to be diseased. Immunohistochemistry methods were then performed on the three control and three diseased leaflets to detect the presence of α-smooth muscle actin and CD31. Results show that many regions with increased myofibroblast density co-express CD-31, αSMA, and TGF- β1. These findings indicate that the myofibroblasts seen in degenerative mitral valves express endothelial cell markers, implicating EndMT as the pathologic process in degenerative mitral valve disease.

Characterization of the Salivary Antibody Response in FIV-infected Domestic Cats

Craig Miller, Karen Boegler, Scott Carver, and Susan VandeWoude

Feline immunodeficiency virus (FIV) is believed to be transmitted primarily by bite wounds via salivary secretions, although mechanisms associated with salivary transmission have not been well studied. Human immunodeficiency virus (HIV) is also known to be present in the saliva of infected individuals and has been shown to be genetically, structurally, and biochemically similar to FIV. Studies involving HIV salivary pathogenesis have increased the prospect of alternative antiviral therapies and diagnostic methodologies in endemic areas. Therefore, further elucidation of lentiviral salivary excretion and transmission characteristics may have significant implications in both medical and veterinary research. Other studies investigating antibody kinetics in plasma of FIV-infected cats and in saliva from HIV-infected humans have shown that total IgG antibodies increase over time, while IgA antibodies remain static or are inconsistent. To characterize the salivary antibody response in FIV infection of domestic cats, microsphere immunoassays (MIAs) were performed on saliva from naïve and infected animals to quantitate Total IgA and IgG and to detect FIV-specific antibodies (α-FIV Capsid and α-FIV Surface Protein (SU)) at various time points. We demonstrate that on average, Total, Capsid and C36 SU positive-background IgA and IgG antibodies increased over time. Significant average differences were also detected for IgG Capsid, IgG C36 SU positive-background, and IgA C36 SU positive-background and negative-background. Significant effects of treatments over time were detected for IgG Capsid and IgG C36 SU positive-background, with antibody levels being greater in positive individuals in both cases. Though not statistically significant, there was also a notable trend for Total IgG to be increasingly elevated for positive over negative individuals. Results suggest that the feline salivary antibody response to FIV infection mirrors kinetics observed in plasma and human lentiviral disease, and that the role of salivary antibodies in viral pathogenesis may be crucial to transmission via saliva.

Pathogenic consequences of flavivirus-mediated suppression of the cellular RNA decay machinery

Stephanie L Moon, John R Anderson, Shelton S Bradrick, Mary K Dozier, Benjamin Dodd, Jeffrey Wilusz

How viruses protect their transcripts from the general RNA decay machinery in the cell is an understudied aspect of host-virus interactions. We recently showed that flaviviruses including Dengue virus (DENV) and West Nile virus (WNV) shut down the major cellular exoribonuclease Xrn1 through the generation of a subgenomic flavivirus RNA (sfRNA). Conserved structural elements in the 3' untranslated regions (UTRs) of these viral RNAs stall Xrn1 and act as reversible, competitive inhibitors of this important exonuclease. Because Xrn1 is inhibited as sfRNA is formed, all arthropod-borne flaviviruses likely suppress cellular RNA decay machinery through this mechanism. We hypothesized that other viruses in the Flaviviridae also suppress Xrn1 via highly structured RNAs. Hepatitis C virus (HCV) and Bovine viral diarrhea virus (BVDV) do not form an sfRNA from their 3' UTRs, but they do contain highly structured conserved RNA elements in their 5' UTRs. Excitingly, we find that these RNA elements known as internal ribosome entry sites (IRES) stall and inhibit Xrn1. Because cells infected with DENV or WNV exhibit several signs of Xrn1 dysfunction correlated with increased levels of pro-inflammatory factors, we hypothesize that pathogenesis may be enhanced by Xrn1 suppression. Hepatitis C virus and BVDV are known to cause cancer and developmental defects (respectively) through unknown molecular mechanisms. We have determined that short-lived mRNAs encoding oncogenes including FOS and JUN are significantly stabilized in HCV and BVDV infected cells. Importantly, these genes are more highly expressed at the mRNA and protein levels in infected cells. Our findings support the hypothesis that Xrn1 suppression may lead to over-expression of oncogenes and contribute to virus-induced cancer and/or developmental abnormalities. Virus-mediated suppression of host RNA decay may be a conserved mechanism by which all members of the Flaviviridae induce pathogenesis.

Phase II evaluation of VDC-1101 in canine cutaneous T cell lymphoma

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Introduction: Canine cutaneous T cell lymphoma (CTCL) is an uncommon disease for which efficacious therapies are lacking. The novel anticancer nucleotide prodrug VDC-1101, formerly known as GS-9219, has shown considerable efficacy in dogs with multicentric lymphoma. One of the observed adverse effects with this drug was a skin change characterized by hair loss, erythema and pruritus, implying significant delivery of VDC-1101 to the skin. The purpose of this study was to identify the objective response rate to VDC-1101 in canine CTCL; secondary endpoints included progression free survival and adverse effects. Materials and Methods: Dogs with chemotherapy-naïve or relapsed histologically confirmed CTCL were enrolled. Dogs received VDC-1101 as a 30-minute IV infusion once every 21 days. Prednisone (1 mg/kg QOD) was administered concurrently. Tumor response was assessed using standard RECIST criteria. All toxicities were graded according to the VCOG CTACAE v1.0. Progression free survival was calculated using the Kaplan-Meier method. Results: Eleven dogs are evaluable to date. Responses included 1 CR, 4 PR, 2 SD, and 4 PD for an objective response rate of 45% and disease control rate (CR/PR/SD) of 64%. The median response duration was 37.5 days (26-399+ days), which includes 1 durable and ongoing CR (>1 year). Gastrointestinal and hematologic adverse effects were mild: no dogs developed grade 3 or 4 toxicity. Three dogs (25%) developed dermatologic toxicity, one of which was dose limiting.Conclusions: GS-9219 has activity against canine CTCL. One patient has experienced long term remission.

Interferon-gamma enhancement of antibiotic activity against Burkholderia is mediated by induction of reactive oxygen species

Kara L Mosovsky, Ediane Silva, Ryan Troyer, Katie Propst-Graham, and Steven W Dow

Burkholderia pseudomallei, a facultative intracellular pathogen, causes both potentially lethal acute infections and chronic systemic infections in humans and is inherently refractory to most antibiotic therapies. Therefore new treatments must be identified which can supplement or enhance existing ones. Previous studies have shown that interferon-gamma (IFN-γ), which is indispensible for protection against B. pseudomallei, can interact with the routinely administered antibiotic ceftazidime to enhance reduction of intracellular bacteria. In the present study we aimed to identify the underlying mechanism of the interaction so as to better protect against acute infections. Using flow cytometry, we first discovered that IFN-γ induces reactive oxygen species (ROS) generation in macrophages, leading us to hypothesize that this increased ROS may be what is actually responsible for enhancing the antibiotic reduction of intracellular bacteria. To test this hypothesis we used a macrophage infection model to show that the interaction of IFN-γ with ceftazidime can be potentiated with pro-oxidant compounds or diminished with certain antioxidants which supports our hypothesis that the effect is mediated by ROS. Next we used fluorescent microscopy to investigate visible changes in Burkholderia pathogenesis due to IFN-γ stimulation and ROS induction. We found that bacteria inside IFN-γ-treated macrophages fail to escape the phagolysosome and form actin tails, both of which are deemed necessary for efficient spread of the infection. These effects were recapitulated with another pro-oxidant drug suggesting ROS involvement. Therefore we conclude that IFN-γ enhances antibiotic activity through generation of ROS. Furthermore, due to increased intracellular ROS, bacteria fail to escape the phagolysosome and form actin-tails which may ultimately limit spread of the infection to neighboring cells. Our results have implications for enhancing existing therapies against Burkholderia with the use of other pro-oxidant drugs which may also interfere with Burkholderia pathogenesis.

Evaluation of the clinical utility of a Lig based ELISA for early and inexpensive diagnosis of leptospirosis in dogs

Kristen Neisler, Julia K Veir, Michael R Lappin

Leptospirosis is a significant zoonotic disease in dogs. Early diagnosis and treatment improves outcome and decreases the potential for zoonotic transmission. Therefore it is in the best interest of public and patient health to quickly and accurately diagnose Leptospira infections.Tests providing an accurate diagnosis early in the course of disease are limited. The microscopic agglutination test (MAT) is the standard diagnostic serology. The method is cumbersome, results are variable amongst labs, and may lack specificity. Additionally, MAT relies on detection of antibodies which may not be detectable early in disease. PCR methods detect pathogenic organisms shortly following infection; however, PCR requires specialized equipment and is far more expensive than ELISA-based assays. Letospira immunoglobulin-like protein (Lig) shows promise as a new target for diagnostic ELISA testing. Lig is a virulence factor of pathogenic Leptospira which is only expressed in vivo during infection; thus, non-pathogenic serovars and vaccinations do not interfere. It has been shown in experimental models that reaction to Lig proteins is a much earlier event than traditional antibody testing, allowing for earlier seroconversion. We are developing a Lig-based ELISA using PCR-confirmed positive samples from dogs with documented clinical signs and duration of illness against a group of PCR confirmed negative samples. Sensitivity and specificity of the assay will be compared to MAT and PCR. If the assay is more sensitive than MAT and as sensitive as PCR for diagnosis of a Leptospira infection early in the course of disease, it will significantly improve early diagnosis of disease.

Evaluation of Live Bp82 Vaccination Efficacy in Goats

Heather Newett, and Torsten Eckstein

A key to the consideration of biological warfare agents is the availability of vaccines against these weapons. The bacterium, Burkholderia pseudomallei is classified as a category B priority select agent and as a Tier 1 pathogen with biothreat potential in the United States. No licensed vaccines are currently available to prevent pulmonary or systemic melioidosis, the main diseases caused by B. pseudomallei. Researchers at Colorado State University recently developed an auxotroph strain of B. pseudomallei, Bp82. It is nearly non-pathogenic in mice and hamsters, and has potential for use as a whole strain vaccine. Previous vaccination with heat-killed Bp82 in goats failed to stimulate significant immune responses, so this project will evaluate the efficacy of vaccinating goats with live Bp82. Two kids will be vaccinated at eight weeks of age, and two will remain uninfected for sample comparison. Blood collections will occur before immunization and at one and three weeks after immunization to determine cellular and humoral immune responses to B. pseudomallei total protein antigens and selected polar immunogenic lipids. The kids will then be challenged via inhalation with virulent B. pseudomallei to evaluate the efficacy of live Bp82 vaccination. Immunization will be performed end of July, immune responses will be evaluated in August, and challenge with virulent B. pseudomallei will occur at the end of this year. Results from this project will augment the development of a working vaccine against B. pseudomallei infection.

Use of filter paper to quantify polychlorinated biphenyl (PCB) in bottlenose dolphins whole blood

Lily Ngai, Cristina Hansen, J. Margaret Castellini, Karsten Hueffer and Todd O'Hara

Whole blood is used to assess many types of contaminants, but handling and shipping samples can be difficult and expensive, especially when the samples are collected in remote areas. Utilization of filter paper may be an inexpensive and relatively easy solution to these problems. Prior studies quantifying total mercury from bottlenose dolphin and harbor seal whole blood showed that Advantec Nobuto filter papers soaked in whole blood and air-dried yield nearly identical total mercury concentrations in comparison to the whole blood. Based on these studies, we hypothesized that Advantec Nobuto filter papers can also be used to quantify total polychlorinated biphenyl (PCB) concentration in bottlenose dolphins whole blood. Our goals were to determine whether the Nobuto filter paper can detect PCBs in dolphin whole blood and if they do, are they comparable with the whole blood. Whole blood and Nobuto filter paper soaked in whole blood were freeze-dried prior to extraction in an Accelerated Solvent Extractor and then analyzed via gas chromatography. Preliminary data demonstrates that many PCBs can be detected and comparable in concentration to whole blood. However, these concentrations were just above the detection limit of the gas chromatography.

Prion seeding activity in peripheral tissues of primary passage and host-adapted murine chronic wasting disease

Sarena A Olsen, Davis M Seelig, Kristen A Davenport, Scott Carver, Glenn C Telling, Nicholas J Haley and Edward A Hoover

Chronic Wasting Disease (CWD) is an important infectious prion disease affecting cervids which has been shown to transmit efficiently between cervid species through excreta (urine, saliva, and feces) and is also capable of transmission across species barriers in experimental models by both intraperitoneal and oral inoculations. The pathogenesis of CWD infection is thought to initiate in peripheral lymphoid and nervous tissues, later progressing into the central nervous system (CNS), and ultimately disseminating to peripheral excretory tissues. A detailed study on the pathogenesis and adaptation of this slowly progressing disease in multiple organ systems has yet to be undertaken with amplification assays such as real time quaking-induced conversion (RT-QuIC), however. We hypothesized that CWD accumulation in peripheral tissues would occur in parallel to central nervous system adaptation in a murine host. To that end, tongue, salivary gland, intestines, kidney, urinary bladder, spleen, and brain from groups of Tg[CerPrP] mice inoculated with primary or 4th passage elk CWD (E2) were analyzed and scored to determine PrPCWD burden using seeded-amplification via RT-QuIC. We found no significant differences between tissue burden and distribution at terminal disease among treatment groups, despite much shorter incubation periods in mice inoculated with 4th passage E2. This implies that peripheral accumulation is able to keep pace with CNS adaptation and may be indicative of peripheral adaptation, and indicates that prion shedding in excreta is likely to be maintained despite shorter incubation times. These findings represent the first evaluation of peripheral prion accumulation in primary passage and host-adapted prion disease, and extend our understanding of trans-species adaptation of CWD.

Prions in plants: potential assay for detection of PrPres in grasses from Rocky Mountain National Park

Aimee Ortega, Jan Leach, and Mark Zabel

Chronic wasting disease (CWD) affects cervids such as elk, deer, and moose and has become endemic over the last decade. The disease is one of many transmissible spongiform encephalopathies which occur due to the accumulation of an abnormally folded, proteinase K resistant, form of the normal cellular prion protein PrPC. This abnormally folded form, PrPRes, seeds conversion of PrPC into PrPRes and eventually forms amyloid fibrils. The exact mechanisms behind transmission and spread of CWD are unknown but research has shown that it can be spread through direct animal to animal contact or via indirect exposure to contaminated feed and water sources. We want to further explore the latter and determine whether prions can be detected in grasses and other plants by use of the protein misfolding cyclic amplification assay (PMCA). Here we describe the optimization and ability of PMCA to detect PrPRes in rice grass samples spiked with known concentrations of prions. In the spring we plan to test grasses and other plants from Rocky Mountain National Park to establish whether plants could be serving as a vector for CWD.

Using Conservation Genetics to Improve ex-situ Management of the Critically Endangered Buffon Macaw (Ara ambiguus guayaquilensis)

Justin S Lee, Rafaela Orrantia, Elisa Bonaccorso, Jessica R Eberhard, Kristy Pabilonia

The Guayaquil subspecies of the Buffon Macaw (Ara ambiguus guayaquilensis), which now exists in only two small areas of western Ecuador, is estimated at less than one hundred individuals in the wild. A captive population consisting of 16 founder individuals and 35 captive-born birds, is being managed by a non-profit conservation organization in southern Ecuador (Fundación Jambelí). We performed a comprehensive evaluation of the genetic characteristics of captive A. a. guayaquilensis in order to inform future captive breeding and reintroduction management decisions aimed at maintaining the existing level of genetic diversity and limiting, to the extent possible, potential problems with inbreeding. All captive individuals were genotyped using three mitochondrial genes and 10 microsatellite loci. Here, we provide the pedigree of the captive population, estimates of population genetic diversity, and recommendations for future breeding and release decisions based on pairwise relatedness values and allele frequencies.

Development of a harness to facilitate a novel method of canine gait analysis utilizing inertial motion sensors.

Alex Pauls, Felix Duerr

Purpose: Design a forelimb harness that does not impede gait for the placement of inertial motions sensors/ units (IMUs) for the measurement of kinematic data.Methods: Clay IMU models were attached to the forelimbs of two dogs with a combination of Velcro straps, hinges, plastic bars, and double-stick tape. A vest secured the wireless transmitter to the thorax. Dogs wearing the harness were walked and trotted for approximately five minutes to allow for adjustments of the harness's fit and to permit the animal to become accustomed to wearing the harness. After this period, the dog's gait and the relative motion of the IMUs to their attachment sites was evaluated by a board-certified surgeon to assess for gait alteration resulting from the harness. Adjustments to the harness were made until no visual gait impairment was observed. Results: The first prototype consisted of IMUs attached to bars along the scapula and humerus and elastic bands for the antebrachium and metacarpus. This prototype caused a hypermetric gait in the dog and the proximal bars did not move with their respective bones. The second prototype added elastic stabilizers for the scapular and humeral bars and used thinner bands distally to minimize the effect on gait. This prototype did not affect gait; however, the bars continued to move with the skin and moved excessively relative to their respective bones. The final prototype did not include proximal bars and consisted of IMUs attached to mid-radius/ulna, mid-metacarpus and the vest. This prototype did not affect gait and the sensors subjectively correlated with bone movement.Conclusions: IMUs cannot be stably positioned on the scapula and humerus without affecting gait. Kinematic data collection for the shoulder and elbow joints is not feasible with currently available IMUs. Future studies will utilize IMUs placed midway on the radius/ulna and metacarpus only.

Detecting SNPs by Deep Sequencing in the Insecticide Resistance Genes of Aedes aegypti

Rosa Patricia Penilla Navarro, Farah Zamira Vera Maloof, Corey Rosenberg, Karla Saavedra Rodriguez, William C. Black IV

Random mutations that naturally occur in wild Aedes aegypti populations can be selected by continuous usage of pyrethroids, the main insecticides used for dengue vector control. Some mutations in the pyrethroid target site, the voltage gated sodium channel gene (para), have been found. These cause a molecular conformational change in the protein, impeding pyrethroid binding. With the advent of next generation sequencing we are now able to sample the whole genome association to detect single-nucleotide polymorphisms (SNPs) associated with insecticide resistance. This study seeks to identify SNPs associated with pyrethroid survival in natural populations of Aedes aegypti collected in the Viva Caucel and Vergel populations from Yucatan, Mexico. Four library sequences were built from the DNA of 25 mosquitoes. Two replicate libraries contained DNA from mosquitoes that had survived one hour exposure to a predetermined LC50 (25 µg a.i./bottle) and two contained DNA of mosquitoes that died from the same exposure. Sequences were obtained from an Illumina HiSeq2000/2500 Sequencer. Alignments of paired read data were run in the Gsnap software, interrogating each library sequence with an insecticide resistance library of reference containing 307 genes with 4,039,599 nucleotides. Samtools and Varscan were also used. SNPs with coverages <25 or >1000 were excluded as were SNPs that didn't occur in all four libraries. Log Likelihood Ratio Tests were then used to identify SNPs associated with resistance. More than a million novel as well previously identified genes were found to be associated with resistance.

Evaluation of coliphage dynamics in bighorn sheep, domestic sheep and cattle: implications for bacteriophage therapy

Sheridan L. Potter, Claudia R. Gentry-Weeks, and Michael W. Miller

Mannheimia haemolytica is a significant bacterial pathogen of the ruminant respiratory disease complex contributing to bighorn sheep population declines. Novel bacteriophages targeted for treatment and prevention are being developed in our laboratory as a means of therapy that is safe, non-invasive and efficient, due to the self-propagating ability of phages. However, little data are available regarding phage population dynamics within ruminant microbiota and transmissibility between herd members, and the presence of bacteriophages in bighorn sheep has never been documented. Therefore, this study was conducted to assess the presence of "natural" coliphages of domestic sheep, cattle, and bighorn sheep, along with their surrounding habitats, to better understand phage ecology and transmissibility before conducting experiments with M. haemolytica therapeutic phage. Nasal and rectal swabs were collected from domestic sheep (n = 35) and cattle (n = 56) housed at the CSU Agricultural Research Development and Education Center (ARDEC), captive bighorns (n = 32) at the Wildlife Research Center (WRC) of Colorado Parks and Wildlife, and wild bighorns (n = 21) near Granite, CO. Soil, feed, vegetation, and water samples were also collected from each habitat. Isolation of viable coliphages from these samples was determined by visualization of plaque forming units (PFU) using an established host E. coli strain in a double agar overlay technique. ARDEC livestock contained a significantly greater prevalence of coliphages than captive bighorns in both nasal and rectal samples ($p<0.001$). This corresponded strongly with the presence of environmental coliphages, the majority of which were isolated from feed and vegetation, indicating that delivery of the M. haemolytica therapeutic phages via livestock feed may be a highly efficient and practical method. Overall this study has demonstrated that high levels of bacteriophage within ruminants are attainable with a sufficiently abundant and ubiquitous titer in their surrounding habitat.

Effect of 2-aminoimidazole Compounds on Advanced Glycation End Products

Mike A Richardson, Brendan Podell, Forrest Ackart, Roberta Melander, Christian Melander, Randall Basaraba

The formation of Advanced Glycation End Products (AGEs) – unregulated, non-enzymatic modification of host proteins with sugar aldehydes – occurs under normal physiologic conditions but transpires at an accelerated rate during states of chronic hyperglycemia and chronic inflammation. Elevated AGEs lead to most diabetes related pathologies through vascular dysfunction and basement membrane thickening, however, diabetic individuals concurrently infected with Mycobacterium tuberculosis (Mtb) are invariably predisposed to greater amounts of AGE accumulation due to chronic inflammation. AGE accumulation in Mtb infection occurs not only within the localized granulomatous lesions, but is reflected by elevated circulating levels in the serum. Locally, AGE formation and accumulation causes tissue damage and impaired wound healing through collagen cross-linking. Because of the deleterious effects of AGEs, developing novel compounds to block the formation, or disrupt preexisting AGEs is an important therapeutic target for diabetic and Mtb infected patients. We characterized a small library of 2-aminoimidazole (2AI) based compounds for their in vitro anti-AGE activity. In our study, bovine serum albumin (BSA) or bovine collagen type IV was incubated with methylglyoxal (MGO) with or without the addition of a 2AI compound for 7, 14 and 21 days. AGE formation and inhibition was quantified by fluorescence spectrophotometry at wavelengths (λexc 370nm; λem 440nm for veserperlysine AGEs and λexc 335nm; λem 385nm for pentosidine AGEs). Efficacy of 2AI compounds was defined as 50% inhibition of veserperlysine or pentosidine based AGEs. The 2AI compound 2c8 was effective at preventing the formation and at breaking preexisting AGEs when compared with carrier treated controls. Based on these results, we believe these 2AI compounds to be an effective anti-AGE candidate for further study.

Paracrine and Endocrine action of Conceptus-derived Interferon-Tau during Early Pregnancy in Ewes

Jared J Romero, Terry M Nett, Jason E Bruemmer, Fuller W Bazer, Russ V Anthony and Thomas R Hansen

Interferon-tau (IFNT) has antiviral activity and is released from ruminant conceptuses to attenuate prostaglandin release so that the corpus luteum (CL) survives, produces progesterone and prepares the uterus for nurturing the embryo. Ewes have increased antiviral activity in uterine vein serum (UVS) during early pregnancy. When day 15 UVS from pregnant (P) ewes is preadsorbed with anti-IFNT antibodies, antiviral activity is blocked. Thus, it was hypothesized that IFNT causes paracrine-induced gene expression in the endometrium and endocrine-induced gene expression in extrauterine tissues such as the CL and liver by entering peripheral circulation. Blood was collected from non-pregnant (NP; cycling) and ewes on Days 12-15. Serum progesterone concentrations remained >1.7 ng/ml in P and NP ewes P on Days 12-13, remained high in P ewes, but declined to concentrations <0.6 ng/ml by Day 15. A highly specific (no cross-reaction with other type I IFNs) and sensitive (71.25 pg/ml in uterine flushing) IFNT radioimmunoassay (RIA) was validated herein demonstrated that IFNT was not detected in NP, but could be detected from Days 13-16 in P uterine flushings. The detection of IFNT in uterine flushings correlates with the paracrine induction of ISGs in the endometrium during pregnancy and precedes the up-regulation of endometrial estrogen receptor (ESR1) and oxytocin receptor (OXTR) on Day 14 in NP ewes. Endocrine induction of IFN stimulated gene mRNA concentrations occur in jugular vein white blood cells, liver and CL by Day 14. Also, IFNT signal transduction genes were upregulated in P compared to NP CL by Day 14. It is concluded that paracrine action of conceptus-derived IFNT on attenuating uterine PGF release coincides with detection of IFNT in uterine flushings and endocrine action of IFNT on the CL and other peripheral tissues.

Comparison of accelerated hydrogen peroxide and per oxygen disinfectants as misting applications

Nadia T Saklou, Brand A Burgess, Paul S Morley, Dave C Van Metre, Katlin Hornig, Stacey R Byers

Mitigating nosocomial outbreaks in veterinary teaching hospitals is important as these occurrences are economically devastating and disruptive to the normal operations of these hospitals. The purpose of this study was to compare the efficacy of two disinfectant solutions [5.8% accelerated hydrogen peroxide (AHP) and single and double applications of 2% peroxygen] for decontamination of a veterinary hospital environment. We hypothesized that mist applications of these solutions have similar efficacies for reducing bacterial contamination. Transparencies (n=78) were inoculated with known concentrations of Staphylococcus aureus, Salmonella enterica and Pseudomonas aeruginosa (26 transparencies per organism). Five transparencies each served as positive and negative controls. After cleaning and disinfection of the hospital environment, all surfaces were allowed to dry overnight and transparencies were then fastened on vertical surfaces in 25 locations, chosen at random. One at a time, each disinfectant was applied, a contact time of 30-mintues was observed, transparencies were collected, individually placed into 25mL Dey-Engley broth, and transported to the laboratory for processing. Six ten-fold dilutions of each sample were plated onto tryptic soy blood agar for bacterial enumeration. Bacterial counts from the control transparencies were compared to results from transparencies exposed to disinfectant to quantify the percent bacterial reduction. Through regression analysis, a significant reduction was observed when applying a 2% peroxygen once and twice for all organisms evaluated; there was no significant reduction in colony count detected when using the AHP product. This study indicates that for the organisms evaluated, a 2% virkon solution effectively reduced the colony counts in a veterinary hospital environment.

Dietary fatty acids do not predict insulin resistance in the presence of similar body weight and visceral adiposity

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Introduction: Insulin resistance is associated with increased risk for cardiovascular disease. Obesity and Western diet consumption are associated with development of insulin resistance, while docosahexaenoic acid (DHA) intake appears to have insulin sensitizing effects. Less is known about the isolated effects of dietary fatty acids on the development of insulin resistance in the absence of increased body weight and visceral adipose mass. We hypothesized that rats fed a Western diet would develop insulin resistance compared to control animals of similar weight and adiposity, and that supplementation with DHA would mitigate this effect. Methods: Weanling male Wistar rats were divided into four treatment groups (n = 12): control, control + DHA, Western, Western + DHA. Intravenous glucose tolerance testing was performed in separate groups at three and six months. A 2-way ANOVA was used to quantitate glucose and insulin serum concentrations over time. Correlation studies were performed to determine relationships between serum measurements and body morphometry. Results: Body weights and visceral adipose mass were similar across dietary treatment groups at both time points. There was no effect of diet on the glucose or insulin response over time at three or six months. At three months, baseline and peak insulin as well as peak glucose concentrations were positively correlated with body weight. At six months, baseline and peak insulin levels were positively correlated with body weight and adipose mass. Conclusion: Body weight and visceral adipose mass are better predictors of the development of insulin resistance than fatty acid composition of the diets in the present study.

A yeast colony morphology phenotypic transition associated with loss-of-heterozygosity

Nadia M V Sampaio, Aline Rodrigues, Theodore M Gurol, Mary J Chapman, Guadalupe M Aguirre, Pedro A Tizei, Gonçalo A G Pereira, Juan L Argueso

The Saccharomyces cerevisiae strain JAY270/PE-2 is a heterozygous diploid strain used in biofuels production. It is favored by many distilleries in part because it does not normally display flocculation or other mechanisms of cellular co-aggregation. In pure cultures of JAY270, we identified the spontaneous appearance of colonies displaying altered rough morphology in agar media and fast sedimentation in liquid culture analogous to flocculation. The rough colonies were abundant (~1 in every 3,000 normal smooth colonies), a very high frequency that was inconsistent with de novo nucleotide mutagenesis. We investigated the genetic control of this phenotype, and found that JAY270 is heterozygous for a frameshift mutation in the ACE2 gene (ACE2/ace2-A7). ACE2 encodes a transcription factor that regulates the expression of genes required for the degradation of the septum that links mother and daughter cells post-budding. All five spontaneous rough colony JAY270-derived isolates analyzed displayed copy-neutral loss-of-heterozygosity (LOH) at this locus (ace2-A7/ace2-A7). We determined the genotypes at several heterozygous markers on chromosome XII (Chr12) where ACE2 is present, and identified continuous LOH tracts that extended for hundreds of kilobases, but did not span the centromere. We introduced a counter-selectable cassette near ACE2 in JAY270 and at three other regions of the genome. The observed rates of LOH for these strains were high and similar at all four loci (~10-7 LOH events / cell / cell division / Kb), and were compatible with the rates measured in a conventional wild type S. cerevisiae laboratory strain. We also analyzed the spectrum of LOH events selected at the ACE2 locus on both Chr12 homologs, revealing that the primary mechanism was allelic mitotic crossing-over. Taken together, these results indicate that while the genome of JAY270 is highly dynamic and prone to mitotic recombination, it is not less stable than that of other yeast strains.

Mechanism of vertical transmission of Chronic wasting disease (CWD) in animal models

Anca I Selariu, Amy V Nalls, Erin McNulty, Jeanette Hayes-Klug, Stephenie Fullaway, Kelly Anderson, Jenny Barfield, Davis M Seelig, Clare Hoover, Nicholas J Haley, Edward Hoover, Candace Mathiason

Chronic wasting is a highly transmissible prion disease affecting wild ranging and captive cervids of North America. Studies of CWD in a small deer animal model, the Reeves' muntjac, revealed that 1) unborn offspring of clinical and subclinical animals harbor infectious prions; 2) the stillbirth rate of offspring born to CWD positive dams is 60% higher than in naïve animals; 3) all viable animals born to CWD positive dams are themselves CWD positive (Nalls et al., 2013). Evidence of various degrees of mother to offspring prion transmission has been shown in other species, i.e. sheep, cows and cats, although none in humans as yet. In this study we aimed to determine the cellular effectors and the mechanism by which prion conversion occurs at the feto-maternal interface. Based on previous work in sheep placentomes (Lacroux et al., 2007), we hypothesize that a specialized subset of chorioectodermal cells, the trophoblasts, are the principal port of entry of PrPCWD into the fetal side in cervid models (muntjac deer and transgenic mouse expressing elk PrPc). We employ immunohistochemical analysis in conjunction with immunofluorescence labeling to demonstrate that PrPCWDcolocalizes with trophoblasts, thus proving that these cells are indeed both the platforms of PrP conversion and the effectors of maternal-to-chorionic transfer. These results may bring us closer in understanding the pathophysiological connection between placentome PrPCWD accumulation, fetal development and the increased chance of stillbirths.

Copy number variation mediated by dispersed repeats in yeast

Rabab S.Sharif, Mary Chapman, Keerthi Vemulapalli, Kaitlyn Calhoon, Ane Zeilder, Jackie Stanton, Christopher Puccia, Guilherme Mattos, Juan Lucas Argueso

Copy number variation (CNV) is a substantial and under-appreciated source of genetic diversity. We have optimized an assay for CNV detection in yeast cells that takes advantage of two genes, SFA1 and CUP1, that confer gene dosage-dependent tolerance to formaldehyde (FA) and copper (Cu), respectively. Diploid cells carrying a single chromosomal insertion of this SFA1-CUP1 CNV reporter were plated on media containing high levels of FA and Cu, and only the rare individuals carrying a genome rearrangement resulting in two or more copies of the reporter were able to grow. The selected clones were then analyzed by PFGE karyotyping and array-CGH to characterize the spectrum of CNV-associated genome rearrangements. The majority of the CNVs were mediated through non-allelic homologous recombination (NAHR) between dispersed repetitive DNA sequences, primarily Ty retrotransposon insertions. We analyzed a panel of strains with insertions of the CNV reporter at sites of interest on chromosomes 4, 5, and 15. These sites varied in their host chromosome size, and neighboring repetitive DNA content. Differences in genomic context resulted in a 15 fold range in CNV rates (from 0.16 to 2.45 x 10-6); and exposure to non-lethal doses of hydroxyurea, camptothecin, methyl methanesulfonate, and ionizing radiation stimulated the CNV rates by 3 to 17 fold. The spectra of CNV events were also heavily influenced by the chromosomal context of the reporter. The primary mechanisms of amplification on Chr5 were whole chromosome aneuploidy and segmental duplication between Ty repeats, whereas on Chr4 and Chr15 most CNVs resulted from Ty-mediated translocations involving loss of a terminal segment of one chromosome and gain of an extra copy of the chromosome arm containing the reporter. A specific chromosome arm loss (Chr7R) was over-represented in our dataset, suggesting an unusually high propensity for NAHR in this region.

The Role of the C2A Domain of Synaptotagmin in Asynchronous Release

Mallory C Shields and Noreen E Reist

Understanding the mechanisms mediating information transmission across a chemical synapse is essential to understanding brain function. During synaptic transmission, membranous vesicles within neurons are loaded with neurotransmitter, docked to the presynaptic cell membrane, primed for release, and fused with the presynaptic membrane to release neurotransmitter onto the next, or postsynaptic, cell. The vesicle membrane and proteins are recycled back into the presynaptic cell to be utilized later. There are three types of neurotransmitter release, two of which are Ca2+-dependent: fast, synchronous release, and a more prolonged, asynchronous release. Asynchronous release has recently been proposed to play a role in synaptic plasticity, the basis for learning and memory mechanisms. These release processes are tightly regulated by a number of key synaptic proteins, including Ca2+ sensors and the fusion machinery complex. One such protein, synaptotagmin, is proposed to be the low-affinity Ca2+ sensor that, upon Ca2+ binding, triggers fast, synchronous release of neurotransmitter. In addition, studies have shown interplay between synaptotagmin and a currently unidentified and controversial high-affinity Ca2+ sensor responsible for the prolonged, asynchronous neurotransmitter release mechanism. At this time, it is postulated that synaptotagmin is directly inhibiting the asynchronous Ca2+ sensor. However, what this interplay entails is currently unknown and poorly understood. Using specific point mutations in vivo, the role of synaptotagmin in modulating asynchronous release will be investigated.

Survivin inhibition via EZN-3042 in canine lymphoma and osteosarcoma

Jenette K Shoeneman, EJ Ehrhart III, JB Charles, Douglas H Thamm

Canine lymphoma (LSA) and osteosarcoma (OS) have high mortality rates and remain in need of more effective therapeutic approaches. Survivin, an IAP family member protein that inhibits apoptosis and drives cell proliferation, is commonly elevated in human and canine cancer. Survivin expression is a negative prognostic factor in dogs and humans with LSA and OS. The objective of our research was to determine the effects of survivin inhibition using a locked nucleic acid antisense oligonucleotide (EZN-3042) on canine LSA and OS cell lines, with respect to cell proliferation, apoptosis, and chemosensitivity in vitro. Furthermore, we sought to determine the efficacy of EZN-3042 on inhibition of survivin transcription, survivin protein production, and tumor growth in subcutaneous and orthotopic canine OS xenografts. Survivin inhibition in canine LSA and OS cells via EZN-3042 resulted in 34-72% decrease in survivin protein expression and a 1.3-3.4 fold decrease in endogenous survivin mRNA expression. When EZN-3042 treated cells were compared to controls, total and viable cell numbers were decreased, and apoptosis was increased. Survivin inhibition via EZN-3042 enhanced canine LSA and OS cell lines sensitivity to DOX. IHC and qRT-PCR analysis of subcutaneous and orthotopic canine OS xenografts confirmed decreased tumor survivin expression in EZN-3042 treated mice. Mice treated with EZN-3042 in combination with DOX had significantly decreased tumor growth when compared to single agent and control groups. These results demonstrate that survivin inhibition via EZN-3042 decreased LSA and OS cell proliferation, increased cellular apoptosis and chemosensitivity to DOX, and EZN-3042 treatment inhibited tumor survivin expression in vivo and significantly decreased tumor growth when combined with DOX. Survivin-directed therapies may be highly effective in treatment of both canine and human LSA and OS.

Evaluation for associations of Bartonella species with azotemia and hematuria in cats.

Sarah B Shropshire, Melissa Brewer, Jennifer R Hawley, Michael R Lappin

Purpose: The purpose of this study was to determine if there are associations between Bartonella species and azotemia and/or hematuria in experimentally inoculated cats. Materials/Methods: Specific pathogen free cats were divided into two groups of five cats and inoculated IV with B. clarridgeiae or B. henselae infected blood. Whole blood, sera, and urine were collected before inoculation and then once weekly for four weeks. Each whole blood and urine sample was assessed for B. henselae and B. clarridgeiae DNA. A limit of detection experiment was performed with both agents and showed the sensitivity of the assay using urine to be similar to PBS. Serum creatinine concentrations, urine specific gravity, and urine RBCs/hpf were determined weekly. A paired t-test was used to evaluate for changes in creatinine concentrations over time with $P < 0.05$ considered significant. Results: While all of the cats became infected with the respective Bartonella spp., none of the cats developed azotemia and the urine specific gravity of all samples was greater than 1.045. After inoculation there was a statistically significant ($p = 8.6 \times 10\text{-}4$, $p = 2.5 \times 10\text{-}3$) increase in creatinine concentrations in both groups over time when compared to the pre-inoculation values. B. clarridgeiae DNA was amplified from the urine of one cat on one date; a threefold increase in hematuria (20-50 RBCs/hpf) compared to pre-inoculation was detected concurrently. Conclusions: The increasing creatinine concentrations over time in Bartonella spp. infected cats suggests that additional studies should be performed assessing the role Bartonella spp. infections may play in chronic kidney disease in cats. The concurrent detection of hematuria with B. clarridgeiae DNA in the blood and urine suggests further studies of Bartonella spp. infections in cats with hematuria should be performed.

Induction of Oxidative Stress during Flavivirus Infection Enhances RNA Replication.

J Jordan Steel, Elnaz Soltani, Becky C Gullberg, Stephanie L Moon, Brian J Geiss

Flaviviruses are positive-strand RNA viruses that replicate their RNA within modified vesicles in the host cell. As obligate intracellular parasites, the viruses are dependent on cellular conditions and resources to replicate. Flaviviruses have been shown to induce cellular oxidative stress late in infection, which has always been assumed to be a simple by-product of infection; however, we report that the flavivirus RNA replication is enhanced with oxidation, indicating that the cellular oxidative stress may a direct effort to manipulate the cell in order to enhance virus replication. Treatment of Kunjin infected cells or dengue-replicon cells with the antioxidant BHA to block oxidative stress resulted in a significant reduction in viral RNA replication. We observed that oxidizing agents enhanced NS5 capping enzyme guanylation activity in vitro while reducing agents decreased guanylation activity, each without significantly affecting GTP binding. Conserved methionines and cysteines, which are amino acids sensitive to oxidation, were mutated to determine their role in the oxidative enhancement of NS5 activity. We have been able to determine that flavivirus RNA replication is sensitive to oxidation conditions, specifically by enhancing the NS5 capping enzyme during infection. These findings provide the first demonstration that flaviviruses induce oxidative stress to support their RNA replication.

Novel gammaherpesviruses in mountain lions and domestic cats: variations in prevalence and predictor variables

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Gammaherpesviruses, such as Epstein-Barr virus and Kaposi's sarcoma herpesvirus in humans, are infectious agents often associated with lymphoproliferative syndromes. We recently identified two novel felid gammaherpesviruses: Felis catus gammaherpesvirus 1 (FcaGHV1), which infects domestic cats; and Puma concolor gammaherpesvirus 1 (PcoGHV1), which infects mountain lions. In order to detect and quantify infection with these viruses, real-time quantitative PCR assays were developed for each virus. These assays were used to test DNA extracted from blood samples of domestic shelter cats (n=135) and captured/released mountain lions (n=89) in California, Colorado and Florida. FcaGHV1 had 15.6% prevalence among shelter cats tested, with higher prevalence in California and Florida than in Colorado. Additionally, presence of FcaGHV1 was positively associated with being male, adult, and co-infection from feline bacterial pathogens. PcoGHV1 had 5.6% prevalence among mountain lions tested and all infected cats were located in a specific region of Southern California. These data indicate that FcaGHV1 infection of domestic cats is widespread in the continental U.S. while PcoGHV1 infection of mountain lions is less prevalent and possibly localized to a small geographic region in California. These preliminary data advocate further investigation into potential disease associations and other impacts on host species.

Effect of dexamethasone concentration on chondrogenic differentiation of equine bone marrow-derived mesenchymal stem cells

Suwimol Tangtrongsup, John D. Kisiday.

Dexamethasone (Dex) is widely used during transforming growth factor-β-induced chondrogenesis of bone marrow mesenchymal stem cells (MSCs) in laboratory models. Dexamethasone is an anti-inflammatory drug, although it is not known if this is the mechanism by which Dex supports chondrogenesis. As a first step in exploring the anti-inflammatory effect of Dex on MSCs chondrogenesis, we evaluated the effects of Dex concentration on chondrogenesis and secretion of prostaglandin E2 (PGE2). Adult equine MSCs were encapsulated in agarose and cultured in chondrogenic medium containing Dex at 1 nM, 100 nM or without Dex. After 14 days, total accumulated glycosaminoglycan (GAG) and hydroxyproline were quantified, and type II collagen accumulation was evaluated. Culture media were collected every third day and analyzed for PGE2 level. Data were analyzed for ANOVA and least squares means. There was no significant difference in GAG or hydroxyproline content between 1 nM and 100 nM Dex, although GAG and hydroxyproline accumulations in Dex-free cultures were significantly lower. There was highly variable of GAG accumulation in Dex-free group across MSCs from 13 horses. Type II collagen was detected in all groups in the pericellular area. The pilot results of PGE2 levels from a single horse showed similar trend between 1nM Dex and Dex-free groups, which were relatively higher at the beginning and continually lower by time. However, the PGE2 level was very low in 100 nM Dex compared to 1 nM Dex and Dex-free groups. Chondrogenesis of MSCs was partially suppressed in the absence of Dex. One nanomolar Dex is capable of achieving chondrogenesis in a similar manner as 100 nM and overcoming the animal-to-animal variability that occurs without Dex. However, 1nM Dex did not suppress the inflammatory indicator PGE2 relative to 100nM Dex, which suggested that Dex may not influence chondrogenesis through an anti-inflammatory pathway.

Early detection of clinical disease in guinea pigs experimentally infected with Mycobacterium tuberculosis.

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Tuberculosis (TB) is a problematic disease across the globe with millions of people currently infected, many new infections each year, and high rates of morbidity and mortality. Currently there is worldwide, ongoing research to develop a safe and effective, widely-accepted vaccine for TB. One of the most common animal models used in immunology studies and vaccine research is guinea pigs—due to their exquisitely similar immune systems to humans. Although guinea pigs show very similar pathologic changes as humans infected with TB, they do not show similar clinical signs. Guinea pigs are stoic by nature and often times mask their disease until it is extremely severe, or in rare cases they are found dead. Our lab has focused on identifying biomarkers of disease in guinea pigs infected with TB, and it was our hope to find parameters that could serve as indicators of severity of disease which could alter the course of treatment. Guinea pigs were either BCG-vaccinated or sham-vaccinated and infected with TB. Blood and urine were collected serially as well as at necropsy for analysis of serum biochemistry, hematology, blood gasses, and urinalysis. Results indicate that sham-vaccinated guinea pigs show more severe signs of disease starting at 30 days post infection, including leukocytosis, acidemia, and decreased survival times. Data also indicate that blood collection in guinea pigs is a useful tool for evaluating progression of TB related disease, thus potentially resulting in earlier intervention or euthanasia.

β-Amyloid- and proinflammatory cytokine-induced cofilin-actin rod formation requires prion-dependent activation of NADPH oxidase.

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Persistent exposure of neurons to hypoxia/ischemia, excitotoxic glutamate, and physiologically relevant forms and amounts of the β-amyloid peptide (Aβ) all provoke a remodeling of the neuronal actin cytoskeleton into rod-shaped cofilin-saturated actin filament bundles (rods). These rods disrupt synaptic function by blocking transport and/or sequestering cofilin from dendritic spines. Rod inducers generate reactive oxygen species (ROS) and rod formation requires cofilin oxidation to form an intermolecular disulfide bond. Oxidative stress is an early indicator of pathology linked to Alzheimer Disease (AD). Here we show that proinflammatory cytokines (e.g. TNFα) induce rods that form in the same population (~20%) of hippocampal neurons that respond to SDS-stable Aβ dimer/trimer (Aβd/t), a physiologically relevant species in AD. Neurons lacking the cellular prion protein (PrPC) do not form rods in response to Aβd/t or cytokines, but PrPC-null neurons form rods after treatment with glutamate or mitochondrial inhibitors, suggesting at least two different pathways. This finding was confirmed by inhibiting isoforms 1 and 2 of NADPH oxidase (NOX) by expressing dominant interfering gp22PHOX or by using a pharmacological inhibitor, each of which blocked neuronal rod formation in response to Aβd/t and TNFα but not in response to glutamate or mitochondrial inhibitors. Because cognitive impairment in Aβ-overproducing AD mice is also PrPC-dependent, we suggest rod formation mediates this loss in synaptic plasticity. To study rod formation and their impact to neural circuit function, we have developed adeno-associated viruses to infect neurons in vivo to express fluorescent cofilin chimeras that can be visualized using two-photon microscopy. Local treatment with endothelin-1, a potent vasoconstrictor, induces significant rod pathology within hours and was accompanied by deficits in treadmill running behavior monitored during imaging. Cofilin-actin rods could explain the common pathologies of familial and sporadic Alzheimer disease (AD), as well as synaptic dysfunction in other neurological disorders.

Expression and function of polo-like kinase in canine cancer

Kristen M Weishaar, Barbara J Rose, Jared S Fowles, and Douglas H Thamm

Polo-like kinases (Plks) are a family of serine/threonine kinases, and Plk1 plays a role in multiple steps in mitosis as well as cancer progression. The goal of this study was to evaluate the effects of a Plk1 inhibitor (GSK461364A) on tumor cell growth and mitosis in canine cancer cell lines. 72-hour growth inhibition assays were performed using 27 canine tumor cell lines treated with serial dilutions of GSK461364A. Cell cycle distribution and apoptosis were assessed with propidium iodide (PI) and annexin V-PI staining and flow cytometry, respectively. Gene expression profiling and pathway analysis were performed to examine pathways differentially expressed between resistant and sensitive cell lines. Expression of Plk1 among all cell lines was also measured. Growth inhibition assays showed that canine tumor cell lines appear to be less sensitive to the Plk1 inhibitor compared to previous reports on human tumor cell lines, with IC50's less than 300 nM in 11 out of 27 cell lines and less than 100 nM in only 7 cell lines. In sensitive cell lines, G2/M arrest occurred at 24 hours as detected by cell cycling, and induction of apoptosis occurred at 48 hours as confirmed by annexin V. Apoptosis caused by the Plk1 inhibitor was dose-dependent. Several cancer-related pathways were significantly over-represented in sensitive cell lines compared to resistant. There was no difference in Plk1 expression in sensitive and resistant cell lines. The Plk1 pathway has the potential to be a druggable target in a variety of canine neoplasms. In vivo studies are needed to further assess possible utility.

Comparing the effect of docosahexaenoic acid (DHA) supplementation of western and low-fat diets on myocardial fatty acid composition

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Introduction: Western diet intake is associated with structural and functional changes of the heart (cardiomyopathy), while consumption of DHA appears to attenuate this pathology. We recently observed that DHA supplementation did not improve Western diet-associated left ventricular hypertrophy in rats that underwent dietary treatment for three months. Enrichment of myocardial DHA with increased oral intake is well documented; however, it is unknown whether concomitant Western diet consumption interferes with tissue incorporation of DHA, compared to concurrent intake of a low-fat diet, possibly lending partial explanation for the apparent absence of antihypertrophic effect. To answer this question and also examine the effect of treatment duration, we characterized the myocardial phospholipid fatty acid profile in rats in response to Western and low-fat diet intake, with and without supplemental DHA. Methods: Thirty-two weanling male Wistar rats received one of four dietary treatments for either three or six months (n = 4): control (CON), CON+DHA, Western (WES) and WES+DHA. At terminal sample collection, perfused myocardial septum was isolated and snap frozen in liquid nitrogen. Myocardial phospholipids were isolated from tissue homogenates using methanol-hexane extraction, and quantitated using gas chromatography. Results: There was an interaction effect of diet and treatment duration on myocardial DHA content. At three months, rats fed the WES+DHA diet had higher myocardial phospholipid DHA (in mean± SE area%, 29.53±0.77) compared to those fed the CON+DHA diet (24.80±0.77; $p < 0.01$). At six months, myocardial DHA content was similar in these groups (WES+DHA 25.96±0.96, CON+DHA 24.46±0.96; $p = 0.70$). At both timepoints, WES+DHA and CON+DHA feeding was associated with greater myocardial DHA compared to both unsupplemented dietary groups. Conclusion: There was robust enrichment of myocardial phospholipid DHA regardless of base diet. Interference with DHA incorporation into the myocardium does not appear to be a mechanism of high-fat diet-induced blunting of DHA cardioprotection.

Assessing mother to offspring transmission of chronic wasting disease using transgenic mouse models

Kassandra Willingham, Erin McNulty, Kelly Anderson, Jeanette Hayes-Klug, Candace Mathiason

Infectious prions found in the bodily fluids (urine, saliva, and blood) of infected animals contribute to the efficient horizontal transmission of chronic wasting disease (CWD) among free-ranging and captive cervids. Recently it has been recognized that transmission from mother to offspring may also play a role in the spread of CWD. The mechanism of maternal transmission has yet to be elucidated. Placental trafficking and/or prion secretion in milk are two means by which vertical/maternal transmission may occur. Our studies will explore these avenues through the use of a transgenic mouse model (TgCerPRP) expressing cervid prion protein. We will determine if CWD prions are transmitted from mother to offspring at time points associated with early or late CWD-infection by: 1) observing offspring born to naïve and CWD-infected mice for clinical TSE disease progression (end stage TSE disease or 500 dpi) . 2) analyzing in utero harvested fetal and maternal tissues, and 3) evaluating milk collected from lactating dams. Tissues and bodily fluids harvested will be analyzed for PrPCWD (immunohistochemistry), PrP converting activity (sPMCA, and RT-QuIC), and infectious prions (bioassay), We have successfully bred CWD positive transgenic mice at time points associated with early and late infection. We have collected milk from several of these dams, and are generating a video library of the offspring to assess disease progression. These studies will provide insight to the mechanisms associated with prion mother to offspring transmission.

Modulation of coagulation and fibrinolysis by carbon monoxide and nitric oxide in dogs: a thromboelastographic analysis

Seung Yoo, Vance G Nielsen, Christine S Olver

Purpose: Inflammatory conditions cause hemostatic abnormalities via a number of mechanisms, including the production of carbon monoxide (CO) and nitric oxide (NO). Both CO and NO have been shown to affect coagulation and fibrinolysis in vitro by redox modulation of heme molecules associated with fibrinogen, plasmin, and α2-antiplasmin. An improved understanding and characterization of the association between inflammation and coagulation is of critical importance for recognizing risk factors for thrombosis. Objectives: We aimed to characterize the effects of NO or CO on coagulation and fibrinolysis. We tested the hypothesis that CO induces hypercoagulable and hypofibrinolytic changes and NO induces hypocoagulable and hyperfibrinolytic changes in normal canine plasma. Methods: Using a plasma-based clot lifespan thromboelastographic method, we established the effects of carboxyheme (CO exposed) and metheme (NO exposed) states on tissue factor activated citrated plasma with tissue plasminogen activator (tPA) (to assess fibrinolysis) or without tPA (to assess coagulation) in 10 healthy dogs. Results: With a carboxyheme state, both clot strength and velocity of clot growth were significantly increased. With a metheme state induced by PHA, both clot strength and velocity of clot growth were significantly decreased. With the addition of tPA, a carboxyheme state significantly increased clot strength, decreased rate of fibrinolysis, increased clot lysis time, and increased clot lifespan. A metheme state did not significantly change clot strength, fibrinolysis, clot lysis time, or clot lifespan. Conclusions: We conclude that coagulation is modulated by a balance between carboxyheme and metheme states in plasma from dogs as evaluated with thromboelastography. In contrast, fibrinolysis is primarily modulated by NO since the lack of change in fibrinolytic parameters with addition of NO suggests a pre-existing metheme dominated state in vivo. Future studies are needed to characterize these effects in dogs with specific inflammatory diseases such as immune-mediated hemolytic anemia, sepsis, pancreatitis, and neoplasia.

Regulation of H19 lncRNA by RNA-binding proteins in muscle cells.

Annie Zhang, Jerome Lee, Jeffrey Wilusz and Carol Wilusz

The CELF1 RNA binding protein plays important roles in muscle and its over-expression in mice induces symptoms resembling muscular dystrophy. In order to gain further insights into CELF1 function we undertook a global analysis of transcripts associated with CELF1 in mouse muscle cells. Among the mRNAs bound by CELF1 we identified H19, a long non-coding RNA required for normal muscle differentiation. H19 abundance was reduced in CELF1 KD cells (~1.5 fold) and dramatically decreased in cells lacking the PARN deadenylase, which interacts directly with CELF1 protein. We are currently investigating how CELF1 and other RNA-binding factors modulate H19 expression using mouse C2C12 cells as a model.

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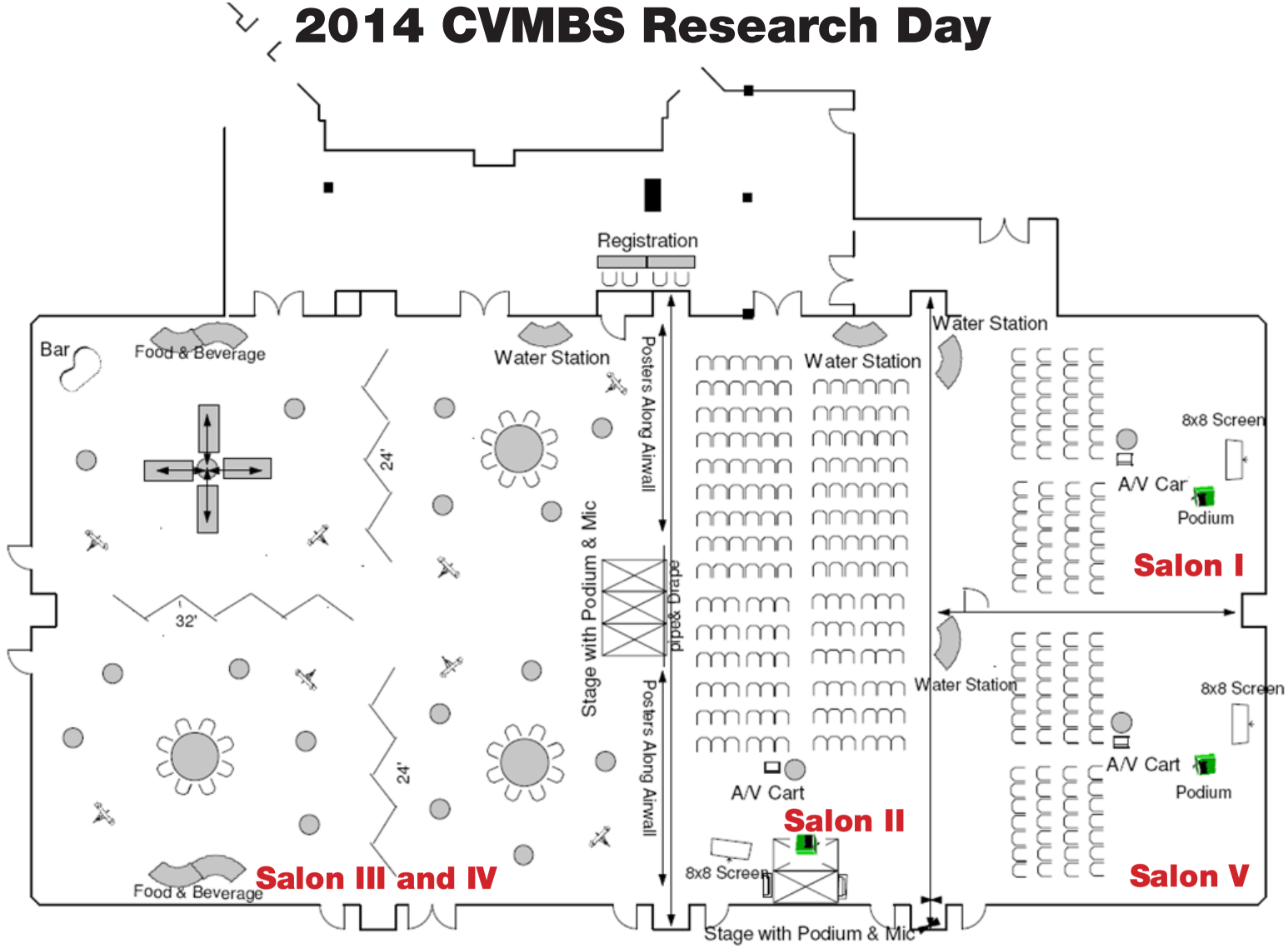

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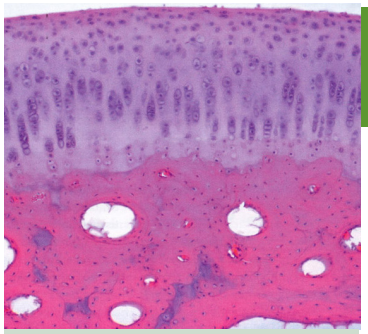

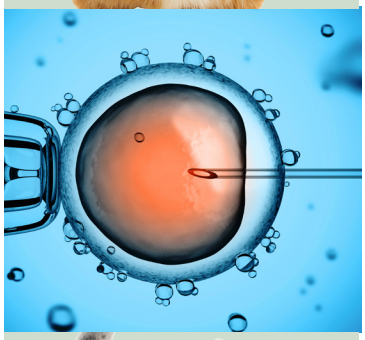

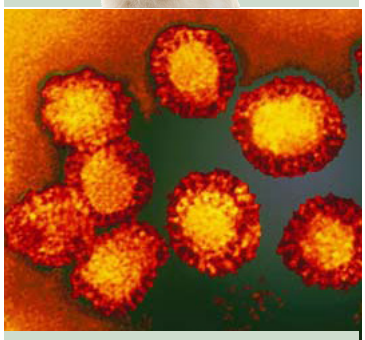

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