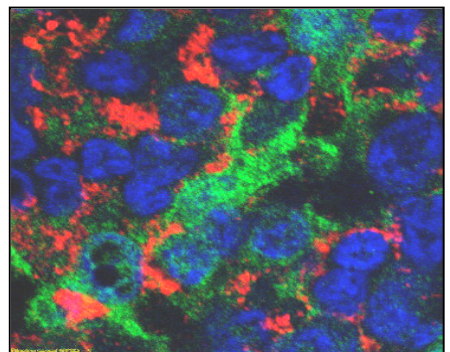
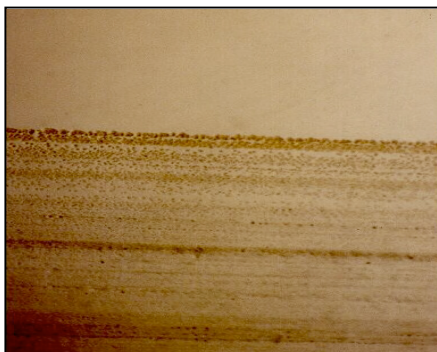


Colorado State University

**College of Veterinary Medicine and
Biomedical Sciences**

**9TH ANNUAL CVMBS RESEARCH DAY
SCIENTIFIC PROCEEDINGS**

**The Hilton Hotel
February 16, 2008**



CVMBS Research Day 2008

Schedule Of Events		Room
11:30-12:00	Poster set up	Oklahoma
12:00	Opening remarks – Dr. William Dernel	Idaho
12:05	Keynote speaker – Dr. Richard Bowen <i>“Opportunities and Challenges for Research with Zoonotic Pathogens”</i>	Idaho
12:50	Pfizer Research Award Winner – Dr. Doug Thamm <i>“Spontaneous Canine Cancer as a Translational Model: From the Laboratory to the Labrador”</i>	Idaho
1:15	Break	
1:30-5:45	Oral Presentation I: Basic Sciences	Idaho
1:30-5:45	Oral Presentation II: Clinical Sciences	Michigan
3:00-4:30	Poster Session I Judging: Basic Sciences	Oklahoma
4:00-5:30	Poster Session II Judging: Clinical Sciences	Oklahoma
5:45-6:30	Social Hour, Remove Posters	Oklahoma
6:30	Awards	Oklahoma

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

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

KEYNOTE SPEECH

CVMBS Research Day
Saturday, February 16th, 2008

Richard A. Bowen, DVM, PhD

Opportunities and Challenges for Research with Zoonotic Pathogens

Idaho State Ballroom
The Hilton Hotel
Fort Collins, CO

Dr. Bowen is a Professor in the Department of Biomedical Sciences. His research focus is on infectious diseases, particularly those transmitted from animals to humans, and he is a strong proponent of the "One Medicine" initiative. A large majority of his studies are translational in nature, and involve investigating host-pathogen interactions and developing methods for control of infectious disease. This work involves studies with a number of microbial agents, including West Nile, rabies and avian influenza viruses. To understand and test control strategies for the diseases these pathogens induce in natural hosts, he and his colleagues have utilized a broad range of animals, including horses, bats, alligators, and birds ranging from emus to sparrows.

PFIZER RESEARCH AWARD WINNER

CVMBBS Research Day
Saturday, February 16th, 2008

Douglas H. Thamm, VMD, DACVIM

**Spontaneous Canine Cancer as a Translational
Model: From the Laboratory to the Labrador**

Idaho State Ballroom
The Hilton Hotel
Fort Collins, CO

Dr. Thamm is an Assistant Professor of Oncology at the Colorado State University Animal Cancer Center, within the College of Veterinary Medicine and Biomedical Sciences. He is also a full member of the Developmental Therapeutics Section of the University of Colorado Comprehensive Cancer Center and the Cell and Molecular Biology Graduate Program at Colorado State University. Dr. Thamm received his Bachelors and V.M.D. degrees from the University of Pennsylvania. He completed a Residency in Medical Oncology at the University of Wisconsin, and was employed as a postdoctoral researcher there for 5 additional years. Dr. Thamm's research interests center around the translational evaluation of novel cancer therapeutics in pet animals with cancer and correlative laboratory studies to support these clinical trials. His laboratory employs techniques such as cell proliferation/apoptosis/migration/invasion/differentiation assays, RT-PCR, Western analysis, and immunohisto- and immunocytochemistry.

Oral Presentations

SESSION 1: BASIC SCIENCE

1:30-5:45PM

Idaho Room

1:30	Amanda Guth	Role of MCP-1 in Regulation of Recruitment of Monocytes and Neutrophils to Tumors	CS
1:45	Gopinath Palanisamy	Role of oxidized low-density lipoprotein in the formation of foam cells and pathogenesis of tuberculosis	MIP
2:00	Kevin Sokoloski	Sequence Elements Within the 3'UTRs of Alphaviruses Repress Deadenylation In Vitro	MIP
2:15	Scott Hafeman	Depletion of Phagocytic Cells Using Liposome Encapsulated Bisphosphonates	CS
2:30	Chris Cirimotich	Involvement of RNA interference in Sindbis virus infection of <i>Aedes aegypti</i>	MIP
2:45	Krystle Reagan	Identification and Characterization of D7 salivary proteins in <i>Culex tarsalis</i>	MIP
3:00	Greg Wilkerson	Development of a human breast cancer carcinogenesis model in mice using adult stem cells from the mammary gland	MIP
3:15	Nate Denkers	Aerosol and Intranasal Exposure to CWD Prions in a Transgenic Mouse Model	MIP
3:30	Joanne Tuohy	Evaluation of regional lymphatic drainage and uptake of paclitaxel following delivery into the mammary tissue of non-tumor bearing mice	CS
3:45	BREAK		
4:00	C. Christina Daniels	Walleye dermal sarcoma virus Orf B functions through receptor for activated C kinase (RACK1) and protein kinase C (PKC)	MIP
4:15	Jerome Lee	Regulation of TNF mRNA stability by CUGBP1 in muscle cells and myotonic dystrophy	MIP
4:30	Ron Carsten	Radiation-induced bone marrow cell chromosome aberration frequency is reduced by resveratrol	ERHS
4:45	Candace Mathiason	Bioassay of CWD prions in the saliva, blood, and excreta of deer	MIP
5:00	Anne Skope	CXCR4 Expression and Function in Canine Lymphoma	CS
5:15	Natalee Holt	IPEC J2 cells provide an excellent system for analysis of enterotoxigenic secretion	CS
5:30	Jesse Thompson	Using Chimeric FIV Constructs to Assess Virulence Determinants	MIP

Oral Presentations

SESSION 2: CLINICAL SCIENCE

1:30-5:45PM

Michigan Room

SESSION 2: CLINICAL SCIENCE 1:30-5:45PM Michigan Room			
1:30	Courtney Ikuta	Does Radiography Permit Accurate Measurement of Femoral Angulation Across a Broad Range of Femoral Conformations?	CS
1:45	Alanna Kirby	Assessment of infectious disease control practices on Colorado equine boarding facilities	CS
2:00	Katie Steneroden	Regional survey of animal shelters on infection control policies and zoonotic disease awareness	CS
2:15	Sonya Wilsterman	Central Venous Pressure Measurement Technique in Normal Horses	CS
2:30	Robert Rebhun	Prognostic and comparative analysis of survivin expression in naïve and relapsed canine lymphoma	CS
2:45	Sara-Lesley Rasmussen	Pregnancy rates of dairy cattle artificially inseminated with sexed and control semen	BMS
3:00	Willem Becker	Retrospective evaluation of anesthetic and patient factors on the development of dysphoria after stifle surgery in dogs	CS
3:15	Michelle Turk	Characterization of Neural Factors in the Murine Ovary and Follicular Dynamics	BMS
3:30	Courtney Steinhauer	Maximum tolerated dosing and metronomic based dosing of docetaxel, vandetanib and radiation therapy in human head and neck squamous cell carcinoma xenografts	CS
3:45	BREAK		
4:00	Christie Mayo	Flock-level Prevalence of Bluetongue in Colorado Sheep	MIP
4:15	Hussain Abdullah	Detection of Mycoplasma Mastitis using SCC, culture, sELISA and PCR in Large Dairy Farms in Saudi Arabia	CS
4:30	Kendra Miller	Durability of Disposable Overboots Under Simulated Field Conditions	CS
4:45	Bunita Eichelberger	Does Dynamic Contrast Enhanced MRI Predict Percent Tumor Necrosis in Spontaneous Canine Osteosarcomas?	ERHS
5:00	Sangeeta Rao	An Epidemiological Investigation of Antimicrobial Use and Occurrence of Antimicrobial Resistant Bacteria in Alberta Feedlots	CS
5:15	Clara Goh	In vitro biomechanical evaluation of semi-contoured, locking plate/rod versus anatomically-contoured, conventional plate/rod fixation in a canine femoral defect model	CS
5:30	Jane Shaw	Does the gender of the client and the veterinarian influence communication in veterinary visits?	CS

Poster Presentations

SESSION 1: BASIC SCIENCES 3:00-4:30PM

#1	Jennifer Fletcher	Pharmacodynamic endpoints of toxicity following oral metronomic dosing of CPT-11 with ZD1839 or ZD6474 in mice.	CS
#2	Scott Purcell	Periattachment factor is required for conceptus elongation in sheep.	BMS
#3	Debora Stump	Evidence of a Putative Lentivirus in Captive Lemur catta.	MIP
#4	Natalia Smirnova	Up-regulation of type I interferon response and down-regulation of SDF-1/CXCR4 signaling in blood cells from pregnant heifers carrying fetuses infected with BVDV.	BMS
#5	Aida Ulloa	Reduction in TRPC4 expression specifically attenuates G-protein coupled receptor-stimulated calcium entry in human myometrial cells.	BMS
#6	Janet Petty	Antiproliferative Effects of 2-Deoxyglucose in Canine Osteosarcoma.	CS
#7	Maggie Clark	Indoor air pollution from cookstove smoke and adverse health effects among Honduran women.	ERHS
#8	Shayna Warner	The acquisition and utilization of the plasminogen activating system by Francisella tularensis.	MIP
#9	Curtis Cline	Isolation and analysis of the Equine Kisspeptin receptor, GPR54.	BMS
#10	Jaclyn Scott	Characterization of Small RNAs Produced During Dengue Virus Infection of Mosquito Cells.	MIP
#11	Andrea Kudwa	Estrogen Receptor β and Oxytocin Interact to Modulate Anxiety-like Behavior in a Sexually Dimorphic Manner.	BMS
#12	David Higgins	Relative levels of macrophage-colony stimulating factor and granulocyte macrophage-colony stimulating factor influence the specific generation of macrophage populations during infection with Mycobacterium tuberculosis.	MIP
#13	Airn Tolnay	Highly Pathogenic Avian Influenza A (H5N1) Virus Dynamics in the Respiratory Tract of Cynomolgus Macaques (Macaca fascicularis) Compared to Human H1N1 Virus Containing 2 or 3 Genes From the 1918 Pandemic Flu.	MIP
#14	Dilyar Murtazina	Functional involvement of the plasma membrane PKA/AKAP interaction in signaling events in uterine smooth muscle.	BMS
#15	Daesuk Chung	Involvement of Signal-Regulated Calcium Entry in Store Refilling in Myometrial Cells.	BMS
#16	Brandon Wadas	Developmental effects of vinclozolin on the GnRH neuronal system in rabbits.	BMS
#17	Beth Spizziri	In Vitro Capacitation of Frozen/thawed Stallion Spermatozoa.	BMS

#18	Hend Ibrahim	The oncoprotein nucleophosmin is a component of a polyadenylation mark.	MIP
#19	Amber Troy	Airway Immune Responses to Inhaled Liposome-Based Immunotherapeutics.	MIP
#20	Eric Lee	The Effects of Mannose Capped Lipoarabinomannan on Dendritic Cell Function.	MIP
#21	Rebeka Klingler	Isolation of Primary Mammary Stem Cells Using a Modified Mammosphere Culture Technique: A Novel Approach to Identifying the True Stem Cell.	ERHS
#22	Abby Williams	Characterization of Lymphoblastoid Cell Lines from Breast Cancer Patients in a Radiation Technologist Cohort.	ERHS
#23	Katie Torley	MicroRNAs in Gonad Development.	BMS
#24	Reem Al-Mubarak	Analysis of a putative <i>M. leprae</i> lipoprotein; LpqE.	MIP
#25	Wei Li	Analysis of the lysine depressed regulatory regions of <i>Escherichia coli</i> and <i>Mycobacterium</i> .	MIP
#26	Craig Miller	Perceived hypoxia in <i>Mycobacterium tuberculosis</i> infection and the role of mycobactin.	MIP
#27	Liza Pfaff	Differential gene expression in canine osteosarcoma predicts tumor aggressiveness.	CS
#28	Timothy Kurt	Analysis of the Potential for Cross-Species Transmission of Chronic Wasting Disease by Protein Misfolding Cyclic Amplification.	MIP
#29	Joseph Sottnik	Exploring the Link between Infection and Tumor Inhibition.	CS
#30	Nicholas Haley	Detection of Prion Infectivity in Saliva and Urine from CWD+ Deer Using a New Transgenic Mouse Bioassay.	MIP
#31	Davis Seelig	New insights from enhanced immunohistochemical detection of PrPres in a transgenic mouse model of Chronic Wasting Disease.	MIP
#32	Katie Propst	Activation of Innate Immunity Inhibits <i>Francisella</i> Infection of Alveolar Macrophages.	MIP
#33	Ryan Hansen	Pharmacokinetics of ZD1839 (gefitinib, Iressa) in adult and geriatric mice.	CS
#34	Luke Wittenburg	Sodium Valproate Enhances Doxorubicin Sensitivity in Osteosarcoma Cells.	CS
#35	Stacy Fuchs	Evaluation of Cytotoxic Effects of Pokeweed Antiviral Toxin and Diphtheria Toxin In Chinese Hamster Ovarian Cells	BMS
#36	Cathrine Denton	Rates of proliferation of human melanoma may predict sensitivity to small molecule MEK inhibition	CS
#37	Abby Jones	Early innate immune response to <i>Burkholderia mallei</i> infection	MIP
#38	Kevin O'Halloran	Expression and activity of indoleamine 2,3 dioxygenase in feline bone marrow derived dendritic cells	MIP
#39	Kristen Pauken	Effects of sand fly salivary component maxadilan on murine dendritic cell migration	MIP

SESSION 2: CLINICAL SCIENCES**4:00-5:30PM**

#40	Adrian Rosas-Taraco	XCL1-targeting siRNA intratracheal delivery therapy in mice challenged with Mycobacterium tuberculosis had decreased numbers of CD4 and CD8 T cells and IFN-gamma production and presented changes in the granuloma's structure.	MIP
#41	Lauren Falkowski	Oxidative Stress and Phagocytic Cell Function in Cats with Type 2 Diabetes Mellitus Compared to Controls: Assessing the Impact of Nutrition.	CS
#42	Erica Gee	Efficacy of medroxyprgesterone acetate in estrus suppression of cycling mares.	CS
#43	Kristin Manning	The Association Between Basophilia and Mast Cell Neoplasia in a Retrospective Case Study of 488 Dogs.	BMS
#44	Danielle Bayliss	Association between Feline Pancreatic Lipase Immunoreactivity Concentration and the Presence of Serum Antibodies against Toxoplasma gondii and Bartonella species.	CS
#45	Jenny Powers	Effects of GnRH Immunization on Reproduction and Behavior in Female Rocky Mountain Elk.	BMS
#46	Samantha Hollingshead	The Expression of Growth Factors in Equine Airway Smooth Muscle and Their Role in Recurrent Airway Obstruction.	BMS
#47	Lauren Kloer	Prevalence of Dirofilaria immitis in dogs and cats in an animal shelter in Larimer County, Colorado.	CS
#48	Eric Garcia	Experiences with Spine Surgery Research in a Sheep Model.	CS
#49	Kelly O'Neill	Regulatory T Cell Responses in Dogs with Cancer.	CS
#50	Jennifer Newquist	Effect of Diet on Blood pH, Serum Electrolytes, and Bone Turnover in Horses.	CS
#51	Kate Vickery	Ezrin Expression in Canine High-Grade Soft Tissue Sarcoma.	CS
#52	William Dernell	Technetium-99M-Sestamibi Scans to Predict Outcome in Canine Osteosarcoma	CS

Departmental Abbreviations

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

Thank you moderators and judges!!

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Oral Presentations

**Session I ~ Idaho Room
1:30-5:45PM**

BASIC SCIENCE

Role of MCP-1 in Regulation of Recruitment of Monocytes and Neutrophils to Tumors

AM Guth, S Dow

Purpose: Increased numbers of tumor-associated macrophages (TAM) in tumors are generally correlated with a poorer disease outcome. TAM may promote tumor growth by stimulating angiogenesis and inhibiting adaptive immune responses against the tumor. TAM are thought to arise from monocytes, and possibly neutrophils (PMN), which are recruited from the blood into tumor tissues. While it is known that monocytes are recruited into tumor tissues under the influence of chemotactic factors, the role that specific chemokines play has not been carefully examined. In tumor-bearing animals, the chemokine CCL2 is produced by either the tumor cells and/or tumor-associated stromal cells. Therefore, we hypothesized that CCL2 was one of the major chemokines responsible for recruiting monocytes into tumors. **Materials and Methods:** Wild type C57Bl6 mice and mice lacking CCR2 (CCR2^{-/-}) were injected subcutaneously with MCA205 fibrosarcoma cells. Tumor growth was monitored using two-dimensional measurements. Monocytes were labeled in vivo using fluorescent microbeads and the uptake of beads in monocytes, macrophages, and PMN in tumor tissues was assessed by flow cytometry. Total numbers of these cells in tumor tissues, spleen, and tumor draining lymph node were also assessed by flow cytometry. **Results:** Tumor growth was decreased significantly in CCR2^{-/-} mice as compared to the wildtype controls. CCR2^{-/-} mice had significantly fewer monocytes and PMN in the peripheral blood and a significant decrease in monocytes and macrophages in the spleen and tumor as compared to wildtype mice. Use of bead-labeled monocytes and PMN allowed us to calculate the rate of recruitment of these cells into tumors. **Conclusions:** CCL2 appears to play a key role in recruiting monocytes into tumors and leading to their differentiation into TAM. Approximately 1.5% of TAM are replaced by recruited monocytes (within 3 days) largely under the influence of CCL2.

Role of oxidized low-density lipoprotein in the formation of foam cells and pathogenesis of tuberculosis

GS Palanisamy, CA Shanley, EE Smith, H Bielefeldt-Ohmann, IM Orme, RJ Basaraba.

Purpose: Tuberculosis, a chronic inflammatory disease, is accompanied by continuous phagocytic events and elevated proinflammatory cytokine levels. Both of these characteristics result in excessive free radical generation which contributes to oxidative stress. One consistent feature in both naturally occurring tuberculosis in humans, and experimental infections in animals, is the presence of foamy macrophages that have numerous clear cytoplasmic lipid containing vacuoles. The importance of lipid-loaded macrophages in tuberculosis is unclear but they often contain numerous bacilli. These foam cells are thought to have decreased bacterial killing capacity and are possibly an important source of proinflammatory cytokines that worsen lesion pathology. We hypothesize that oxidative stress conditions in tuberculosis result in the accumulation of oxidized lipids that contribute to the formation of foam cells. **Materials and methods:** Oxidized low-density lipoprotein (oxLDL) level in serum from guinea pigs infected with *M. tuberculosis* by low-dose aerosol was assayed using a commercial competitive ELISA kit. The oxLDL and its uptake receptors were detected in lung lesions using immunohistochemistry. **Results:** We show that the oxidative stress conditions exist in *M. tuberculosis* infected guinea pigs resulting in elevated serum levels of oxLDL compared to non-infected controls. The oxLDL accumulated in lipid-loaded macrophages. Moreover the scavenger receptors that are involved in the uptake of oxLDL such as CD36 and lectin-like oxidized low-density lipoprotein receptor (LOX-1) were expressed at highly throughout the granulomatous lesions in the foam cells. **Conclusion:** Free radical generation during *M. tuberculosis* infection through oxidation of LDL contributes to lipid-loaded foam cell formation that could support enhanced bacterial growth. Our hypothesis is further supported by the fact that the scavenger receptors involved in oxLDL uptake are highly expressed in foam cells.

Sequence Elements Within the 3'UTRs of Alphaviruses Repress Deadenylation In Vitro

KJ Sokoloski, CJ Wilusz, J Wilusz.

Cellular mRNA decay is a robust regulatory mechanism responsible for ~50% of observed gene regulation. It has been previously shown that elements present within the 3'UnTranslated Regions (UTRs) of many cellular mRNAs regulate the pathway and rate of decay. The genomes of RNA viruses, for instance members of the Togavirus family, often strongly resemble cellular mRNAs in that they are capped and polyadenylated. This similarity dictates that the genomes and transcripts of RNA viruses should be under control of the cellular RNA decay system. Therefore without a manner by which to evade or usurp aspects of this cellular defense mechanism, replication would be severely impaired. Using a mosquito cell cytoplasmic extract system, we have evaluated the roles of elements within the 3'UTRs of several Alphaviruses with respect to mRNA decay. This system reliably recapitulates many aspects of cytoplasmic mRNA decay in vitro, including processes such as deadenylation, decapping, as well as 5' and 3' exonuclease degradation. The Alphavirus 3'UTR consists of several elements: a series of Repeated Sequence Elements, a U-Rich Element and a 19nt Conserved Sequence Element immediately adjacent to the poly(A) tail. We have shown that elements within the Alphavirus 3'UTRs from either Sindbis virus or Venezuelan Equine Encephalitis Virus are capable of conferring resistance to deadenylation on an unstable reporter transcript. Furthermore, stability appears to be mediated by the binding of cellular trans-acting factors. Through the addition of specific competitors we have observed a 38kD cellular factor whose binding correlates with stabilization of the poly(A) tail. Excitingly, the mechanism of stability appears to be highly similar between the two viruses as demonstrated by cross-competition analysis, despite the fact that the 3'UTR sequences have diverged considerably. We are currently characterizing the underlying mechanism(s) imparting stability to the Alphavirus transcripts.

Depletion of Phagocytic Cells Using Liposome Encapsulated Bisphosphonates

SD Hafeman, RM Troyer, SW Dow

Purpose: Tumor-associated macrophages help promote tumor growth and increased numbers of these cells have been associated with a poor prognosis in many tumors. Liposomal clodronate (LC; dichloromethylene bisphosphonate encapsulated in phosphatidylcholine liposomes) has been effectively used for macrophage depletion in the treatment of IMHA in dogs, and in anti-tumor studies. However, the influence of the type of liposome used for delivery of clodronate on the efficiency of macrophage depletion has not been extensively studied. Therefore, we assessed the effects of LC prepared with different characteristics on the effectiveness of macrophage killing both in vitro and in vivo with the goal of finding the most effective formulation. Methods: Liposomes with different charges (positive, negative, neutral) as well as those containing the mannose targeting ligand were prepared containing either clodronate or phosphate buffered saline. The effects of the different liposomes on macrophage killing were assessed using murine macrophage cell lines and the MTT assay to assess cell viability. We also used fluorescently labeled liposomes and flow cytometry to assess the efficiency of macrophage uptake of liposomes. The effectiveness of in vivo depletion of phagocytic cells was also assessed by flow cytometry after i.v. administration of different LC types. Results: The liposome formulation had a significant impact on efficiency of macrophage killing in vivo, as did the origin of the macrophage cell line (monocyte-derived vs mature macrophages). Neutral, negatively charged, and mannosylated LC showed the most consistent cell killing in vitro. All three LC formulations also demonstrated significant depletion of all phagocytic cells in vivo, with the mannosylated LC being the most effective. Conclusions: Mannosylated LC elicits the most effective macrophage depletion. This suggests that mannosylated LC would be used for depletion of macrophages in anti-tumor studies.

Involvement of RNA interference in Sindbis virus infection of *Aedes aegypti*.

CM Cirimotich, BJ Geiss, AT Phillips, I Sanchez-Vargas, KE Olson.

Mosquito-borne viruses cause significant morbidity and mortality throughout the world. The viruses establish persistent infection in the mosquito vector and do not cause significant pathology during infection. Virus and vector genetics have been implicated in mosquito infection by arboviruses, but little is known about the interactions between the virus and mosquito in the establishment of persistence. RNA interference has been implicated in insect antiviral immunity against pathogenic viruses and may play an important role in mosquito vector competence for transmitting RNA viruses. Experiments in our lab show that transient knockdown of key components of the RNAi pathway increases the ability of arboviruses to disseminate in and be transmitted by *Aedes aegypti* mosquitoes. To examine the direct involvement of RNAi in viral infection of a mosquito vector, we have engineered a Sindbis virus to express B2 protein, a known viral inhibitor of the RNAi pathway from the insect-pathogenic flockhouse virus. This system allows us to examine the direct effects of RNAi knockdown on virus infection because only infected cells will have impaired RNAi. We show that virus expressing the B2 protein infects the midgut of female *Ae. aegypti* mosquitoes and disseminates into the mosquito hemocoel more efficiently than virus containing no insert when fed in an infectious bloodmeal. We also show that RNAi may be directly involved in the establishment of persistent infection in the mosquito; B2 virus killed significantly more mosquitoes during a 21-day mortality assay. Our results suggest that RNAi is a determinant of Sindbis virus infection of *Ae. aegypti* mosquitoes and may play a role in establishment of persistent infection in the mosquito vector.

Identification and Characterization of D7 salivary proteins in *Culex tarsalis*

KL Reagan, C Machain-Williams, CD Blair, T Wang

Blood feeding arthropods have the ability to transmit a variety of pathogens to their hosts upon taking a blood meal. The saliva of several haematophagous arthropods, including mosquitoes, is known to contain immunomodulatory factors. Vaccination targeting salivary proteins has been shown to induce protective immunity upon infection by vector-transmitted pathogens. The D7 family proteins are the most abundant group of proteins excreted in the mosquito saliva. In this study, we have identified and characterized two different sizes of D7 proteins present in the sialome of *Culex tarsalis*, an efficient vector of West Nile virus (WNV) in the western United States. Saliva was first collected from adult female *Culex tarsalis* and immunoprecipitated with serum against *Aedes aegypti* D7 proteins. Proteins which cross reacted with anti-*Aedes aegypti* D7 serum were excised from protein gel and subjected to mass spectroscopy and MALDI-TOF analysis. A 37 kDa protein, was identified as a D7-related protein by a search against mosquito protein databases. It was submitted for Edmond degradation and N-terminal sequencing. Based on the amino acid sequence obtained, we designed degenerate primers to further identify D7 gene by using 3' and 5' rapid amplification of cDNA ends (RACE). The cDNA sequence identified was next cloned and expressed in a pBAD/TOPO-Thio bacterial expression system,. The DNA sequence obtained has 85% identity to the *Culex pipiens* published D7 gene. We plan to test whether recombinant D7 protein in conjunction with various adjuvants could protect host against WNV infection by mosquito challenge in vaccination studies.

Development of a human breast cancer carcinogenesis model in mice using adult stem cells from the mammary gland

GK Wilkerson, MM Weil, RL Ullrich

Purpose: The role of adult stem cells in the genesis of human breast cancer is currently of great interest. Previous attempts to create mouse models from single adult stem cells have success rates reported between 12-32% . We have worked to develop a more reproducible, mouse mammary gland, stem-cell model to serve as a basis for carcinogenesis research in human breast cancer. **Materials/methods:** Mammary tissue collected from donor mice was processed into single-cell suspensions and then allowed to form free-floating spheroid multicellular colonies (primary mammospheres) on non-adhesive plates. These mammospheres were dissociated into single-cell suspensions and replated to form stem-cell derived secondary mammospheres. Secondary mammospheres were then placed in laminin-rich extracellular matrix to bud ductal and alveolar structures. The mammary gland fat pads of 12 histocompatible mice had their endogenous mammary epithelium removed and then budding mammospheres were transplanted into the fat pads. 4 weeks later the animals were euthanized and the glands collected. **Results:** 19 of 24 mammary glands had mammary epithelial growth. 11 of the 24 (45.8%) had definite outgrowth of donor mammary epithelium. 4 glands had definitive ingrowth of the endogenous mammary epithelium (clearing failure) thereby obscuring any growth of donor epithelium. The final 4 glands had epithelial growth that could not be differentiated between donor and endogenous origin. **Conclusion:** The percentage of definitive donor outgrowths using this method is within the reported range (40-85%) of the more well-established, mixed-cell mammary gland transplants and exceeded the reported success rates of other techniques using stem cells specifically. As such, this first attempt at this method is considered a success. We believe the number of questionable and obscured epithelial growths can be greatly limited in subsequent runs with slight modifications to the transplant technique.

Aerosol and Intranasal Exposure to CWD Prions in a Transgenic Mouse Model

ND Denkers, EA Hoover

Purpose: Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) that affects cervids (deer, elk and moose). While it appears CWD is horizontally transmitted, the exact mechanism of transmission, entry, and trafficking of the CWD prion has not been elucidated. Moreover, little is known regarding the potential transmissibility of prion infections by the aerosol or nasal routes. Given the potential for the above pathways of CWD infection in nature, we have undertaken studies to determine whether CWD prions are infectious by aerosol or intranasal exposure. **Materials and Methods:** Forty-eight transgenic Tg(cerPrP) mice expressing the normal cervid PrPC protein (previous work has shown these mice susceptible to CWD infection after intracerebral inoculation) were inoculated intranasally (IN) with 10⁻¹ of a 10% w/v brain homogenate from either CWD-positive (n=24) or negative (n=24) deer. Another cohort of Tg(CerPrP) mice were exposed by aerosolizing approximately 0.5 ml of comparable 5% w/v brain homogenate into only the nasal passages. To simulate activation of the vomeronasal organ (the Flehmen response), epinephrine was administered post inoculation. The mice were observed for >400 days post inoculation (dpi). Western blot (WB) analysis and immunohistochemistry (IHC) were used to detect the CWD abnormal prion protein (PrPCWD) in nasal mucosa, vomeronasal organ, lymphoid tissue, and the brain of mice developing clinical illness judged to be terminal. **Results:** At 422 and 411 dpi respectively, 1/8 surviving IN-inoculated and 1/2 aerosol-exposed Tg(cerPrP) mice developed clinical signs of neurologic dysfunction and were euthanized. Necropsies were performed and tissues were analysed for PrPCWD. Both mice were positive for PrPCWD by immunohistochemistry and western blot. The remaining exposed mice are currently under observation at 452 dpi (aerosolized) and 575 dpi (IN). No evidence of PrPCWD was detected in the Tg(cerPrP) mice examined at early time points pi. **Conclusions:** These preliminary results indicate for the first time that CWD can be transmitted by both the aerosol and intranasal exposure. The results of pending studies are needed to develop significant numbers to substantiate attack rates and determine where and when PrPCWD can be detected after respiratory mucosal exposure.

Evaluation of regional lymphatic drainage and uptake of paclitaxel following delivery into the mammary tissue of non-tumor bearing mice

J Tuohy, L Chubb, W Dernell.

Purpose: Regional lymph node metastasis is the primary site of breast cancer metastasis and remains the major point of failure for breast cancer patients following conservative surgery. Lymphatic uptake and drainage to locoregional lymph nodes has not been evaluated for local or systemic administration of chemotherapy. Local intracavitarily administered chemotherapy may result in significant chemotherapeutic levels reaching regional lymph nodes. Recent evaluation of intracavitary chemotherapy using gel polymer delivery systems for control of breast cancer has shown control of local tumor regrowth and metastasis. We propose to further refine and develop polymer/chemotherapy systems within human breast tumor mouse models to evaluate lymphatic uptake and control of lymphatic metastasis. **Methods:** Phase I of the project involved the evaluation of regional lymph node drainage and uptake of paclitaxel (Taxol), which was injected into the mammary fat pad of non-tumor bearing mice. Bilateral inguinal lymph nodes, mammary adipose tissue and plasma were harvested at selected time points up to 24 hours post injection of drug. Taxol levels were measured in the collected samples using tandem liquid chromatography/mass spectrometry. **Results:** Taxol was detected in lymph node, adipose tissue and plasma, with levels generally higher in tissues ipsilateral to the side of injection. After achieving peak levels of Taxol, both tissue and plasma levels of drug dropped consistently to negligible concentrations. **Conclusions:** Results of phase I demonstrate that local injection of Taxol results in uptake by local lymphatics and subcutaneous tissues with maximal levels reached 6 hours post injection. These levels were sustained up to 12 hours post injection, and steadily declined until 24 hours post injection. Phase II will use similar methods to evaluate locoregional and lymphatic uptake of Taxol following injections of gel polymer/Taxol systems.

Walleye dermal sarcoma virus Orf B functions through receptor for activated C kinase (RACK1) and protein kinase C (PKC)

CC Daniels, J Rovnak, and SL Quackenbush

Purpose: Walleye dermal sarcoma virus is a complex retrovirus that is associated with walleye dermal sarcomas that are seasonal in nature, providing a unique model with which to study molecular mechanisms of tumor development and regression. Fall developing tumors contain low levels of spliced accessory gene transcripts A and B, suggesting a role for the encoded proteins, Orf A and Orf B, in oncogenesis. The purpose of this study was to determine the functional outcome of the interaction of Orf B with RACK1 and contribution to tumorigenesis. **Materials/Methods:** Immunofluorescence assays were utilized to evaluate Orf B expression. Immunoprecipitation assays were used to verify the interactions of Orf B with cellular proteins. MTS based assays were used to measure cell viability of Orf B-expressing and control cells. Membrane and cytosol components were separated from total cell lysates by ultracentrifugation. **Results:** In explanted tumor cells the 35 kDa Orf B protein is localized to the cell periphery in structures consistent with focal adhesions, and along actin stress fibers. Similar localization was observed in cultured cell lines. The cellular protein, receptor for activated C kinase 1 (RACK1), bound Orf B in yeast two-hybrid assays and in cell culture. Sequence analysis of walleye RACK1 demonstrated high conservation to other known RACK1 sequences. RACK1 binds to activated protein kinase C (PKC). Orf B associates with PKC α , which is constitutively activated and localized at the membrane. Activated PKC promoted cell survival and proliferation of Orf B-expressing cells. Treatment of cells with the PKC inhibitor, bisindolymaleimide I, significantly diminished proliferation of Orf B-expressing cells when cultured in serum deprived conditions. **Conclusions:** Activation of the PKC signaling pathway in Orf B-expressing cells is responsible for cell proliferation and survival and likely contributes to tumor formation.

Regulation of TNF mRNA stability by CUGBP1 in muscle cells and myotonic dystrophy.

JE Lee, L Zhang, J Wilusz, CJ Wilusz

Myotonic Dystrophy Type 1 (DM1) is an autosomal dominant disorder caused by a triplet repeat expansion in the 3' UTR of the DMPK gene. Production of the repeat-containing mRNA triggers protein loss, contractile dysfunction, muscle wasting, insulin resistance and elevated serum TNF. DM1 cells exhibit aberrant expression of several RNA-binding proteins, one of which, CUGBP1, has been the focus of our research. We hypothesize that altered function of CUGBP1 in DM1 may affect the understudied roles of this protein in the cytoplasm. CUGBP1 has been implicated in the first step of mRNA degradation, namely deadenylation. We demonstrated that CUGBP binds specifically to the 3' UTR of TNF mRNA in vitro and characterized the sequence elements involved in this interaction. CUGBP1 also directly binds the PARN deadenylase, recruiting this enzyme to the RNA substrate to induce poly(A) shortening. We utilized shRNA-mediated knockdown of CUGBP1 and PARN to examine the role of these two proteins in decay of TNF mRNA in myoblasts. In C2C12 myoblasts TNF mRNA decays with a half life of around 9 min. However, stable knockdown of either CUGBP1 or PARN protein results in a two fold increase in TNF half life. Moreover, expression of CUG-repeat RNA in C2C12 cells also results in stabilization of TNF mRNA. Finally, treatment of C2C12 cells with phorbol ester (TPA), which activates the PKC pathway and induces dramatic changes in CUGBP1 expression, also results in TNF stabilization. Significantly, TPA does not further stabilize TNF mRNA in CUGBP1 knockdown cells. Taken together our results have significant implications for myotonic dystrophy, suggesting that aberrant CUGBP expression may lead to overproduction of TNF in DM1 patients through slower deadenylation and decay of the TNF mRNA. Future research is aimed at determining whether TNF poly(A) tail length and translation are affected in CUGBP knockdown cells, and also identifying other targets of this pathway.

Radiation-induced bone marrow cell chromosome aberration frequency is reduced by resveratrol

RE Carsten, AM Bachand, SM Bailey, RL Ullrich

PURPOSE: The ability of resveratrol to reduce radiation-induced chromosome aberrations in bone marrow cells was investigated. **MATERIALS/METHODS:** Ten week old, male CBA mice were divided into groups of 10 mice for treatment: 1) no treatment, 2) resveratrol only, 3) radiation only (RAD), 4) resveratrol before radiation (Res>RAD), and 5) radiation prior to resveratrol initiation (RAD>Res). Irradiated mice received one 3-Gy dose of gamma-radiation. The Res>RAD group received resveratrol (100 mg/kg) daily for 2 days prior to irradiation and continued in drinking water at 100 mg/kg daily. Bone marrow was collected at 1 and 30 days. The RAD>Res group received resveratrol (100 mg/kg) by a) a single gavage dose 2 hours after irradiation, b) initiated by gavage 2 hours post-irradiation and continued in water, or c) started 2 days after radiation in the water. Bone marrow was collected at 1, 7, and 30 days post-irradiation. Bone marrow metaphase cells were scored for chromosome aberrations. **RESULTS:** Resveratrol started before or after irradiation significantly reduced chromosome aberration frequencies at all time points. Significant differences ($p < 0.05$) were found on day 1 and 30 for the mean total chromosome aberration frequencies between the Res>RAD and RAD groups. Initiation of resveratrol post-irradiation also resulted in significant ($p < 0.05$) reductions in chromosome aberration frequencies at 1, 7, and 30 days for each of the resveratrol start times vs. the respective RAD group. At day 30, resveratrol pre-irradiation was not more beneficial than a single dose 2 hours post-irradiation ($p > 0.05$) and borderline more beneficial than continued resveratrol started 2 hours post-irradiation ($p \sim 0.05$). On day 30, resveratrol started pre-irradiation was more effective ($p < 0.05$) than resveratrol started 2 days post-irradiation. **CONCLUSION:** Resveratrol significantly reduces radiation-induced chromosome aberrations in bone marrow when initiated before or after whole body irradiation.

Bioassay of CWD prions in the saliva, blood, and excreta of deer

CK Mathiason, SA Hays, SJ Dahmes, DA Osborn, J Langenburg, GL Mason, J Hayes-Klug, CJ Sigurdson, EA Hoover.

Purpose: To determine whether infectious CWD prions are present in body fluids and excreta of CWD exposed, symptomatic and pre-symptomatic deer. **Materials/Methods:** In bioassay study A, three cohorts of n=3 deer/cohort, were exposed orally to either: (a) saliva (50 ml), (b) urine (50 ml) plus feces (50 grams), or (c) a whole blood transfusion (250 ml) from CWD-positive symptomatic deer. Study B examined body fluids and excreta from CWD+ but pre-symptomatic deer infected in study A. Controls included a positive control cohort of (n = 8) deer exposed to CWD-positive deer brain and a negative control cohort of deer receiving inocula from CWD-negative donors. The recipient animals were maintained under rigorous indoor isolation conditions to exclude potential adventitious prion exposure and were monitored for CWD infection for a minimum of 18 mos. post infection (pi) by serial tonsil biopsy and terminal necropsy. **Results:** Study A confirmed the results of the original white-tailed deer bioassay (Mathiason et. al., Science 2006) in that infectious prions capable of transmitting CWD were detected in saliva (by the oral route) and in blood (by transfusion). Study B inoculations using materials from donor deer in study A demonstrated that CWD+ but asymptomatic deer also shed prions in saliva and are prionemic. In both assays, PrPCWD was first detected in tonsils between 3 and 12 mos. pi. And in both studies, no deer fed urine and feces from CWD-positive donors developed detectable CWD infection, despite multiple exposures. **Conclusions:** (1) CWD-infected deer shed infectious prions in saliva; this may explain the efficient transmission of CWD in nature. (2) Infectious prions circulate in the blood of CWD-positive deer; which both establishes a basis for developing antemortem blood-based detection assays and emphasizes the widespread distribution of infectivity in CWD-positive deer.

CXCR4 Expression and Function in Canine Lymphoma

AM Skope, A Avery, B Rose, L Wittenburg, D Thamm.

Purpose: The chemokine receptor CXCR4 and its ligand stromal cell-derived factor-1 (CXCL12/SDF-1) are involved in trafficking of lymphocytes. In humans, neoplastic lymphocytes appear to use CXCR4 to access niches that are normally restricted to progenitor cells, and thereby reside in a microenvironment that promotes their growth and survival. CXCR4 neutralization by monoclonal antibodies has profound in vitro effects on human non-Hodgkin's lymphoma cells including inhibition of migration, enhanced apoptosis, and decreased proliferation. In this study, we investigated the expression and function of CXCR4 in two lymphoma cell lines and in clinical samples. **Materials/Methods:** Flow cytometry was performed on peripheral blood, bone marrow aspirates or lymph node aspirates from patients with confirmed lymphoproliferative disorders using an anti-CXCR4 antibody. Two canine lymphoma cell lines were also evaluated for CXCR4 expression via flow cytometry and RT-PCR. CXCR4 function was confirmed via a migration assay. **Results:** A total of 108 clinical samples were analyzed. Fifty-one dogs had large B cell lymphoma/leukemia, 18 had small B cell lymphoma/leukemia, 20 had CD8+ T cell leukemia, and the remaining 18 fell into other subsets based on cell surface markers. Ten large B cell lymphomas (19.6%), 14 small B cell lymphomas (77.8%) and 4 CD8+ T cell lymphomas (21.1%) expressed CXCR4. Both cell lines, 1771 and OSW, expressed mRNA and protein for CXCR4 and migrated in response to CXCL12. **Conclusions:** CXCR4 is expressed by the majority of small B cell lymphomas/leukemias and may be a therapeutic target.

IPEC J2 cells provide an excellent system for analysis of enterotoxigenic secretion.

N Holt, BD Schultz

Enterotoxigenic diarrhea is a significant cause of morbidity and mortality in the swine industry. IPEC J2 cells were derived from neonatal pig jejunum and have been characterized as an *in vitro* model for bacterial pathogenesis in swine. In this study, IPEC J2 cell monolayers were exposed to a variety of physiological and pharmacological agents to elucidate the components of secretory pathways present in the cell line. IPEC J2 cells exposed to forskolin yielded a fifteen-fold increase in intracellular cyclic AMP compared to control. IPEC J2 cell monolayers in a modified Ussing chamber respond with increased *I_{sc}* (a sensitive indicator of net anion secretion) when exposed to forskolin, 3-isobutyl-1-methylxanthine (IBMX) and 8-bromoadenosine- 3', 5'- cyclic monophosphate (8-Br-cAMP). Additionally, *I_{sc}* increased when IPEC J2 cell monolayers were exposed to 8-(4-chlorophenylthio) guanosine 3',5'-cyclic monophosphate (CPT-cGMP), A21387 (a Ca²⁺ ionophore), norepinephrine, adenosine triphosphate (ATP), N-ethylcarboxamidoadenosine (NECA), carbachol, and guanylin. These results show responsiveness of ion transport systems to cAMP-, cGMP, and Ca²⁺-mediated second messenger pathways and demonstrate the presence of surface receptors for adrenergic, cholinergic, and purinergic agonists. All of the increases in *I_{sc}* were reduced when the monolayers were exposed to bumetanide, an inhibitor of the Na⁺/K⁺/2Cl⁻ cotransporter, and DASU-02, a blocker of the apical anion channel, CFTR. IPEC J2 monolayers also respond with an increase in *I_{sc}* when exposed to *Escherichia coli* heat labile (LT) and heat stable (STa) enterotoxins. The increase in IPEC J2 *I_{sc}* when exposed to forskolin or LT is sensitive to potassium channel blockers clotrimazole and BaCl₂ in a concentration dependant manner, suggesting that small (SK) or intermediate (IK) potassium channels are required for the secretory response. These data parallel outcomes reported for native porcine intestinal tissues. The results demonstrate that IPEC J2 cells are an excellent *in vitro* model to elucidate secretory mechanisms present in intestinal epithelial cells. Furthermore, the model system can be used to identify targets for therapeutic interventions to prevent or treat enterotoxin-induced intestinal secretion.

Using Chimeric FIV Constructs to Assess Virulence Determinants

J Thompson, S de Rozieres, J Gruber, K Anderson, E McNulty, D Stump, J Elder, and S VandeWoude

Purpose: Relatively minor variations in lentiviral genotype can result in substantial differences in pathogenicity to the host. Feline immunodeficiency virus (FIV) is a naturally occurring immunodeficiency-inducing lentivirus of cats that provides a useful animal model to study the mechanisms of this phenomenon. For instance, while infection with the clade A isolate FIV-PPR results in a disease course marked by acute viremia followed by a long asymptomatic phase, infection with the clade C clone FIV-C36 leads to rapid immunodeficiency marked by CD4⁺ T cell depletion in domestic cats. **Materials and Methods:** To test the hypothesis that the envelope gene is a determinant of viral pathogenesis, a chimeric virus, FIV-PC.Env, was constructed by inserting the 3' region of C36, including *vif*, *orfA*, *env*, and *rev1*, into the PPR background. An additional chimera, FIV-PC.3_LTR, containing the C36 3' long-terminal-repeat and *rev2* in the PPR background was also constructed. To determine the pathogenicity of these chimeras *in vivo*, groups of 5 specific-pathogen-free felines were infected with parental or chimeric FIVs. **Results:** Viral kinetics and virulence characteristics of C36 and PPR molecular clones were similar to those observed previously and for uncloned field isolates. PC.Env demonstrated a delayed replication and plateau phase with regard to proviral and circulating virus levels, and resulted in neutropenia similar to C36 infections by study end. PC.3_LTR was attenuated, with viremia levels below detectable levels in some cats at timepoints throughout the study. **Conclusions:** These results indicate that substitution of the 3' envelope region from a phenotypically virulent FIV onto a less virulent strain does not result in altered phenotype, thus not fully explaining the rapid growth phenotype of C36. To further pinpoint molecular determinants of virulence, FIV-PPR/C36 accessory gene chimeras are currently being constructed and assessed *in vitro* for infectious potential.

Oral Presentations

**Session II ~ Michigan Room
1:30-5:45PM**

CLINICAL SCIENCE

Does Radiography Permit Accurate Measurement of Femoral Angulation Across a Broad Range of Femoral Conformations?

CI Ikuta, RH Palmer, JM Cadmus

Femoral corrective ostectomy has been proposed for treatment of medial patellar luxation in patients with excessive femoral angulation. Accurate assessment of limb alignment is essential to this procedure, not only to determine whether a patient is a candidate, but also for surgical planning and post-operative evaluation. However, the radiographic technique commonly used for evaluating femoral angulation has not yet been validated and previous work has suggested it may be inaccurate. The purpose of this study was to determine whether or not there is a significant difference between direct anatomic and radiographic measurements of the anatomic lateral distal femoral axis (D-aLDFA vs. R-aLDFA) over a broad range of femoral angles. **MATERIALS & METHODS:** Ventro-dorsal, hip-extended radiographs were made of each femur of four fresh large-breed (>18 kg) canine cadavers positioned in dorsal recumbency. A range of distal angular deformities was created by serial implantation of custom-milled wedges of 3, 6, 9, 12, 15, or 18 degrees into a standardized supracondylar osteotomy in each femur. Femora were harvested for direct measurement of anatomic lateral distal femoral angle. R-aLDFA was measured on each of the randomized blinded radiographs and compared to D-aLDFA by analysis of variance and correlation coefficient. **RESULTS:** n=59 radiographs. There was no significant difference between readers for R-aLDFA ($p=0.12$), D-aLDFA ($p=0.63$), or for the deviation of their R-aLDFA measurement from their D-aLDFA ($p=0.07$). Mean difference between measurements was 3.38 degrees ($p<0.05$), and the correlation coefficient between measurements (r^2) was 0.77. **DISCUSSION:** The results support cautious use of well-positioned radiographs of the femur in the planning of corrective ostectomy. **ACKNOWLEDGMENT:** Funding provided by CSU CVMBS Research Council.

Assessment of infectious disease control practices on Colorado equine boarding facilities

AT Kirby, JL Traub-Dargatz, AE Hill, PS Morley, L Kogan, J Heird.

Purpose: The purpose of this project was to assess infection control practices used by Colorado equine boarding facilities using a mail survey with in-person validation of selected items at a randomly selected subset of facilities. **Materials/methods:** Previously published equine operation biosecurity assessment protocols were used to develop an equine facility assessment survey. Three experts in equine biosecurity sorted the questions on the survey into categories of importance in prevention and containment of equine infectious diseases. The survey was mailed to a list of equine boarding facilities in Colorado compiled from internet phone listings. Facilities were sent a reminder post card after 3 weeks, another survey form after 5 weeks, and received a phone call after 7 weeks to request their participation. To validate the responses made by facilities, a member of the research team visited a randomly selected sample of participating facilities to independently evaluate management related to key sections of the survey. Agreement between mailed survey responses and in-person validation is being analyzed. **Results:** The overall response rate by facilities with a valid address was 37%. Based on response to the survey, 46% of responding facilities quarantine new horses to the facility, 52% educate personnel regarding infection control strategies such as washing hands between horses, and 88% transport resident horses on and off the facility. **Conclusions:** Although a majority of Colorado equine boarding facilities are at risk of infectious disease via introduction of infected animals, fewer than half have satisfactory infection control practices.

Regional survey of animal shelters on infection control policies and zoonotic disease awareness.

K Steneroden, A Hill

Purpose: The objective of this project was to determine the level of infection control and zoonotic disease awareness practiced by animal shelters in Colorado, Wyoming, Utah, Montana, North Dakota and South Dakota and compare shelters by size, shelter type and location. **Methods:** A needs assessment survey was developed and mailed to 157 animal shelters. Survey questions focused on shelter demographics, infection control practices and policies, awareness and concern over infectious and zoonotic diseases, staff and volunteer training relating to infection control and zoonotic disease awareness, utilization of diagnostic tools, and communication with the public. **Results:** We received a 50% response rate from a wide variety of shelter types, sizes and locations. Infectious diseases of greatest concern include feline upper respiratory disease, canine parvovirus and feline panleukopenia. Zoonotic diseases of greatest concern include ringworm, and fecal parasites. Zoonotic diseases of slight or no concern include plague, tularemia and leptospirosis. Approximately 25% of shelter staff and volunteers receive no training in infection control principles and practices. Approximately 30% receive no training in infectious disease identification and up to 50% receive no training in zoonotic disease identification. Overall volunteers receive less training in these areas than staff members. Ninety percent of shelters said they would benefit from training in infectious and zoonotic disease. **Conclusion:** Animal shelters in our six state area may benefit from training particularly in infection control practices, zoonotic disease awareness and cleaning and disinfection.

Central Venous Pressure Measurement Technique in Normal Horses

S Wilsterman, ES Hackett, TB Hackett

Central venous pressure (CVP) is an important measurement in critically ill horses, both as a diagnostic aid and in dictating and monitoring response to treatment. The purpose of this study was to investigate a technique of CVP measurement using a newly developed long line catheter in normal horses. Twenty horses were studied following approval from the CSU Institutional Animal Care and Use Committee. The jugular vein in the mid-cervical region was aseptically prepared. A 2 inch 14 gauge short-term venous catheter was used to introduce a long line catheter of 19-gauge diameter (Mila #LL1990). The long line catheter was first inserted roughly 60cm in an attempt to catheterize the pulmonary artery. It was then withdrawn until presence in the cardiac right atrium was confirmed ultrasonographically. Insertion distance and pressure were measured at this location with a disposable manometer in cm of water. From this location the catheter was withdrawn in 5cm increments until exiting the jugular insertion site and pressure measured at each location. All pressure measurements were taken with the manometer zero position at the point of the shoulder. Pressure measurements were taken in triplicate and recorded at the lowest pressure of oscillation, if present, correlating to peak expiration. The three pressure measurements were mathematically averaged. Pulmonary artery catheterization was successful in 16 of 20 horses. Right atrial pressure was confirmed and compared to pressures recorded at sequential insertion distances. Insertion distance required for central venous pressure measurement was determined based on this comparison. Insertion distance in the 20 horses was used to standardize the recommended catheter insertion length. This catheter measurement technique is well tolerated. Routine clinical use of this long line catheter will improve our ability to monitor patients and may improve patient care and outcomes of sick horses in hospital.

Prognostic and comparative analysis of survivin expression in naïve and relapsed canine lymphoma

RB Rebhun, SE Lana, EJ Ehrhart, JB Charles, DH Thamm.

Purpose: Survivin, a member of the Inhibitor of Apoptosis family of proteins, plays a critical role in cell proliferation and resistance to apoptosis. Expression of survivin is an independent poor prognostic parameter in several human cancers including diffuse-large B-cell lymphoma. The purpose of this investigation was to 1) determine expression of survivin in canine lymphoma patients, 2) assess whether survivin expression may serve as a prognostic factor, and 3) determine if survivin expression is upregulated in relapsed canine lymphoma. Materials/methods: Immunohistochemical analyses were performed on patient matched, naïve (N=31) and relapsed (N=16) samples from canine lymphoma patients treated identically with an abbreviated CHOP-based chemotherapy protocol. Survivin expression was determined using a semi-quantitative scoring method incorporating percent of cells staining positive, intensity of staining, and the product of the two scores for each sample. Results: Survivin was expressed in 29 of 31 (~94%) pre-treatment, and 14 of 16 (~88%) biopsies obtained at relapse. In the absence of known concurrent negative prognostic factors, dogs with B-cell lymphoma that had high survivin immunoreactivity scores experienced a significantly ($P < 0.01$) shorter median disease free interval than did dogs with low survivin immunoreactivity scores (171 days vs. 321 days respectively). Conclusions: Survivin is expressed in the majority of canine lymphomas. Furthermore, high expression of survivin is a negative prognostic factor in dogs with B-cell lymphoma. There was no significant difference in the expression of survivin in patient matched naïve and relapsed canine lymphomas.

Pregnancy rates of dairy cattle artificially inseminated with sexed and control semen.

S Rasmussen, Z. Brink, K McSweeney, A Shiflett, G E Seidel, Jr

Use of sexed semen in the dairy industry can be beneficial. The higher price of sexed semen must be justified by its benefits, particularly if pregnancy rates are somewhat lowered, for it to be considered a useful assisted reproduction technique. Increasing the percentage of heifer calves born on a dairy offsets replacement costs and reduces the risk of disease introduced by outside replacement heifers. Heifers also are smaller than bull calves at birth, and thus may decrease the incidence of dystocia during calving. Sexed and nonsexed semen from each of three bulls was used for artificial insemination at a commercial dairy in Colorado. Estrous cycles of cows were synchronized as follows: cows to be bred for the first time after calving were scanned for a corpus luteum (CL) using ultrasound and injected with 100 ug GnRH i.m. Seven days later cows were scanned again for a CL and follicle, and given 25mg of PGF2? i.m. if a CL was present. Two and a half days later cows received 100 ug GnRH i.m. Cows were inseminated artificially 18 hours later with control or sexed semen. Bulls HO40 (n=52), HO47 (n=52) and HO6682 (n=41) were used for the trial. Pregnancy status was determined 35 days after insemination by ultrasound. Cows with dead or dying fetuses were considered non pregnant. There was no difference ($p>0.1$) in pregnancy rate between sexed 36% (26/72) and nonsexed semen 42% (31/73). We conclude that sexed semen technology may be beneficial on dairy farms with well managed reproduction programs when increased heifer calves are the desired result.

Retrospective evaluation of anesthetic and patient factors on the development of dysphoria after stifle surgery in dogs

W Becker, KR Mama, S Rao, AE Hill.

Our impression is that a number of dogs anesthetized at Colorado State University Veterinary Medical Center (CSU-VMC) are dysphoric during recovery. The purpose of this retrospective was to document the incidence of dysphoria and determine if post-anesthetic dysphoria is correlated with anesthetic or patient factors. We examined the records of 96 dogs that underwent general anesthesia for stifle surgery at the CSU-VMC between February 2006 and August 2007. Information obtained from anesthetic records included signalment, weight, preoperative and postoperative temperature, packed cell volume, total protein, blood urea nitrogen, duration of surgery and anesthesia, dose and frequency of all anesthetic drugs. Patients were categorized retrospectively as dysphoric if they received an opioid antagonist (naloxone), tranquilizer (acepromazine) or sedative (medetomidine) within 30 minutes of extubation. Chi-square testing was used to determine if breed, sex or surgical procedure were associated with dysphoria. Independent t-tests were used to evaluate the influence of continuous variables; age, temperature, blood values, duration of surgery and anesthesia. The influence of administration of individual drugs and drugs grouped by class (e.g., opioids) were analyzed using Fisher's Exact test. Non-parametric analysis (Wilcoxin Sign Rank test) was performed to assess influence of drug dose. Animals ranged from 1 to 12 years; males and females were equally represented. Among the 26 breeds, Labrador Retrievers represented the highest number (31.5%). Dysphoria was recorded in 32 of 96 dogs (33%), but was not influenced by patient factors or administration of individual or grouped anesthetic drugs. Although only 2 dogs received intra-operative hydromorphone, the dose was significantly different between the dysphoric and non-dysphoric dogs (Z-statistic=1.99; p= 0.046). Results of this retrospective study indicate a high incidence of post-anesthetic dysphoria. Intraoperative hydromorphone administration increased the probability of this.

Characterization of Neural Factors in the Murine Ovary and Follicular Dynamics

ML Turk, SK Garber, JG Knoll, SA Tobet

The mammalian ovary contains both endocrine and neural components, but little work has been done to characterize the neural side of this equation. Therefore, it is desirable to explore the role ovarian innervation may play in events such as follicular development and ovulation. Identifying neural inputs could aid in recruitment of preantral follicles for assisted reproductive techniques and help explain the pathologic processes underlying such disorders as polycystic ovarian syndrome. Using immunocytochemistry in fixed murine ovaries (50um) we have localized the catecholamine synthetic enzyme, tyrosine hydroxylase (TH), indicative of adrenergic innervation and norepinephrine (NE) to multiple positive fibers surrounding antral follicles. In the ovary, nitric oxide, a gaseous neurotransmitter, is produced by the vasculature, neurons, granulosa, theca, and luteal cells. Nitric oxide is synthesized by NO synthase (NOS), an enzyme with three isoforms (inducible, endothelial, or neuronal _ iNOS, eNOS, nNOS, respectively). The expression of iNOS and eNOS, both stimulated by gonadotropins, may participate in various ovarian functions. Gamma-aminobutyric acid (GABA) has been implicated as having an inhibitory effect on progesterone production from luteal cells. Immunocytochemistry localizes the GABA synthetic enzyme glutamate decarboxylase (GAD) and GABA itself to ovarian interstitial compartments. This project also focuses on characterizing follicular dynamics and inducing ovulation through the examination of live murine ovarian slices (200um) using a brain slice protocol (Tobet et al., 2003) adapted to the ovary. Thus far, we have demonstrated an FSH dose dependent increase in the number of follicles that appear ready to ovulate within a 48-hour period in vitro. LH appears to cause oocyte mobilization within 1 hour of exposure in vitro. Creating a live slice model for the ovary in vitro provides a paradigm to determine the role(s) of NE, NO, and GABA in relation to ovarian dynamics.

Maximum tolerated dosing and metronomic based dosing of docetaxel, vandetanib and radiation therapy in human head and neck squamous cell carcinoma xenografts

C Steinhauer, EJ Ehrhart, B Charles, E Bradshaw-Pierce, D Gustafson.

Purpose: The goal of this study is to investigate the anti-endothelial and anti-tumor effects of docetaxel, vandetanib and radiation therapy as sole agents and combination therapy. **Materials/methods:** Thirty-six mice with established human head and neck squamous cell carcinoma (HNSCC) tumor xenografts were placed into 1 of 12 treatment groups and received therapy over 28 days. Docetaxel was administered at both maximal tolerated doses and metronomic based doses. Tumor samples were collected on day 10 of treatment. Apoptosis, proliferation and microvessel density were investigated through immunohistochemistry. Markers include Ki67 (cell proliferation), caspase3 active (apoptosis) and von Willebrand Factor (endothelial cells). **Results:** The mice that received the triple combination therapy of docetaxel, vandetanib and radiation therapy had no tumor tissue to analyze due to a robust anti-tumor response. Cell proliferation analysis yields a significant difference ($P < 0.01$) between the treatment groups and the vehicle. There is a positive correlation between Ki-67 and tumor growth rate ($P = 0.0333$) across all treatment groups, suggesting growth is from an increase in proliferation rather than a decrease in apoptosis. Amongst samples analyzed, the combination of docetaxel and vandetanib has the longest tumor doubling time, highest rate of apoptosis and lowest proliferation rate, yielding the best survival advantage. Proliferating endothelial cell analysis yields a significant difference ($P < 0.05$) for all treatment groups besides radiation therapy alone, when compared to the vehicle. **Conclusions:** The combination therapy of docetaxel, vandetanib and radiation therapy shows promising effects of tumor response by enhanced cytotoxicity on tumor cells and growth coupled with a decrease in endothelial proliferation. Human HNSCCs commonly have elevated levels of epidermal growth factor receptor and poor prognosis making it an ideal candidate for anti-angiogenic therapy.

Flock-level Prevalence of Bluetongue in Colorado Sheep

CE Mayo, AE Hill

Purpose: The objectives of this study are to determine flock-level seroprevalence and virus prevalence, and identify environmental factors associated with BTV infections among Colorado sheep. Bluetongue (BT) is caused by Bluetongue virus (BTV), family Reoviridae, genus Orbivirus, transmitted by *Culicoides* spp. gnats with highest vectorial capacity from July until November. Bluetongue causes significant morbidity in sheep; clinical signs include weight loss during acute infection, decreased wool production due to wool break, lost income from ram sales due to interstate trade restrictions on BTV test positive animals, and decreased reproductive rates due to consequences of in utero infections and transient ram infertility. **Materials and Methods:** During the July-November vectorial season, blood is being obtained from ten ewes, ten lambs, and five rams from flocks in Colorado for cELISA and PCR testing in conjunction with collection of GPS data. **Results:** After four months of data collection, 33% of the flocks have been antibody positive, 22% have been PCR positive, and 19% have demonstrated clinical signs consistent with BTV. **Conclusion:** Bluetongue virus affects a substantial number of sheep flocks in Colorado, and environmental factors are likely to be associated with BTV.

Detection of Mycoplasma Mastitis using SCC, culture, sELISA and PCR in Large Dairy Farms in Saudi Arabia

H A AL-Abdullah and A A Fadel almullah

Bovine mycoplasma mastitis is, an important disease affecting the economic viability of the dairy industry in the Kingdom of Saudi Arabia. Identification of mycoplasma infected dairy cows is a critical aspect of control of mycoplasma mastitis. Current single test methods for diagnosis of mycoplasma mastitis do not effectively identify all carrier and clinical cases. The objective of this study was to develop a diagnostic routine that uses four bovine mycoplasma mastitis detection methods: somatic cell count (SCC), culture, sELISA, and PCR in order to control mycoplasma mastitis. Individual cow milk samples were collected from 4 separate dairies known to be endemic for mycoplasma. Somatic cell counts (SCC), mycoplasma culture, and mycoplasma sandwich antigen capture ELISA tests were run on all milk samples. Mycoplasma bovis PCR was performed on all culture or ELISA positive milk samples. Results showed that the SCC of all mycoplasma positive milk samples was >700,000cells/ml. Hence, the elevation of SCC can be use as a very good marker for identifying the onset of a mycoplasma outbreak. While the culture method is commonly recommended pre and post onset of a mycoplasma outbreak, the sELISA test was found to be comparable to the culture method in detecting mycoplasma infection in cattle when it predispose by enrichment procedure. In fact, sELISA was found to be essential as a screen test during an outbreak. The sensitivity of PCR was found to be necessary as a confirmatory test for strains isolated or non-speciated by sELISA

Durability of Disposable Overboots Under Simulated Field Conditions

K Miller, C Zadina, J Traub-Dargatz, D Dargatz

Problem-Ambulatory clinicians are often called to handle infectious and contagious equine cases. To achieve biocontainment in these situations, veterinarians should wear protective garments. Durability of protective clothing in the veterinary setting has not been adequately evaluated, making selection of optimal products difficult. Objectives-The objective of this study was to evaluate the durability of four different similarly priced disposable overboots when worn under simulated field conditions. Materials/Methods-Four different disposable overboots were selected for the study. A 265 foot walking course was designed that covered three surfaces: gravel, cement and rubber stall matting. Ten third-year professional veterinary students participated. The order in which boots were worn was randomized and each participant wore each type of boot three times. Large or extra large boots were provided based on participants shoe size. After walking the course, the porosity of the boots was measured by pouring two liters of water into the boot and measuring the amount of water that leaked into a collection container in one minute. Participants also selected their preferred boot. Results-The porosity by boot type differed significantly ($p < 0.05$). The mean volume of water recovered by boot type was 209mL (blue), 58mL (clear), 0mL (heavy yellow), and 1mL (light yellow). Among the 4 types of boots, the number with no leakage and 10 mL or less were 5 and 14 for blue boots, and 10 and 18 for clear boots, respectively. None of the heavy yellow boots leaked and only 1 of the light yellow boots leaked. Conclusions-Porosity was different across boot types. Based on this study veterinarians can make a more informed decision in selection of overboots. a. Clear boot (Continental Plastic), blue boot (Jorgensen), heavy yellow (Lab Safety Co.), light yellow (Onguard Industries)¹. Animal Population Health Institute, College of Veterinary Medicine and Biomedical Science, Colorado State University.

Does Dynamic Contrast Enhanced MRI Predict Percent Tumor Necrosis in Spontaneous Canine Osteosarcomas?

BM Eichelberger, E Ashton, EJ Ehrhart, D Thamm, W Dernell, S LaRue, S Plaza, S Withrow, A Bachand, SL Kraft.

Percent tumor necrosis (%TN) of primary osteosarcoma has been shown to predict tumor control and response to treatment. An in vivo method of indirectly estimating percent tumor necrosis would be advantageous for evaluating early response to treatment and tailoring therapy. The dynamic contrast enhanced (DCE-MRI) pharmacokinetic K_{trans} , is a contrast media uptake rate constant that reflects vascular volume, flow and permeability. We hypothesized that a decrease in K_{trans} could be associated with increasing %TN. We assessed this hypothesis by doing DCE-MRI as part of a clinical oncology trial using novel treatment of a group of dogs with osteosarcoma. DCE-MRI was performed on 10 dogs receiving single high dose radiation with or without neoadjuvant immunomodulator of liposomal muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE), before and again 3-4 weeks after radiation prior to amputation. Compartmental analysis of the DCE-MRI signal intensity-time curves were done by regions of interest drawn of the entire tumor volume using 3D geometrically constrained region growth (3D GEORG). Pathology-derived %TN was derived from surgically amputated limb specimens. Data were statistically analyzed and DCE-MRI results compared against the pathologically based %TN. There were no statistical differences between treatment and control groups for indices of local tumor control. With combination of treatment and control groups, the contrast uptake rate constant (K_{trans}) decreased in 6/10, with no change in 2/10 dogs and increased in 2/10. The correlation between K_{trans} and pathology derived % TN was 0.52 which is not a strong correlation coefficient. There was a trend for the means of K_{trans} to decrease and the percentage of non-enhancing tumor volume, which corresponds to necrosis, to increase after radiation. DCE-MRI does seem to provide information regarding radiation effects on tumor vascularity because of the trend for K_{trans} to decrease in many dogs after radiation. Future studies should explore the relationship between K_{trans} and %TN.

An Epidemiological Investigation of Antimicrobial Use and Occurrence of Antimicrobial Resistant Bacteria in Alberta Feedlots

S Rao, J Van Donkersgoed, V Bohaychuk, T Besser, X Song, B Wagner, D Hancock, D Renter, D Dargatz, PS Morley

Antimicrobial resistance (AMR) in bacteria from livestock and their products has been a significant concern of human medical profession and Health Canada. The purpose of this study was to determine whether AMR in foodborne pathogens (*E. coli* O157:H7, *Salmonella* and *Campylobacter*) and generic *E. coli* obtained from fecal samples from floor of feedlot pens were associated with antimicrobial (AM) use in Alberta feedlots. Twenty-one feedlots with >5000 head capacity were randomly selected during March-December 2004. All samples were subjected to microbiological isolation of the bacteria and their antimicrobial susceptibility determined. Prevalence was estimated by season (spring, fall) and type of pen (newly arrived, pre-slaughter animals). Defined Daily Doses (DDD) for each drug class was calculated based on the treatment records of feedlots. In-feed medication and injectables were considered as separate variables. Generalized Linear Mixed models were used to investigate the relationship between each AMR and antimicrobial drug use. Generic *E. coli* was commonly found in fecal samples (98%) showing resistance to Tetracyclines (53%), Streptomycin (28%) and Sulfadiazine (48%). Among the pathogenic bacteria, *E. coli* O157 was recovered from 7% of fecal samples, 16% of which were resistant to Tetracyclines, 16% to Sulfazoxazole, 5% to Chloramphenicol, 2% to Trimethoprim/ Sulfamethoxazole and 0.7% to Streptomycin. *Campylobacter* was found in 76% of fecal samples but *Salmonella* was rarely isolated. The prevalence estimates of bacterial population and their AMR in the feedlots varied between seasons and pen type. The drug that was significantly associated with Tetracycline resistance in generic *E. coli* was Tetracycline. However, further analysis taking `_feedlot_` as a random effect in the model would give more information to assess the risk of AMR in bacteria with use of AM drugs in feedlots.

In vitro biomechanical evaluation of semi-contoured, locking plate/rod versus anatomically-contoured, conventional plate/rod fixation in a canine femoral defect model

CSS Goh, BG Santoni, CM Puttlitz, RH Palmer

These experiments were designed to compare the mechanical behavior of a semi-contoured, locking plate/rod (LP/rod) construct to an anatomically-contoured, limited-contact dynamic compression plate/rod (LC-DCP/rod) construct in a canine femoral defect model. **MATERIALS and METHODS:** Eight matched pairs of canine femora were utilized; one randomly assigned to LP/rod and matched pair assigned to LC-DCP/rod group. Construct stiffness and ostectomy gap subsidence were determined prior to and after cyclic axial loading at 20%, 40% and 60% of body weight for 6,000 cycles each (total 18,000 cycles). Three constructs from each group were subsequently exposed to an additional 45,000 cycles at 60% body weight (total 63,000 cycles). Following cycle loading, mode of failure was documented for each construct loaded to failure at 5 mm/minute. **RESULTS:** No significant differences in global construct stiffness were detected between treatment groups in either short-term (18,000) or long-term (63,000 cycle) protocols. There was no evidence of implant failure in any of the six constructs tested to 63,000 cycles. Ostectomy gap subsidence increased with progressive increases in axial load for both treatment groups and a difference was not detected between them. No change in gap subsidence was identified for either treatment group following the 63,000 cycle protocol. No significant difference in mean load (+/- SEM) at failure was detected between treatment groups; 1493.83 (200.12) and 1276.05 (156.11) Newtons for LP/rod and LC-DCP/rod, respectively. The primary failure event for both constructs was documented at the screw hole immediately distal to the ostectomy. **CONCLUSIONS:** The semi-contoured LP/rod construct is biomechanically similar to the anatomically-contoured LC-DCP/rod system. **ACKNOWLEDGEMENTS:** The authors acknowledge Synthes Veterinary, Inc. and IMEX Veterinary for providing hardware and CSU's College Research Council for financial support.

Does the gender of the client and the veterinarian influence communication in veterinary visits?

JR Shaw, BN Bonnett, CL Adams, DL Roter.

The purpose of this study is investigate the role of client and veterinarian gender in communication during veterinary visits and to characterize gender communication based on verbal dominance, relationship-centered care, communication patterns and length of visit. A descriptive cross-sectional study of veterinarian-client-patient communication was conducted with a random sample of 50 veterinarians and a convenience sample of 300 clients and their pets in South Western Ontario. Six appointments were videotaped for each veterinarian, including three wellness and three problem appointments. The Roter Interaction Analysis System, a quantitative communication assessment tool, was utilized to describe visit communication. Individual gender effects and dyadic relationships were investigated, as well as differences in wellness and problem appointments. Concordant gender dyads were more likely to display a collaborative approach reflected in communication pattern use and mean relationship-centered care scores. The percentage of wellness appointments with the biolifestyle-social communication pattern was highest in concordant dyad visits (MV-MC 87%, FV-FC 82%). In wellness appointments, female veterinarians were more likely to use a relationship-centered approach with female clients (mean RCC = 1.11) than with male clients (mean RCC = 0.83, $p = 0.04$). In problem appointments for female veterinarians mean RCC score (0.46) was 1.5 times greater than that of male veterinarians (0.30, $p = 0.002$). There was no significant gender difference in verbal dominance scores or length of visit. Findings of this study will inform the development of outcomes-based studies and communication curricula. Understanding gender-related communication is important, since concordant or discordant communication may impact quality of care for the client and pet.

Poster Presentations

Session I

3:00-4:30PM

BASIC SCIENCE

Pharmacodynamic endpoints of toxicity following oral metronomic dosing of CPT-11 with ZD1839 or ZD6474 in mice

JM Fletcher, RJ Hansen, DL Gustafson

Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) are promising targets for treating cancer. Coadministration of ZD1839 (gefitinib), an EGFR inhibitor, with CPT-11 (irinotecan), a topoisomerase I inhibitor, shows reasonable antitumor activity in colorectal cancer at the maximum tolerated dose; however, this combination results in severe diarrhea and neutropenia. Metronomic dosing (lower more frequent doses) may reduce these side effects while retaining effectiveness. In addition, ZD6474 (vandetanib) is a novel small molecule inhibitor of VEGFR-2 and EGFR that appears not to exacerbate CPT-11-induced. Purpose: The aim of this study was to determine whether oral metronomic dosing with ZD6474 or ZD1839 increases CPT-11-induced toxicity. Methods: Pharmacodynamic endpoints of toxicity in non-tumor bearing mice following administration of CPT-11 at three dose levels (20, 40 or 60 mg/kg) alone or in combination with ZD1839 or ZD6474 (each at 25 mg/kg) p.o. qD x 4 days x 2 cycles. Results: Significant weight loss was observed only when CPT-11 (60 mg/kg) was combined with ZD1839. With the exception of ZD6474 plus CPT-11 (20 mg/kg), all treatments resulted in significant intestinal toxicity (villus length: crypt depth ratio) compared to vehicle. Interestingly, mice treated with ZD1839 plus CPT-11 had enhanced toxicity (reduced villus length: crypt depth ratio) compared to CPT-11 alone or CPT-11 plus ZD6474 ($P < 0.001$). Immunohistochemical analysis revealed that mice receiving ZD1839 plus CPT-11 had a significant decrease in proliferation and increase in apoptosis compared to CPT-11 alone ($P < 0.05$). ZD6474 did not significantly alter any of these endpoints as compared to CPT-11 alone. Conclusions: Overall, metronomic oral dosing of all treatments was well tolerated at the doses and schedules tested in this study. Also, ZD6474 does not enhance CPT-11-induced toxicity to the same extent as ZD1839.

Periattachment factor is required for conceptus elongation in sheep

SH Purcell, JD Cantlon, RV Anthony

Periattachment factor (PF) is a nuclear protein expressed in the elongating ruminant conceptus. Previously, we determined that sheep PF mRNA becomes detectable at d 13, before increasing to peak concentrations at d 15-16, and then declining through d 30 of gestation. This pattern of expression corresponds with the time of rapid conceptus elongation and attachment to the endometrium. Furthermore, we have identified PF within the invasive cytotrophoblasts of the first trimester human placenta, as well as in carcinoma cell lines. PF has been immunolocalized to the nucleus in all tissues studied, and we hypothesized that it may play a role in regulating trophoblast proliferation and/or migration. Our current objective was to ablate PF expression in sheep conceptuses by in vivo RNA interference to determine its role and importance during conceptus development. Superovulated ewes were naturally mated and embryos were collected by hysterectomy and uterine flushing on d 8 post mating. Upon collection, zona-free blastocysts were infected with a lentivirus (pLL3.7-shRNA3) expressing a short-hairpin (sh) RNA designed to target PF mRNA degradation, or with a lentivirus (pLL3.7) expressing no shRNA. Following a 6 h infection period, the blastocysts were surgically-transferred into the uterus of synchronized recipient ewes. On d 15 of gestation, relative to the time of blastocyst donor-ewe mating, recipient ewes were euthanized and conceptuses were collected. For the 6 recipient ewes receiving blastocysts infected with pLL3.7, all of the conceptuses harvested had undergone normal elongation. For the 6 recipient ewes receiving blastocysts infected with pLL3.7-shRNA3, conceptuses were recovered from only 2 recipients, and those conceptuses had not undergone elongation. These results indicate that PF is required for conceptus elongation and survival in sheep, and may play a similar role regulating the migration and invasion of cytotrophoblasts during human pregnancy.

Evidence of a Putative Lentivirus in Captive Lemur catta

DS Stump, L Villers, ML Sauther, S VandeWoude.

Background: Ring-tailed lemurs endemic to Madagascar have been previously shown to exhibit seroreactivity to lentiviral antigens. SIV, origin of HIV, has been identified as a natural infection in many primate species of continental Africa, but is not naturally-occurring in Asian species. The identification of a lentivirus specific to lemurs would be of great interest with respect to conservation efforts as well as the elucidation of primate lentiviral origins. Materials/Methods: Whole blood taken from 16 ring-tailed lemurs at the Indianapolis Zoo was tested for seroreactivity to SIV, LLV, PLV, and FIV lentiviral antigens by immunoblot. Lemur PBMC culture supernatants were evaluated for reverse transcriptase activity using standard techniques, and SIV p28 capsid concentration was determined by ELISA. Universal degenerate primers, LPQG and YMDD, designed to amplify a conserved pol sequence, were used in PCR reactions with genomic DNA from three lemurs; amplicons were cloned and sequenced. Specific primers were designed from one of the cloned sequences and used in reactions with lemur, macaque, and human genomic DNA. Results: Immunoblot assay revealed that captive lemurs were 100% seroreactive to SIV. Seventy-two percent were seroreactive to 2 lentiviral antigens, while 33% had antibodies that recognized 3 lentiviral antigens. No appreciable reverse transcriptase activity was noted in culture supernatants, but SIV p28 ELISA detected capsid production. One of the cloned sequences was 73% homologous to the betaretrovirus Simian type D virus 1. Specific primers designed from this clone amplify the expected 97 bp sequence from DNA of fifteen lemurs, but in no human or macaque samples. Conclusions: These results further support the presence of a retroviral agent with lentiviral characteristics that is lemur specific. Further expansion of the lemur specific retroviral pol sequence will be attempted for further identification and characterization.

Up-regulation of type I interferon response and down-regulation of SDF-1/CXCR4 signaling in blood cells from pregnant heifers carrying fetuses infected with BVDV

NP Smirnova, H Bielefeldt-Ohmann, H Van Campen, K J Austin, AA Ptitsyn, TR Hansen

Purpose: Infection with Bovine viral diarrhea virus (BVDV) leads to economic losses in cattle industries. The transplacental infection with non-cytopathic (ncp) BVDV in early gestation (< d150) results in fetal persistent infection (PI) with chronic life-long viremia; infection of the fetus in late gestation (> d150) or after birth causes a transient infection (TI) that is cleared by a competent immune system. We hypothesized that acute BVDV infection and the presence of an infected fetus would affect gene expression in blood cells of pregnant heifers. Materials/methods: Naïve pregnant heifers were inoculated with ncpBVDV2 virus on d 75 (PI fetuses) or d 175 (TI fetuses) of gestation, or were kept uninfected. Samples of maternal blood during time course of infection were collected. Microarray analysis of gene expression in blood cells on d 160 and d 190 of pregnancy was completed by using the Affymetrix bovine chip. GeneSifter and GEDA were used for data analysis. Results: 1. Microarray analysis of gene expression on d 190 revealed up regulation of type I interferon (IFN) stimulated genes (ISGs) in the transient BVDV infection. High antiviral activity detected by IFN bioassay in blood of TI heifers (3-7 days p.i) was accompanied by up regulation of ISG 15kDa (ISG15) mRNA (a marker of the IFN response), measured by qRT-PCR. ISG15 expression returned to baseline level by day 45 p.i. 2. Expression of chemokine receptor 4 (CXCR4) and several genes of SDF-1/CXCR4 signaling pathway were down regulated in the blood of heifers with PI fetuses on d 160 of gestation. RT-PCR confirmed rapid onset (by d 7 p.i.) and prolonged (for ~90 days) downregulation of CXCR4 expression. Conclusions: 1. Infection with ncpBVDV2 induces a vigorous type I IFN response in acutely infected animals. 2. Presence of the PI fetus causes downregulation of SDF-1/CXCR4 signaling in blood of the dam, which could have deleterious consequences on fetal development and immune response. Evaluation of the effects of single, high-dose radiation with or without an immunomodulator for canine appendicular osteosarcoma

Reduction in TRPC4 expression specifically attenuates G-protein coupled receptor-stimulated calcium entry in human myometrial cells.

A Ulloa, M Zhong, YS Kim, J Clanton, C Clay, CY Ku, and BM Sanborn

Canonical transient receptor potential (TRPC) proteins may play a role in regulating changes in intracellular calcium ($[Ca^{2+}]_i$) and thus contractility. Human myometrium expresses TRPC4, TRPC1 and TRPC6 mRNAs in greatest abundance relative to other TRPCs. Specific contributions of TRPC4 to signal-regulated calcium entry (SRCE) were assessed in PHM1 and primary human myometrial (UtSMC) cells using RNA interference (RNAi). Four shRNAs elicited 60-90% knockdown of a TRPC4 target construct in the psiCHECK-2 reporter system and TC4sh1 was selected for construction of an adenoviral vector that infected PHM1 cells with 90% efficiency. Adenovirus expressing TC4sh1 induced both mRNA and protein TRPC4 knockdown, whereas infection with empty vector had no effect and expression of other TRPCs were unchanged. PHM1 cells were treated with 100nM oxytocin to examine effects on receptor-stimulated Ca entry, 100nM thapsigargin for store-operated Ca entry, and 100mM OAG for diacylglycerol-stimulated Ca entry, measured as increases in $[Ca^{2+}]_i$ using Fura-2. Cells infected with vector expressing TC4sh1 exhibited attenuated oxytocin-mediated SRCE, but there was no effect on thapsigargin- or OAG-stimulated calcium entry. Furthermore, the SRCE elicited by two additional G-protein coupled receptor (GPCR) stimulants, ATP and PGF2a, were also attenuated by TC4sh1 vector. Interestingly, TRPC4a overexpression induces no significant changes in response to any of the SRCE stimulants tested. Similar results were obtained in primary UtSMC. These data show that adenoviral constructs expressing TC4sh1 induce a specific knockdown in TRPC4 mRNA and protein expression. In myometrial cells, such knockdown specifically induces a decrease in GPCR-stimulated increases in $[Ca^{2+}]_i$, but not in thapsigargin or OAG-stimulated increases in $[Ca^{2+}]_i$. These data indicate that Ca entry in response to cellular signals can be specifically affected by attenuation of a specific TRPC in myometrial smooth muscle cells.

Antiproliferative Effects of 2-Deoxyglucose in Canine Osteosarcoma

JC Petty, EJ Ehrhart, SE Lana, DH Thamm.

Purpose: Some cells within a tumor may be poorly perfused, and therefore less susceptible to traditional chemotherapy. Cancer cells, especially hypoxic cells distant from stromal blood vessels, require more glucose than normal cells as they utilize glycolysis, rather than oxidative phosphorylation, to survive. 2-deoxy-D-glucose (2-DG) is a glucose analog that blocks the first step of glycolysis, leading to growth arrest and/or apoptosis in cells dependent on glycolysis for ATP generation. We hypothesized that treatment with 2-DG would block glycolysis in canine osteosarcoma (OSA) cells, leading to growth arrest or apoptosis. Since Glucose Transporter-1 (GLUT-1), a glucose transporter, is expressed in canine osteosarcoma, we hypothesized that simulated hypoxia would up-regulate GLUT-1 and increase sensitivity to 2-DG. **Materials/Methods:** 6 canine OSA cell lines were grown in 96 well plates under normoxic and simulated hypoxic (100 uM desferrioxamine) conditions and exposed to concentrations of 2-DG ranging from 0 to 10,000 ug/mL. Relative viable cell number was then determined using a bioreductive fluorometric assay. Fluorescence cytochemistry was used to evaluate GLUT-1 expression and evaluated under normoxic and simulated hypoxic conditions. **Results:** The OSA cell lines showed varying dose-dependent sensitivities to 2-DG. The IC₅₀ ranged from 183 ug/mL to 10,000 ug/mL. Simulated hypoxia increased GLUT-1 expression in canine OSA cells. However, we could detect no increase in 2-DG sensitivity in cells concurrently exposed to desferrioxamine. **Conclusion:** 2-DG induces dose-dependent growth inhibition in canine OSA cell lines in vitro. Different cell lines have widely differing sensitivities to 2-DG. GLUT-1 appears to be up-regulated in vitro under conditions of hypoxia.

Indoor air pollution from cookstove smoke and adverse health effects among Honduran women.

ML Clark, JL Peel, TL Nelson, AM Bachand, S Conway, JB Burch, SJ Reynolds.

Purpose: Elevated indoor air pollution exposures associated with the burning of biomass fuels in developing countries are well established. Studies have demonstrated the value of estimating exposures by evaluating stove type and household parameters. Adverse health endpoints have been associated with cookstove exposures, although little research has been performed on cardiovascular health endpoints in these settings. Materials/Methods: We conducted a cross-sectional survey among 79 non-smoking Honduran women. Thirty-eight women cooked with traditional stoves and 41 with improved stoves. For a subgroup of these women (N=54-58), carbon monoxide and particulate matter (PM_{2.5}) levels were assessed via eight-hour indoor monitoring, as well as eight-hour personal PM_{2.5} monitoring. Stove quality, using a four-level scale, and ventilation factors, such as the volume of the kitchen, area of the windows, and materials of the walls, were assessed. Forced expiratory volume in one second and peak expiratory flow, as well as respiratory symptoms, were measured. Finger-stick blood samples were collected and dried on filter paper in order to assess a biomarker of inflammation, C-reactive protein (CRP). Results: The stove scale and ventilation factors predicted more than 50% of the variation in personal and indoor PM_{2.5} and 85% of the variation in indoor carbon monoxide. Women exposed to cookstove exposures reported symptoms of cough, phlegm, wheeze, headache, and shortness of breath more frequently than those not exposed. Associations consistent with a null association were observed between cookstove exposures and lung function and CRP. Conclusions: Results of the exposure assessment could provide a cost-effective alternative to air quality monitoring. The ease and convenience of collecting, storing, and transporting finger-stick blood samples, could prove to be a useful tool for larger community-based investigations, especially in developing countries.

The acquisition and utilization of the plasminogen activating system by *Francisella tularensis*.

SL Warner, D Crane, CM Bosio

Francisella tularensis the causative agent of pneumonic tularemia is a gram-negative, facultative intracellular bacterium. This bacterium has recently become of interest as potential biowarfare agent due to: its ability to cause rapid, lethal disease following inhalation with as few as 10 organisms, its availability in the environment and, its previous weaponization. Although it is widely accepted that this organism spends the majority of its life cycle in the intracellular environment, recent data suggests that the more virulent strains can exist in the extracellular milieu as well. Thus, it is possible that Schu4 co-opts host components present in the extracellular environment to enhance its infectivity and pathogenesis. Here, we provide evidence that Schu4 utilizes the host plasminogen system to increase its pathogenicity and replication in peripheral tissues. Specifically, Schu4 binds both plasminogen and, using host derived urokinase-plasminogen activator (uPA), also binds active plasmin. Plasmin bound Schu4 is capable of degrading the extracellular matrix protein vitronectin, and to a lesser extent laminin and fibronectin. Finally, mice lacking uPA had significantly fewer bacteria in the spleen and liver, and nearly undetectable pathological changes in lung compared to wild type controls following low dose aerosol challenge with *F. tularensis* Schu4. Together this data suggests that Schu4 may be utilizing the host plasminogen system while in the extracellular environment as a mechanism to evade host immune responses while enhancing dissemination and replication following inhalation.

Isolation and analysis of the Equine Kisspeptin receptor, GPR54.

CR Cline, JD Whitesell, C Magee, CM Clay

The mammalian G-protein coupled receptor GPR54 is a receptor present on specific neurons in the hypothalamus including Gonadotropin Releasing Hormone (GnRH) neurons. The ligands for GPR54 are peptides known as kisspeptins, which are secreted from KiSS-1 neurons, also located in the hypothalamus. Various stimuli, such as gonadal steroid feedback and photoperiod, control the expression of KiSS-1 mRNA in KiSS neurons. The binding of Kisspeptin to GPR54 results in a downstream signaling cascade which ultimately leads to GnRH release. Manipulation of the Kisspeptin-GPR54 relationship in mares may provide a unique avenue to influence their reproductive cycle. The gene sequence encoding GPR54 is conserved between species, specifically mouse, human, rat and bovine, but the sequence of equine GPR54 has not been determined. Here, a comparison of GPR54's gene sequence between species using Basic Local Alignment Search Tool (BLAST) available from NCBI's website indicates GPR54 consists of five exons. Exons 1- 5 for the mouse, human, rat and bovine GPR54 sequence were aligned separately using the Gene Runner v3.05 program. Reading frame was determined in each exon using the human amino acid sequence and PCR primers were created for each of the five exons based on highly conserved sequences near or at the end of each exon. Primers were analyzed to obtain ideal annealing conditions. PCR was then performed separately for each exon, using equine genomic DNA as the template. The goal is to determine the coding sequence for equine GPR54. The full-length cDNA encoding for Equine GPR54 will then be cloned into a plasmid and a cell line transformed to create an in-vitro model. This model will allow for high through-put screening of Kisspeptin agonists and antagonists in order to identify candidate ligands that can ultimately be used to exogenously influence reproductive cyclicity in mares through GPR54 stimulation and GnRH secretion.

Characterization of Small RNAs Produced During Dengue Virus Infection of Mosquito Cells

JC Scott, J Wilusz, KE Olson, I Sanchez-Vargas, CD Blair

The resurgence of mosquito-borne diseases such as dengue is an important global health concern, with 50 to 100 million dengue fever cases globally each year. New methods for control of virus transmission by the vector mosquito are being examined by our laboratory, including enhancement of the evolutionarily conserved RNA interference (RNAi) pathway. RNAi has been found in plants, insects and mammals and in some organisms serves as a defense mechanism that recognizes and degrades double-stranded RNA (dsRNA), such as some forms of viral RNA, to produce small interfering RNAs (siRNAs). Studies in our lab indicate that mosquitoes have an active RNAi pathway and that manipulation of this pathway can prevent viral infection and transmission. Still, little is understood about what occurs in the RNAi pathway in mosquitoes at a molecular level during an arboviral infection. We are examining how virus replication is targeted by the RNAi pathway, by looking at the siRNAs produced from the viral RNA during an infection. These siRNAs can be detected a few days post infection using northern blotting. Cloning and sequencing of the siRNAs indicates that they are derived from all parts of the genome, but there may be some areas that are targeted more often. This could indicate that the viral dsRNA trigger may be secondary structure of single-stranded virus genome itself. This information will be used to design better strategies to interrupt virus transmission by mosquitoes.

Estrogen Receptor β and Oxytocin Interact to Modulate Anxiety-like Behavior in a Sexually Dimorphic Manner

AE Kudwa, J Fromm, and RJ Handa

Oxytocin (OT) is a neuropeptide most studied for its role in maternal behavior and its actions are modulated by gonadal steroid hormones such as estrogen. Oxytocin also has anxiolytic properties, attenuates stress-induced hypothalamic-pituitary-adrenal activity and is localized in estrogen receptor beta (ER β) containing neurons in stress-relevant brain nuclei. While ER β modulates the adult stress response, the role of OT is relatively unknown. Here we test the hypothesis that OT and ER β interact to decrease the display of anxiety-like behaviors. Using both synthetic and endogenously-derived ER β -selective compounds, we tested whether OT and ER β interact in a sexually dimorphic manner to reduce anxiety-related behaviors. Young adult rats were gonadectomized and one week later received 5 daily injections of diarylpropionitrile (DPN, 2mg/kg), dihydrotestosterone (DHT; 1mg/kg), 5 α -Androstane-3 β , 17 β -Diol dipropionate (3A β diol; 1mg/kg) or vehicle (VEH; β -cyclodextran). On the fifth day, animals were tested on the elevated plus maze (EPM). Results showed that 3A β diol-treated females entered, spent significantly more time on, and displayed more head dips from the open arm of the EPM compared to all groups except DPN-treated females. These findings show that selective activation of ER β reduces adult anxiety-like behavior in a sexually dimorphic manner. To examine whether oxytocin is involved in the anxiolytic properties of ER β , additional animals were implanted with cannulae directed at the right lateral ventricle. Animals received 5 daily injections of either DPN or VEH, and 30 minutes prior to behavioral testing, animals were infused with an OT antagonist (des Gly-NH₂ d(CH₂)₅ [Tyr(Me)₂, Thr₄] OVT; 1 μ g/5 μ l aCSF). Preliminary results indicate that DPN-treated animals displayed fewer anxiety-like behaviors compared to VEH and OT antagonist treatment reversed the anxiolytic effects of DPN. The findings support a possible interaction between ER β and OT in adult HPA regulation.

Relative levels of macrophage-colony stimulating factor and granulocyte macrophage-colony stimulating factor influence the specific generation of macrophage populations during infection with Mycobacterium tuberculosis

DM Higgins, J Sanchez-Campillo, AG Rosas-Taraco, JR Higgins, E Lee, IM Orme and M Gonzalez-Juarrero

Purpose: Members of the colony stimulating factor cytokine family play important roles in macrophage recruitment and activation. However, the role of M-CSF in pulmonary infection with Mycobacterium tuberculosis (M. tuberculosis) is not clear. Therefore, this study evaluated the role of macrophage-colony stimulating factor (M-CSF) during pulmonary infection with M. tuberculosis and compared it to granulocyte macrophage-colony stimulating factor (GM-CSF). Materials/Methods: Using a low dose aerosol infection of M. tuberculosis in C57BL/6 mice we determined the levels of M-CSF and GM-CSF by Enzyme-Linked ImmunoSorbent Assay (ELISA) during the first 60 days post challenge. We gave repeated intratracheal installations of M-CSF to M. tuberculosis infected mice and analyzed lungs by flow cytometry, ELISA, immunohistochemistry, Greiss reaction and plating for bacterial load. Additionally bronchoalveolar lavage was performed on uninfected and infected mice and cells obtained were analyzed by flow cytometry, microscopy and used in a mixed lymphocyte reaction. Results: In this study we show the lungs of mice infected with M. tuberculosis displayed a progressive decrease in M-CSF in contrast to increasing levels of GM-CSF. Restoring pulmonary M-CSF levels during infection resulted in a significant decrease in the presence of foamy macrophages and increased expression of CCR7 and MHC class II, specifically on alveolar macrophages. In response to M-CSF, alveolar macrophages also increased their T-cell stimulating capacity and expression of DEC-205. Conclusions: In this study we show that the levels of expression of M-CSF and GM-CSF participate in the progression of macrophages into foamy cells and that these cytokines are important factors in the differentiation and regulation of expression of dendritic cell associated markers on alveolar macrophages. In addition, these studies demonstrate that M-CSF may have a role in the adaptive immune response to infection with M. tuberculosis.

Highly Pathogenic Avian Influenza A (H5N1) Virus Dynamics in the Respiratory Tract of Cynomolgus Macaques (*Macaca fascicularis*) Compared to Human H1N1 Virus Containing 2 or 3 Genes From the 1918 Pandemic Flu.

AE Tolnay, CR Baskin, TM Tumpey, PJ Sabourin, CL Sabourin, JP Long, JA Pyles, R Albrecht, A Garcia-Sastre, MG Katze, H Bielefeldt-Ohmann

Purpose: human influenza A viruses cause a limited respiratory tract infection that is non-fatal in otherwise healthy adults, whereas infections with highly pathogenic avian influenza H5N1 viruses result in severe, rapidly progressing pneumonia, systemic symptoms, and high mortality. Reasons for this are still not understood. Comparison of infection with an H5N1 isolate and viruses containing genes from the 1918 pandemic flu was carried out in macaques to clarify virulence factors. Methods: Cynomolgus macaques (n=34) were infected by the respiratory route with either: A/Vietnam/ 1203/2004 (H5N1) virus, A/Texas/36/91 (H1N1) virus or reassortants of this virus containing either 2 (HA, NA) or 3 (HA, NA, NS) genes from the 1918 strain. Animals were killed 1, 2, 4, or 7 days pi. Respiratory tract tissues were processed for histopathology and immunohistochemistry for viral proteins, dendritic cells (DC), macrophages (Mo), and neutrophils (PMN). Results: While viral antigen and pneumonia was evident in animals infected with either H5N1 or reassortant viruses by 24 hours pi, both features were most severe in the H5N1-infected animals, and progressed to diffuse virus replication and pathology through day 7 pi. In contrast, viral antigen load peaked at day 2 in animals infected with reassortant viruses and was cleared by day 7, although residual pathology persisted. Viral antigen was detected in tonsils and lymph nodes of H5N1-infected animals, while these tissues were negative in all other groups. A severe influx of Mo and PMN was seen in both H5N1 and reassortant virus-infected groups, however, there was a notable, yet unexplained depletion of DC from lungs and lymphoid tissues in the H5N1 infected animals. Conclusions: immune functions may be subverted during an H5N1 infection, resulting in impaired viral clearance and resolution of the inflammatory process, leading to irreversible tissue damage, acute respiratory distress syndrome, and deaths in infected human patients.

Functional involvement of the plasma membrane PKA/AKAP interaction in signaling events in uterine smooth muscle

D Murtazina, CY Ku, M Zhong, A Ulloa, BM Sanborn

In rat uterine smooth muscle (myometrium), a fall in plasma membrane protein kinase A (PKA) without a fall in AKAP150 is associated with loss of the ability of cAMP to inhibit contractant (oxytocin)-stimulated phospholipase C activation (Dodge et al, Mol. Endo 12: 1977, 1999). The effect of cAMP was reversed by SHT31, an AKAP interaction inhibitor. Progesterone treatment prolonged rat pregnancy and attenuated the decline in myometrial plasma membrane PKA (Ku et al, BOR 67:605, 2002). The antiprogestins RU486 and onapristone induce a fall in rat myometrial plasma membrane PKA with no change in membrane AKAP150 at mid-pregnancy. In the latter case, the fall in membrane PKA accompanies the onset of electrical and contractile activity prior to the onset of labor (R. Garfield). Phospholipase C β 3-Ser1105 in the oxytocin signaling cascade is a PKA target. Phosphorylation of PLC β 3-Ser1105 occurs in myometrial cells in response to uterine relaxants and is attenuated by PKA inhibitors. An AKAP5 shRNA construct produces 90% suppression of a psiCHECK-2 luciferase reporter in AD293 cells. Because myometrial cells are difficult to transfect, we inserted this construct into an adenoviral vector, achieving ~100% infection. To date, AKAP5 mRNA and protein knockdown have ranged 65% and 55%, respectively. Phenotype is being explored. These data point to a role for progesterone in regulating the myometrial plasma membrane PKA/AKAP interaction and to the potential importance of this interaction in negative crosstalk between relaxant and contractant pathways in the myometrium during pregnancy. Supported by HD09618.

Involvement of Signal-Regulated Calcium Entry in Store Refilling in Myometrial Cells

D Chung, CY Ku, BM Sanborn

Increases in intracellular Ca ($[Ca^{2+}]_i$) regulate contraction in uterine smooth muscle and different types of Ca channels contribute to the increase in $[Ca^{2+}]_i$. The purpose of this study was to determine if signal-regulated Ca entry (SRCE), which increases $[Ca^{2+}]_i$, plays a role in endoplasmic reticulum (ER) Ca refilling. The simultaneous measurement of changes in Ca, in cytoplasm and ER was accomplished using Fura-2 to measure changes in $[Ca^{2+}]_i$ and Mag-fluo-4 to measure changes in ER Ca ($[Ca^{2+}]_L$). Oxytocin (100 nM) elicited a transient increase in $[Ca^{2+}]_i$ in PHM1 and a sustained decrease in ER Ca in the absence of extracellular Ca. Thapsigargin (100 nM), which irreversibly inhibits Sarco/Endoplasmic Reticulum Ca^{2+} -ATPase (SERCA), increased $[Ca^{2+}]_i$ and produced a greater Ca store depletion than oxytocin. The addition of 1 mM extracellular Ca after thapsigargin resulted in an increase in $[Ca^{2+}]_i$ but only a small increase in $[Ca^{2+}]_L$. CPA (10 μ M), a reversible SERCA inhibitor, showed similar changes in $[Ca^{2+}]_i$ and $[Ca^{2+}]_L$ except that wash-out of CPA before the addition of 1 mM extracellular Ca resulted in complete ER store refilling. These data are consistent with the premise that Fura-2 and Mag-fluo-4 are simultaneously measuring changes in $[Ca^{2+}]_i$ and $[Ca^{2+}]_L$, respectively. We then used Ca channel inhibitors to assess the contribution of different types of Ca channels to ER store refilling. Oxytocin-induced SRCE and ER refilling were not inhibited by nifedipine, a L-type channel blocker. Mibefradil, a T-type channel blocker, also did not affect oxytocin-induced SRCE and ER refilling. Two inhibitors for SRCE (SKF96365 or gadolinium), inhibited oxytocin-induced SRCE and ER refilling in a concentration-dependent manner. These data suggest that there is a close correlation between the oxytocin-induced increase of $[Ca^{2+}]_i$ and ER Ca depletion and between SRCE and ER refilling. The data also provide evidence for a possible role for Ca entry through SRCE/receptor-operated channels in myometrial ER store refilling.

Developmental effects of vinclozolin on the GnRH neuronal system in rabbits

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Previous work has demonstrated that developmental exposure to a commonly used agricultural fungicide, vinclozolin, impairs male reproductive function in experimental animals. Recently, we reported that 6-week-old Dutch-Belted rabbits exposed prenatally to vinclozolin had fewer cells containing immunoreactive gonadotropin-releasing hormone (ir-GnRH) in the region of the organum vasculosum of the lamina terminalis and preoptic area/anterior hypothalamus (Bisenius et al., 2006). GnRH neurons, which are the central nervous system controllers of the reproductive axis, reach their final destinations in the mammalian forebrain early in development. In the current study, pregnant rabbits were orally dosed with vinclozolin (10 mg/kg/day) or vehicle for the last two weeks of gestation (gestation length: 30 days) and offspring were analyzed for ir-GnRH either at postnatal day 1 or at 24 (male) or 30 (female) wk of age. In vinclozolin-treated rabbits the number of ir-GnRH neurons was decreased in a region-dependent manner both at birth and in adulthood. However, in block-dissected brain regions of females total GnRH (by radioimmunoassay) was increased at 6 weeks of age. Further work is in progress to elucidate the potential mechanisms underlying disruption of the GnRH neuronal system following developmental exposure to vinclozolin. Supported by NIEHS ES013810.

In Vitro Capacitation of Frozen/thawed Stallion Spermatozoa

BE Spizziri, JK Graham, MR Hudson

Inability to effectively capacitate stallion sperm in vitro limits equine assisted reproductive technologies including gamete intrafallopian transfer and IVF, which will be necessary to maximize offspring using cryopreserved sperm from stallions with low cryosurvival or with very limited sperm numbers. Experiments were conducted to develop artificial capacitation methods for frozen/thawed stallion sperm, using dilauroylphosphatidylcholine (PC12), calcium ionophore A23187 (ION), or methyl- β -cyclodextrins (MBC). Capacitation status was confirmed by evaluating their ability to penetrate bovine oocytes. Frozen/thawed sperm were washed through Percoll to remove egg yolk particles and suspended in Medium 199 (0.6% BSA and 5mM CaCl) to 50 million sperm/mL. Thirty in vitro matured bovine oocytes per treatment were transferred to 0.1 mL droplets and fertilized with 250,000 sperm treated with PC12 (15, 20, 30, 40 μ M), ION (1, 1.5, 2, 2.5 μ M), or MBC (0.4, 0.6, 0.8, 1 μ M). Zygotes were cultured for 30h and evaluated for cleavage. Data were analyzed by Chi-Square Analysis. Sperm treated with capacitation agents produced higher cleavage rates than control sperm ($P < 0.05$). Overall cleavage rates among treatment levels were similar ($P > 0.05$). The PC12 induced significantly higher cleavage rates (39%, $P < 0.05$) than ION or MBC (30, 31%, respectively). In conclusion, frozen/thawed stallion sperm can be artificially capacitated with PC12, ION, or MBC treatment, and this may lead to practical in vitro equine assisted reproductive technologies.

The oncoprotein nucleophosmin is a component of a polyadenylation mark

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Nucleophosmin (NPM) is a shuttling, multifunctional nucleolar phosphoprotein that is frequently over-expressed in human cancer cells. Recent studies suggest that about one third of adult acute myeloid leukemias exhibit aberrant NPM expression in the cytoplasm. 50-60% of all AML cases with a normal karyotype contain a characteristic frameshift mutation in the NPM gene that blocks transport of the protein to the nucleus. NPM can act as a tumor promoter or tumor suppressor depending on its level in the cell. Previous work from our laboratory shows that NPM is deposited on the 3' UTR of mRNAs that have undergone 3' end processing by the nuclear polyadenylation machinery. NPM may play a key role in the post-transcriptional control of gene expression. Since 3' end formation is the last step in the production of an mRNA, we hypothesize that NPM may be a mark for a properly processed mRNA and perhaps assists in the quality control of gene expression. We are currently addressing two key questions. First, is NPM the only component of this polyadenylation or is a complex of proteins deposited on mRNAs in a manner similar to the Exon Junction Complex that is deposited on mRNAs after successful splicing? We have recently observed a ~14 kDa protein is also deposited on mRNAs following polyadenylation. We speculate that this protein could be the p15 mRNA export factor, or ARF - a 14kDa, tumor suppressor that is known to interact with NPM. Efforts are underway to purify and identify the 14 kDa protein. Second, shRNA-mediated knockdown of NPM, as well as the addition of a peptide that interacts with NPM, result in a significant hyperadenylation of RNAs in vitro. Hyperadenylation of mRNAs is often a signal for the nuclear quality control machinery that an mRNA is aberrant and should be destroyed. We are currently assessing the mechanism of regulation of poly(A) tail length by NPM and investigating its association with the oncogenic properties of this protein.

Airway Immune Responses to Inhaled Liposome-Based Immunotherapeutics

AR Troy, RM Troyer, SW Dow

Purpose: Previous studies have shown that inhalational immunotherapy with cationic liposome-DNA complexes (CLDC), potent activators of innate immunity, can induce protective immunity in mouse models of infection. However, in many cases the immunological mechanisms by which CLDC elicit protection in the lungs are not known. Therefore, we investigated innate immune responses in the airways to inhalation of CLDC and related immunotherapeutics. **Materials/methods:** Mice were anesthetized and 20 µl of CLDC were administered intranasally. At various time points post-administration, the mice were euthanized and the airways were lavaged (bronchoalveolar lavage, BAL) with PBS to obtain cell and cytokine samples. Cytokine concentrations in the BAL fluid were determined by ELISA. The cellular response in BAL fluid was assessed using flow cytometry. **Results:** We found that i.n. administration of CLDC elicited strong activation of innate immune responses in the airways of mice, including strong induction of IFN-γ, IL-12, CCL2, TNF, and IL-8 production, which persisted for up to 24h post-administration. In addition, administration of CLDC also elicited a unique inflammatory response in the airways of mice, including a pronounced infiltrate of inflammatory monocytes. This response was distinct from that observed following administration of other stimuli, including live attenuated bacteria. Additional experiments are planned to compare and contrast the immune response to CLDC to that elicited by other inflammatory stimuli. **Conclusions:** These results indicate that CLDC are very potent activators of pulmonary innate immunity following mucosal administration. Ongoing experiments suggest that local production of IFN-γ may be one of the key protective cytokines for inducing protection from bacterial infection. In addition, the ability of CLDC to attract inflammatory monocytes to the airways may also account for the strong protective activity of CLDC immunotherapy.

The Effects of Mannose Capped Lipoarabinomannan on Dendritic Cell Function

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Purpose: Mycobacterium tuberculosis (Mtb) derived mannose capped lipoarabinomannan (ManLam) is a major component of the bacterial cell wall. ManLam has been shown to have a negative effect on the function of dendritic cells (DCs), thus it is the purpose of this study to examine how ManLam affects DC function through IL-10, an anti-inflammatory cytokine, and nitric oxide (NO), an anti-microbial product. **Materials/Methods:** Bone marrow derived DCs were cultured for 8-10 days in cRPMI and 20ng/mL GM-CSF. Cells were treated with 1µg/mL Mtb purified ManLam, 20ng/mL LPS, or left untreated. DC phagocytic capacity was measured using 1µm fluorescent beads and CD4⁺ T cell stimulatory capacity was measured by mixed lymphocyte reaction. In addition cells were analyzed by immunohistochemistry and flow cytometry for IL-10 and NO production. **Results:** DCs treated with ManLam show reduced phagocytic capacity and an inability to stimulate CD4⁺ T cells in a mixed lymphocyte reaction, while LPS stimulated DCs are highly phagocytic and can stimulate CD4⁺ T cell proliferation. In response to ManLam, DCs show positive staining for intracellular IL-10, though only a small amount of IL-10 is secreted. ManLam treated DCs exhibit a short lived production of small amounts of NO. **Conclusions:** These experiments show that ManLam alters the ability of DCs to activate CD4⁺ T cells and to initiate a proper immune response. Even though IL-10 is not secreted by ManLam treated DCs, its intracellular production serves an autocrine function. IL-10, a major anti-inflammatory cytokine, serves to alter the functionality of DCs preventing proper activation and expression of stimulatory receptors on the cell surface.

Isolation of Primary Mammary Stem Cells Using a Modified Mammosphere Culture Technique: A Novel Approach to Identifying the True Stem Cell

RH Klingler, TS Magers, RL Ullrich.

The target cells for carcinogenesis are generally believed to be tissue stem cells, but that remains to be directly tested. Numbers of tissue stem cells are ill-defined, having been estimated using a variety of relatively indirect assays. A clear understanding of changes in numbers of stem cells as a function of age and the renewal characteristics of these cells are likely to significantly impact understanding of mechanisms of carcinogenesis and also provide insight into prevention strategies. Recently, multiple techniques have been developed to isolate a population enriched for mammary stem cells; however, this population also contains progenitor cells in various stages of differentiation. Progenitor cells exhibit many of the characteristics of true stem cells but lack the capacity to create a fully developed and functional mammary gland. To date, there are no techniques capable of specifically isolating mammary stem cells from the mammary gland. As a result, our ability to answer even the most basic questions about mammary stem cells is severely lacking. In response to this problem, we have used a modified mammospheres culture technique to develop novel methods of characterizing mammary stem cell growth, propagation and differentiation in vitro. We have developed criteria that allow us to identify primary mammospheres containing true stem cells by using a combination of growth characteristics and cell-specific immunofluorescence. The criteria created here will be used in future studies to examine the radiation response of mammary stem cells, as well as the role of stem cells in mammary cancer initiation.

Characterization of Lymphoblastoid Cell Lines from Breast Cancer Patients in a Radiation Technologist Cohort

AJ Williams, AJ Sigurdson, RL Ullrich, SM Bailey

Breast cancer is one of the most frequently diagnosed cancers and a leading cause of death among women today. Genetic factors contribute to breast cancer risk, however, most breast cancer cases are in fact sporadic - their underlying mechanisms and contributing elements being not well understood. We are using radiosensitivity, DNA repair deficiency and telomere instability as biomarkers to further understand the etiology of breast cancer. Telomeres, the physical ends of linear chromosomes, serve to protect these natural DNA ends from inappropriate damage response and repair. Shortening of telomeres has been linked with aging, as well as with increased radiosensitivity. Radiosensitivity can be evaluated using gamma-H2AX foci, phosphorylated histone variants that mark sites of DNA double-strand breaks (DSBs). Utilizing lymphoblastoid cell lines (LCLs) established from human breast cancer patients and controls, all of who are radiation technologists, we investigated gamma-H2AX foci formation following radiation exposure. We also determined relative telomere length in each individual cell line. Evaluating lymphoblasts from patients' peripheral blood, a non-invasive and cost-effective procedure, may provide valuable mechanistic and prognostic information. In addition to shedding light on what roles telomere instability and radiation sensitivity play in breast cancer occurrence, these studies will also help better define the contribution of occupational doses of radiation in breast carcinogenesis.

MicroRNAs in Gonad Development

KJ Torley, RV Anthony, GJ Bouma

Approximately one in every one thousand human births is diagnosed with some sort of gonadal or genital defect. The underlying causes of these developmental defects are often unknown. Proper gene expression and function is critical in fetal gonad formation and differentiation. MicroRNAs (miRNAs) have been shown to regulate gene expression in tissues throughout the body and most likely have a role in mammalian gonadal development. MiRNAs are small non-coding RNAs, ~22 nucleotides in length. Through modification by Drosha and Dicer and assembly within a ribonucleoprotein complex called RNA-induced silencing complex (RISC), miRNAs regulate gene expression and function at the post-transcriptional level. It is the hypothesis of this study that miRNAs are expressed in mammalian fetal ovaries and testes, and regulate gene expression. Expression profiling of 128 selected miRNAs was conducted using real time PCR. A total of 47 miRNAs were identified as significant differentially expressed between fetal ovaries and testes. Using a bioinformatics approach, a summation of the predicted targets for these miRNAs yielded over 11,000 possible target genes, including known sex determining genes. Furthermore, new miRNAs are identified by cloning of miRNA from mammalian fetal gonads collected at specific time points during development. Results from this study provide important new insight into the complex process of fetal gonadal development and its regulation by miRNAs.

Analysis of a putative *M. leprae* lipoprotein; LpqE

RA Al-Mubarak, VD Vissa, R Sakamuri.

The causative agent of leprosy is *Mycobacterium leprae*, a pathogen which has not been cultivable in vitro or amenable to genetic manipulation. The genome sequence of *M. leprae* is available and through in silico comparative genomics; allows for the identification of various gene families. Our study is focused on lipoproteins which are implicated in immunity and infection in leprosy and other bacterial infections. A typical bacterial lipoprotein is characterized with an N-terminal signal sequence with a lipobox motif. This motif is followed by a conserved cysteine residue to which the lipid moieties get attached. The genome of *M. leprae* encodes 31 putative lipoproteins; while that of *M. tuberculosis* which causes tuberculosis has 99 genes. The goal of this study is to experimentally verify that the putative lipoprotein signatures of *M. leprae* proteins are functionally active and to characterize lipid modifications. To this end, we have used cloning, expression and fusion protein purification techniques. The first gene we selected is LpqE (ML0319), which has not been studied in any mycobacteria. The N-terminal signal of LpqE was fused to the C-terminus of LprG, a known lipoprotein of *M. tuberculosis*. A C-terminal six histidine fusion tag was engineered to enable protein purification. The fusion protein was expressed in *M. smegmatis*, a fast growing avirulent mycobacterial host system. The full length *M. tuberculosis* LprG was also studied in parallel. The two recombinant proteins were subjected to biochemical analyses. Lipid analysis using GC/MS and mass-spectroscopy confirmed that the proteins were lipidated with stearic (C18) and tuberculostearic (C19) fatty acids. These approaches are therefore suitable for further study of lipid modifications of *M. leprae* proteins. Further experiments are in progress for the detection and comparison of lipid modifications and to provide tools for investigations of their biological significance.

Analysis of the lysine depressed regulatory regions of Escherichia coli and Mycobacterium

W Li, VD Vissa

In order to elucidate the regulation mechanism of 5'-UTR of aspartokinase gene and construct genetic tools for studying functions of Mycobacteria genes in Mycobacteria, a series of Escherichia coli-mycobacteria shuttle vectors were constructed. These vectors contain LacZ or His-tag fused ML2649 as reporter genes and assessed 5'-UTR sequence from Escherichia coli, M. tuberculosis or M. leprae fusions to LacZ or ML2649. β -galactosidase activity was measured. The expression of ML2649 in mycobacteria smegmatis was tested by Western blots and real-time PCR results. From a previous study, 5'-UTR of lysC gene of Bacillus subtilis carries a conserved RNA element that serves as a lysine-responsive riboswitch. Our results show aspartokinase gene promoters from these three species can initiate genes expression in mycobacterium, which are repressed by 20mM lysine. We can map a significant secondary structure of lysine riboswitches in 5'UTR of aspartokinase gene from E.coli but not from M. tuberculosis and M. leprae, although these two 5'UTR sequences also have responses to the repressed regulation of lysine. Western blots and real-time PCR results showed this regulation happened after the transcription level. These series vectors can be used as genetic tools for expression and studying functions of Mycobacteria genes.

Perceived hypoxia in Mycobacterium tuberculosis infection and the role of mycobactin

C Miller, D Ackart, A Tolnay, H Bielefeldt-Ohmann, I Orme, R Basaraba

Purpose: Mycobacterium tuberculosis, the etiologic agent of tuberculosis, requires iron to infect and replicate within host macrophages. The bacterium utilizes a virulence factor called mycobactin that allows it to scavenge, or chelate host iron. Desferrioxamine, a known chemical iron chelator, has been shown in vitro to induce hypoxia-inducible factor (HIF-1), a transcription factor that regulates transcription of hypoxia-inducible genes. HIF-1 normally functions as an oxygen sensor that responds to hypoxic conditions, however, it can also be expressed by non-hypoxic mechanisms such as iron chelation. The aim of this study was to compare the ability of mycobactin and desferrioxamine to induce HIF-1 stabilization and function through iron chelation. **Methods:** An in vitro study was conducted employing a human myelomonocytic cell line (THP-1) and guinea pig alveolar macrophages. Cells were treated with mycobactin or desferrioxamine and analyzed for HIF-1 and hemoxygenase-1 (HO-1) expression by immunohistochemistry (IHC) and Western blot. **Results:** IHC revealed nuclear staining for both HIF-1 α and HO-1 in cells treated with mycobactin or desferrioxamine. Western blot of extracted nuclear proteins confirmed expected bands of 93kDa and 43 kDa when stained for HIF-1 α and HO-1, respectively. Elevated levels of IL-8 were found in supernatants from cultures of mycobactin or desferrioxamine treated cells. **Conclusions:** Mycobactin induces accumulation of cytoplasmic HIF-1 α in a dose-dependent manner, followed by its translocation to the nucleus. Furthermore, HIF-1 α upregulation resulted in a significant amount of HO-1 translocation to the nucleus, as well as production of a pro-inflammatory cytokine, IL-8. These results are consistent with the role of mycobactin and HIF-1 expression contributing to "perceived" hypoxia by macrophages during M. tuberculosis infection.

Differential gene expression in canine osteosarcoma predicts tumor aggressiveness

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Osteosarcoma (OSA) is the most common bone tumor in dogs and roughly 80% of OSA tumors arise in the appendicular skeleton of large and giant breed dogs. The majority of patients experience rapid lung metastasis following tumor removal and chemotherapy while a minority remains disease-free for six months or longer. The ability to identify gene markers of tumor aggressiveness and drug resistance at the time of limb amputation would increase prognostic accuracy and would allow for a tailored chemotherapeutic approach. Thus, we analyzed gene expression in archived OSA tissue from dogs that had undergone treatment with limb amputation followed by doxorubicin or platinum-based drug chemotherapy. Tumor samples were archived at the time of limb amputation and were divided into two groups: dogs which had a disease free interval (DFI) of less than 90 days (n=3) and those with a DFI greater than 300 days (n=3). Gene expression was screened with Affymetrix Canine 2.0 genome chips and analyzed with a two-sample t-test with a false discovery rate correction for multiple comparisons following Probe Logarithmic Intensity Error (PLIER) estimation with a log₂ transformation. This analysis identified two genes with statistically relevant changes in expression between the groups. We also performed an otology analysis to identify genes that were up- or downregulated more than 3-fold. We observed 3- fold changes in approximately 800 genes including: upregulation of the Wnt/beta-catenin pathway, IGF-II mRNA binding protein (IMP-1, an mRNA stabilizing factor), Interleukin-8 and others in the DFI300 group. These findings suggest that gene expression has a strong predictive value for metastatic potential in OSA tumors. Furthermore, identification of deranged pathways and subsequent chemotherapeutic targeting of key elements may improve treatment success.

Analysis of the Potential for Cross-Species Transmission of Chronic Wasting Disease by Protein Misfolding Cyclic Amplification

TD Kurt, MD Zabel, EA Hoover.

Purpose: Chronic Wasting Disease (CWD) of deer is associated with conversion of the normal prion protein, PrPC, to a misfolded isomer termed PrPCWD (or abnormal prion protein). Whether non-cervid species may become naturally infected is not known. To predict species susceptibility to CWD we are using an in vitro PrPC-to-PrPCWD conversion assay, termed protein misfolding cyclic amplification (PMCA) to assess whether CWD prions can be propagated in non-cervid species brain. The PMCA methodology has three potential impacts: (1) replacement of expensive and long term in vivo studies, (2) elucidation of mechanisms of prion propagation and (3) identification of potential non-cervid reservoirs for CWD infection. Methods: As sources of PrPC, normal brain homogenates (NBH) (10% w/v) were prepared from multiple species. Each NBH was then spiked with brain from a CWD-positive deer and incubated at 37C for 48 hrs in a cup-horn sonicator with pulses delivered every 30 min (to fragment PrPCWD fibrils, creating seeds for conversion). Any remaining PrPC was degraded with proteinase K; newly formed protease-resistant PrPCWD was detected by immunoblotting. To correlate in vitro findings with susceptibility in vivo, we have conducted in vivo bioassays in a subset of these non-cervid species. Results: Using PMCA, we show that NBH from several species including ferrets, prairie voles, guinea pigs and transgenic mice expressing cervid PrPC can propagate PrPCWD in vitro. Several of these species are also susceptible to CWD in vivo. Species that do not support PrPCWD propagation by PMCA (e.g. cats, hamsters) have been resistant to CWD in vivo. Other species (e.g. crows, coyotes, sheep and non-human primates) are currently under investigation. Conclusion: Using PMCA, we have demonstrated the conversion of PrPC from several non-cervid species to PrPCWD. These results offer the opportunity to predict CWD-susceptibility in vitro--something not previously possible in prion biology.

Exploring the Link between Infection and Tumor Inhibition

JL Sottnik, LW U'Ren, DH Thamm, SW Dow.

Purpose: Osteosarcoma (OS) is the most prevalent bone tumor in humans and dogs, and the high metastatic rate of these tumors leads to high morbidity. Limb salvage, through the use of cortical allografts, has helped patients with OS retain their limbs; however, metastases remain the primary cause of death. It was discovered that patients developing a bone infection at their surgical site lived twice as long, and their metastases took twice as long to develop. The goal of this project is to develop a mouse model of bacterial osteomyelitis that can be used to probe the mechanisms underlying these observations. **Materials/Methods:** C3H mice were infected with a strain of *S. aureus* transfected to express luciferase. Bacteria were grown as biofilms on suture segments before being implanted into the intermedullary cavity of the tibia 3 days before challenge with a syngeneic OS cell line, DLM8. Serial imaging of the infection, and serial tumor measurements were used to track the extent of disease. Mice were sacrificed and terminally bled when their tumors reached 1 cm maximal diameter. Natural killer (NK) cell depletion with anti-asialo GM1 was also performed in a separate group of mice to elucidate the mechanism of tumor growth inhibition. Immunohistochemistry (IHC) and flow cytometry were used to elucidate a mechanism of action. **Results:** Mice bearing osteomyelitis had a significantly increased survival over mice undergoing a sham operation with sterile suture implanted. Depletion of NK cells led to blocking of the infection mediated growth inhibition. IHC and flow cytometry results are pending. **Conclusions:** Mice with localized bone infections had a significant tumor growth delay, and increased survival compared to non-infected mice; closely resembling clinical observations. NK depletion led to inhibition of the antitumor effects of osteomyelitis, and will be studied further to dissect specific mechanisms of action.

Detection of Prion Infectivity in Saliva and Urine from CWD+ Deer Using a New Transgenic Mouse Bioassay.

NJ Haley, EA Hoover

Chronic Wasting Disease (CWD) is a naturally occurring transmissible spongiform encephalopathy (TSE) of cervids. Transmissible spongiform encephalopathies are characterized by the accumulation of protease resistant prion protein (PrPres) generated by conversion of the host's normal cellular prion protein (PrPc) by an infectious prion "seed". In CWD this infectious "seed" appears to be transmitted via bodily fluids.¹ Here we report use of a transgenic mouse expressing the cervid PrP gene (Tg[CerPrP] mice), as an alternative to live cervids to bioassay a variety of excreta (saliva, urine, or feces) that may play a role in the transmission of CWD between susceptible species. Because the amount of infectious prion protein in bodily fluids is beyond the level of detection in traditional assays, such as western blot and ELISA, it was necessary to develop a strategy for concentrating potential infectivity from bodily fluids for mouse bioassay. Saliva and urine samples from deer in the terminal stages of CWD infection were first lyophilized, then reconstituted and dialyzed to neutral pH, prior to intracranial inoculation into Tg(CerPrP) mice. The recipient mice have been observed for up to 400 days, during which time 4/9 saliva-inoculated mice and 2/9 urine-inoculated mice have developed neurologic signs consistent with clinical TSE, including unexplained pruritis, a lumbering, hunched gait, torticollis, circling, listlessness, and general unthriftiness. Remaining mice are under continued surveillance. Definitive diagnosis of TSE was established in these mice by western blot and immunohistochemical assays demonstrating PrPres. These results confirm in a more facile bioassay the presence of infectious prions in the saliva of CWD-infected deer, and demonstrated for the first time infectious prions in urine, thus expanding the proven routes of prion shedding and transmission of CWD.

1. Mathiason CK, Powers JG, Dahmes SJ et al. (2006) *Science*, 314(5796):133-6.

New insights from enhanced immunohistochemical detection of PrPres in a transgenic mouse model of Chronic Wasting Disease.

DM Seelig, GL Mason, GC Telling, and EA Hoover

Chronic Wasting Disease (CWD) is a naturally occurring prion disease of cervids. In this, and other prion diseases, the normal prion protein isoform (PrP^c) is transformed to the disease-associated isoform (PrP^{Sc}), which accumulates in nervous- and non-nervous tissues. We are utilizing cervid prion protein (CerPrP) expressing transgenic (Tg[CerPrP]) mice to determine the tissue tropism and pathogenesis of CWD. Here we describe a modified immunohistochemical (IHC) technique, which provides enhanced sensitivity in the detection of PrP^{Sc} in CWD-infected transgenic mice. In previous work with this model system, we were able to detect PrP^{Sc} by IHC only in the brains of intracerebrally- and intraperitoneally-inoculated mice. We were not able to convincingly detect PrP^{Sc} in intravenously (IV)-inoculated mice or in non-nervous tissues from any animal. To enhance the sensitivity of our IHC, we have altered our protocol in the following two ways: (1) substitution of an HRP-conjugated primary antibody (BAR 224) for the previous un-conjugated anti-PrP antibody (R505.5), and (2) incorporation of tyramide-based signal amplification (TSA). This methodology now allows us to detect PrP^{Sc} in the brains of mice inoculated by all routes of exposure (including IV) and to detect PrP^{Sc} in non-nervous tissues, including pancreas, salivary gland, and bone marrow. Detection of PrP^{Sc} in the salivary gland provides the first histologic basis for the presence of infectious prions in saliva of infected deer (see abstracts of Mathiason and Haley). Detection of PrP^{Sc} in bone marrow may provide a basis for previously reported prionemia and non-neural prion trafficking to the nervous system.

Activation of Innate Immunity Inhibits Francisella Infection of Alveolar Macrophages

K Propst-Graham, R Troyer, H Schweizer, S Dow

Purpose: Cationic liposome-DNA complexes (CLDC) are potent stimulators of Th1-type innate immune response. Our lab has previously shown that intranasal administration of CLDC elicits significant protection of mice from *Francisella tularensis* (Ft) infection. However, the precise mechanism(s) for this effect remains incompletely defined. The purpose of this research was to develop an *in vitro* model to investigate the mechanisms by which CLDC immunotherapy may affect infection of the alveolar macrophage by Ft. **Materials and Methods:** We used the alveolar macrophage cell line (AMJ) and Ft LVS strain. Spleen supernatants were generated from mice stimulated by CLDC. AMJ cells were then pre-treated with the supernatants to assess the effects this treatment had on Ft intracellular replication. The macrophages were infected with Ft and bacterial replication was assessed. Macrophage uptake of LVS was also examined at early time points by flow cytometry. **Results:** Ft readily infected AMJ cells and replicated intracellularly. Pre-treatment of AMJ cells with spleen supernatants from mice given CLDC suppressed LVS intracellular replication and increased bacterial killing by greater than 4 logs. Recent *in vivo* experiments suggest that activation of the lungs by CLDC induces high levels of TNF, IFN- γ , and nitric oxide formation, which may enhance antibacterial defense. For example, we demonstrated the protective effects of CLDC treatment were completely eliminated in mice lacking a functional IFN- γ gene. *In vitro* neutralization experiments are being conducted to assess the relative role of these mechanisms. We also found that pre-treatment of AMJ cells with CLDC elicited supernatants partially suppressed macrophage uptake of Ft. **Conclusions:** Cytokines elicited by CLDC treatment appear to be potent inhibitors of Ft replication in alveolar macrophages. The suppressive effects appear to be mediated by IFN- γ and possibly other pro-inflammatory cytokines.

Pharmacokinetics of ZD1839 (gefitinib, Iressa) in adult and geriatric mice.

RJ Hansen, BJ Samber, DL Gustafson

Some drugs, including chemotherapy, are dose reduced for certain protocols when used in the elderly to reduce the incidence of unwanted side effects; however, there is no evidence that the susceptibility of colon cancer differs in younger and older patients. Therefore, attempts should be made to modify the dosing of chemotherapy to decrease toxicity without compromising efficacy as it's related to identifiable pharmacokinetic (PK) and pharmacodynamic differences in various populations. To this end, a model representing an aged population for use in pre-clinical studies could prove beneficial. Purpose: Determine if adult (10 mo) and aged (18 mo) mice show differences in the PK of ZD1839 (gefitinib, Iressa), an EGFR inhibitor in clinical trials for colon cancer. Methods: The PK of ZD1839 (20, 40 or 60 mg/kg p.o.) was determined in adult and geriatric mice. Plasma and tissues were collected 0.25, 0.5, 1, 2, 4, 8, 24 and 48 hrs post dose with feces collected from mice sacrificed at 8, 24 and 48 hrs. Results: Area under the curve (AUC) and peak plasma levels (Cmax) increased in a dose dependant fashion in both age groups. Both plasma AUC and Cmax were higher and Tmax was later in geriatric mice compared to adult mice at all doses tested. Decreased clearance (CL) was also observed in aged mice. Consistent with decreased CL, less ZD1839 was measured in the feces of geriatric mice. Similar metabolic profiles were observed in the feces between age groups, but lower levels of the metabolites were measured in geriatric mice, possibly a result of reduced metabolism. Conclusion: The use of aged mice (18 mo) reasonably modeled a geriatric population dosed with ZD1839 and may also be useful for studying PK differences between adult and geriatric populations for other drugs alone or in combination.

Sodium Valproate Enhances Doxorubicin Sensitivity in Osteosarcoma Cells

LA Wittenburg, L Bisson, BJ Rose, D Thamm

Osteosarcoma (OS) remains an incurable and ultimately fatal disease in many patients, and novel forms of therapy are needed. The histone deacetylase (HDAC) enzymes are powerful new targets for epigenetic cancer therapy. The acetylation of histones, controlled by multiple histone acetyltransferases and HDACs, is important in governing chromatin structure and thereby can modulate expression of genes associated with cellular proliferation, differentiation and survival. Pharmacologic inhibition of HDAC has been shown to have multiple anti-tumor effects. One available drug with HDAC inhibitory activity is the anticonvulsant sodium valproate (VPA). We hypothesized that VPA can be used to inhibit HDAC and potentiate the anti-tumor effects of doxorubicin (DOX) in OS cells in vitro and in vivo. Canine and human OS cell lines were incubated with and without VPA alone and in combination with DOX, and antiproliferative effects were evaluated using bioreductive assays. Apoptotic effects were evaluated using TUNEL assay. Immunofluorescence cytochemistry and western analysis were used to evaluate changes in histone H3 acetylation. In vivo efficacy was evaluated in a canine OS xenograft model in athymic mice. Treatment with VPA resulted in significant increases in acetylated histone H3. Incubation with VPA alone had modest antiproliferative effects, while pre-incubation with VPA followed by DOX exposure resulted in significant chemosensitization. Exposure to VPA also resulted in increased nuclear DOX accumulation and potentiated DOX-induced apoptosis. Mice receiving combination therapy had statistically significant reductions in tumor growth and improvements in overall survival compared to mice receiving either agent alone. In conclusion, pretreatment of canine and human OS cells with clinically relevant doses of VPA results in histone hyperacetylation, enhanced nuclear DOX accumulation, and sensitization of these cells to the antiproliferative and proapoptotic effects of DOX.

Evaluation of Cytotoxic Effects of Pokeweed Antiviral Toxin and Diphtheria Toxin In Chinese Hamster Ovarian Cells

S.L. Fuchs, M.C. Allen, E.R. Weber and T.M. Nett

The ligand-receptor signaling system can be used to deliver toxins to cells expressing a specific receptor to cause cell death. One example is the GnRH-PAP conjugated ligand that targets gonadotropes in the anterior pituitary to eliminate gonadotropin secretion and achieve chemical castration. This toxin-conjugated ligand may also be used as a chemotherapeutic agent against certain types of cancer cells expressing receptors for a specific ligand. However, two toxins that interfere with different steps of the protein synthesis pathway when used simultaneously may yield a synergistic effect that permits an overall reduction in therapeutic dose of each toxin. We are conducting a clonogenic assay to test the hypothesis that two toxins that disrupt protein synthesis by different mechanisms in Chinese hamster ovarian (CHO) cells; pokeweed antiviral protein (PAP), a ribosome inhibiting protein from *Phytolacca americana*, and diphtheria toxin (DT), will have a synergistic effect and be much more toxic than either alone. CHO cells were plated in six-well plates, established for 2 days, and treated with PAP, DT and a combination of the two toxins at varying concentrations. On the sixth day following treatment, the living cells that adhered to well bottoms were fixed and stained. Each well was then individually photographed with a digital camera. Digital photographs were then calibrated and analyzed using the Image Pro Plus program to measure the number and size of colonies. Data from each replicate was then graphed and reviewed to evaluate whether CHO cell cytotoxic response to PAP and DT in combination was additive or synergistic.

Rates of proliferation of human melanoma may predict sensitivity to small molecule MEK inhibition

CL Denton, DL Gustafson.

Purpose: Melanoma is the most common fatal skin cancer worldwide. Regarding malignant melanoma cell lines and the crucial Ras-Raf-MEK-ERK signaling cascade, approximately 60% harbor activating mutations in the BRAF gene. Therefore, inhibitors of this pathway may prove to be valuable treatment options in melanoma therapy. AR389 (Array Biopharma, Boulder, CO) and AZD6244 (ARRY-142886; AstraZeneca, Macclesfield, United Kingdom) are potent, selective, noncompetitive MEK1/2 inhibitors. Binding of this class of molecules to the MEK protein-ATP complex prevents downstream phosphorylation of ERK1/2 and thus impedes MAPK signaling. To better understand as well as employ these agents in the clinic, cellular and molecular characterization of small molecule MEK inhibitors is absolutely imperative. **Materials/methods:** The effects pharmacological MEK inhibition on a panel of six human melanoma cell lines with varying BRAF and NRAS mutations were characterized. Methods used include proliferation assays, effects on MAP kinase pathway signaling, cell cycle, clonogenic potential and apoptosis and finally effects on cell growth kinetics. **Results:** We demonstrate that AR389 and AZD6244 inhibit phosphorylation of ERK1/2 and induce a profound and sustained cell cycle arrest in G1, with a substantial decrease in % cells in S-phase, and a corresponding decrease in proliferation of all cell lines tested. However, AR389 did not increase apoptosis nor decrease cellular clonogenic potential, indicating that the main therapeutic effect of these drugs is to inhibit cell cycle and proliferation. **Conclusions:** Sensitivity to small molecule MEK inhibitors can be closely correlated with potential doubling time ($r^2=0.7667$) in human melanoma cell lines. Additionally, fraction of cells in S-phase decreases linearly with dose in MEK inhibitor treatment groups. We hypothesize that sensitivity to MEK inhibition may be predicted based on cellular growth rates (T_{pot}) and fraction of cells in S-phase.

Early innate immune response to Burkholderia mallei infection

A Jones, A Goodyear, R Troyer, S Dow

Purpose: Burkholderia mallei is an intracellular bacterium that can cause either severe acute pneumonic disease or chronic disseminated disease. Without antibiotic treatment the disease is nearly 100% fatal. B. mallei is a category B select agent and was used previously as a bioweapon. The aim of this study was to analyze the early immunologic events following aerosol B. mallei infection. **Materials/ Methods:** Mice were infected intranasally with high or low doses of B mallei. Mice were sacrificed serially post-infection. Lung, liver, spleen and bronchial alveolar lavage (BAL) fluid were analyzed for bacterial colony counts. Serum and BAL fluid were analyzed for cytokine production by ELISA, and cells were stained and analyzed by flow cytometry. **Results:** Mice infected with a high dose of B. mallei show high levels of bacteria in the lungs with no dissemination to liver and spleen until 24 hours. Serum samples contained significant amounts of CCL2 beginning at 24 hours. Mice infected with a low dose of B. mallei showed bacterial replication in lung and BAL but minimal dissemination to liver and spleen over a 72 hour period. Based on the strong CCL2 response observed, we next examined and compared the kinetics and immune response between Wt and CCL2 -/- mice. CCL2 -/- mice died significantly earlier than WT mice following a low-dose intranasal challenge and developed significantly higher bacterial burdens in all organs examined. Significant differences in nitrite, IFN-gamma, TNF-alpha and IL-8 levels were observed in the BAL fluid between Wt and CCL2 -/- mice. **Conclusions:** Our data indicate that B. mallei aerosol infection induces strong production of several cytokines, particularly CCL2, IFN-gamma and nitric oxide. Our results also suggest that CCL2 and recruitment of monocytes plays a significant role in B. mallei infection and protective immunity.

Expression and activity of indoleamine 2,3 dioxygenase in feline bone marrow derived dendritic cells

KP O'Halloran, SA Fallon, TL Lehman, PR Avery

Background: Dendritic cells (DC) play a crucial role in the regulation of cell mediated immune responses. Indoleamine 2,3 dioxygenase (IDO) expression is induced in DC during a wide variety of diseases. IDO catalyzes the degradation of the essential amino acid tryptophan (TRP) via the kynurenine (KYN) pathway. The local depletion of TRP and accumulation of TRP catabolites results in immunosuppression. **Purpose:** The present study examines the effects of feline immunodeficiency virus (FIV) on levels of IDO expression and activity in cats. **Materials/methods:** Cultured DC from naïve and FIV-infected cats were incubated for 48 hours with or without maturation cocktail. The cells were then washed and incubated with TRP for 4 hours. Expression of IDO mRNA was quantified using real time PCR. IDO enzymatic activity was determined indirectly by colorimetric measurement of KYN concentration. TRP and KYN levels were measured by high performance liquid chromatography in serum collected from cats pre- and post-infection. **Results:** IDO mRNA expression and IDO activity were increased in DC from FIV-infected cats compared to naïve DC. No difference was found in serum TRP concentrations from naïve and infected cats. However, serum KYN concentrations were significantly increased and the KYN/TRP ratio was significantly decreased as early as 3 weeks post-FIV infection. **Conclusions:** These results demonstrate that FIV infection leads to a marked increase in IDO expression and activity by feline DC. The elevated levels of KYN in the serum of FIV-infected cats indicate increased TRP metabolism. Comparable observations have been made in HIV infection. Future studies will use the FIV model to investigate the significance of IDO in immunosuppressive mechanisms of chronic lentiviral infection using inhibitors such as 1-methyl-TRP.

Effects of sand fly salivary component maxadilan on murine dendritic cell migration

KE Pauken, WH Wheat, RG Titus

Vector-borne diseases such as leishmaniasis are a significant world health issue. The etiological agents of leishmaniasis are protozoan parasites of the genus *Leishmania*, which are transmitted by phlebotomine sand flies. Successful transmission of parasites such as *L. major* from arthropods to mammalian hosts require vector salivary components that modulate host immune responses ensuring parasitic entry, survival and resulting pathogenesis. One such component is the peptide maxadilan (MAX) derived from sand fly (*L. longipalpis*) saliva. Evidence has emerged showing that MAX alters several aspects of murine dendritic cells (DCs). Migration of antigen-primed DCs from the site of vector-mediated parasite inoculation to secondary lymphoid organs is essential to initiation of an adaptive immune response. We sought to determine whether MAX modulates anti-leishmanial immunity by impeding the course of epithelial DC migration to draining lymph nodes. This work has shown that MAX treatment of LPS-stimulated murine bone-marrow derived DCs prevents optimal up-regulation of the chemokine receptor CCR7. We hypothesize that phenotypically altered DCs fail to adequately mobilize to T cell areas of draining lymph nodes, stalling the initiation of adaptive immune responses against leishmanial antigens. Transwell tissue culture chambers were utilized to simulate migration in vitro: DC migration was facilitated through the membrane by supplementation with CCR7 chemokine ligands CCL19 and CCL21. Using this approach, we show that MAX treatment resulted in significantly reduced numbers of migrating cells, suggesting that reduced surface expression of CCR7 correlates with decreased migration. Comparable results were obtained using DCs derived from either an *L. major*-resistant (C3H) or _susceptible (BALB/c) murine strain. These data suggest that MAX modulates migration from the epithelium to the secondary lymphoid organs, a critical step in successfully eliminating *L. major* parasites.

Poster Presentations

Session II

4:00 - 5:30PM

CLINICAL SCIENCE

XCL1-targeting siRNA intratracheal delivery therapy in mice challenged with Mycobacterium tuberculosis had decreased numbers of CD4 and CD8 T cells and IFN-gamma production and presented changes in the granuloma's structure.

AG Rosas-Taraco, D Higgins, J Sanchez, E Lee, I Orme, M Gonzalez-Juarrero

Purpose: XCL1 is an important chemokine involved in T-cell, B-cell and NK cell migration. It is known that T lymphocytes, found in association with the tuberculoid granuloma, are essential in the containment of bacterial growth during an M. tuberculosis pulmonary infection. Previously, we demonstrated that XCL1 was produced by activated CD8 T cells and participated in the immunity against M. tuberculosis. The aim of this work was to elucidate the role of XCL1 in chronic M. tuberculosis infection. Materials and Methods: Mice were challenged with a low dose aerosol infection of Mycobacterium tuberculosis, and after sixty days post-challenge the mice were treated by intrapulmonary delivery with PBS, unspecific-siRNA, or XCL1-targeting siRNA. Results: Therapy resulted in a 48-55 % reduction in XCL1 protein expression after 3 and 5 days post-therapy. At five days post therapy with XCL1-targeting siRNA, the total numbers of CD4 and CD8 positive T cells, as well as IFN-gamma-producing CD4 and CD8 T cells in the lungs were reduced when compared to the control groups. Likewise, total IFN-gamma production in these mice was also decreased 33-42% when compared to controls. The histological findings indicated that mice treated with XCL1-targeting siRNA had lower lymphocytic infiltration and increased fibrosis which resulted in disrupted tuberculoid granulomas. Conclusions: XCL1 is an important chemoattractant factor for lymphocytes involved in the M. tuberculosis growth control and granuloma maintenance in chronic tuberculosis.

Oxidative Stress and Phagocytic Cell Function in Cats with Type 2 Diabetes Mellitus Compared to Controls: Assessing the Impact of Nutrition.

LB Falkowski, CB Webb.

Purpose: The purpose of this study was to test the hypothesis that oxidative stress is increased and neutrophil function is decreased in cats with Type 2 Diabetes Mellitus (DM), and both will improve with consumption of a diabetes-specific diet. Material and Methods: Body weight, serum fructosamine level, complete blood count, and parameters of oxidative stress and phagocytic cell (PC) function were measured in 15 cats with DM and 20 control cats. Erythrocyte superoxide dismutase (SOD), blood glutathione peroxidase (GPx), malondialdehyde (MDA), and reduced glutathione (GSH):oxidized glutathione (GSSH) ratio were evaluated spectrophotometrically. PC phagocytosis and subsequent respiratory burst activity were evaluated using flow cytometry. Cats were then fed a commercially available diet designed for feline diabetics (Purina Veterinary Diets® DM®) for 8 weeks, after which all assays were repeated. Results were compared both between groups and within groups over time. Results: The diabetic cats were significantly older and heavier than the controls. Cats with DM had significantly less plasma SOD than controls. Other oxidative stress parameters and PC function measures were not significantly different between the two groups. Following 8 weeks of consuming Purina DM®, the control group gained a significant amount of weight, the mean fructosamine value was significantly decreased in both groups, and GPx increased significantly in both groups. PC function did not change significantly in either group. Conclusions: Type 2 Diabetes Mellitus may be associated with an increase in oxidative stress in cats. Diet appears to significantly impact some parameters of oxidative stress, and antioxidant dietary supplementation may be targeted towards specific deficits. A diabetes-specific diet may be beneficial in non-diabetic cats prone to developing the disease, but the potential for weight gain needs to be considered.

Efficacy of medroxyprogesterone acetate in estrus suppression of cycling mares

EK Gee, PM McCue, CA DeLuca, JL Stylski

Abstract: character count - 1938 Purpose: To evaluate the effects of medroxyprogesterone acetate (MPA) on follicular activity and estrous behavior in mares. Materials and methods: Eighteen cycling Quarter Horse-type mares were randomly assigned to one of three treatment groups: MPA, saline or altrenogest (Regumate). Treatments began on day 7 post-ovulation. Mares in the MPA treatment group (n=6) were injected intramuscularly (IM) with 1600mg MPA (Wedgewood Pharmacy) initially, then 400mg weekly for 5 weeks. Saline treatment mares (n=6) were injected IM with saline weekly for 6 weeks. Altrenogest treatment mares (n=6) received 10ml altrenogest orally each day for 48 days. For the treatment period and 18 days after treatment ceased, mares were teased daily and categorized as displaying behavioral estrus, diestrus or anestrus. Transrectal ultrasounds were performed three times weekly, or daily when a 35mm follicle was identified until ovulation. Blood samples were harvested weekly and serum frozen for analysis of progesterone concentration. Assessors of estrous behavior and follicular activity were blinded to the treatment each mare received. Results: Mares treated with saline or MPA showed normal intervals of behavioral diestrus and estrus (14 and 8 days, respectively) for the duration of the study. All altrenogest treated mares showed signs of behavioral diestrus during the treatment period and 4 resumed behavioral estrus 6-18 days after cessation of treatment. All mares in the saline and MPA treatment groups showed normal follicular development and ovulations for the duration of the study. No mares in the altrenogest treatment group ovulated during the treatment period; 4 of the 6 mares resumed normal follicular development after treatment ceased. Progesterone analyses showed agreement with the transrectal ultrasonographic ovarian activity for all mares. Conclusion: MPA is not effective in suppression of estrus in normal cycling mares.

The Association Between Basophilia and Mast Cell Neoplasia in a Retrospective Case Study of 488 Dogs

KK Manning, AA Bohn

Basophilia in association with mast cell neoplasia has been reported in humans, but the association is inadequately documented in veterinary patients. While canine basophilia is not as rare as sometimes described, it is poorly characterized and often overlooked. This research examined the incidences of different diseases associated with basophilia in canines, in particular the incidence of mast cell neoplasia. Data was compiled and analyzed from a total of 488 canine patients with an absolute basophil count greater than 100/uL and/or a basophil percentage greater than 1% that presented to the Veterinary Medical Center between January 2006 and August 2007. The data showed a wide variety of causes of basophilia, with neoplasia accounting for 57.8% of cases, inflammatory causes accounting for 21.9% of cases, and 20.3% of cases attributed to multiple disease processes or other etiologies. There were a total of 68 cases of mast cell neoplasia (13.9%); however, basophilia was also seen in comparable numbers of other types of neoplasia. Chemotherapy as a causative agent of basophilia was also explored, and no association was demonstrated. Surprisingly, there were very few cases of parasitism resulting in basophilia, but this may be due to a lack of fecal screening tests on this population of patients. Overall, these findings contribute to the sparse information available about basophilia in canine patients, and suggest that all types of neoplasia, not just mast cell neoplasia, may be associated with basophilia.

Association between Feline Pancreatic Lipase Immunoreactivity Concentration and the Presence of Serum Antibodies against *Toxoplasma gondii* and *Bartonella* species

DB Bayliss, MM Brewer, AK Morris, JR Hawley, S Radecki, JM Steiner, MR Lappin

Background: Feline pancreatitis is commonly diagnosed; however the cause is often unknown. It has been proposed that some cases may be caused by infection with *Toxoplasma gondii* or *Bartonella* species. Feline pancreatic lipase immunoreactivity (fPLI) is a sensitive, specific, non-invasive test for pancreatitis. Purpose: To determine if there is an association between fPLI concentration and presence of serum antibodies against *T. gondii* or *Bartonella* spp. Methods and Materials: Serum samples from 464 cats for which fPLI concentration had been determined were obtained from Texas A&M University's gastroenterology lab. fPLI concentration >12 mcg/L was considered evidence of pancreatitis. Samples were assayed by ELISA for the presence of *T. gondii* IgG and IgM, and *Bartonella* spp. IgG antibodies. Logistic regression analysis was used to determine the association between fPLI concentration and *T. gondii* or *Bartonella* spp. antibodies, with fPLI considered as both a binomial and continuous variable. Odds ratios and 95% confidence intervals were calculated, and significance defined as $p < 0.05$. Results: Of 179(38.6%) cats with fPLI concentration >12 mcg/L, 15(8.4%), 13(7.3%), and 34(19%) were seropositive for *T. gondii* IgG, *T. gondii* IgM, and *Bartonella* spp. IgG antibodies, respectively. Of 285 cats with fPLI concentration <12 mcg/L, 19(6.7%), 20(7.0%), and 54(18.9%) were seropositive for *T. gondii* IgG, *T. gondii* IgM, and *Bartonella* spp. IgG antibodies, respectively. fPLI positive cats were no more likely to be seropositive for *T. gondii* or *Bartonella* spp. than fPLI negative cats, and no statistically significant association was found between the magnitude of fPLI concentration and the presence of antibodies against *T. gondii* or *Bartonella* spp. Conclusion: Failure to find an association between fPLI concentration and antibodies against *T. gondii* or *Bartonella* spp. suggests that serological tests for these organisms may not be useful in all cases of feline pancreatitis.

Effects of GnRH Immunization on Reproduction and Behavior in Female Rocky Mountain Elk

JG Powers, DL Baker, MM Conner, AH Lothridge, TL Davis, TM Nett

Purpose: There is an increasing need in protected environments, for non-lethal methods of managing overabundant wild ungulates. Immunocontraception using gonadotropin releasing hormone (GnRH) vaccination is one approach. This study evaluated the safety and efficacy of GnRH immunization in captive female elk. Methods: Pregnant females were immunized with either a GnRH vaccine (GonaCon) ($n = 10$) or control vaccine ($n = 7$). We studied the effects of active GnRH vaccination on; maintenance of pregnancy, neonatal survival, dam health, reproductive behaviors, and subsequent pregnancy rates. Results: There were no differences in serum progesterone concentrations and calving rates or neonatal survival and growth rates. Two females had large purulent abscesses at the site of injection up to 18 months after vaccination. Reproductive behavior measurements showed no differences in general breeding/herding behaviors. Male pre-copulatory behaviors directed towards treated females were nearly twice those directed towards control females ($P=0.07$), while differences in female pre-copulatory behavior rates were not statistically different over the breeding season ($P=0.5$). The vaccine resulted in a significant reduction in pregnancy rates 8 months post vaccination. GnRH vaccinated animals had a 10% pregnancy rate (1.1 - 38.1%, 95% C.I.) while control animals had a 100% pregnancy rate (70.8-100%, 95% C.I.). Conclusion: Active immunization of female elk during mid-gestation using a single dose of GonaCon does not affect success of the current pregnancy, significantly reduces pregnancy rates the following breeding season, and induces minimal changes in social breeding behaviors; however, individual breeding behaviors may be altered. Ongoing studies are investigating the duration of vaccine efficacy in treated females, prevalence of vaccine related injection site reactions, and potential long-term reproductive effects in developing calves born to treated females.

The Expression of Growth Factors in Equine Airway Smooth Muscle and Their Role in Recurrent Airway Obstruction

SN Hollingshead, R Leguillette

Purpose: Currently there are no permanent treatments for RAO. Many of the current therapies are also associated with side effects such as corticosteroid-induced adrenal suppression. Previous research has shown that horses with RAO have a 3-fold increase in ASM mass which contributes significantly to obstruction of airways. To localize which cells in equine lung tissue produce platelet derived growth factor (PDGF) and express epidermal growth factor receptor (EGFR). **Materials/Methods:** Indirect immunohistochemistry with signal amplification was performed on peripheral equine lung samples that were embedded in paraffin. Samples were incubated with either anti-EGFR IgG rabbit polyclonal and anti-PDGF IgG rabbit polyclonal primary antibodies, then samples were incubated in anti-rabbit IgG biotinylated secondary antibody. Bound antibody was visualized according to standard protocols for the avidin-biotin-alkaline phosphatase complex method. Negative controls were made by omitting the primary antibodies. To identify smooth muscle cells in the samples a smooth muscle myosin (SMM) IgG mouse monoclonal antibody was used as a specific marker of airway smooth muscle (ASM). **Results:** Epithelial cells and ASM cells stained positively for EGFR. SMM, EGFR and PDGF immunoreactivity was strongly expressed in the smooth muscle cells around bronchi, bronchioles and vessels but not among other cell types within the lung tissue from a normal horse. Negative controls showed an absence of SMM, EGFR and PDGF immunoreactivity. **Conclusion:** Both EGFR and PDGF were present on equine ASM cells as well as on the apical surface of the epithelial cells surrounding bronchus and bronchiole airway lumens. This data provides a starting point for further exploration into how EGFR and PDGF can be used as sensitive markers for RAO and as a future target for therapy.

Prevalence of *Dirofilaria immitis* in dogs and cats in an animal shelter in Larimer County, Colorado.

L Kloer, A Hill, N Wilkerson

PURPOSE: The purpose of this study was to determine the prevalence of *Dirofilaria immitis* (heartworm disease) in dogs and cats in Larimer County, CO. The most recent assessment of *D. immitis* prevalence in dogs in this area occurred nearly 16 years ago. This study determined the point prevalence to be low (0.3%), however, with warmer temperatures year-round and with the increased migration of dogs from heartworm endemic areas to the west-central United States, the occurrence of this disease in Larimer County is expected to rise. By gaining a better understanding of the prevalence of *D. immitis* in this area, veterinarians can make more informed decisions regarding testing, prevention, and treatment. **MATERIALS & METHODS:** One milliliter of blood was drawn from dogs and cats at the Larimer Humane Society between May 22, 2007, and Aug. 15, 2007. Canine plasma was tested using Heska_s SoloStep heartworm antigen test. Feline plasma was tested using Heska_s SoloStep heartworm antibody test. Any feline samples that tested positive were sent to the Heska veterinary diagnostic lab for confirmatory testing. **RESULTS:** 295 cats were tested, and 321 dogs were tested. Of the feline samples tested, 16 were SoloStep positive (5.42%; 95% CI: 3.13%-8.66%) and of those, 5 were confirmed positive (1.69%; 95% CI: 0.55%-3.91%) Of the canine samples tested, 2 were SoloStep positive (0.62%; 95% CI: 0.08%-2.23%). Microfilaremia was documented in both of these samples. **CONCLUSIONS:** The authors conclude there is a high enough prevalence in dogs to recommend yearly heartworm testing and monthly prophylaxis. There are large stray cat populations in Larimer County which may help to explain the seroprevalence findings. Regarding testing and prophylaxis in pet cats, the authors recommend owners keep their cats indoors to lessen mosquito exposure. Symptomatic cats should be tested. Non-symptomatic cats allowed outdoors should be tested annually and receive monthly prophylaxis.

Experiences with Spine Surgery Research in a Sheep Model

EB Garcia, TL Sarrafian, AS Turner

Biomedical research for human spine application requires a relevant model and guidelines for selecting animal models have been established. Sheep spines are anatomically and biomechanically acceptable for spine research. Sheep are inexpensive, tractable, large enough to accept human sized implants and are useful for osteoporosis research. Spine studies in sheep have included intervertebral stabilization, treatments for vertebral fracture, and prevention of post-operative peridural adhesions. Intervertebral stabilization surgery targets instability from disc disease and facet arthritis, vertebral fractures, often incorporating bone morphogenetic proteins (BMP) to improve success rates. Studies have demonstrated increased rates of fusion when BMP is used as an autograft substitute in arthrodesis. Polyetheretherketone interbody fusion devices in sheep improve radiographic evaluation of vertebral fusion because they are radiolucent. Bisphosphonates aid in prevention of bone resorption in osteoporosis. Pseudoarthrosis, adjacent level degeneration, and donor graft site pain have stimulated development of stabilizing non-fusion implants. Current research is underway to evaluate stabilization methods in sheep. Osteoporotic vertebral compression fracture repair decreases instability and pain in patients. Vertebral augmentation with vertebroplasty or kyphoplasty uses a bone void filler such as polymethylmethacrylate, calcium sulfate, calcium phosphate or composites. In the ovariectomized ewe, osteogenic protein-1 may increase osteopenic vertebral strength, while ongoing projects aim to compare several osteoconductive bone void fillers. Failed back surgery syndrome is sometimes attributed to peridural adhesion following epidural fibrosis. Following failure of certain bioresorbable barriers to peridural adhesion, a new efficacious polylactide film has been developed. Other anti-adhesive products are currently being tested in our lab using an ovine model.

Regulatory T Cell Responses in Dogs with Cancer

K O'Neill, A Guth, B Biller, R Elmslie, S Dow

Purpose: Regulatory T cells (Tregs) are a subset of CD4+ T cells that play a key role in suppressing abnormal T cell responses and maintaining T cell homeostasis in healthy animals. Treg abnormalities may play a role in a number of disease states, including cancer. We hypothesized that abnormal Treg numbers would be found in dogs with cancer and certain cancer types would be associated with higher Tregs. To test this, we analyzed the blood of healthy dogs and dogs with cancer using flow cytometry and FoxP3 expression. Materials/methods: The cancer patient population consisted of dogs evaluated at the CSU Veterinary Teaching Hospital and at the Veterinary Referral Center of Colorado, and control animals came from the same region. Peripheral blood mononuclear cells (PBMC) were obtained from EDTA blood by lysis of RBC. Leukocytes were immunostained with antibodies (CD4, CD8, CD44, CD14, CD 5, B cells, and FoxP3). Cells were also immunostained with appropriate isotype controls. Cells were analyzed by flow cytometry and the numbers and percentages of Tregs and other leukocytes were determined. Patient data were obtained by chart review. Results: The percentage of Tregs was significantly ($p = 0.003$) increased overall in dogs with cancer compared to control dogs (6.5% vs. 4.1%). When tumor types were compared, Treg percentages were significantly increased in dogs with carcinoma. The number of CD8+ T cells and B cells was decreased in dogs with cancer. The Treg/CD8 ratio was markedly elevated in a subset of dogs with lymphoma, notably those with T cell lymphoma. Conclusions: Tregs are increased in dogs with cancer, particularly in dogs with carcinoma and T cell lymphoma. These findings suggest that tumor-specific factors may drive Treg expansion. Strategies designed to deplete or suppress Tregs, such as metronomic chemotherapy with cyclophosphamide, should be considered for treatment of dogs with cancer, particularly those with the highest levels of Tregs.

Effect of Diet on Blood pH, Serum Electrolytes, and Bone Turnover in Horses

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Purpose: Investigate the possible association between grain consumption and acidosis, alterations in serum electrolytes, and bone remodeling in horses. High dietary acid load (DAL) has been associated with metabolic acidosis and accelerated bone loss in many species. Racehorses are typically fed grain-rich diets which represent high DAL that may decrease blood pH and result in calcium mobilization from bone as a buffer. High DAL may hinder appropriate bone development and predispose young racehorses to injury. **Materials/Methods:** 39 racehorses, 11 pastured horses in pilot study. Data collected include grain intake, work status, age, gender, breed, blood pH, iCa, HCO₃, TCO₂, BE, BE_{ecf}, K, Cl, and PTH. 10 sedentary horses in following study, 5 fed grass hay, 5 fed grain and grass hay. Data collected include blood pH, iCa, HCO₃, TCO₂, BE, SID, K, Cl, Na, glucose and OC. **Results:** Study 1: Grain intake was not significantly associated with lower pH, but lower mean pH and increased HCO₃ and TCO₂ were observed. Significant differences were found between working and sedentary horses in iCa, BE, BE_{ecf}, HCO₃ and TCO₂. Higher blood pH after exercise and a tendency for higher grain intake to result in increased osteocalcin were noted. Study 2: Mean blood pH in the grain group was significantly lower after eating and significantly higher at later collections. Grain consumption was significantly associated with lower HCO₃, TCO₂ and BE. SID (increased anions relative to cations) was significantly lower in the grain group near the time of decreased buffer and pH. **Conclusion:** These data support our hypothesis that high DAL significantly effects blood pH and acid-base balance. Grain-induced acidosis may be offset by exercise-induced alkalosis.

Ezrin Expression in Canine High-Grade Soft Tissue Sarcoma

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Ezrin is a cytoskeleton linker protein that is involved in regulating the metastatic capacity of cancer cells. Reports have demonstrated a strong correlation between ezrin expression and poor prognosis for pediatric and canine osteosarcoma as well as human soft tissue sarcoma (STS). Canine high grade STS (HG-STS) are locally aggressive tumors, half of which will metastasize. There are no reliable predictive markers for metastasis in HG-STS. The purpose of this study was to evaluate ezrin expression as a prognostic marker in canine HG-STS. Formalin-fixed, paraffin-embedded tumor tissue sections from 47 dogs with HG-STS were evaluated for ezrin expression using standard immunohistochemical techniques. Ezrin expression was evaluated using a semiquantitative scoring system and correlated with outcome measures including disease free interval (DFI), median survival time (MST), and presence or absence of metastasis. 47 primary tumors, 11 patient-matched recurrences, and 8 patient-matched metastases were evaluated. The overall DFI and MST were 279 and 575 days, respectively. 97.9% (46/47) of the primary tumors and 100% (19/19) of the patient-matched recurrent and metastatic tumors expressed ezrin. Ezrin expression was numerically higher in recurrent and metastatic tumors compared with patient-matched primary tumors. Patients with high ezrin expressing tumors had a shorter DFI compared with patients with low ezrin expressing tumors, this difference was not significant. There was no significant difference in MST between tumors with low ezrin expression and tumors with high ezrin expression. High ezrin immunoreactivity may be associated with poor patient outcome. There was no significant correlation with ezrin immunoreactivity and DFI, MST, or metastatic frequency as there is in humans with HG-STS. Statistical significance may be improved with a larger sample size. Supported by an ACORN grant from the American Kennel Club Canine Health Foundation.

Technetium-99M-Sestamibi Scans to Predict Outcome in Canine Osteosarcoma

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Introduction: Despite recent advances in the treatment of osteosarcoma (OSA), locoregional and distant metastatic control are still difficult. Combinations of radiotherapy and immunotherapy have the potential to improve both local tumor control and metastasis. This study was designed to evaluate local tumor pathologic in addition to local and systemic response to single high-dose radiation with or without an immune modulating agent in dogs with OSA.**Methods:** Dogs presenting with appendicular OSA were staged to local disease and offered inclusion within the study. Pre-treatment magnetic resonance imaging (MRI) was performed and all dogs received 15 Gy single-dose radiation to the tumor. Dogs were randomized to receive liposomal muramyl tripeptide-phosphatidylethanolamine (L-MTP-PE) or placebo twice weekly until amputation at 3 weeks. A second MRI was performed immediately preceding amputation. Tumor macrosections were used to estimate percent necrosis and compared to MRI. Dogs were given L-MTP-PE twice weekly for 8 weeks postoperatively and followed until failure.**Results:** Both single, high-dose radiation and L-MTP were well tolerated by all dogs and allowed a 3-week delay in amputation. Median percent necrosis was 51% (range 19-83%) with no apparent effect of L-MTP-PE. MRI uptake and DCE rate constants, as well as volume of extracellular/vascular space, and percent non-enhancing voxels all decreased following radiation. However, these changes were not correlated with percent necrosis and were not related to preoperative L-MTP-PE. Overall median disease free interval (DFI) was 179 days and median survival 182 days.**Conclusions:** Single high-dose radiation and L-MTP-PE appear to be well tolerated and result in reasonable degree of local tumor response and systemic disease control. Further evaluation of ultra-high-dose radiation and immunotherapy combinations with or without amputation is warranted.

