Colorado State University
College of Veterinary Medicine and Biomedical Sciences

7th Annual CVMBS Research Day

Scientific Proceedings

The Hilton Hotel
February 4, 2006

Presented by:

The Society of Phi Zeta
The Honor Society of Veterinary Medicine

College of Veterinary Medicine and Biomedical Sciences

CVMBS Research Day

Schedule of Events		Room
1:00	Opening Remarks – Dr. Terry Nett	Idaho/Michigan
1:05	Keynote Speaker – Dr. Mike Glode *Lessons from an Academic Career*	Idaho/Michigan
1:50	Pfizer Research Award Winner, Dr. Sandra Quackenbush *Walleye Dermal Sarcoma Virus: Mechanisms of Oncogenesis and Tumor Regression*	Idaho/Michigan
2:15-5:45	Oral Presentations Session I Moderators: Scott Hafeman	Idaho
2:15-5:45	Oral Presentations Session II Moderators: Stacey Henderson, Josie Mallincrodt	Michigan
2:15-3:00	Poster Set Up	Oklahoma
3:00-4:00	Poster Session I Judging: PVM & Graduate Student/Post Doc Clinical Sciences	Oklahoma
4:00-5:00	Poster Session II Judging Graduate Students/Post Doc Clinical Sciences	Oklahoma
3:00-6:00	Posters on Display & Sponsor Exhibits	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. 4 from 2:15-3:00 in the **Oklahoma Ballroom**. Individuals presenting the poster must be in attendance to discuss their materials with judges from 3-5 pm.

Keynote Speech
CVMBS Research Day
Saturday, February 4th, 2006

Dr. L. Michael Glode

"Lessons from an Academic Career"

Idaho/Michigan Ballrooms
The Hilton Hotel
Fort Collins CO

Dr. L. Michael Glode is the Robert Rifkin Professor for prostate cancer research at the University of Colorado Cancer Center. He joined the faculty in 1978, having been an undergraduate at the University of Nebraska, medical student at Washington University in St. Louis and post-doctoral training at UT Southwestern, NIH, and Harvard. Dr. Glode, along with colleagues at TAPP pharmaceutical, developed the initial human trials of the GnRH analog Lupron, which became a $1.5 B "blockbuster" drug. Subsequently he has focused his clinical career on prostate cancer. He was the founding editor of ASCO OnLine, the official website of the American Society of Clinical Oncology, and has also been recognized by the American Association of Cancer Research for initiation of a molecular biology course for clinical oncologists held each year in Aspen. He has authored more than 100 articles and book chapters and is an active collaborator with CSU scientists in attempting to develop novel therapeutics. His presentation will focus on prostate cancer as a vehicle for an academic career.

Oral Presentations

SESSION 1: Idaho Room Moderators: Scott Hafeman & Jayme Lauderdale			
PVM			
2:15	April Davis	The Response Of Bats And Mice To Aerosolized Rabies Virus	MIP
2:30	Brittney Fierro	IL-10 Knockout (Ko) Mice Of Various Genetic Backgrounds Develop Non-Protective Isotype Specific Anti-Campylobacter Jejuni IGg Antibodies	Ntnl Food Safety & Toxicology Center, MSU, East Lansing MI
2:45	Amanda Guth	Airway Antigen Presenting Cells and Influence of the Lung Environment	MIP
Graduate Student/Post Doc Basic Science			
3:00	Kelvin Kow	Characterization Of Telomeres And Telomerase In Canine Osteosarcoma	ERHS
3:15	Indira Gujrai	West Nile Virus Health Behaviors in Northern Colorado, 2003	ERHS
3:30	Keith Nelson	Leishmania Major gp63 Surface Protein Interaction With Complement Component C3 Affects The Progression Of Disease In Mice	MIP
3:45	Ryan Ashley	Identification Of A Putative Ovine Membrane Progesterone Receptor	BMS
4:00	Melinda Frye	Chronic beta1,3-Adrenergic Antagonism Increases Serum Free Fatty Acids But Preserves Endothelium-Dependent Vasodilation In Fat-Fed Rats	CS
4:15	BREAK		
Graduate Student/Post Doc Basic Science			
4:30	Nicole Garneau	How Do RNA Viruses, Such As Sindbis, Evade Decay Machinery In Host Cells?	MIP
4:45	Emily Kampf	Immunogenicity of Sub-Cellular Fractions from Francisella tularensis	MIP
5:00	Brendan Mangan	Retinal Ganglion Cell Damage in Young DBA/2J Mice	CS
5:15	Lance U'ren	Phosphatidylserine Expression By Tumor Cells Stimulates VEGF Production By Tumor Associated Macrophages	MIP
5:30	Ying Zhang	Partial Deficiency Of DNA-Pkcs Increases Ionizing Radiation Induced Mutagenesis, Cell Killing, And Telomere Instability In Human Cells	ERHS

Oral Presentations

SESSION 2: Michigan Room Moderators: Stacey Henderson & Josie Mallincrodt			
Graduate Student/Post Doc Clinical Science			
2:15	Jennifer Fontenelle	Effect Of Topical Cidofovir On Experimentally Induced FHV-1 Conjunctivitis	CS
2:30	Barbara Biller	Foxp3 Expression By Regulatory T Cells In Dogs With Cancer	MIP
2:45	AmyButler	Minimally Invasive Lithium Dilution Cardiac Output Monitoring And Oxygen Delivery In Conscious, Critically Ill Dogs	CS
3:00	Matthew Miller	The Diagnostic Utility Of Bone Marrow Cytology In Canine Thrombocytopenia	CS
3:15	Break		
3:30	Sarah Coburn	Bighorn Sheep Plasma Cortisol, Catecholamines, And Fecal Glucocorticoid Metabolites In Response To Stress	CS
3:45	Katja Duesterdieck	RNA Isolation From Microscopic Tissue Samples	CS
4:00	Nick Bacon	Primary Re-Excision Following Unplanned Resection Of Soft-Tissue Sarcomas In Dogs	CS
4:15	Aurora Villarroel	Comparison of Random and Cohort Sampling to Evaluate the Effect of Antimicrobial Use on Resistance	CS
PVM			
4:30	Timothy Kurt	In Vitro Amplification of CWD PrPres in Deer and Ferrets	MIP
4:45	Katrina Easton	A Multiple Dye Staining Technique To Evaluate Change In Metacarpophalangeal Joint Contact Area Under Load	CS
5:00	Alison Hurwitch	Epidemiological Study Of Adult Dairy Cow Removals On A Colorado Dairy Farm	BMS
5:15	Sarah Jensen	Evaluation Of Fecal Culture Pooling Methods For Detection Of Mycobacterium Paratuberculosis In A Beef Herd	CS
5:30	Loni Queer	IgE and Inflammatory Responses In The Lavage Fluid And Peripheral Circulation In Guinea Pigs Infected With Mycobacterium Tuberculosis	MIP

Poster Presentations

PVM			
Name	**Department**	**Research Type**	**Abstract Title**
Erin Quist	National Institutes of Health Summer Student Program	Basic Science	Craniofacial And Axial Skeletal Defects In A Mouse Mutant With A P53 Binding Protein Mutation
Corrie Welch	Microbiology, Immunology & Pathology	Basic Science	Detection Of Prion Protein (PRPcwd) In Deer Urine Using Methanol Precipitation
Jennifer Mayo	Clinical Sciences	Clinical Research	The Affect Of Grain Intake On Blood Ph, Electrolytes And Parathyroid Hormone In Fit And Sedentary Horses
Tami Reynolds	Biomedical Sciences	Basic Science	Molecular Cloning Of Zebrafish Calcium Channel B4 Subunit Splice Variants
Melanie Spoor	Laboratory of Immunology, NIAID/ National Institutes of Health, Bethesda, MD	Basic Science	Inhibition Of Naïve T Cell Th1/Th2 Differentiation By CD4+CD25+ Regulatory T Cells In Vitro
Skye Dobberstein	Clinical Sciences	Clinical Research	Efficacy Of Daily Rectal Temperature Monitoring Vs. Visual Observation Of Dairy Cows For Detection Of Post-Partum Disease
Liam Bisson	Clinical Sciences	Clinical Research	Histone Deacetylase Inhibition To Increase Osteosarcoma Chemosensitivity
Jason Eberhardt	Clinical Sciences	Clinical Research	Prevelence Of Select Infectious Disease In Cats From Arizona
Erin Pedersen	Environmental & Radiological Health Sciences	Clinical Research	Impact Of Prevalence Of Escherichia Coli O157 On The Prevalence Of Salmonella In Feedlot Beef Cattle Immediately Prior To Shipping And After Lairage At The Abattoir.
Sherry Hill	Clinical Sciences	Clinical Research	Antibody Responses To West Nile Virus In Vaccinated And Unvaccinated Pronghorn
Holly Tuttle	Environmental & Radiological Health Sciences	Clinical Research	Regional Metabolic Heterogeneity Of Canine Spontaneous Tumors Using 1H And 31P Nuclear Magnetic Resonance.
Eric Hutchinson	Microbiology, Immunology &Pathology	Basic Science	The Effects Of Super-Enriched Environments On Murine Immunology, Health, And Behavior

Christopher Anderson	Clinical Sciences	Clinical Research	Impact Of An Alternative Injectible Colostral Supplement On Morbidity And Mortality Of Preweaned Dairy Calves With Failure Of Passive Transfer.
Jennifer Campbell	Microbiology, Immunology & Pathology	Basic Science	Characterization Of Toll-Like Receptor Ligand Responses In Bone Marrow-Derived Feline Dendritic Cells
Samuel Franklin	Microbiology, Immunology & Pathology	Basic Science	Cross-Species Transmission Of Lentivirus Among Felids In Southern California
Marti Shearin	Clinical Sciences	Basic Science	Comparison Of Gross Pathologic, Histologic, And Subchondral Density Changes In Racing Horses

Poster Presentations

Grad Student/Post Doc			
Name	**Department**	**Research Type**	**Abstract Title**
Marcela Henao-Tamayo	Clinical Sciences	Basic Science	Establishment Of Chronic Mycobacterium Abscessus Infection In The Gamma-Interferon Knockout Mice
Rena Saito	Environmental & Radiological Health Sciences	Basic Science	GC/MS Analysis And Biological Assay For Endotoxins In Agricultural Dusts
Kristin Askin	Environmental & Radiological Health Sciences	Basic Science	Using Knock-In Mice To Determine The Relevance Of Prkdc(BALB) In BALB/C Susceptibility To Radiation-Induced Mammary Carcinogenesis
Julita Ramirez	Other - Biochemistry and Molecular Biology	Basic Science	Tax And Pcreb Bind The KIX Domain Of CBP/P300 At Two Distinct Sites
Abby Williams	Environmental & Radiological Health Sciences	Basic Science	The DNA Repair Protein Rad51d Plays A Role In Mammalian Telomere Function
R. TannerHagelstrom	Environmental & Radiological Health Sciences	Basic Science	WRN-Deficient Cells Exhibit Unusually High Rates Of Telomeric Recombination
Tonya Sirisalee Magers	Environmental & Radiological Health Sciences	Basic Science	Using An Embryonic Stem Cell Mutagenesis Model To Identify Radiation Sensitivity Genes
Krystle Reagan	Microbiology, Immunology & Pathology	Basic Science	Comparative Potential Of Ae. Triseriatus, Ae. Albopictus, And Ae. Aegypti To Transovarially Transmit La Crosse Virus
HyungJin Eoh	Microbiology, Immunology & Pathology	Basic Science	Characterization Of 4-Diphosphocytidyl-2-C-Methyl-D-Erythritol Synthase (Ispd) From Mycobacterium Tuberculosis
Julia Veir	Clinical Sciences	Basic Science	Effect Of An Enteroccocus Faecium (SF68) Enhanced Diet On Immune Responses To A Feline Herpesvirus 1, Feline Calicivirus, And Panleukopenia Vaccine In Cats
Iman ElKiweri	Biomedical Sciences	Basic Science	Modulation Of Opioid Pharmacokinetics And Pharmacodynamics, In Vivo
Kristy McClellan	Biomedical Sciences	Basic Science	A Live View Of Hypothalamic Development Using Transgenic Thy-1 YFP Mice

Emily Thorp	Clinical Sciences	Basic Science	Characterization Of Production Practices, Environmental Parameters, And Selected Infectious Diseases In Catfish Aquaculture - A Retrospective Study
Kevin Sokoloski	Microbiology, Immunology & Pathology	Basic Science	Repeat Sequence Elements In The 3'UTR Of Alphaviruses Stabilize Rnas Against Decay In Mosquito Cell Extracts
Angela Morrison	Microbiology, Immunology & Pathology	Basic Science	Experimental Validation Of In Silica-Identified Regulatory Elements Involved In Human Mrna Polyadenylation
Rodman Tompkins	Microbiology, Immunology & Pathology	Basic Science	O'nyong-Nyong Virus Infection In Anopheles Gambiae
Aida Ulloa	Biomedical Sciences	Basic Science	Contributions Of Specific Htrpcs To Myometrial Ca2+ Entry
Gopinath Palanisamy	Microbiology, Immunology & Pathology	Basic Science	B Lymphocyte Influx Into Lung Granulomas Induced By Mycobacterium Tuberculosis Infections In Naïve And Vaccinated Mice
Kindra Orr	Clinical Sciences	Basic Science	Joint Tissue Mrna Expression In An Equine Osteoarthritis Model To Evaluate Extracorporeal Shockwave Therapy
Deborah Stump	Microbiology, Immunology & Pathology	Basic Science	Alloimmunization As An HIV Vaccine Strategy In A Macaque Model
Beth Stallman	Microbiology, Immunology & Pathology	Basic Science	Characterization Of Yersinia Pestis Culture Filtrate Proteins
Julia Stangel	Clinical Sciences	Basic Science	Hydrogel Selection Of Mesenchymal Stem Cells For Chondrogenic Potential
Wendy Kuhne	Environmental & Radiological Health Sciences	Basic Science	Transgenerational Radiation Genetics: Low Dose-Rate Effects And Adaptive Response In Japanese Medaka (Oryzias Latipes)
Daesuk Chung	Biomedical Sciences	Basic Science	2,2'-DCB Decreases Amplitude And Synchronization Of Uterine Contractions Through MAPK-Mediated Phosphorylation Of Cx43
Jennifer Taylor	Microbiology, Immunology & Pathology	Basic Science	A Role For Matrix Metalloproteinase-9 In Resistance To Pulmonary Mycobacterium Tuberculosis Infection
Joseph Anderson	Microbiology, Immunology & Pathology	Basic Science	Engineered Expression Of TRIM5αrh By Lentiviral Vector Transduction Restricts HIV-1 Infection In CD34+ Stem Cell Derived Macrophages

Joseph Anderson	Microbiology, Immunology &Pathology	Basic Science	Potent Suppression Of CCR5 Expression And HIV-1 Infection By Synthetic And Lentiviral Vector Expressed Shrnas
Jennifer Troyer	Microbiology, Immunology & Pathology	Basic Science	The Effects Of Cytosine Deaminase Activity On Lentiviral Persistence In Vitro And In Vivo: Development Of Feline Immunodeficiency Virus (FIV) As A Relevant Model System
Karen Moraes	Microbiology, Immunology & Pathology	Basic Science	CUG-BP Binds ARE-Containing RNA Substrates And Recruits PARN Deadenylase
Brian Geiss	Microbiology, Immunology & Pathology	Basic Science	Computer-Aided Identification Of Novel Dengue Antiviral Compounds

Poster Presentations

Grad Student/Post Doc -- continued

Jacqueline Whittemore	Clinical Sciences	Clinical Research	Association Of Microalbuminuria With Systemic Disease In Dogs
Heather Low	Clinical Sciences	Clinical Research	Quantification Of FHV-1 DNA From The Conjunctiva Of Cats With And Without Conjunctivitis
Rebecca Kerscher	Clinical Sciences	Clinical Research	Hypoinflammatory In Septic And Critically-Ill Dogs
Aurora Villarroel	Clinical Sciences	Clinical Research	Longitudinal Study On Isolation And Resistance Patterns Of Salmonella Spp. And E. Coli Obtained From Dairy Cattle
Nichole Logan	Clinical Sciences	Clinical Research	Superovulation Of Mares With Equine FSH
Jacquelin Lawler	Clinical Sciences	Clinical Research	Interaction Of Di-Tri-Octahedral (DTO) Smectite With Equine Colostral Antibodies In Vitro
Andrew Goodyear	Microbiology, Immunology & Pathology	Clinical Research	Activation Of Pulmonary Immunity By Liposome-DNA Complexes
Jesse Thompson	Other - Cellular and Molecular Biology	Clinical Research	IMPDH Drug Resistance Gene To Select For HIV-1 Resistant Macrophages From Lentiviral Vector Transduced CD34 Cells
Anne Skope	Clinical Sciences	Clinical Research	Clinical Usefulness Of Echocardiography For Detection Of Atrial Masses In Dogs With Primary Splenic Or Subcutaneous Hemangiosarcoma
Debra Kamstock	Microbiology, Immunology & Pathology	Clinical Research	Vaccination With RhVEGF For Inhibition Of Angiogenesis In Dogs With Soft Tissue Sarcoma

Poster Presentations

Faculty			
Name	**Department**	**Research Type**	**Abstract Title**
Bill Dernell	Clinical Sciences	Basic Science	Computed Tomography Imaging Of Ovone Pulmonary Adenocarcinoma Disease Progression
Susan Lana	Clinical Sciences	Clinical Research	Proteomic Profiling Using SELDI-TOF Mass Spectrometry In Canine Mast Cell Tumors
Josie Traub-Dargatz	Clinical Sciences	Clinical Research	Impact Of Antimicrobial Use On Susceptibility Of Commensal Enteric Bacteria In Horses
D. N. Rao Veeramachaneni	Biomedical Sciences	Basic Science	Rampant Testicular Dysgenesis In Sitka Black-Tailed Deer On Kodiak Island, Alaska
Jane Shaw	Clinical Sciences	Clinical Research	Does The Gender Of The Client And The Veterinarian Influence Communication In Veterinary Visits?
Douglas Thamm	Clinical Sciences	Basic Science	Epidermal Growth Factor Promotes The Malignant Phenotype In Canine Mammary Carcinoma
Kristy Dowers	Clinical Sciences	Clinical Research	The Association Of Bartonella Spp. Infection With Chronic Stomatitis In Cats
Chester Moore	Environmental & Radiological Health Sciences	Basic Science	West Nile Virus Surveillance In Northern Colorado 2004
Miyako Kimura	Microbiology, Immunology & Pathology	Basic Science	Methods For Strain Typing Of Mycobacterium Leprae
John Wenz	Clinical Sciences	Clinical Research	Association Between Local Clinical Signs And Important Outcomes Of Clinical Mastitis Episodes In Dairy Cattle
Paul Morley	Clinical Sciences	Clinical Research	Re-Examination Of The Etiology Of Fatal Undifferentiated Fever / Bovine Respiratory Disease Of Feedlot Cattle
Lorene Martinez	Microbiology, Immunology & Pathology	Basic Science	Strain Typing Of North American M.Bovis Isolates
Herbert Schweizer	Microbiology, Immunology & Pathology	Basic Science	Melioidosis : Novel Therapies For An Emerging Disease

David Vail	Clinical Sciences	Basic Science	Expression And Pharmacologic Inhibition Of Anti-Apoptotic Bcl2 Family Members In Canine Lymphoma
Moises Barceló-Fimbres	Biomedical Sciences	Basic Science	Effects Of Fetal Calf Serum Or Phenazine Ethosulphate (PES) And Fructose Or Glucose On Embryonic Development And Lipid Accumulation Of Bovine Embryos
Susan Kraft	Environmental & Radiological Health Sciences	Clinical Research	A Prospective Study Evaluating Whole Body MRI For Staging Lymphoma In Dogs

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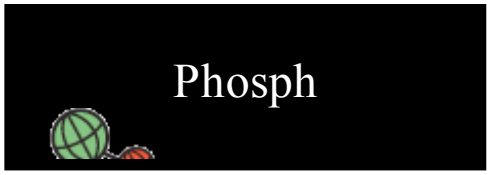

Session I

Oral Presentations

Veterinary Students
PVM I

THE RESPONSE OF BATS AND MICE TO AEROSOLIZED RABIES VIRUS

Author List: AD Davis and RJ Rudd

Abstract:
The conundrum of rabies virus transmission through aerosolization has remained unsolved for decades. The suspicion that four humans became infected with rabies through aerosol transmission in the 1950's and 1970's heightened the concern of the public health community while stimulating interest in aerosol rabies transmission in caves. However, each of these human cases has a more plausible explanation. Nonetheless, experiments in the 1960's were able to document the transmission of rabies to wild animals when placed for one week or more in a cave where millions of Tadarida brasiliensis bats were present. In our study, laboratory mice, Mus Muscus and two species of bats, Tadarida brasiliensis and Eptescus fuscus were exposed to aerosolized rabies virus. Six months post aerosol exposure all animals were challenged intramuscularly with a virulent rabies virus. The serological response was followed for twelve months after the initial aerosol exposure. All animals developed antirabies viral neutralizing antibodies (VNAs) following aerosol exposure. However, VNAs produced after exposure to aerosolized rabies virus did not protect the animals when challenged intramuscularly.

IL-10 KNOCKOUT (KO) MICE OF VARIOUS GENETIC BACKGROUNDS DEVELOP NON-PROTECTIVE ISOTYPE SPECIFIC ANTI-CAMPYLOBACTER JEJUNI IgG ANTIBODIES

Author List: BR Fierro, AJ Murphy, JA Bell, VK Rathinam, LS Mansfield

Abstract:
Campylobacter jejuni is a globally distributed human pathogen, is a leading cause of food borne enteritis, and has been linked to chronic neurological and joint diseases. To combat this pathogen, it is important to develop murine models of enteritis induced by primary *C. jejuni* challenge employing mice with a genetic background and immune bias that enhances susceptibility. We hypothesized that a particular genetic background enhances susceptibility to colonization and enteritis when inbred IL-10 KO mice are infected with *C. jejuni*. To test this hypothesis, we experimentally infected C57BL/6, NOD, and C3H/HeJ wildtype (WT) mice and their corresponding IL-10 KOs with *C. jejuni* and followed the course of infection by clinical exam, gross- and histopathology, immunohistochemistry, and anti-*C. jejuni* isotype specific ELISA serology. All IL-10 KO and wildtype mice were colonized by *C. jejuni* at 35 days. Colonization was necessary but not sufficient for enteritis lesions. 11/20 C57BL/6 KO, 8/10 NOD KO, and 7/10 C3Bir KO mice had enteritis on necropsy. 0/10 C57BL/6 WT, 4/10 NOD WT, and 0/10 C3H/HeJ WT mice had enteritis on necropsy. All *C. jejuni* infected KO and wildtype mice had significant anti-*C. jejuni* specific IgG antibody by ~30 days after infection. We conclude that mice of these backgrounds react to *C. jejuni* producing specific IgG antibody that in the context of an IL-10 KO does not protect against enteritis.
(Supported by NIH contract MI004-04.)

Airway Antigen Presenting Cells and Influence of the Lung Environment

Author list: A. Guth1, C. Bosio1, and S. Dow1,2.

Abstract:
Background. The antigen presenting cells (APC) located in the small airways and alveoli are critical in mounting early responses to inhaled antigens and in responding to mucosally delivered vaccines. Two recent reports from our lab indicate that bronchoalveolar lavage cells (BAL) have a unique phenotype such that they resemble dendritic cells (DC) rather than true alveolar macrophages. We conducted studies therefore to more fully assess the phenotype and function of these major resident APCs in the airways of mice. **Methods**. BAL cells were collected from naive mice and phenotyped using flow cytometry. The phenotype of BAL cells was compared to that of peritoneal lavage (PL) cells. Macropinocytosis and antigen presentation ability was compared between BAL cells and PL cells. The development of adoptively transferred and labeled bone marrow cells in the lung and peritoneal environments was compared. **Results**. BAL cells fromnaive mice were expressed high levels of CD11c and DEC-205 and did not expressthe typical macrophage markers CD11b or F4/80. BAL cells were highly pinocytotic and supported mixed lymphocyte reaction better than resident PL cells. After adoptive transfer into the airways, a small population of labeled bone marrow precursor cells upregulated expression of CD11c and DEC-205, whereas transfer into the peritoneal cavity did not result in upregulation of DC marker expression. **Conclusions**. Our data suggest that the majority of BAL cells, rather than being macrophages, are actually more likely actually immature DC. These results suggest that the airway microenvironment favors the preferential development of DC and not macrophages.

Session I

Oral Presentations

Graduate Student
Post Doctoral – Basic Science

Characterization of telomeres and telomerase in canine osteosarcoma

Author List: K Kow, E Williams, S Bailey, S Withrow, S Bailey, S Lana.

Abstract:
Introduction: Telomeres are highly specialized DNA-Protein complexes that cap the ends of chromosomes. Telomere attrition occurs with each round of replication, ultimately leading to cellular senescence. The enzyme telomerase facilitates telomere length maintenance by acting as a reverse transcriptase for addition of telomeric sequences. Telomerase is not active in most somatic tissues, but is widely reactivated in tumors. Some tumors lack telomerase activity (TA) and maintain telomere length by an alternative lengthening mechanism known as ALT. The purpose of this study is to characterize telomerase activity versus the presence of the ALT pathway in canine osteosarcoma (OSA)

Methods: Telomeric repeat amplification (TRAP) assays were performed on six canine OSA cell lines and six canine clinical OSA samples to assess the presence or absence of telomerase activity. As a control, two telomerase positive human cell lines were also assayed

Results: TRAP assays revealed the presence of telomerase activity in 5/6 canine osteosarcoma cell lines as well as 5/6 clinical osteosarcoma samples. The human controls for telomerase activity were both positive.

Conclusion: Telomerase activity is present in 5/6 (83%) of canine OSA cell lines and clinical samples assayed to date. Our future goals are to determine the predominant telomere maintenance mechanism in clinical tumor samples, correlate the telomere maintenance mechanism with clinical outcome and to develop a real-time PCR method for telomere length measurement/ALT determination.

West Nile Virus Health Behaviors in Northern Colorado, 2003

Author List: IB Gujral, EC Zielinski-Gutierrez, A LeBailly, MD

Abstract:
Background Larimer county, in northern Colorado, experienced an outbreak of West Nile virus (WNV) disease in 2003. Age-adjusted neuroinvasive disease rates varied in the county; 12.1/100,000 in Fort Collins and 34.6 in Loveland. Fort Collins had higher mosquito populations compared to Loveland; however, mosquito infection rates were not significantly different. A survey was done to identify whether personal prevention and risk practices differed by city. **Methods** Between May and June of 2004, a random digit dial telephone survey was conducted among residents of Fort Collins and Loveland to assess knowledge/beliefs about WNV and to describe personal preventive behaviors during 2003. Unconditional logistic regression models were built to predict: 1) DEET usage; 2) use of protective clothing (long sleeves/pants); 3) dusk to dawn exposure during the week; and, 4) exposure during weekends. **Results** 957 interviews were completed. Models controlled for age, sex, income, risk perception, education, ethnicity and city of residence. Compared to Fort Collins residents, Loveland residents were 30% more likely to report seldom/never using insect repellent and approximately 30% more likely to report spending time outdoors from dusk to dawn during the week and on weekends. Additional significant factors will be discussed. **Conclusions** Results suggest that the higher rates of WNV severe disease in Loveland may be due, in part, to lower use of insect repellent and greater dusk to dawn outdoor exposure. While the complex ecology of WNV must be taken into account, data suggest that use of personal protection can have an impact on the level of illness experienced in a community

Leishmania Major gp63 Surface Protein Interaction With Complement Component C3 Affects The Progression Of Disease In Mice

Author List: KG Nelson, WR McMaster, R Titus.

Abstract:
Leishmania spp. are important protozoan parasites of humans and domestic animals throughout the world. An understanding of the interaction between their most prevalent surface molecules and host immune responses is vital to understanding the pathogenesis and potential control of these pathogens. We investigated the role of gp63, a metalloprotease that is the most prevalent protein on the surface of Leishmania major (Lm), in both the pathogenesis of disease and interaction a primary component of the complement cascade, C3. Susceptible, resistant, and C3KO mice were infected with LV39 (wild-type) Lm or gp63KO Lm and lesion development, parasite growth, and cytokine response were assessed over time. Lesion size& parasite numbers in C3KO mice infected with LV39 Lm and in resistant control mice infected with gp63KO Lm were similar and demonstrated a pattern of development that was temporally slowed and prolonged. Similar changes were seen in cytokine profiles, particularly for IFN-gamma. The temporal lag in response of the C3 KO mice to LV39 Lm infection indicates that C3 opsonization is one of the more significant routes by which the parasite is recognized and phagocytosed by macrophages and possibly one of the mechanisms that induces the host's innate/adaptive immune response. Similarly, the lag in response to gp63KO Lm in control resistant mice suggests that gp63 is a biologically important molecule for interactions of the parasite with complement and that the lack of gp63 may effect the murine host in an equivalent manner to the lack of C3, affecting opsonization and clearance of the parasites in a temporal manner. This suggests an important, yet not vital, and interacting role for gp63 and C3 in Leishmania survival, proliferation, and pathology and will affect potential avenues for leishmaniasis treatment and prevention in the future.

Identification of a putative ovine membrane progesterone receptor.

Author List: RL Ashley, CM Clay, T Farmerie, GD Niswender, TM Nett

Abstract:
Classically, progesterone (P4) functions through the well-known genomic pathway involving hormone binding to nuclear receptors (PR) and subsequent modulation of gene expression. Alternatively, there is increasing evidence for rapid, nongenomic P4 effects in a variety of tissues in mammals and the likelihood of a membrane PR (mPR) causing these events is quite plausible. The objective of this study was to isolate and characterize an ovine mPR distinct from the intracellular PR. Gene specific primers were generated for PCR and a cDNA clone was isolated from ovine genomic DNA similar to recently reported mammalian mPRs. The ovine mPR is a 350 amino acid protein that based on computer structural analysis possesses seven transmembrane domains and is distinct from the nuclear PR. RT-PCR was used to determine tissue distribution of mRNA for mPR in sheep. Message for the ovine mPR was detected in hypothalamus, pituitary, uterus, ovary and corpus luteum. Confocal microscopy studies in CHO cells overexpressing a mPR-GFP fusion protein revealed the ovine mPR is uniquely localized to the endoplasmic reticulum and not the plasma membrane. Also, P4 and 17-Hydroxy-P4 stimulate intracellular Ca^{2+} mobilization in CHO cells expressing ovine mPR in Ca^{2+}-free medium ($P < 0.05$) but not in CHO cells transfected with empty vector. This rise in intracellular Ca^{2+} is believed to be from the endoplasmic reticulum as intracellular Ca^{2+} mobilization is absent when mPR transfected cells are first treated with thapsigargin, a drug that depletes Ca^{2+} stores from the endoplasmic reticulum. This is, to our knowledge, the first report of the cellular localization as well as a functional response for any of the recently reported mammalian mPRs.

Chronic beta1,3-adrenergic antagonism increases serum free fatty acids but preserves endothelium-dependent vasodilation in fat-fed rats

Author List: M Frye, K Fagan, I McMurtry, K Morris, S Golembeski, E Carter, J Weil, C Orton

Abstract:
INTRODUCTION Obesity is a risk factor for vascular disease, and endothelial dysfunction is common to both. The mechanism linking obesity and endothelial dysfunction is unclear. Serum free fatty acids (FFAs) are elevated in obesity, and impair endothelium-dependent vasodilation (EDV) by inflammatory and pro-oxidant means. Elevated FFAs are partly due to enhanced triglyceride lipolysis, which is increased by sympathetic stimulation of the beta-adrenoreceptor. There is evidence of enhanced sympathetic tone in obesity. HYPOTHESIS Beta-antagonism will reduce serum FFAs and improve EDV in fat-fed rats. Reduced FFAs will be associated with decreased inflammation and oxidative stress. METHODS Male Sprague-Dawley rats (n = 8) were fed a high-fat diet for 12 weeks. During the final 4 weeks rats received beta1,3-antagonists (atenolol, SR59230A) by subcutaneous slow-release pellets. EDV was measured using transit time flowmetry of the femoral artery in vivo to measure the flow-time integral (i.e. area under the flow curve) in response to 0.25, 0.75, and 2.5 mcg/kg intra-arterial acetylcholine. Serum FFAs and markers of inflammation (CRP, IL-6, TNF-alpha) and oxidative stress (TBARS) were measured. RESULTS Beta-antagonism increased serum FFAs (placebo mean ± SE = 0.60 ± 0.04 meq/L; treated = 0.78 ± 0.06 meq/L; $p < 0.01$) but did not alter the flow-time integral (placebo = 112 ± 18 mLs; treated = 142 ± 21 mLs; $p = 0.18$). Beta-antagonism was associated with reduced CRP, IL-6 and TNF-alpha and increased TBARS. CONCLUSIONS Beta-antagonism may increase serum FFAs by attenuating brown adipose fatty acid oxidation. Treatment preserved EDV despite increased FFAs, suggesting that favorable effects of beta-antagonists on the endothelium are not mediated by FFA-lowering properties. Beta-antagonism did not decrease oxidative stress but did reduce inflammation despite elevated FFAs. Because FFAs are pro-inflammatory, beta-antagonism may preserve EDV by attenuating FFA-mediated inflammation.

How do RNA viruses, such as Sindbis, evade decay machinery in host cells?

Author List: NL Garneau, M Opyrchal, KJ Sokoloski, CJ Wilusz & J Wilusz

Abstract:
Eukaryotic cells have efficient machinery designed to provide both regulated turnover of mRNAs to control gene expression as well as to degrade aberrant mRNAs. As many viral genomic RNAs resemble cellular mRNAs, we hypothesize that host cells may use this intrinsic decay machinery as an anti-viral defense. Accordingly, viruses themselves likely have evolved a way in which to avoid or exploit the cellular mRNA decay machinery. In support of this idea, the 3'UTR of Sindbis virus RNA is able to inhibit poly(A) tail removal and thereby stabilize a reporter RNA in extracts from *Aedes albopictus* cells. We are developing an innovative system to measure the rate of decay of Sindbis virus RNA in both vertebrate and invertebrate cell lines. This system will allow us to confirm our *in vitro* results, as well as take steps towards understanding mechanistically exactly how viral RNAs are turned over within the host cell.

Immunogenicity of Sub-Cellular Fractions from Francisella tularensis

Author List: EE Kampf, JT Belisle, SL Warner, KG White, and CM Bosio

Abstract:
Francisella tularensis is an obligate intracellular bacterium and the causative agent of tularemia. Inhalation of *F. tularensis* can cause rapid, lethal, pneumonic disease. Vaccination with a highly attenuated strain of *F. tularensis* (Live Vaccine Strain [LVS]) efficiently protects mice and humans against lethal pneumonic infections. Unfortunately, vaccination with LVS can have severe side effects. Coupled with the ability of LVS to undergo a spontaneous attenuating phase shift, these traits have eliminated LVS as a vaccine against tularemia. Thus, a vaccine that embodies the protective characteristics of LVS without the inherent risks of a live vaccine is desirable. Sub-unit vaccines, developed using LVS sub-cellular fractions, would overcome obstacles associated with LVS. LVS sub-cellular fractions were first examined for their immunogenicity in vitro and in vivo. Both cytosolic and membrane fractions from LVS elicited production of TNF-α from primary bone marrow derived dendritic cells. Membrane fractions were routinely more stimulatory for dendritic cells compared to cytosol fractions. This difference in immunogenicity was also reflected in vivo. Mice vaccinated either intranasally (i.n.) or sub-cutaneously (s.c.) with LVS membrane fractions elicited stronger systemic humoral responses compared to cytosol fractions. To determine if these immunogenic LVS fractions could protect against lethal pneumonic tularemia, vaccinated mice were infected with a low dose aerosol of *F. tularensis* strain Schu4, a highly virulent isolate of *F. tularensis*. Vaccination with membrane fractions of LVS significantly increased the mean time to death of mice infected with Schu4 compared to mice immunized with cytosolic fractions and sham vaccinated controls. Together this data suggests that LVS fractions, specifically membrane fractions, may serve as effective vaccines for pneumonic tularemia.

Retinal Ganglion Cell Damage in Young DBA/2J Mice

Author List: BG Mangan, JE Madl, JR Gionfriddo, CC Powell.

Abstract:
The DBA/2J mouse is an important model for a type of spontaneous glaucoma, in which neurodegenerative changes in retinal ganglion cells occur prior to elevations in intraocular pressure. Damaged retinal ganglion cells can be identified in 9-week-old DBA/2J mice, while elevated intraocular pressure is first detected in 6-month-old mice. Changes in their retinal ganglion cells follow a pattern of hypoxic-ischemic damage leading to redistribution of glutamate and gamma-aminobutyric acid. Furthermore, retinal ganglion cell damage typically occurs in a patchy manner, which is suggestive of retinal circulatory compromise. Retinal microvessels in the retinal ganglion cell layer exhibit changes that appear to be associated with regions of patchy damage. Thus, early changes in retinal circulation may be responsible for neurodegenerative changes in retinal ganglion cells prior to elevations in intraocular pressure. The standard medical therapy for glaucoma is aimed at lowering intraocular pressure. By identifying early pathophysiologic events in glaucoma, such as retinal vascular damage, it may be possible to intervene with neuroprotective therapies at an earlier stage of the disease.

Phosphatidylserine Expression By Tumor Cells Stimulates VEGF Production By Tumor Associated Macrophages

Author List: L. U'Ren1,2, P. Henson3, Shyra Gardhi3, and S. Dow1,2.

Abstract:
Background. Phosphatidylserine (PS) is normally expressed on the inner leaflet of living cells and is translocated to the outer leaflet as cells becomes apoptotic. Surface expression of PS stimulates engulfment of cells by macrophages. Previous studies have found that engulfment of PS^+ apoptotic cells by macrophages triggers release of anti-inflammatory cytokines. It is also known that tumor-associated macrophages (TAM) are associated with increased tumor growth and angiogenesis. Therefore, we investigated the effects of PS^+ tumor cells on production of the pro-angiogenic factor VEGF by macrophages. **Methods**. Tumor cell lines were screened by flow cytometry for expression of PS, using Annexin V staining. Normal acrophages were obtained from the peritoneal cavity of mice, while tumor associated macrophages (TAM) were sorted from enzymatically digested tumor tissues of mice. VEGF production was measured by elisa. **Results**. Incubation of macrophages with PS+ tumor cells (live cells or apoptotic cells) triggered release of significant quantities of VEGF. This response could be partially inhibited when the apoptotic tumor cells were pre-incubated with Annexin V. We also observed that exosomes derived from a PS expressing tumor cell line (MCA2.1-1) could significantly increase VEGF production by peritoneal macrophages, and that this response could be completely blocked by the addition of Annexin V. The addition of exosomes derived from a non-PS expressing tumor cell line (B16) did not induce macrophage VEGF production. **Conclusions**. PS appears to play an important role in regulating the production of VEGF by tumor associated macrophages and may therefore promote tumor angiogenesis. Our results indicate that PS expressed on tumor cells or on tumor exosomes can both stimulate production of macrophage VEGF. Thus, tumor cells or their secreted membranes may promote tumor angiogenesis via their interaction with tumor associated macrophages.

Partial deficiency of DNA-PKcs increases ionizing radiation induced mutagenesis, cell killing, and telomere instability in human cells

Author List: Ying Zhang,Junqing Zhou, Xiaofan Cao, Qinming Zhang, Chang UK Lim, Susan M. Bailey, and Howard L. Liber

Abstract:
The correct repair of DNA double strand breaks (DSBs) is essential to maintaining the integrity of the genome. Misrepair of DSBs is detrimental to cells and organisms, leading to gene mutation, chromosomal aberration, and cancer development. Nonhomologous end-joining (NHEJ) is one of the principal rejoining processes in most higher eukaryotic cells. NHEJ is facilitated by DNA dependent protein kinase (DNA-PK), which is composed of a catalytic subunit, DNA-PKcs, and the heterodimeric DNA binding regulatory complex Ku70/86. Null mutation of DNA-PKcs leads to immunodeficiency, chromosomal aberration, gene mutation, telomeric end-capping failure, and cancer predisposition in animals and cells. However, it is unknown whether partial deficiency of DNA-PKcs as might occur in a fraction of the population (e.g., heterozygotes), influences cellular function. Using small interfering RNA (siRNA) transfection, we established partial deficiency of DNA-PKcs in human cells, ranging from 4-85% of control levels. Our results reveal for the first time, that partial deficiency of DNA-PKcs leads to increased IR-induced mutagenesis, cell killing, and telomere dysfunction. Radiation mutagenesis was increased inversely with DNA-PKcs protein level, with the most pronounced effect being observed in cells with protein levels below 50%. Increased IR-induced cell killing was observed over the entire range of decreased DNA-PKcs levels. Frequencies of IR-induced telomere-DSB fusion and telomeric sister chromatid exchange (T-SCE) were increased at levels of DNA-PKcs as low as ~50%, similar to what would be expected in heterozygous individuals. Taken together, our results suggest that partial deficiency of DNA repair proteins may represent a considerable risk to genomic stability.

Session II

Oral Presentations

Graduate Student
Post Doctoral –
Clinical Sciences

Effect of Topical Cidofovir on Experimentally Induced FHV-1 Conjunctivitis

Author List: JP Fontenelle, CC Powell, JR Gionfriddo, S Radecki, MR Lappin

Abstract:
Purpose To examine the effects cidofovir eye drops on FHV-1 DNA copy numbers by use of real-time PCR and to determine whether the drug lessens clinical signs of conjunctivitis in cats with experimentally-induced FHV-1 infection. **Methods** Twelve FHV-1 negative cats were inoculated OU with FHV-1 and randomly assigned to 1 of 2 groups. Treatment cats received 1 drop of 0.5% cidofovir in carboxymethyl cellulose and placebo cats received 1 drop of carboxymethyl cellulose, OU BID for 10 days. Standardized clinical scoring was used to evaluate each cat for 24 days. Pre-treatment (days 0-3), treatment (days 4-14), and post-treatment (days 15-24) ocular scores were averaged for each cat. Samples were collected OU on days 0, 3, 6, 9, 12, 15, 18, 21, and 24, were kept at room temperature for 2-3 hours, then stored at -70°C until analyzed. DNA was extracted and assayed for FHV-1 DNA using an adaptation of a previously published protocol. Repeated measures ANOVA was used to evaluate a statistical model containing treatment group, period, and the period by group interaction. Within period effects of treatment were evaluated if significance was found ($p<0.05$). **Results** The interaction between period and group was statistically significant for ocular scores and viral quantity. The within period effects for clinical scores showed statistically significant lower scores in the treated group compared to the placebo group during the treatment period ($p=0.03$). Due to large differences in the pretreatment mean viral copy numbers between the treated and placebo group, these values were included in the statistical model as a covariate. The within period effects for viral quantity were also significantly lower in the treated group compared to the placebo group during the treatment period ($p=0.003$). **Conclusions** Twice daily topical application of 0.5% cidofovir eye drops decreases clinical signs of ocular disease and FHV-1 shedding in cats with experimentally induced FHV-1.

Foxp3 Expression By Regulatory T Cells In Dogs With Cancer

Author List: BJ Biller1, R Elmslie2, RC Burnett1, AC Avery1, and SW Dow1.

Abstract:
Background: Regulatory T cells (Treg) play an important role in the prevention of autoimmunity and maintenance of self-tolerance. In cancer patients, however, expanded numbers of Treg may promote tumor growth through suppression of anti-tumor immune responses. Expression of the transcription factor Foxp3 (a member of the forkhead/winged family) is limited to the Treg subset of T cells and thus serves as a unique Treg marker in humans and mice. Therefore, we evaluated expression of Foxp3 mRNA and protein in T cells from dogs with cancer to determine whether Foxp3 can be used as a marker of canine Treg. **Materials and Methods:** PBMC were isolated from cancer-bearing dogs and age-matched healthy dogs. Quantitative analysis of mRNA for canine Foxp3 was determined using real time-PCR. Expression of Foxp3 protein was assessed by intracellular staining and flow cytometry, using a cross-reactive anti-mouse Foxp3 antibody. **Results:** Foxp3 mRNA expression was detected in PBMC of normal dogs and dogs with cancer. Flow cytometric analysis revealed Foxp3 protein expression in $CD4^+$ T cells and also in other lymphocytes, but not in neutrophils or monocytes. Lymph nodes contained significantly higher numbers of $Foxp3^+$ T cells than blood. The percentage of $Foxp3^+/CD4^+$ T cells was significantly greater in dogs with cancer than in normal dogs. **Conclusions:** The ability to detect expression of the Foxp3 transcription factor, both at the mRNA and protein levels, in PBMC of dogs will greatly facilitate the study of the role of Treg in cancer and other diseases. More detailed characterization of Foxp3 expression is underway in our laboratory including correlation of $Foxp3^+/CD4^+$ T cells with other markers of Treg, such as expression of the IL-2 receptor (CD25) and IL-10 production.

MINIMALLY INVASIVE LITHIUM DILUTION CARDIAC OUTPUT MONITORING AND OXYGEN DELIVERY IN CONSCIOUS, CRITICALLY ILL DOGS

Author List: A Butler, VL Campbell, AE Wagner, C Sedacca, TB Hackett

Abstract:
Objective: To determine cardiac index (CI) and oxygen delivery (DO2) in conscious, critically ill dogs compared to healthy dogs. Procedure: Study group: Thirteen client owned dogs meeting clinical criteria of the systemic inflammatory response syndrome (SIRS) and weighing > 10kg were evaluated. SIRS criteria included 3 or more of the following: respiratory rate >20 breaths/minute; heart rate > 120 beats/minute, rectal temperature < 100.4°F or > 104°F, and WBC < 5000 or > 18,000 cells/mm3. The study was approved by IACUC and informed client consent was obtained prior to enrollment in the study. Lithium dilution cardiac output (CO) was measured using the PulseCO Plus cardiac output monitor at times 0, 4, 8, 16, and 24 hours after admission to the Critical Care Unit. CI (L/min/m2) was calculated from CO to allow for variation in patient size. At each time period, arterial partial pressure of oxygen, arterial oxygen saturation, and hemoglobin concentration were measured to calculate oxygen content. DO2 was calculated by multiplying CO and oxygen content. Control group: Eight healthy owned dogs were used as a control group. Dogs did not meet criteria of SIRS based on physical examination and CBC. Arterial blood gases and three measurements of cardiac output over 15 minute intervals were obtained as described above. Statistics: Cardiac index and oxygen delivery data were analyzed using a 2-sample comparison of unequal variances, a 2-tailed distribution and a p-value of <0.05. Results: Complete data was collected from thirteen SIRS patients at all five time periods. Mean CI in SIRS patients was 3.32 ± 0.99 L/min/m2 and was significantly lower compared to healthy controls at 4.175 ± 0.22 L/min/m2 ($p<0.05$). Mean oxygen delivery index in SIRS patients was 422.02 ± 158 ml O2/min/m2 and was significantly lower from healthy controls at 809.82 ± 51 ml O2/min/m2/ ($p<0.05$). Conclusions: CI and DO2 in conscious dogs meeting criteria of SIRS are significantly lower than healthy control dogs.

THE DIAGNOSTIC UTILITY OF BONE MARROW CYTOLOGY IN CANINE THROMBOCYTOPENIA

Author List: MD Miller, K Lunn

Abstract:
Bone marrow cytology has been recommended in canine patients with non-regenerative anemia, persistent thrombocytopenia, persistent neutropenia, and atypical cells on peripheral blood smears. To our knowledge, the diagnostic value of bone marrow cytology in canine thrombocytopenia has not been investigated. The most common cause of severe thrombocytopenia is immune-mediated platelet destruction. Bone marrow cytology in these patients is predicted to show megakaryocytic hyperplasia as an appropriate response to peripheral platelet destruction. Bone marrow disorders that could lead to thrombocytopenia include myelophthisis and dysthrombopoiesis. Our hypothesis was that bone marrow cytology does not commonly identify a cause of severe thrombocytopenia. The medical records database at the Colorado State University Veterinary Teaching hospital was searched from 1999 through 2004 for canine patients with thrombocytopenia that had bone marrow cytology performed. Cases were excluded which had neutropenia or had received previous therapy with immune-suppressive drugs. 48 cases met the selection criteria. The cases were divided into dogs with severe thrombocytopenia (<20,000 platelets/μL) and mild to moderate thrombocytopenia (>20,000 but <200,000 platelets/μL). The diagnostic utility of the bone marrow cytology was compared between groups. 31 dogs had severe thrombocytopenia: in none of these dogs did bone marrow cytology demonstrate the cause of thrombocytopenia, such as myelophthisis or dysmyelopoeisis. 19 dogs had mild to moderate thrombocytopenia. Bone marrow cytology in 4 of these dogs showed myelophthisis. Significantly fewer dogs with severe thrombocytopenia had evidence of myelophthisis on bone marrow cytology ($p=.02$). Based on the results of this study, we conclude that bone marrow cytology rarely provides a specific diagnosis in dogs with severe thrombocytopenia. Bone marrow cytology may not be an efficient use of resources in the workup of these patients.

Bighorn sheep plasma cortisol, catecholamines, and fecal glucocorticoid metabolites in response to stress

Author List: S Coburn, MD Salman, J Rhyan, T Keefe, T Spraker

Abstract:
This study used plasma epinephrine, norepinephrine, and fecal glucocorticoid metabolites (FGM) to compare the stress response to a novel environment and repeated handling between captive-raised and wild-caught Rocky Mountain bighorn sheep (*Ovis canadensis canadensis)*. Stress has been proposed as one factor limiting wild bighorn sheep population growth by predisposing them to disease outbreaks. Hormones from 20 bighorn sheep born and raised in captivity (CR) were compared to 14 bighorn sheep captured in the wild and brought into captivity (WC). The first part of the study used a one-time dropnet event to elicit an acute stress response. Blood samples were collected at approximately five minute intervals from each animal for the determination of plasma cortisol, epinephrine, and norepinephrine. For the second part, a fecal sample was collected from the rectum of the sheep once to twice per month for the determination of baseline FGM. Fecal samples were collected from the pen at approximately 24 hour intervals for three days following every handling event to monitor the cortisol response to handling. Epinephrine was generally higher for the CR group as compared to the WC group in response to the dropnet sampling. No significant differences were detected between the CR and WC groups in their plasma cortisol or norepinephrine response. The patterns of fecal cortisol levels for the WC sheep were different initially and eventually parallel to that of the CR sheep in response to handling events. Overall, our results support FGM as a potentially useful tool for monitoring stress levels in free-ranging populations, but interpretation of FGM levels must be done carefully. Our results corroborate previous studies that found a difference depending on which stress response pathway was monitored. Future studies should continue to look for correlations between FGM levels and impacts to essential biological functions.

RNA isolation from microscopic tissue samples

Author List: KF Duesterdieck, DD Frisbie, JD Kisiday, CE Kawcak, CW McIlwraith

Abstract:
Osteoarthritis (OA) remains a common and debilitating disease in horses, despite advances in diagnosis and treatment. Thus, it is important to develop new methodologies to investigate the pathophysiology of OA. Gene expression analysis of articular cartilage is hampered by its layered nature, resulting in samples containing phenotypically heterogeneous cells. Our goal was to develop a protocol to isolate RNA from microscopic tissue samples to allow the investigation of gene expression patterns from phenotypically homogeneous cells. RNA was isolated from 3 equine tissues/cells (muscle, cartilage, chondrocytes cultured in agarose). Samples were snap frozen in OCT and sectioned with a cryostat. RNA was extracted on a spin column from complete frozen sections and from samples obtained by laser capture microdissection (LCM) of the frozen sections. Extracted RNA was evaluated by capillary electrophoresis and, after reverse transcription, with quantitative real-time PCR (rtPCR). Sample processing prior to LCM was crucial for successful cell capture with LCM. Optimal section thickness was found to be 6-7µm, and sections had to be completely flat. Capture of 100-120 cells was achieved within 20 minutes of thawing the frozen section. RNA was successfully isolated with a commercially available kit. The presence of matrix components such as proteoglycans or agarose did not influence RNA isolation. Capillary electrophoresis revealed the isolated RNA to be of inferior quality. RNA from whole frozen sections was of slightly better quality compared to LCM samples. However, rtPCR showed specific target amplification. Relative abundances of GAPDH, MMP-3 and collagen II were within expected ranges. The use of random hexamer or poly-A primers for reverse transcription did not influence relative transcript abundances. In conclusion, RNA isolation from microscopic samples with and without LCM is feasible, and rtPCR is a possible method to investigate gene expression in these samples.

Primary re-excision following unplanned resection of soft-tissue sarcomas in dogs

Author List: NJ Bacon, WS Dernell, N Ehrhart, BE Powers, SJ Withrow

Abstract:
Unplanned or inadequate surgery of soft-tissue sarcomas typically leaves residual microscopic malignant cells in the wound bed, and local recurrence rate is high. Primary re-excision (PRE) is the wide resection of the scar in these patients, aiming to remove all remnants of tumor and so avoiding further local treatment, in particular radiation therapy. The success of PRE for soft-tissue sarcomas in dogs is unreported. The significance of finding tumor cells in the resected specimens is also unknown - in humans conflicting studies have shown it to be both a negative prognostic indicator for recurrence or to be unimportant. This study aimed to determine the presence and significance of residual disease in resected scars; to assess the effectiveness of PRE in terms of local recurrence; and to assess patient-, disease-, and treatment-related factors for survival prognosis. 39 dogs that presented to the CSU-VTH (1999-2004) with incompletely resected soft-tissue sarcomas were retrospectively studied long-term. All were treated with curative intent re-resection of the scar. 6/39 (15%) dogs suffered a local recurrence following PRE. Median time to recurrence was 144 days. 4/39 (10%) died of pulmonary metastatic disease. No correlation (by Log Rank Analysis) was found between age, sex, breed, tumor size, tumor grade, anatomical site, duration before first surgery, extent of first surgery, interval between surgeries, PRE margin taken, and presence of tumor in resected tissue, and local recurrence. A significant correlation between tumor type and local recurrence was found, with a greater risk seen for both lipo- and fibrosarcomas. A significant correlation between tumor recurrence and decreased patient survival was identified.

Session II

Oral Presentations

Veterinary Students
PVM II

In Vitro Amplification of CWD PrPres in Deer and Ferrets

Author List: TD Kurt, MR Perrott, CJ Wilusz, J Wilusz, S Supattapone, EA Hoover

Abstract:
Chronic wasting disease (CWD) of cervids is a prion disease increasing in prevalence and geographic distribution in the US. A central event in the transmission of prion diseases is conversion of the normal prion protein, PrP^{c}, to its protease-resistant conformer, PrP^{res}. To determine whether the protease-resistant prion protein of CWD (PrP^{CWD}) can be amplified *in vitro*, thereby increasing sensitivity of detection, we used a non-denaturing amplification protocol and brain tissue from either deer (*Odocoileus* spp.), the native host species, or ferrets (*Mustelidae putorius futo)*, a species susceptible to CWD (1), as sources to amplify PrP^{CWD}. We obtained PrP^{CWD} amplification of up to 10-fold without cyclic sonication. Moreover, the efficient in vitro amplification of PrP^{CWD} in normal ferret brain substrate is consonant with the trans-species transmission and relatively short incubation period of CWD in ferrets vs. deer *in vivo*. *In vitro* amplification of PrP^{CWD} in both deer and ferret brain was inhibited by degradation of single-stranded RNA molecules, consistent with similar findings in the hamster scrapie system and suggesting that host co-factor molecules may also be needed for PrP^{CWD} conversion. These results support the use of *in vitro* amplification to investigate species barriers, mode of transmission, and pathogenesis of chronic wasting disease.

A multiple dye staining technique to evaluate change in metacarpophalangeal joint contact area under load

Author List: KL Easton, CE Kawcak.

Abstract:
Osteochondral disease is a chronic fatigue injury in racehorses that can result in irreversible joint damage and catastrophic failure. Previous research has shown that in the metacarpophalangeal joint (MCP) the subchondral density pattern in the third metacarpal condyle may dictate the type of injury that results. Some horses show a sharp density gradient at the site of third metacarpal condylar fractures, and others do not. Therefore, the site and type of injury may be related to how the joint surfaces articulate. The objective of this study, therefore, was to develop a method of assessing the change in joint surface contact area with loading. Six equine forelimbs were loaded on a materials testing system (MTS) until the MCP was at 150° extension. The MCP was then injected with safranin-O. The dye was flushed and the joint was then loaded to 120° extension and injected with toluidine blue. After flushing, the limb was disarticulated and the third metacarpal condyle was removed and digitized using a Microscribe G2 and ProEngineer. Computed tomography (CT) data were obtained for 5 of these limbs before the MTS testing. There was a significant increase in contact area ($p=0.007$) from 63% ± 9.2% at 150° to 77% ± 1.0% at 120°. Approximately 46% of the contact was with the proximal sesamoid bones and ~53% was with the proximal phalanx at both loads. Areas of contact appeared to have a higher density as shown on the CT scans. Further studies and analysis investigating load and its correlation to subchondral bone density are warranted. The results from this study show that the multiple dye staining technique is a valid method for assessing change in contact area under different loads. This method can be used to evaluate clinically useful methods such as magnetic resonance imaging for determining contact area with the ultimate goal of ascertaining a technique for determining a horse's predisposition to injury based on the loading characteristics of the joint.

Epidemiological Study of Adult Dairy Cow Removals on a Colorado Dairy Farm

Author List: A Hurwitch, J Severidt, F Garry, J Lombard.

Abstract:
Dairy cow mortality represents a serious economic loss for dairy farmers. It is estimated that mortality rates are as high as 4-12% nationwide and cull rates are approximately 20-30% nationwide. The objective of this study was to evaluate removal rates on a modern dairy and determine any health or production factors that precluded an increased association with removal from the milking herd. Cows were selected from a 1300 cow free stall dairy in Colorado. Serology samples were collected from cows 3-5 days in milk. The cows were followed for 100 days and subsequent health events such as mastitis, metritis and lameness were recorded. There were 1093 cows enrolled in the study that freshened between March and November 2005. 3% of the cows died (34/1093), 9% (97/1093) of the cows were sold within 100 days in milk. First calf heifers were 41.7% (456/1093) of the fresh cows during the study. Of the 456 first calf heifers enrolled, 11 (2.4%) died and 32 (7%) were sold. Analysis of the health events showed that 18% (337/1839) of the health events were mastitis, 6% (122/1839) were metritis, 4.2% (79/1839) were pneumonia and 3.9% (72/1839) were retained placentas. Serum Chemistry panels were evaluated for ten cows that died within ten days of parturition, ten cohorts that remained in the herd for at least 100 days were selected and matched for lactation and calving date. Analysis was done using logistic regression. The serum panels indicated that all cows had at least one result that was outside the normal range. CK and AST showed increased levels in the cows that died and were statistically associated with death. This data indicates that certain health events may be related to a higher risk of culling or death in a dairy herd.

Evaluation of fecal culture pooling methods for detection of Mycobacterium paratuberculosis in a beef herd

Author List: SM Jensen, JE Lombard, FB Garry.

Abstract:
Due to the substantial cost of whole herd fecal culture for detection of Mycobacterium paratuberculosis (M. paratb) infection, studies evaluating fecal pooling in dairy cattle have been conducted. The objective of this study was to compare the use of individual fecal samples, strategically pooled samples, and collection order pooled samples for detecting M. paratb infected animals within a beef herd. Whole herd sampling was performed on a 174 head beef herd previously evaluated for Johne's infection. Individual samples were collected and divided into 3 aliquots for individual testing, strategic pooling and ordered pooling. Cultures were performed concurrently via radiometric methods. Individuals were selected for strategic pools based on their ranked age whereas order pooled samples were based on order of collection. Each pool included 4-5 individual samples. Nineteen of the 174 individual samples, 6 of the 35 strategic pools, and 2 of the 35 ordered pools were culture positive for M. paratb. Four of the 6 strategic pools and 1 of the 2 ordered pools that were positive contained at least 1 of the 19 positive individual samples. Both individuals classified as heavy shedders were detected by strategic pooling, while only 1 heavy shedder was detected by ordered pooling. Of the positive pools, 2 strategic pools and 1 ordered pool contained no samples found to be positive on individual culture. One pool in each pooling method was found to contain 2 positive individuals.The results of this preliminary beef study suggest that bacteriologic culture of strategically pooled samples may provide a more reliable method for detection of M. paratb infected animals compared to ordered pooling. However infected individuals remain unidentified because sample pooling results in decreased sensitivity compared to individual culture.

IgE and inflammatory responses in the lavage fluid and peripheral circulation in guinea pigs infected with Mycobacterium tuberculosis.

Author List: LM Queer, M Ostmeyer, CA Shanley, EE Smith, IA Orme and RJ Basaraba

Abstract:
The guinea pig (*Cavy porcellus*) is highly susceptible to human isolates of *Mycobacterium tuberculosis* (Mtb) and develops lesions similar to that of humans following aerosol exposure. The pulmonary and extra-pulmonary granulomas consist of coalescing foci of mixed mononuclear inflammatory cells and fewer granulocytes, that progress to necrosis and mineralization. In the guinea pig model, eosinophils are a prominent cell type that accumulate in foci of necrosis as well as fill and obstruct the lumens of small airways. Elevated airway eosinophilic inflammation and elevated IgE levels are characteristic of airway inflammatory diseases such as asthma that are predominated by Th2 type immune response. We hypothesized that eosinophils and total IgE levels would increase in the bronchoalveolar lavage (BAL) fluid and peripheral circulation in guinea pigs following aerosol infection with Mtb. In addition we investigated whether vaccination with BCG would prevent or delay eosinophilic inflammation and elevated serum IgE levels. Total and differential cell counts in BAL and blood were compared between non-vaccinated and BCG vaccinated guinea pigs from 5 to 60 days post infection. Total IgE levels in BAL and serum were measured by ELISA. The increases in eosinophils in blood and BAL over the course of Mtb infection were not different from non-infected controls. There was however, a significant increase in serum total IgE that was delayed by BCG vaccination. There was a significant increase in heterophils in the peripheral circulation, as well as BAL that was significantly suppressed by BCG vaccination. We concluded that the increase in eosinophils seen histologically in Mtb infected guinea pigs was not reflected in the BAL or peripheral circulation. However the progressive increase in serum total IgE levels may reflect an inappropriate Th2 immune response to Mtb that warrants further investigation.

Poster Presentations

Veterinary Students

Craniofacial and axial skeletal defects in a mouse mutant with a p53 binding protein mutation.

Author List: Erin Quist1*, Qing Yu1, Amanda Boyce2, Gina Jiang2, Cheryl Gray1, Charles Kauffman1, Bishwanath Chatterjee1, Rocky Tuan2, and Cecilia Lo1

Abstract:
We recovered a recession mutation from a N-ethyl-N-nitrosourea mouse mutagenesis screen that exhibited phenotypes very similar to those seen in humans with velo-cardio-facial/DiGeorge syndrome (VCFS/DGS). Homozygous mutants exhibit heart outflow tract septation defects, thymus hypoplasia and craniofacial anomalies. In addition, we also observed axial skeletal and limb anomalies. This mutation was mapped to mouse chromosome 2, and more recently, we identified the mutation as a missense mutation in the p53 binding protein, TRP53bp1, a protein known to have an important role in DNA repair and mitotic check point control. To examine the nature of the craniofacial and skeletal anomalies, we prepared newborn offspring from this ENU mutant line for Alizarin red and Alcian blue staining. These studies showed that mutants had VCFS/DGS craniofacial abnormalities such as micrognathia, cleft palate, blunted nasal bones and broad nasal bridge. In addition, mutants had fused ribs and vertebrae, scoliosis, kyphosis, dwarfism and limb dysplasia. These findings suggest that TRP53bp1 may play a significant role in the patterning and developmental regulation of membranous and endochondral bone ossifications, consistent with recent studies that suggest p53 can regulate differentiation of osteoblasts. Observations from this novel ENU induced TRP53bp1 mutation represent the first evidence indicating an important role for TRP53bp1 and the p53 cell signaling pathway in regulating patterning and development of the mammalian embryo.

Detection of prion protein (PrpCWD) in deer urine using methanol precipitation.

Author List: C Welch, G Eliason, CK Mathiason, E Hoover

Abstract:
Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy (TSE) that affects captive and free-ranging deer and elk. Endemic to areas of Colorado and Wyoming, CWD has recently been detected in several states within the Western and Mid-Western regions of the United States and in Canada. TSEs, such as scrapie in sheep and goats, Bovine Spongiform Encephalopathy (BSE) in cattle and Creutzfeldt-Jakob Disease (CJD) in humans, are prion diseases, characterized by the accumulation of abnormal protease resistant prion protein (Prpres) in the brain. Although the method of transmission of CWD is not well understood, it is thought to be transmitted horizontally, most likely through environmental contamination with excreta from affected animals. Current diagnostic testing for CWD in cervid species requires ante mortem tonsilar biopsy or post mortem testing of brain or lymphatic tissue. The development of a pre-clinical diagnostic tool for detecting prion protein in bodily fluids, such as urine, blood or saliva would allow for earlier detection of all TSE diseases. Previous studies in humans with CJD and scrapie sick hamsters have shown that the aberrant prion protein may be present in urine. To date, it is unknown whether this abnormal prion protein is present in deer urine, blood or saliva. A technique has been developed using methanol precipitation and western blotting to recover prion protein from deer urine to which a known amount of CWD-infected brain tissue has been spiked. Further testing will incorporate the concentration and precipitation of CWD positive deer and elk urine. Once developed, this technique will be adapted to detect PrpCWD in deer and elk saliva, blood and other bodily fluids.

The affect of grain intake on blood pH, electrolytes and parathyroid hormone in fit and sedentary horses

Author List: Mayo JM, Enns RM, MacLeay JM

Abstract:
This study examined the impact of grain intake on blood pH, electrolytes and parathyroid hormone (PTH) in racehorses (RH) and sedentary horses (SH). High dietary acid load (HDAL) has been associated with metabolic acidosis and accelerated bone loss in many species. RH are fed grain-rich diets which represent a HDAL which decreases blood pH and may result in calcium mobilization from bone as a buffer. HDAL may hinder appropriate bone development predisposing RH to injury. 39 RH and 11 SH were used. Data collected included grain intake (kg), work status, age, gender, breed, blood pH, iCa, HCO_3, TCO_2, BE, BEecf, K, Cl, and PTH. Grain intake was not significantly associated with lower pH, but lower mean pH and increased HCO_3 and TCO_2 were observed. Significant correlations between high iCa and low PTH, and iCa and K were observed. Significant differences were found between RH and SH in iCa, BE, BEecf, HCO_3 and TCO_2. A tendency was seen for pH to decrease and serum Cl to increase with increasing age. Thoroughbreds had significantly lower K than Quarter Horses, and mares tended to have higher K than geldings. Results of this study suggest that grain-induced acidosis might be offset by exercise-induced alkalosis as suggested by higher pH after exercise and differences in iCa, BE, BEecf, HCO_3 and TCO_2 between RH and SH. Persistent decrease in iCa in RH compared to SH is also suggested.

MOLECULAR CLONING OF ZEBRAFISH CALCIUM CHANNEL B4 SUBUNIT SPLICE VARIANTS

Author List: TN Reynolds, AM Ebert, MD Terry, N Iverson, DM Garrity, WA Horne.

Abstract:
Calcium channel gating allows for precise regulation of calcium influx at nerve terminals and play a major role in regulating neurotransmitter release. Voltage-gated calcium channels consist of at least four subunits (α_1, α_2/δ, β, and γ). Traditionally, the major function of the β subunits (four subtypes, β_1- β_4) have been thought to be regulating gating kinetics and voltage sensitivity of the pore-forming α_1 subunit. Recently, the β subunit has been shown to resemble the membrane-associated guanylate-kinase (MAGUK) family of proteins that serve as scaffolding proteins and regulate a variety of protein-protein interactions. Mutations of the β_4 subunit have been associated with juvenile myoclonic epilepsy, generalized epilepsy, ataxia, and dyskinesia epilepsy in humans and therefore have been the focus of our research studies. Our laboratory has isolated several alternatively spliced variants of the β_4 subunit from human nervous tissue; these variants have differentiable effects on channel gating. As an extension of our studies with human calcium channel genes, the purpose of this project was to characterize the β_4 subunit genes from zebrafish (*Danio rerio*). Zebrafish serve as an excellent model system for the study of nervous system development and genetics. Zebrafish cDNA was obtained from A. Srinivasan (Harvard University). The β_4 sequences were amplified using RACE-PCR and subcloned into a pCR2.1 TA cloning plasmid (Invitrogen) for sequencing. Sequence results show that two β_4 subunit N-terminal splice variants (β_{4a} and β_{4b}) are expressed in zebrafish and are highly homologous to the proteins found in human and mouse cerebellum. Cloned sequences of each splice variant will be used for in situ hybridization experiments throughout zebrafish development to determine temporal and tissue specific expression as well as for electrophysiological analysis using the *Xenopus* oocyte transient expression system. Supported by Merck-Merial and NIH grant RO1 NS42600 to WAH.

Inhibition of Naïve T Cell Th1/Th2 Differentiation by CD4+CD25+ Regulatory T Cells In Vitro

Author List: MS Spoor, GL Stephens, and EM Shevach

Abstract:
While CD4+CD25+ regulatory T cells (Tregs) are widely believed to play an essential role in attenuating immune responses in vivo, relatively little is known regarding the targets of their suppressive abilities. Although the function of Treg is most often evaluated in terms of their ability to inhibit responder T cell proliferation in co-cultures, evidence suggests that Tregs do not inhibit the proliferation of naive T cells in vivo. Mounting in vivo evidence suggests that Tregs primarily function by inhibiting the differentiation of CD4 and CD8 T cells into effectors. Based on these in vivo observations, we have evaluated an alternative and potentially more accurate way to assay Treg function in vitro. By measuring effector cytokine production under optimal stimulation/polarizing conditions, we find that Tregs efficiently inhibit the differentiation of naive CD4 T cells into TH1 or TH2 effectors, while not appreciably affecting their expansion, as is seen in vivo. In similar experiments, we find that Tregs inhibited both the acquisition of effector function and the expansion of CD8 T cells. However, in contrast to Th cells, the addition of exogenous IL-2 to co-cultures restored CD8 T cell effector function, while proliferation was still measurably diminished. These experiments demonstrate that Tregs can directly affect the acquisition of effector function by CD4 T cells and CD8 T cells in vitro, and thus, suggest that measurement of T cell differentiation may be a reliable alternative assay for measuring Treg function, which closely resembles the known consequences of suppression in vivo.

Efficacy of Daily Rectal Temperature Monitoring vs. Visual Observation of Dairy Cows for Detection of Post-Partum Disease

Author List: SE Dobberstein, SM Scott, JR Wenz, W Wailes

Abstract:
Post-parturient cows are highly susceptible to disease, which may result in decreased milk production, death, and culling. Early disease detection increases cure rates and minimizes production losses. Rectal temperature monitoring (RTM) for the first 10 days in milk (DIM) has been advocated as a critical management tool, however, there are no studies evaluating the efficacy of this labor-intensive program. This study compares the efficacy of visual observation (VO) to RTM for detection of post-partum disease. Rectal temperature and milk production was recorded for the first 10 and 30 DIM respectively of 208 Holstein cows. Cows with temperatures >103.0ºF (FEVER) were further examined for metritis (MET). Health events (HEVNT) diagnosed by dairy staff using VO, calving ease, 5-25 DIM production (5-25dMLK) and first service conception rate (CR1) were also recorded. During their first 10 DIM, 39% of cows had a fever. More cows with dystocia (DYS) had FEVER (50%) than those without (34%) (p=0.0238). Of cows with DYS, 25% had a fever that peaked at 4 DIM, as compared to cows without DYS (15% had a fever that peaked at 3 DIM). Of 79 cows with FEVER, 55% had MET. Thirty-six cows (29%) with no HEVNT had FEVER and half of them had MET undetected by VO. Cows at 30 DIM with FEVER and no HEVNT produced 243 lbs less milk than those without FEVER (p=0.0983). Furthermore, of cows with no HEVNT those with FEVER and a discharge consistent with MET had a lower CR1 (26%) than those without FEVER (35% CR1) and discharge (p=0.0372). These results suggest FEVER identified by RTM is commonly associated with MET. Therefore, disease undetected by VO but identified by RTM results in significant economic losses through decreased milk production and reproductive efficiency. Rectal temperature monitoring is more effective than visual observation for detection of post-partum disease in dairy cows and could significantly improve the health and productivity of post-partum dairy cows.

Histone Deacetylase Inhibition to Increase Osteosarcoma Chemosensitivity

Author List: LA Bisson, S Dreitz, B Rose, DH Thamm

Abstract:
Osteosarcoma (OSA) is characterized by both local tissue destruction and a high metastatic rate. Novel therapies are needed. Histone acetylation, controlled by histone acetyltransferases and histone deacetylases (HDACs), is important for governing chromatin structure and may be key in regulating gene expression associated with cellular proliferation, differentiation and survival. One inexpensive, available HDAC inhibitor is the antiepileptic agent valproic acid (VPA). VPA has been shown to inhibit proliferation and increase chemotherapy and radiation sensitivity in human and mouse cancer models, but its effect on OSA is unknown. The goal of this study was to determine the antiproliferative and chemosensitizing effects of VPA against OSA cells. D17 canine OSA cells were exposed to VPA +/- the antineoplastic drug doxorubicin (DOX) in various doses and schedules. Relative viable cell number was then determined using a tetrazolium-based colorimetric assay (MTS). Apoptosis induction was assessed by Annexin V-FITC staining and flow cytometry. The concentration of VPA necessary to inhibit cell growth by 50% (IC_{50}) for a 72-hour exposure was between 2.3 and 5.7mM. Clinically achievable concentrations of VPA (0.5 and 1 mM) resulted in a minimal reduction in proliferation, however coincubation of D17 cells with DOX and 0.5 or 1 mM VPA for 72 hours profoundly reduced the IC_{50} of DOX. Preincubation of D17 cells with 0.5 and 1 mM VPA for 48 hours, followed by a 4-hour DOX exposure also potently reduced the DOX IC_{50}. Preincubation with VPA also enhanced DOX-induced apoptosis. Western analysis revealed that 1 mM VPA treatment increased acetylation of the histone protein H3. These observations suggest that histone deacetylase inhibition with VPA, at clinically achievable concentrations, is capable of potentiating the antiproliferative and pro-apoptotic effects of DOX in canine OSA cells. [Supported by funds from the CSU Animal Cancer Center and a Merck-Merial Research Scholarship.]

PREVELENCE OF SELECT INFECTIOUS DISEASE IN CATS FROM ARIZONA

Author List: JM Eberhardt, MR Lappin, K Neal, T Shackelford

Abstract:
A number of vector borne infectious diseases cause morbidity and mortality in the domestic feline, with some infectious agents being of zoonotic concern. An *Ehrlichia canis*-like organism is known to infect cats but documented cases are minimal. Because *E. canis* infection is common in dogs in Arizona (12% seroprevalence rate), we hypothesized that it would be a logical state to perform a prevalence study in order to obtain more *E. canis* infected cats to study. The objective of this study was to determine the prevalence of *Ehrlichia ssp*., *Anaplasma phagocytophilum, Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum', and *Bartonella ssp.*, DNA in blood of cats in Arizona. Between March 2004 to July 2004, blood was collected from feral and relinquished cats in Phoenix and Nogales, AZ, placed into EDTA tubes, and stored at -20ºC. The samples were shipped on cold packs to Colorado State University and stored at -20ºC until assayed. Previously published PCR assays for amplification of DNA of *Ehrlichia spp.*, *A. phagocytophilum*, *Neorickettsia risticii*, *M. haemofelis,* '*Candidatus* M. haemominutum', and *Bartonella spp* were utilized. DNA from one or more of the organisms was amplified from 31 of 112 blood samples (27.7%). DNA consistent with *B. clarridgeiae* 15 (13.4%), *B. henselae* 14 (12.5%), '*Candidatus* M. haemominutum' 9 (8.0%), and *M. haemofelis* 5 (4.5%) were detected. DNA of *Ehrlichia spp.*, *N. risticii*, or *A. phagocytophilum* was not amplified from the blood of any cat. Our results indicate that cats from these regions of Arizona are exposed to *M. haemofelis,* '*Candidatus* M. haemominutum', *B. henselae,* and *B. clarridgeiae*. Failure to amplify DNA of *A. phagocytophilum* may relate to that the tick vector, *Ixodes*

pacificus, is not known to be present in these regions of Arizona. Failure to amplify DNA of *Ehrlichia* spp. suggests that cats were not exposed, exposed but not infected, or infected but the DNA was not detected by the PCR assay used in this study.

Impact of prevalence of Escherichia coli O157 on the prevalence of Salmonella in feedlot beef cattle immediately prior to shipping and after lairage at the abattoir.

Author List: EL Pedersen, G Patterson, G Dewell

Abstract:
Establishing the presence or absence of a pattern in prevalence changes between Escherchia coli O157 and Salmonella during shipping and lairage may help identify critical control points for cleaning lairage environments and shipping trucks. Our focus was to identify and characterize such a relationship.1752 pen samples including environmental, fecal, and hide swabs, were collected from 40 feedlots from Colorado and Nebraska immediately before shipping to various slaughter plants. 1898 corresponding samples were collected at the plant after those cattle were processed. Each sample was tested for E. coli O157 and Salmonella. E. coli O157 was identified by immunomagnetic separation, culture, and latex agglutination and later confirmed by PCR. Samples were also enriched for Salmonella growth, cultured, and identified with latex agglutination, then sent to NVSL at Ames, Iowa for confirmation and serotyping. After initial identification, 10.3 %, 19.6% and 2.2% of feedlot environmental samples were positive for E. coli O157, Salmonella, and both organisms, respectively. 10.5 %, 6.9% and 0.6% of feedlot hide samples and 6.8%, 4.7%, and 0% of feedlot fecal samples were positive for E. coli O157, Salmonella, and both organisms respectively. At the abattoir, 26.6%, 18.9%, and 4.2% of environmental samples tested positive for E. coli O157, Salmonella, and both organisms, respectively. 37.0%, 19.8%, and 6.2 % of hide samples and 7.8%, 6.1%, and 0.5% of fecal samples tested positive for E. coli, Salmonella, and both organisms. These data indicate a slight increase in shedding of both organisms after shipping and lairage, but a clear relationship between the two is not identified. Additional comparisons will be made once the database is complete.

Antibody Responses to West Nile Virus in Vaccinated and Unvaccinated Pronghorn

Author List: SD Hill, NA Wimmer, MD Salman, DS Miller, J Santaella,

Abstract:
The effects of West Nile Virus (**WNV**) in pronghorn (*Antilocapra americana*) is unknown. In May 2004, nine pronghorn less than 2 days of age were obtained from Lakeview, Oregon for research. Oregon was considered WNV negative at this time. Six of the Oregon pronghorn were randomly selected to be vaccinated in a double blind study for WNV using a commercially produced killed vaccine for horses on Day 0 (7-9 days of age) and day 42. The remaining three received saline injections as controls. Blood samples were taken prior to vaccination administration and then weekly for ELISA WNV antibody quantification for 11 weeks. Another group of seven pronghorn, between 1-3 days of age, were obtained from Colorado in June 2004. Colorado was considered WNV positive at this time. Six of the Colorado pronghorn were in twin pairs from non-vaccinated domestic pronghorn. These domestic pronghorn have been supervised by a veterinarian and have never shown clinical signs of WNV infection. The seventh Colorado pronghorn was orphaned in the wild. The Colorado origin animals were never vaccinated. Blood samples were taken on day 78 (8-12 weeks of age), and 148 for ELISA WNV antibody quantification. Initially the group of pronghorn from Oregon had negative antibody titers and all of these animals developed a positive antibody titer after the vaccine booster. The control animals from the Oregon group did not develop a positive titer. The entire Colorado group had positive titers despite never being vaccinated. This preliminary research is suggestive that pronghorn can develop an antibody response to a killed WNV vaccine. It is also suggestive that pronghorn can become infected with WNV without developing clinical disease, and that does are able to pass WNV antibodies in their colostrum to their young.

Regional Metabolic Heterogeneity of Canine Spontaneous Tumors Using 1H and 31P Nuclear Magnetic Resonance

Author List: S Kraft, HL Tuttle, and N Serkova.

Abstract:
Metabolomics has received recent attention with its potential use in further understanding cancer pathophysiology and potential treatments. Nuclear magnetic resonance (NMR) provides a spectral display of the metabolite concentrations of tumor cells both *in vivo* and *ex vivo*. Although it is now widely accepted that cancer cells have a very different cellular metabolism and glucose uptake from normal cells, there is little research on this phenomenon in canine cancer cells. In this study we investigated tumor metabolic concentrations relevant to malignancy from canine spontaneously occurring tumors. Our focus was to evaluate any regional differences biochemically using NMR, since physiological differences have been demonstrated between tumor peripheries and their centers, as well as to determine what metabolic markers are present in canine tumors. Our hypothesis is that the peripheral tumor is characterized by "aggressive" tumor markers to a greater degree than the central (often necrotic) tumor region. To test this hypothesis, we collected *in vivo* samples from dogs of either sex, any breed, any age, and having any tumor type, excluding lipomas. Each sample was collected using a punch biopsy, and was snap frozen in liquid nitrogen. A dual chloroform/methanol extraction was performed, and each sample was analyzed using proton and phosphorus NMR. Those data were then statistically evaluated for regional differences and the influence of tumor size and type. Due to the large biological variation from this sample group, as well as the relatively small sample size, no significant differences were demonstrated between tumor geography or differences in metabolite concentration in varying malignancies. Future studies using larger sample sizes and less variation may provide further insight into metabolite correlations, and may later also be directed towards comparison and validation of *in vivo* MR spectroscopy results with those obtained from *ex vivo* NMR.

The effects of super-enriched environments on murine immunology, health, and behavior

Author List: EK Hutchinson, A Avery, S VandeWoude

Abstract:
Laboratories have come under increasing pressure to focus on the welfare of the mice and rats in their care. The subsequent push to enhance rodent enclosures has outpaced the growing body of research examining the effects of different environmental enrichment (EE) schemes. A previous study found that female mice that were super-enriched, i.e. housed with two or more different EE devices, experienced significant thymic atrophy. This is likely a result of stress induced corticosterone release, suggesting a super-enriched environment is more stressful for certain mice. Animals housed in super-enriched enclosures also had greater variability than un-enriched controls in nearly every parameter examined. The current study was initiated to verify these results, and explore the effects of super-enrichment on a variety of additional parameters, including general health, behavior, and cognition. After being raised and weaned in enclosures supplied with cardboard tubes and paper nesting material, twenty female Balb/c mice were transferred into two groups, one with no enrichment materials and one with a plastic shelter, a cotton nestlet, and two chew balls. Over the next 6 weeks, the animals were tested biweekly in a T-maze alternation task, subjected to a novel object situation, and observed in their home cages for aggression or stereotypic behaviors. At the beginning and end of the study, mice were weighed, examined for skin or coat lesions, and given ophthalmic exams. In week two, half the mice from each condition were inoculated with ovalbumin, and these mice were again boosted in week five. At the conclusion of the study, thymuses were weighed and thymocyte populations characterized by flow cytometry, and histologic samples of ophthalmic structures examined for signs of inflammation. The outcome of this study will provide baseline data for further tests examining untoward or positive effects of environmental enrichment on mice used in biomedical research.

Impact of an alternative injectible colostral supplement on morbidity and mortality of preweaned dairy calves with failure of passive transfer.

Author List: C Anderson, JR Wenz

Abstract:
The most important management issue for neonatal dairy calves is failure of passive transfer (FPT) of immunity from the dam. Because dairy calves routinely never nurse, they can be given dehydrated or frozen colostrums as their first meals. Increasingly, dairy calves are transported off-farm in their first few days of life, which can interfere with their receiving an appropriate volume of high-quality colostrums. Because bull calves are not as valuable as heifer calves, we used bull calves in this study under the assumption they might have a higher rate of FPT. The objective of this study was to evaluate the effectiveness of an injectible IgG supplement (IIgG), on the health of dairy calves with failure of passive transfer. Whole blood was collected from one hundred-eighty 2-day-old calves. One-hundred bull calves with serum total protein 5.0 or less were enrolled into 2 cohort groups. Treated (TX) calves recieved three 25-ml injections of IIgG subcutaneously at 12-hour intervals. Control (CON) calves received no treatment. Morbidity (diarrhea, pneumonia) and mortality were evaluated up to weaning at 60-days of age. Average serum total protein was 4.05 mg/dl and 4.16 mg/dl in the TX and CON groups, respectively. The percent morbidity in the TX group was 60% (28% had diarrhea and 32% pneumonia). In the CON group, there was a 46% occurrence of morbidity (14% diarrhea and 32% pneumonia). Mortality in the TX and CON groups was 18% and 14%, respectively. Of the TX calves that died, 67% had been medicated for a health event. In the CON group, that figures was 43%. IIgG appeared to have no effect on decreasing morbidity or mortality in the "treated" calves. The results of this study suggest a popular extra-label use of an injectible, bovine colostrums IgG product does not improve health or survivability of dairy calves with FPT.

Characterization of Toll-like Receptor Ligand Responses in Bone Marrow-derived Feline Dendritic Cells

Author List: JA Campbell, TL Lehman, KP O'Halloran, PR Avery

Abstract:
As professional antigen presenting cells in peripheral tissues, dendritic cells (DC) play a critical role in the detection of pathogens as well as induction of the acquired immune response via stimulation of T-cells in the regional lymph nodes. The interaction of DC with T-cells highlights their potential importance in the pathogenesis of viral infections such as Feline Immunodeficiency Virus (FIV). Dendritic cells express toll-like receptors (TLR) that are activators of the innate immune system, recognizing conserved molecular patterns shared by particular pathogens. This study attempts to characterize the toll-like receptor profile of feline dendritic cells in uninfected cats, as well as the cytokine production induced by incubation of DC with various TLR ligands. The expression of TLR mRNA was quantified using real-time PCR in DC from naïve cats. Additionally, DC from naïve and infected cats were stimulated for 6 hours with either Poly(I:C), LPS, or Loxoribine, and rt-PCR was used to detect mRNA levels of cytokines interleukin-12 (IL-12), IL-10, IL-6, interferon-alpha(IFNα), and tumor necrosis factor-alpha(TNFα) in a relative quantitation assay. We hypothesized that stimulation of feline DC with TLR ligands will induce characteristic cytokine profiles when compared to unstimulated DC, similar to results seen in human DC. Quantification of the TLR profile of naïve DC showed marked expression of TLR 2, moderate expression of TLRs 4,7,and 8, and minimal expression of TLRs 3, 5, 6, and 9. Stimulation with all three ligands produced characteristic cytokine profiles in uninfected cats, and FIV infected cats had no significant difference in their ability to respond to stimulation. In LPS stimulated naïve and positive cats, FIV positive cats have a significantly greater production of IL-10 relative to IL-12 than naïve cats. More study is necessary to clarify the role of cell signaling pathways affecting IL-12/IL-10 production in infected versus naïve animals.

CROSS-SPECIES TRANSMISSION OF LENTIVIRUS AMONG FELIDS IN SOUTHERN CALIFORNIA

Author List: Samuel P. Franklin,1 Julie A. TerWee,1 Jennifer L. Troyer,1 Lisa Lyren,2 Kevin R. Crooks,3 Christine V. Fiorello,4 Roland W. Kays,5 Walter Boyce,6 Seth Riley,7 Sue VandeWoude1

Abstract:
Wildlife disease has become an increasing concern because of serious impact on endangered wildlife populations and the threat it poses to domestic animal and human health. There is a particular interest in understanding disease ecology in fragmented habitats where wildlife, domestic animals, and humans interact. We evaluated the prevalence and characteristics of lentivirus infection in bobcats (Lynx rufus) and pumas (Felis concolor) in three geographic locations in the Los Angeles metropolitan area. We found a relatively high seroprevalence of infection with rates ranging from 27-100% of study animals depending on the species and study site. Genetic sequencing and phylogenetic analysis of proviral fragments isolated from infected animals indicate that there are two strains of virus present in these populations. One strain has been reported in pumas elsewhere in western North America and was the infective agent in approximately half of the infected pumas in this study. The other strain of virus was isolated from the other half of infected pumas and all infected bobcats from which we were able to sequence virus. These data indicate that cross species transmission of virus has occurred between bobcats and pumas in southern California and is the first report of cross-species lentivirus transmission in non-captive felids. We hypothesize that habitat fragmentation may favor increased contact among bobcats and pumas leading to increased occurrence of infection and potential for cross-species transmission.

COMPARISON OF GROSS PATHOLOGIC, HISTOLOGIC, AND SUBCHONDRAL DENSITY CHANGES IN RACING HORSES

Author List: MG Shearin, CE Kawcak, RW Norrdin, RD Park, CM Les, CW McIlwraith

Abstract:
No direct comparison of quantitated subchondral bone density obtained using CT and histologic or gross pathologic changes have been made in horses. The goal of this study was to determine if subchondral bone density is associated with gross and histopathologic lesions of the distal third metacarpal condyle in racing horses, and that subchondral bone density can predict the presence and severity of lesions.

Metacarpophalangeal (MCP) joints were collected, CT scanned, scored for gross lesions and photographed from 9 racehorses. 4-mm thick sagittal and 30° palmar frontal plane section were cut, decalcified and stained with H&E from the distal third metacarpal bone. Microscopic osteochondral lesions and subchondral bone remodeling were scored on a scale of 0-3. Subchondral bone percent was measured. Lesion severity was the sum of all lesion scores in a section. Three-dimensional computer models of histologic sections were created, and mean density and mean pixel standard deviations (MPSD) measured. Pearson's correlation coefficients were calculated. A split-split plot analysis was also performed with a backwards selection method (α=0.10). Pairwise comparisons for significant variables was performed using Fisher's least significant differences test.

Mean CT density was not a good predictor of lesion severity or number of lesions, nor was there a good correlation with lesion severity or number of lesions. Mean density was weakly correlated with gross score and SCBR, but was not predictive for gross score. There were good correlations of subchondral remodeling score with number of lesions and lesion severity. Subchondral remodeling score was a significant predictor of lesion severity and number of lesions. The present study supported the association of subchondral remodeling with the development of palmar lesions in racing horses. However, the results do not demonstrate a predictive relationship between mean density or MPSD and histologic lesions, but the study could be biased due to the small sample size and subsequently limited number of lesions.

Poster Presentations

Graduate Students
Post Doctoral

Establishment of chronic Mycobacterium abscessus infection in the gamma-interferon knockout mice

Author List: M Henao-Tamayo1, JTroudt1,X Bai3, M Gonzalez-Juarrero1, IM Orme1, and ED. Chan2,3,4, DJ Ordway1

Abstract:
M. abscessus pulmonary disease is an emerging clinical problem due, in part, to drug resistance, reinfection, and recrudescence. To develop a model to study pulmonary *M. abscessus* infection, gamma-interferon knockout (GKO) mice were infected with *M. abscessus* by low dose aerosol exposure. Analysis of cell accumulation in the lungs of these animals showed increases in CD4+ and CD8+ cells producing TNF-α, Gr1+CD11b+granulocytes and B220+Gr1+CD11c+ plasmacytoid dendritic cells as the infection slowly progressed.

GC/MS Analysis and Biological Assay for Endotoxins in Agricultural Dusts

Author List: R Saito, S Reynolds, B Cranmer, J Mehaffy, J Tessari

Abstract:
Endotoxins play an important role in occupational lung disease. However, occupational guidelines for endotoxins do not yet exist. Accurate measurement of endotoxin exposure is critical for establishing appropriate occupational guidelines and controls. The goal of this study is to determine the correlations between the results of GC/MS analysis (3-hydroxy fatty acids of lipopolysaccharide) and biological recombinant Factor C assays for endotoxins in different types of agricultural dusts. Samples for analysis were obtained from two tandem studies. Personal sampling was performed during an Endotoxin Genetic Study (EGS) and area sampling was performed during an Organic Dust Study (ODS). Among all dust types studied in this project, the grain dust has the lowest concentration of endotoxins per quantity of dust. The grain dust contains fewer endotoxins than the livestock dust in both GC/MS and biological assay results. However, the EGS found higher concentrations of endotoxins per volume of air than the ODS. Correlations between overall 3-OH FAs in picomole and assay results in EU vary by environment. The livestock dusts have a better correlation than grain dusts. For different dust types, different patterns of 3-OH FAs exist. The livestock dusts contain more variable 3-OH FAs than grain dusts. Multiple regressions were performed to evaluate relationship between assay and GC/MS results accounting for effects of individual 3-OH FAs at the same time. EGS feedlots have the highest correlation among all. For feedlots, C13, C14 and C18 3-OH FAs explain 96% of the variability in assay results. In general, good correlations exist between biological assay and GC/MS analysis. GC/MS is especially useful to identify important individual 3-OH FA components for endotoxins from various environments.

Using knock-in mice to determine the relevance of Prkdc(BALB) in BALB/c susceptibility to radiation-induced mammary carcinogenesis

Author List: KF Askin, S Bailey, M Weil, R Ullrich

Abstract:
Our lab has been using mouse models to study the effects of polymorphisms in DNA repair proteins on mammary cancer susceptibility. The BALB/c mouse strain is of interest because it has two single nucleotide polymorphisms in the coding region of the DNA repair gene, *Prkdc,* and may be responsible for the BALB/c's deficiency in DNA repair and is susceptible to radiation-induced mammary carcinogenesis. The purpose of this study is to analyze the consequences of the SNPs found in the BALB/c *Prkdc* gene by generating knock-in mouse strains that have one or both SNPs. Knock-in technology is valuable because the base substitutions will be specific and should not affect the rest of the genome. This approach will allow us to determine if one or both of the SNPs are responsible for the BALB/c's DNA repair deficiency and its sensitivity to mammary cancer.

Tax and pCREB Bind the KIX Domain of CBP/p300 at Two Distinct Sites

Author List: J Ramírez and JK Nyborg

Abstract:
The cellular transcription factor CREB participates in transcriptional activation from the human T-cell leukemia virus type 1 (HTLV-1) promoter mediated by the viral oncoprotein Tax. Both CREB and Tax bind to unique cAMP response elements, called viral CREs, located in the viral promoter. Stimulation of the cAMP pathway results in CREB phosphorylation at serine 133. This event facilitates transcription via the recruitment of the cellular coactivators CBP/p300. The interaction between KIX, a domain of CBP/p300, and phosphorylated CREB (pCREB) has been well-characterized. We have performed a variety of *in vitro* binding assays that indicate pCREB and KIX together dramatically enhance Tax binding to the viral CRE. KIX enhances Tax binding to the pCREB/viral CRE complex over 50-fold. Furthermore, efficient KIX recruitment to this complex is strongly dependent on CREB phosphorylation. These molecular events appear to be facilitated by the simultaneous binding of Tax and pCREB to two distinct surfaces on the KIX domain. This is supported by experiments with KIX mutants that block Tax incorporation into the complex, while pCREB binding remains unaffected. These results provide evidence for a model in which Tax and pCREB cooperatively bind distinct surfaces of KIX.

The DNA repair protein Rad51d plays a role in mammalian telomere function

Author List: AJ Williams (1), DL Pittman (2), RL Ullrich (1), SM Bailey (1)

Abstract:
Telomeres, the natural ends of linear eukaryotic chromosomes, consist of tandem arrays of repetitive G-rich DNA as well as a plethora of telomere-associated proteins. Functional telomeres are crucial for maintaining genomic instability, protecting chromosomal termini from degradation and recognition as double-strand break (DSB) ends, thereby preventing inappropriate triggering of damage response pathways. Recent studies have begun to reveal intriguing, unsuspected roles of DNA DSB repair proteins in telomere function. One such protein, Rad51d, commonly regarded as a homologous recombination (HR) repair protein, has also been shown to co-localize at telomeres. Mammalian cells deficient in Rad51d display an increased level of genomic instability. We are further characterizing the telomeric role of Rad51d in mouse cells deficient in Rad51d. Our cytogenetic approach utilizes Fluorescence In-Situ Hybridization (FISH) and Chromosome-Orientation FISH (CO-FISH), a strand-specific modification of standard FISH. The spectrum of chromosomal aberrations present in these cells will be determined and any telomere dysfunction identified. Additionally, CO-FISH analysis allows distinction between telomeres produced via leading- vs. lagging-strand synthesis, revealing any strand specificity for failure or involvement in aberrations. CO-FISH also facilitates investigation of recombination levels, both within genomic DNA (genomic sister chromatid exchange; G-SCE) and within the telomeric DNA itself (telomere sister chromatid exchange; T-SCE). Current results of this study will be presented and how they help shed light on the role of Rad51d at the telomere will be discussed.

WRN-Deficient cells exhibit unusually high rates of telomeric recombination

Author List: S Chang, EH Goodwin, RL Ullrich, SM Bailey

Abstract:
Werner syndrome is a rare autosomal recessive genetic disorder characterized by premature aging, elevated genomic instability, and increased cancer incidence. The WRN gene encodes a RecQ DNA helicase involved in DNA recombination, replication and repair. We have been investigating telomere dysfunction in a mouse model that is null for both Wrn and Terc (the telomerase RNA component) that elicits a classical Werner phenotype. Cells lacking active telomerase are occasionally able to escape senescence due to telomere shortening through an alternative (i.e., telomerase-independent; ALT) pathway that is thought to maintain telomere length via some form of inter- or intrachromosomal recombination. The most common form of intrachromosomal recombination in somatic mammalian cells is sister chromatid exchange (SCE). Due to the fact that standard cytogentetic methods lack the resolution to detect SCE's that occur within a few megabases of chromosomal termini, we utilized the strand-specific hybridization technique of CO-FISH (chromosome orientating-fluorescent in situ hybridization) in order to extend SCE analysis into telomeric DNA. Our results to date reveal that telomere sister chromatid exchange (T-SCE) occurs at unusually high rates compared to the genome as a whole, and that this rate is dependent on genotype. Late generation double knockout mouse cells (Wrn/Terc) exhibited heterogeneous telomere lengths (characteristic of ALT) and hyper recombination within the telomere proper in comparison to a WRN+/- control. Interestingly, throughout the remainder of the genome, including another block of highly repetitive sequence (pericentromeric mouse major satellite DNA) SCE's did not occur at elevated levels. In order to extend these observations, we are currently utilizing RNA interference (RNAi) to knockdown the expression of WRN (and BLM, another RecQ helicase) in telomerase negative normal human fibroblasts. Our results suggest the Wrn protein normally functions to repress inappropriate recombination specifically within telomeric DNA

Using an embryonic stem cell mutagenesis model to identify radiation sensitivity genes

Author List: T Sirisalee (1), FA Ray (1), MM Weil (1), JS Bedford (1), J Schimenti (2), RL Ullrich (1)

Abstract:
There is considerable uncertainty regarding the impact of genetically susceptible subpopulations on low dose risks. Much of this uncertainty relates to the number and nature of genes that might impact risks for radiation-induced cancer. To identify genes associated with radiation sensitivity, we are using a panel of mutagenized mouse embryonic stem (ES) cells to screen for radiation sensitive ES cell clones. This panel of mutagenized ES cell clones was developed using ethyl methanesulfonate (EMS) exposure of a population of ES cells at a dose of 400 ug/ml. This allows a high level of mutagenesis within the population while still permitting individual ES cell clones to be used to develop mice containing specific point mutations in virtually any gene. ES cell clones containing mutations that impact radiation sensitivity are being identified using an assay that examines growth of these clones under continuous low dose rate radiation exposure. This approach has previously been shown to facilitate the identification of radiation sensitive cell lines even under conditions where such sensitivity is not dramatic. Importantly, this assay is not limited to identification of genes in any specific pathway. Studies to date have determined the optimal dose rate at which ES cell clones can be properly screened for radiation sensitive clones. These preliminary studies will allow us to screen and isolate radiation sensitive ES cell clones in order to identify novel genes that are associated with radiation sensitivity and ultimately to determine their role in the development of radiation-induced cancer.

Comparative potential of Ae. triseriatus, Ae. albopictus, and Ae. aegypti to transovarially transmit La Crosse virus

Author List: MT Hughes, KL Reagan, J Gonzales, C Blair, & BJ Beaty

Abstract:
In this study, the vector competence and transovarial transmission amplification potential of *Aedes triseriatus, Aedes albopictus*, and *Aedes aegypti* for La Crosse virus (LAC virus) were determined. While *Ae. triseriatus* and *Ae. albopictus* mosquitoes were significantly more susceptible to oral infection than *Ae. aegypti* mosquitoes, the three species differed in oral and disseminated infection rates. Transovarial transmission rates (TOTR) and filial infection rates (FIR) were greater for *Ae. triseriatus* mosquitoes than either *Ae. albopictus* or *Ae. aegypti* mosquitoes. The relative rates for these characteristics were integrated into a single numerical score termed TOT amplification potential (TAP). Differences in TAP scores were due mainly to differences in DIRs and FIRs between these mosquitoes. The TAP score of *Ae. albopictus* mosquitoes, while lower than that of *Ae. triseriatus,* was found to be 10-fold greater than that determined for *Ae. aegypti*.

Characterization of 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (IspD) from Mycobacterium tuberculosis

Author List: HJ Eoh1, AC Brown2, L Buetow3, WN Hunter3, T Parish2, D Kaur1, PJ Brennan1, and DC Crick1

Abstract:
Many eubacteria, including *Mycobacterium tuberculosis,* utilize the 2-C-methyl-D-erythritol (MEP) pathway for the biosynthesis of IPP, a precursor of all isoprenoid compounds. This pathway is of interest as a source of potential drug targets since it is absent in mammals and disruption of the genes involved in *Escherichia coli* are lethal. In the MEP pathway, 4-diphosphocytidyl-2-C-methyl-D-erythritol is formed from MEP and cytidine 5'-triphosphate (CTP), which is catalyzed by a 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) synthase (IspD in *E. coli*). Blast searches show that the protein encoded by *Rv3582c* in *M. tuberculosis* has significant homology to *E. coli* IspD. *Rv3582c* was cloned and expressed and the purified protein is capable of catalyzing formation of CDP-ME from MEP and CTP. The enzyme is active between pH 6.0 - 9.0 with optimal activity at pH 8.0 and requires divalent cations. Rv3582c has *Km* values of 54 µM and 49 µM for MEP and CTP, respectively. The K*cat* and K*cat*/K*m* values were calculated to be 3.29×10^4 min^{-1} and 6.13×10^5 $M^{-1}min^{-1}$ and 4.28×10^4 min^{-1} and 8.66×10^5 $M^{-1}min^{-1}$ for MEP and CTP, respectively. Allelic disruption of *Rv3582c* in *M. tuberculosis* provided direct evidence that the protein encoded by this gene is essential for *M. tuberculosis* survival. Thus, *Rv3582c* encodes a functional IspD in *M. tuberculosis,* and provides a new target for the development of novel anti-tuberculosis drugs.

Effect of an Enteroccocus faecium (SF68) Enhanced Diet on Immune Responses to a Feline Herpesvirus 1, Feline Calicivirus, and Panleukopenia Vaccine in Cats

Author List: JK Veir, R Knorr, C Cavadini, SJ Sherrill, J Benyacoub, E Satyaraj, MR Lappin

Abstract:
Objective- To evaluate the effect of supplementation with *Enteroccocus faecium* (SF68) on non-specific and antigen-specific humoral and cell mediated immune responses. **Animals-** 18 purpose bred kittens. **Procedure-** Kittens were divided into two groups and were given palatability enhancer with (Treatment) or without (Placebo) SF68 daily for 20 weeks starting at 7 weeks of age. Feline viral rhinotracheitis (FHV-1), calicivirus (FCV), and panleukopenia virus (FPV) vaccine was administered at 9 and 12 weeks of age. Survival of SF68 after ingestion, presence of *Clostridium* spp. products, fecal cultures, CBC, biochemical profiles, CD4+, CD8+ and B cell populations, CD44 and MHC Class II expression, Concanavalin-A and FHV-1-specific proliferation, total serum, salivary and fecal IgG and IgA, FHV-1-specific serum and salivary IgG and IgA, and FCV- and FPV-specific serum IgG were evaluated at 7, 9, 15, 21, and 27 weeks of age. **Results-** No significant differences were seen in developmental parameters. SF68 was demonstrated in feces of seven of nine treatment cats during the study. The percentage of CD4+ lymphocytes was significantly higher in the treatment group at 27 weeks of age. Numerically greater levels of FHV-1-specific IgA levels in serum and saliva in the treatment group were found at 21 and 27 weeks of age, FHV-1-specific IgG levels at 15, 21, and 27 weeks of age, and FPV-specific IgG levels at 15 weeks of age. **Conclusions and Clinical Relevance-** Supplementation of kittens with SF68 does not negatively affect measured developmental parameters. The results suggest that supplementation modulates systemic immune responses but longer supplementation may be needed to reach statistical significance.

MODULATION OF OPIOID PHARMACOKINETICS AND PHARMACODYNAMICS, IN VIVO

Author List: Submitting Author - Iman ElKiweri

Abstract:
Fentanyl, a potent, synthetic opioid analgesic is widely used to supplement general anesthesia and as a primary anesthetic agent during cardiac surgery. Dosing strategies are extremely difficult due to an 8-fold difference in inter-individual response. Approximately 83% of fentanyl is first taken up in the lungs, greatly impacting the concentration of fentanyl reaching the brain. Identification of transport proteins responsible for lung and brain uptake of fentanyl may provide targets for therapeutic interventions aimed at controlling inter-individual response to fentanyl. Based on results from previous *in vitro* experiments, we ***hypothesized*** that P-glycoprotein transporter (P-gp) is responsible for outward transport of fentanyl and organic anion transporter (Oatp) is responsible for inward fentanyl transport in lung and brain, and inhibition of P-gp would alter the central effect of fentanyl, *in vivo*. **Methods.** Male Sprague-Dawley rats received continuous intravenous fentanyl administration, with and without P-gp or Oatp inhibitors verapamil and pravastatin or naloxone, respectively. Plasma, lung and brain concentrations of fentanyl were determined at 0, 5, 6, 8, 10 and 60 minutes (n = 4 per time point, per treatment) and continuous EEG monitoring was performed (n = 4 per treatment). Loperamide, a known opioid substrate of P-gp was used as a control. Resulting pharmacokinetic and pharmacodynamic data were modeled using SAAM II software. **Results.** Inhibition of P-gp efflux slightly reduced brain partitioning of fentanyl and was associated with an attenuated intrinsic central effect, but loperamide brain and lung partitioning and intrinsic central effect were increased. Inhibition of Oatp transport reduced lung and brain partitioning of fentanyl. **Conclusion.** Primary control of fentanyl brain and lung uptake is via Oatp transporters. Transporters are opioid specific, possibly providing a discriminating therapeutic target for controlling opioid uptake.

A Live View of Hypothalamic Development using Transgenic Thy-1 YFP Mice

Author List: KM McClellan, JG Knoll, SA Tobet

Abstract:
The determination of cell position and phenotype within the developing brain occurs through a combination of mechanisms, including differentiation, survival, and migration. To understand influences on cell migration in the hypothalamus we use live video microscopy to track fluorescently labeled cells. Transgenic mice obtained from The Jackson Laboratory were created with the Thy-1 promoter driving yellow fluorescent protein (YFP) expression (Feng et al. 2000). The Thy-1 promoter drives neuronal expression in the brain in a spatially and temporally regulated manner during development including the ventromedial nucleus of the hypothalamus (VMH) and preoptic area (POA). Neurons within the embryonic hypothalamus express GABAA and GABAB receptor subunits, estrogen receptors, nkx2.1, calbindin, islet-1, NPY, and RC-2, all candidate factors for contributing to structural and functional differentiation of the hypothalamus. We are using GABA receptor agonists and antagonists to study GABA's effect on the movement of cells in the region of the VMH using brain slices in vitro. Administration of the GABAB receptor antagonist saclofen increased the rate of cell movements through the medial and central regions of the VMH in agreement with previous data showing decreased cell movement in the presence of the GABAB agonist baclofen. Saclofen reduced the number of cells moving radially and this decreased further when saclofen and the GABAA antagonist bicuculline were co-administered, emphasizing a potential synergistic role for these receptors. We also utilize the Thy-1 YFP mice to study estradiol's effect on movement in the POA. In the POA there are two primary cell groups that express YFP early in development, and estradiol may only influence the movement of one located more dorsally. In summary Thy-1 YFP transgenic mice provide a useful model to study the development of cells in the hypothalamus and determine factors that influence them. Supported by MH61376, MH57748 (SAT).

Characterization of production practices, environmental parameters, and selected infectious diseases in catfish aquaculture - A retrospective study.

Author List: EC Thorp, DC Van Metre, CL Huston, CA Myrick, and MD Salman

Abstract:
Worldwide, the aquaculture (farmed fish) industry ranks second only to the pork industry in animal protein production per year. Infectious disease represents one of the greatest threats to aquaculture productivity; furthermore, reporting, surveillance, and control programs are widely lacking, as is a means of auditing management practices that impact infectious diseases. As a collaborative effort between the Colorado State University - Animal Population Health Institute and Mississippi State University College of Veterinary Medicine, we conducted a retrospective, pond-based cross-sectional study of fingerling production on a catfish aquaculture operation in Northwestern Mississippi. By applying fundamental tools used in epidemiology, this study is the first objective measurement of the association between selected infectious diseases and certain management practices, environmental data, and spatial parameters of individual farm catfish ponds. Preliminary results show catfish stocking density ($p = 0.0086$), wind speed four weeks prior to the weeks of the first recorded and peak mortality ($p = 0.0005$), secondary position of the pond ($p = 0.0007$), and year ($p = <0.0001$) are significantly associated with disease related mortality on this farm. Further investigation is needed to determine the effects of other management practices and environmental parameters in preventing or controlling disease in catfish aquaculture. The results of this study will better define the common management practices within the catfish industry as well as identify areas to be addressed that may effect management and health recommendations. Such information is crucial for aquaculture diagnosticians, researchers, epidemiologists and risk analysts alike.

Repeat Sequence Elements in the 3'UTR of Alphaviruses stabilize RNAs against decay in mosquito cell extracts

Author List: KJ Sokoloski, NL Garneau, M Opyrchal, C Wilusz, J Wilusz

Abstract:
It has been previously shown (both *in vitro* and in cell culture) that the degradation of cellular mRNAs is a potent yet regulated method for the removal of undesired mRNAs from the cytoplasm. Previous evidence has shown that regulatory elements in the 3'UTRs of messenger RNAs are capable of influencing the rate at which a cell is able to degrade the RNA. Using a mosquito cell (C6/36) extract system, the stability of reporter RNAs containing the 3'UTR of either Sindbis (SinV) or Venezuelan Equine Encephalitis (VEE) virus was examined. Interestingly, both viral 3' UTR-containing RNAs were extraordinarily stable in the in vitro system. Specifically, transcripts containing either viral 3' UTR exhibit an ability to resist deadenylation thereby preventing subsequent decay of the RNA. Mutagenesis and deletion mapping of the viral 3' UTRs indicates that the stability of both VEE and SinV RNAs in our RNA decay assays is due to the presence of Repeat Sequence Elements (RSEs) in the 3' UTR. While most alphaviruses have RSEs in their 3' UTRs, these sequences often bear little relation to each other at the primary sequence level. Computed-aided predictions of secondary structure do suggest some conserved higher order features of these RSEs. In summary, while previous observations have clearly demonstrated that alphavirus 3'UTRs have a significant influence on virus replication, the mechanism(s) underlying this effect remains obscure. Our results suggest that the alphaviral 3' UTR, and specifically the RSEs, may represent an evolutionary adaptation of both Sindbis and VEE viruses to evade the degradation of their viral RNA genome by host cell machinery.

Experimental validation of in silica-identified regulatory elements involved in human mRNA polyadenylation

Author List: AL Morrison, J Hu, CS Lutz, B Tian, C Wilusz, J Wilusz

Abstract:
Polyadenylation is an essential step in the maturation of almost all eukaryotic mRNAs. The classic poly(A) site in human mRNAs consists of an upstream AAUAAA sequence and downstream U/GU-rich region. Based on EST databases of mRNAs, however, this poly(A) signal is responsible for only ~1/3 of known polyadenylation events. We therefore hypothesized that additional sequence elements mediate 3' end processing in human mRNAs. We previously developed a computer program named PROBE to identify putative regulatory elements in humans by examining the 100 nucleotides (nt) both upstream and downstream of poly(A) sites. PROBE identified a total of 15 cis elements divided into core (within 41 nt of the poly(A) site) or auxiliary (41-100 nt from the poly(A) site) elements. An auxiliary downstream element (DSE) and a core DSE were selected to test functionality *in vitro*. The auxiliary and core sequences were inserted by PCR at appropriate sites downstream of the SV40 late AAUAAA signal. PCR products were transcribed and the transcripts used in an *in vitro* polyadenylation assay. After 60 minutes, the transcript containing the candidate auxiliary DSE, CCUCCC, was polyadenylated ~3-fold more than the negative control containing only the core polyadenylation elements. The putative core DSE, UGCCU, was polyadenylated ~2-fold more than the positive control containing the native core DSE in the SV40 late poly(A) signal. We conclude that these sequences can act as elements of a functional polyadenylation signal in human mRNAs and may represent binding sites for novel regulatory factors.

O'nyong-nyong virus infection in Anopheles gambiae

Author List: RT Tompkins, KE Olson, BD Foy

Abstract:
O'nyong-nyong virus (ONNV, family Togaviridae, genus *Alphavirus*) is unique among the alphaviruses because transmission occurs through *Anopheles gambiae* and *An. funestus* mosquitoes, which are also epidemiologically important as transmitters of human malaria. We are exploring the potential uses of ONNV to serve as an Alphavirus Transducing System (ATS) to study *Anopheline* mosquitoes through RNAi and through foreign gene expression. However, we have noticed interesting interactions between the SG650 infectious clone of ONNV and *An. gambiae* G3 mosquitoes. After numerous experiments using high-titer virus and large numbers of mosquitoes, we conclude that with this system, orally fed virus fails to spread beyond the alimentary canal. Furthermore, when the midgut is bypassed with direct injection of the virus into the hemocoel, virus disseminates throughout many tissues, including fat body associated with the salivary glands, but the actual salivary glands themselves remain uninfected. These observations differ from those in the literature. Several potential factors could account for these differences, including virus and mosquito strain differences, and differences between wild-type virus and the infectious clone.

Contributions of specific hTrpCs to myometrial Ca2+ entry

Author List: A Ulloa, M Zhong, and B Sanborn

Abstract:
Understanding the mechanisms that regulate contractions in the myometrium during pregnancy may help avoid scenarios such as premature births. During labor, increases in intracellular calcium ([Ca2+]i) have been closely correlated with human myometrium contractions. Extracellular calcium enters the cell through voltage-operated and signal-regulated Ca2+-entry (SRCE) mechanisms and is involved in actions such as stimulating the contractile apparatus and replenishing intracellular Ca2+ stores. In SRCE, activation of some receptors and/or depletion of agonist-sensitive [Ca2+]i-stores stimulate Ca2+-uptake from the extracellular solution. This and other SRCE mechanisms contribute to the regulation of calcium homeostasis. Ion channels potentially responsible for SRCE are the canonical transient receptor potential (TrpC) channels. The seven members (TrpC1-7) are postulated to form hetero- or homotetramers thus allowing for the formation of a variety of channels possessing different physiological properties. Our studies show that human myometrium expresses hTrpC4 and hTrpC1 mRNAs in relative abundance compared to other hTrpCs. Furthermore, previous studies suggest that hTrpC proteins are involved in SRCE pathways when studied in the immortalized myometrial cell line PHM1. We have designed an effective method for inducing RNAi (RNA interference) of specific hTrpC channels in the myometrial cell line PHM1-41. RNAi was performed by using short hairpin RNAs (shRNA) constructs against hTrpC4 channel proteins. hTrpC4-shRNA-containing plasmids were designed, placed into a miRNA (microRNA) backbone, and tested using the psiCHECK-2 luciferase reporter system. Four hTrpC4-shRNA constructs prove to be effective for targeting hTrpC4-mRNA degradation (60-90% reduction). These data show the efficacy of this system to identify potential shRNA sequences which effectively knockdown a targeted mRNA. Effective hTrpC4-shRNA sequences were incorporated into adenoviral vectors for further infection of PHM1-41 cells. Initial studies show ~85% hTrpC4-mRNA reduction by this method and changes in phenotype in response to agents that stimulate SRCE. In conclusion, successful gene targeting of hTrpC4 has been achieved and promises to be useful in elucidating the role of this protein in SRCE in myometrium.
Sponsored by HD38970, T32-HD07031, and HD051037-01.

B lymphocyte influx into lung granulomas induced by Mycobacterium tuberculosis infections in naïve and vaccinated mice

Author List: G Palanisamy

Abstract:
A major curiosity of the lung tuberculosis granuloma in mice is the large number of B cells that comprise part of the overall cellular influx. To determine if this might be altered by prior vaccination, we compared the response of naïve and BCG-immunized mice. We report that B cells are present in comparatively lower numbers in BCG vaccinated mice and they are more densely packed within lesions; they also constitute the majority of inflammatory cells on days 30, 37, and 44 post-infection in when compared to saline vaccinated mice. However, on day 58 post-infection, B cells become disbursed and the granuloma comprises of similar proportions of B cells, macrophages and CD4 and CD8+ T cells resembling the saline vaccinated mice granulomas.

Joint Tissue mRNA Expression in an Equine Osteoarthritis Model to Evaluate Extracorporeal Shockwave Therapy

Author List: KE Orr, DD Frisbie, CE Kawcak, CW McIlwraith

Abstract:
Recent experimental evidence and clinical impressions of extracorporeal shockwave therapy (ESWT) have contributed to the novel use of ESWT to treat proximal suspensory desmitis, navicular syndrome, and osteoarthritis (OA) of the tarsometatarsal and distal intertarsal joints in horses. While ESWT decreased lameness, the mechanism by which this occurs is unknown. The purpose of this study is to use TaqMan® real-time PCR for the investigation of joint tissue mRNA expression in an equine OA model to evaluate the potential mechanism of action of ESWT. Primers and probes have been designed for 17 cytokines and growth factors because of their potential role in OA. In addition, some of these cytokines and growth factors have been shown to be up-regulated in cartilage and bone exposed to shockwaves. Validation occurred by running a standard dilution curve of each primer/probe pair and ensuring the primer and probe amplified at a 90% efficiency when compared to a housekeeping gene. In addition to primer and probe design, a method of RNA extraction from cartilage is being developed. Methods tested included rotor-stator homogenization, Spex-Mill disruption, and pulverization in conjunction with Ivitrogen TRIzol extraction and Qiagen RNeasy extraction kits. Spex-Mill disruption and rotor-stator homogenization followed by Invitrogen TRIzol extraction and a Qiagen RNeasy Mini Clean-Up have yielded the most pure RNA with a 260/280 ratio of 1.7-2.0. Efforts are now focused on increasing the RNA yield with this method. Once a protocol for obtaining highly concentrated and pure RNA is developed, mRNA will be isolated and reverse transcribed from cartilage and synovial membrane, and real-time PCR will be run on the resulting cDNA. Gene expression will then be evaluated among horses treated with ESWT, an intramuscular polysulfated glycosaminoglycan, and a sham therapy for the treatment of OA in the mid-carpal joint.

Alloimmunization as an HIV Vaccine Strategy in a Macaque Model

Author List: DS Stump, C Apetrei, J Terwee, S VandeWoude.

Abstract:
Early HIV vaccine studies carried out in the rhesus macaque demonstrated that animals vaccinated with whole inactivated Simian Immunodeficiency Virus (SIV) grown in human cells were protected against virus infection--but only when the challenge virus was grown in human cells. In subsequent studies, vaccination of macaques with human cells only also afforded protection against challenge virus grown in human cells. These findings suggested the possibility that immune responses to host cell antigens, rather than virally encoded proteins, contributed to resistance to infection. Our study was designed to define humoral and cell mediated components of alloimmunization that correlate with protection from SIV challenge in rhesus macaques. Stimulation Indices (SI) from eight rhesus macaques were used to select three macaques eliciting the highest SI and six animals were alloimmunized with pooled PBMC from these animals by the intravenous and intradermal route. Two animals were selected as controls and received self PBMC vaccines by the same route. All animals received an intrarectal boost with pooled or self PBMC at six weeks post-inoculation (pi). Plasma was collected from all animals at 3 days pi, 2 weeks pi, 3 days post-boost (pb), and 4 weeks pb. Humoral responses to alloantigens measured by western blot, plasma chemokine levels measured by MIP1α, MIP1β, and RANTES cytokine ELISA, and in vitro serum neutralization, were compared in alloimmunized vs self-immunized animals. Preliminary results of this study will be described, along with implications and plans for future experiments. This study was performed in collaboration with colleagues at the Tulane National Primate Research Center, and was funded by the CVMBS College Research Council and the TNPRC pilot grant program.

Characterization of Yersinia pestis Culture Filtrate Proteins

Author List: B. Stallman, A. Jones, S. Warner, C. Bosio, and S. Dow.

Abstract:
Background. *Yersinia pestis* can cause localized infection (bubonic plague) or rapidly fatal pulmonary infection (pneumonic plague). Currently there is no approved vaccine for plague in the U.S. and earlier vaccines failed to provide reliable protection from pneumonic plague. Therefore, we screened culture supernatants from *Y. pestis* cultures to identify secreted proteins that might be used as candidate antigens in new plague vaccines and characterized these proteins by electrophoresis and immunological assays.
Materials and Methods. Virulent *Y. pestis* strain CO92 was grown in shaker cultures in modified GAS medium. Several different media and supplements were screened for their ability to increase the yield of CFP from cultured *Y. pestis*. Supernatants from log phase cultures were collected and then filtered and concentrated. The culture filtrate proteins (CFP) were characterized by gel electrophoresis and further characterized using immune serum from mice infected with *Y. pestis* or with serum from mice immunized with CFP.

Results. Optimal growth of *Y. pestis* in synthetic medium was achieved with culture in GAS medium supplemented with tryptic soybean extract. With this medium, we obtained yields of CFP in the range of 1-2 mg per liter of cultured bacteria. By gel electrophoresis, concentrated CFP was found to contain at least 7 major protein bands. By immunoblotting, one band was identified as the F1 glycoprotein. Serum from infected and CFP immunized mice recognized several major protein bands in the CFP preparation and studies are underway to more fully identify these proteins.

Conclusions. Culture filtrate proteins from *Y. pestis* contain several major proteins. Additional studies to determine whether these proteins can elicit protective immunity against parenteral or inhalational challenge with *Y. pestis* are being conducted.

Hydrogel selection of mesenchymal stem cells for chondrogenic potential

Author List: JC Stangel, JD Kisiday

Abstract:
Bone marrow contains pluripotent mesenchymal stem cells (MSCs) that are capable of differentiating into a chondrogenic lineage. In our lab, we are evaluating the potential of adult equine MSCs to undergo chondrogeneic differentiation as part of an investigation into the potential use of autologous cells for treatment of equine cartilage defects. In vitro MSC chondrogenesis is evaluated by first encapsulating culture-expanded MSCs in a hydrogel scaffold, then culturing in medium containing TGF-β1 for a period of 21 days. We identify chondrogenesis by the synthesis of a dense proteoglycan matrix that is characteristic of cartilage. In previous studies, equine MSCs were found to undergo chondrogenesis. However, histological analysis showed that only subsets of encapsulated cells were surrounded by a pericellular matrix rich in proteoglycans. Subsequent studies showed that cell viability decreases from >95% on day 0 to as little as 10-20% with time in culture in both chondrogenic and control medium, suggesting a potential relationship between survival in the gel and the ability to differentiate. In this study, MSCs were encapsulated in a hydrogel scaffold for 3 days to select for progenitor cells. The 3 days in culture within chondrogenic medium reduced viability from ~95% to ~60%. Viable cells were isolated and reseed into another hydrogel scaffold and cultured in chondrogenic or control medium for 12 days. Initial viability was high (~90%). By day 12 viability in both TGF and control cultures decreased with time, especially in the control medium (~57% TGF, ~23% control). Both cultures showed higher viability than typically seen without the gel selection step. In addition increased viability coincided with elevated levels of biosynthesis relative to previous studies. These data suggest that viability was a good indication of progenitor potential and that cell selection may be achieved with short-term hydrogel culture.

Transgenerational Radiation Genetics: Low dose-rate effects and adaptive response in Japanese Medaka (Oryzias latipes)

Author List: WW Kuhne, FW Whicker, and JS Bedford

Abstract:
Over the past decade or so, one new area of concern that may have an important bearing on the issue of genetic hazard assessment is the so-called adaptive response (AR), meaning a reduction in radiation sensitivity caused by previous low doses of radiation. The intent of this project was to initiate research which can ultimately answer the question as to whether there is a measurable transgenerational adaptive response in Japanese Medaka (*Oryzias latipes*) in response to chronic, low dose-rate irradiation. The experimental plan, involved either chronically irradiating (or not irradiating) populations of F0 wild-type Medaka using low dose-rate exposures in a range of 10^{-5} to 10^{-2} Gy/h. Irradiations were begun with 6-hour post-fertilization embryos using gamma rays from a 370 Ci ^{137}Cs source. Embryos were kept in 250 ml glass jars containing aerated water at 22-24ºC until hatchout. Hatchout fish (~ 2-weeks post-fertilization) were fed an adequate amount of brine shrimp several times daily and supplemented with a flake food and moved to 2.5 L glass containers at a density of 6 fish per container. At 8-weeks post-fertilization adults were paired up for breeding and kept at 26-28ºC in 2.5 L glass containers under a 16 h light:8 h dark light cycle. F1 embryos from the F0 breeding pairs were collected and either kept in the chronic irradiation field for 48-hours giving a dose of 25cGy or in a control environment and then given an acute test dose of 5 Gy (3.9 Gy/min). The presence of an adaptive response will be determined by measuring chromatid-type aberrations using a G2 assay. Results from the experiment will be presented.

2,2'-DCB decreases amplitude and synchronization of uterine contractions through MAPK-mediated phosphorylation of Cx43

Author List: D Chung, R Loch-Caruso

Abstract:
2,2'-dichlorobiphenyl (2,2'-DCB) is an ortho substituted polychlorinated biphenyls (PCBs). Previously, we reported that uterine strips from gestation day (GD) 10 pregnant rats exposed to 2,2'-DCB decreased the amplitude and the synchronization of contraction and that MAPK-induced phosphorylation of connexin43 (Cx43) results in 2,2'-DCB-induced inhibition of gap junction communication between myometrial cells. The present study examined that MAPK-induced phosphorylation of Cx43 is the mechanism for 2,2'-DCB-induced modification of uterine contractions. To examine whether 2,2'-DCB-induced modification of uterine contractions is dependent on the MEK-MAPK pathway, the pattern of uterine contraction was observed in uterine M).μM 2,2'-DCB and the MEK inhibitor PD98059 (5 μstrips cotreated with 100 Decrease in amplitude and desynchronization of contractions reversed significantly 2 h after cotreatment. In order to see whether MAPK-induced phosphorylation of Cx43 is the mechanism for 2,2'-DCB-induced modification of uterine contractions, western blot of Cx43 phosphorylated at ser255 by MAPK was compared in uterine strips untreated or exposed to 0.1% DMSO (solvent control), M PD98059 for 1 h. DensitometricμM 2,2'-DCB and 5 μ M 2,2'-DCB or 100 μ100 analysis of uterine tissue showed about a two-fold increase of Cx43 M 2,2'-DCB.μphosphorylated at ser255 relative to GAPDH after exposure to 100 This 2,2'-DCB-induced phosphorylation of Cx43(S255) was prevented by cotreating M PD98059. Therefore, thisμM 2,2'-DCB and 5 μuterine strips exposed with 100 study suggests that 2,2'-DCB decreases amplitude and synchronization of uterine contractions through MAPK-mediated phosphorylation of Cx43.

A role for matrix metalloproteinase-9 in resistance to pulmonary Mycobacterium tuberculosis infection

Author List: JL Taylor, JM Hattle, SA Dreitz, JM Troudt, LS Izzo, RJ Basaraba, LM Matrisian, IM Orme and AA Izzo

Abstract:
Recent studies have shown that matrix metalloproteinases (MMPs) are induced by *Mycobacterium tuberculosis* during pulmonary infection. Here, expression of MMP-9 during pulmonary *M. tuberculosis* infection was characterized to determine whether its production correlated with disease resistance *in vivo*, and to determine what role, if any, MMP-9 might have in granuloma formation. Following aerosol infection with *M. tuberculosis*, dissemination of bacilli occurred earlier in the C57BL/6 resistant strain compared to the susceptible CBA/J strain, as was evident by an increased number of bacteria in the blood, spleen and liver at day 14 after infection. In addition, early dissemination of the bacilli was associated with early induction of protective immunity as assessed by IFN-γ levels. Nonspecific blocking of MMPs in C57BL/6 mice early during infection reduced hematogenous spread of the bacilli, suggesting that MMPs indeed play a role in facilitating dissemination likely via ECM degradation. The concentration of active MMP-9, specifically, was consistently greater in the lungs of C57BL/6 mice than in the CBA/J mice, but not until day 28, thereby suggesting that MMP-9 is not involved in promoting early dissemination of the *M. tuberculosis*. Instead, however, histological lung sections and flow cytometric analysis of lung cells from MMP-9 knockout mice showed that MMP-9 is involved in macrophage recruitment and granuloma development. These combined data support that early MMP activity is an essential component of resistance to pulmonary mycobacterial infection and that MMP-9, specifically, is required for recruitment of macrophages and tissue remodeling to allow for the formation of tight, well organized granulomas.

Engineered expression of TRIM5αrh by lentiviral vector transduction restricts HIV-1 infection in CD34+ stem cell derived macrophages

Author List: J Anderson, R Akkina

Abstract:
Species specific innate resistance against viral infections offers novel avenues for antiviral therapeutic and prophylactic approaches. The retroviral and lentiviral restriction factors Ref1 and Lv1 are variants of the tripartite motif protein, TRIM5α, a component of cytoplasmic bodies. TRIM5α severely restricts productive retroviral infections at the post-entry and pre-integration steps by destabilizing the incoming viral capsid via ubiquitination. Using this approach, resistance to HIV-1 infection could be conferred by TRIM5α_{rh} expression in otherwise susceptible cells. Here we show that stable expression of simian TRIM5α_{rh} via a lentiviral vector in a permissive cell culture line, Magi-CXCR4, conferred resistance to HIV-1. To translate these findings into a stem cell gene therapy setting, the TRIM5α_{rh} transgene was stably introduced into CD34+ hematopoietic progenitor cells to derive transgenic macrophages. Upon viral challenge, TRIM5α_{rh} expressing macrophages were highly resistant to HIV-1 infection compared to control cells. Human macrophages expressing TRIM5α_{rh} were also found to be phenotypically and functionally normal expressing the characteristic surface markers CD14, CD4, CCR5, CXCR4, MHCII, and B7.1. These results demonstrate that the species-specific restriction factor TRIM5α_{rh} is effective in conferring HIV-1 resistance in a stem cell setting thus paving the way for its application in AIDS gene therapy.

Potent suppression of CCR5 expression and HIV-1 infection by synthetic and lentiviral vector expressed shRNAs

Author List: J Anderson, A Vermeulen, J Karpilow, R Akkina

Abstract:
The recently discovered phenomenon of RNA interference has been shown to be highly potent and sequence specific in targeted gene silencing with great potential for use as a therapeutic in combating HIV infections. By targeting the critical coreceptor CCR5, infection can be inhibited at the level of viral entry. CCR5 has been shown to be dispensable for normal physiology and is, therefore, an excellent target for gene therapy applications. Several reports have so far described the use of CCR5 siRNAs showing varied levels of gene knockdown. In the present study, a new generation of anti-CCR5 siRNAs was designed with a goal of achieving complete silencing. Based on a rational target sequence identification criteria, new and more effective CCR5 siRNAs were identified. Complete knockdown of CCR5 expression as determined by quantitative real-time PCR and FACS was observed. To achieve stable knockdown of CCR5, siRNA coding sequences were cloned individually and in tandem into a lentiviral vector. Vector transduced cells showed similar impressive levels of CCR5 knockdown. When challenged with R5-tropic BaL-1 HIV-1, cells exhibited significant resistance to infection. These results are the first to demonstrate complete knockdown of CCR5 expression after years of intensive efforts in several labs.

The Effects of Cytosine Deaminase Activity on Lentiviral Persistence In Vitro and In Vivo: Development of feline immunodeficiency virus (FIV) as a Relevant Model System

Author List: JL Troyer, J Terwee, M Poss, S VandeWoude

Abstract:
Both HIV-1 and HIV-2 are widely accepted to be the result of cross-species transmission: HIV-1 from SIVcpz and HIV-2 from SIVsm. Examination of experimental and natural lentiviral infection indicates that these successful events are relatively rare and likely require substantial host-virus adaptation. A family of cytosine deaminases has been implicated in the restriction of cross-species transmission. Domestic cat FIV, a naturally occurring infection, results in AIDS-like disease and death; in contrast, species-specific strains of FIV infecting non-domestic cat species result in little or no clinical disease. Laboratory strains of puma virus can productively infect domestic cats, yet do not result in disease. We have characterized a viral stock derived from puma lentivirus field isolate PLV-1695 that results in productive avirulent infection in domestic cats, is cleared from circulation, and persists at low levels in intestinal tissues. We propose that a feline cytosine deaminase (fe3) plays an important role in viral containment in this cross-species transmission model. Viral sequences from domestic cats inoculated with PLV and from FIV and PLV infected puma and domestic cat cells in culture were examined for G to A mutations. In vivo, PLV sequences demonstrate high error burden and significant increase in G to A transitions, suggestive of an active role for fe3 in control of viral persistence. In vitro, viral replicative capacity and cell viability, as well as G to A mutation rate and fe3 expression, varied with cell type and virus strain with clear cross-species restriction in some, but not all, cell types. This model system will be used to examine the importance of intracellular restriction mechanisms in facilitating cross-species lentiviral containment relative to adaptive immune effects. Future studies will determine how such mechanisms can be engineered to restrict or eliminate infection by virulent host-adapted lentiviral strains.

CUG-BP binds ARE-containing RNA substrates and recruits PARN deadenylase

Author List: Karen C.M. Moraes, Carol J. Wilusz and Jeffrey Wilusz

Abstract:
The stability of an mRNA plays an important role in the regulation of gene expression. The major pathways of mRNA decay in eukaryotic cells initiate with the shortening of the 3' poly(A) tail. Elements that regulate the efficiency of mRNA turnover are often found in the 3' untranslated region (UTR) of the transcript. In *Xenopus oocytes*, poly(A) tail length is used to control gene expression as there is no transcription ongoing during oocyte development. Maternal mRNAs undergo a default deadenylation during the maturation process mediated by the PARN deadenylase while post-fertilization deadenylation is dependent on the presence of AU-rich and/or EDEN (embryonic deadenylation element) sequences within the 3'UTR. The ARE elements in *Xenopus* mRNAs appear similar to those in mammalian mRNAs. Numerous ARE-binding proteins in mammals have been identified. However, there has been no direct evidence for an interaction between ARE-binding proteins and the deadenylation machinery that leads to modulation of this process. CUG-BP is the human homolog of the *Xenopus* EDEN-BP which was shown previously to bind to mRNAs that exhibit rapid deadenylation following fertilization of the frog oocyte. While several studies have focused on roles of CUG-BP as a splicing or translation regulator in mammalian cells, its role in mRNA decay has not been examined in detail. Using an *in vitro* deadenylation assay, we demonstrate that CUG-BP binds to ARE-containing RNAs and stimulates poly(A) shortening by PARN.Moreover, CUG-BP interacts with PARN by co-immunoprecipitation. CUG-BP, therefore, is the first RNA-binding protein shown to directly recruit a deadenylase to an RNA substrate.

Computer-Aided Identification of Novel Dengue Antiviral Compounds

Author List: SM Keenan, BJ Geiss, KE Olson

Abstract:
Dengue virus (DEN) infection is a leading cause of death in children in tropical and subtropical regions of the world. A staggering 2.5 billion people worldwide are at risk of infection and ~50 million cases of infection are reported each year. DEN is obviously a major socio-economic and medical problem worldwide, yet antiviral therapeutics to treat DEN infection are not currently available. The recent release of DEN NS5 2'-O-methyltransferase (2'-OMTase) three dimensional coordinates provides structural information that can be exploited in the development of novel antiviral compounds using computer-aided drug design. DEN 2'-OMTase binds the guanosine base of the viral RNA cap and adds a methyl group to the 2'-OH position of the first RNA nucleotide. The 3D-structures provide evidence for the atomic level interactions between the guanosine ligand and binding site amino acids. Mutation of 2'-OMTase guanosine interacting amino acids is lethal to viral replication, suggesting that 2'-OMTase is a good target for antiviral design. Eighteen solvent accessible residues line the guanosine binding site, and five are observed to specifically interact with the non-specific GTP competitor ribavirin. Both hydrogen bond formation and π-π stacking interactions are observed. Lys14, Asn18, and Phe25 are highly conserved among flaviviruses and all are proposed to interact with ligand. We have developed a series of *in silico* binding domain mutants and computationally docked ribavirin monophosphate into the binding sites. In addition to confirming the necessity of Lys14, Asn18, and Phe25 for ligand affinity, we have identified Lys22 as playing an important role in ligand association. These interactions have enabled us to develop a chemical model for use as a query to identify potent and selective inhibitors from our virtual small molecule library containing ~1,200,000 compounds. Candidate compounds will be analyzed for antiviral value using high-throughput screening assays.

ASSOCIATION OF MICROALBUMINURIA WITH SYSTEMIC DISEASE IN DOGS

Author List: JC Whittemore, VL Gill, WA Jensen, SV Radecki, MR Lappin.

Abstract:
Human studies have shown microalbuminuria (MALB) status to be an excellent predictor of disease, morbidity, and mortality. It is unknown whether these relationships are true for dogs. The objectives of the current study were to determine the prevalence of systemic disease in urine dipstick (DpP) negative dogs with and without MALB and to determine the diagnostic utility of a semi-quantitative MALB kit (MALBE, E.R.D.-HealthScreen® Urine Test) and quantitative MALB assay (MALBQ) in dogs. Urine samples from 408 dogs presented to Colorado State University (CSU) and negative on DpP were assessed. Urinalyses were performed at the CSU Clinical Pathology Laboratory. UPC (positive = > 0.5 and 0.1), MALBQ and MALBE (positive = values > 1 mg/dl) were determined. Clinical diagnoses recorded within 3 months of the urine collection were grouped as: healthy, neoplasia, infection/inflammatory/immune-mediated, urinary/renal, endocrine, and other. Sensitivity (Se) and specificity (Sp) were determined for each test using disease status as the standard. The influence of clinical diagnosis, gender, age, BUN, creatinine, blood pressure, urine culture results, temperature, pyuria, hematuria and bacteriuria, on test results was evaluated by logistic regression. The small number of dogs positive by UPC0.5, precluded statistical evaluation of this test. Sensitivity and Sp for presence of disease for the tests are: MALBQ Se 35.6%. Sp 85.4%; MALBE Se 36.9%, Sp 91.7%; UPC0.5 Se 4.5%, Sp 100%; UPC0.1 Se 71.1%, Sp 18.8%. Factors predicting MALB status ($p < 0.05$) were clinical diagnosis, age, BUN, hematuria, urine culture results and bacteriuria. In this study, MALB was associated with the presence of disease. The utility of MALB tests for identifying occult disease will depend on disease prevalence and the Se and Sp of other screening tests. The Se and Sp of MALB tests for systemic disease were lower than would be anticipated if overtly proteinuric dogs were included in the study.

Quantification of FHV-1 DNA from the conjunctiva of cats with and without conjunctivitis

Author List: HC Low, CC Powell, JK Veir, JR Hawley, MR Lappin

Abstract:
Purpose The purpose of this study was to determine if FHV-1 DNA copy numbers, measured by fluorogenic (real-time) PCR, correlated to the presence or absence of conjunctivitis. **Methods** Client owned cats were sampled and included those that had active conjunctivitis (56), those that had a history of conjunctivitis which was resolved for at least 3 months (42), and those that had never had conjunctivitis (45). Cotton tipped applicators were rolled in the ventral conjunctival cul-de-sac and placed in 1.0 mL sterile 0.01 M PBS. Samples were kept 2-3 hours at room temperature then stored at -70°C until analyzed. DNA was extracted using a commercial kit (QIAamp DNA Blood Mini Kit, Qiagen, Inc, Valencia CA) and assayed for FHV-1 DNA using adaptations of a previously published protocol. **Results** By use of real-time PCR, FHV-1 DNA was amplified from 6/56 cats with conjunctivitis (10.7%, 1.61×10^{-1} mean FHV-1 copy/cell equivalent), 3/42 cats with previous conjunctivitis (7.1%, 6.35×10^{-3} FHV-1 copy/cell equivalent), and 3/45 cats that never had conjunctivitis (6.7%, 3.48×10^{-2} mean FHV-1 copy/cell equivalent). There were no significant differences in mean FHV-1 copy number/cell equivalent among the groups. **Conclusions** There were not enough positive samples to confirm that there are increased copy numbers of FHV-1 DNA in cats with conjunctivitis, compared to carriers or healthy cats.

Hypoinflammatory State in Septic and Critically-Ill Dogs

Author List: R Kerscher, K McCord, S Dow, C Webb

Abstract:
Neutrophils (PMNs) are a central component of the innate immune response to bacterial infection, and yet in patients with severe bacterial infection PMNs appear to enter a hypoinflammatory state. This hypoinflammatory state maybe critical to patient morbidity. The ability of PMNs to eliminate pathogens depends on their production of reactive oxygen species (ROS) and effective phagocytosis; both of these properties can be assayed using flow cytometry. DHR 123 is used to determine ROS production in PMNs stimulated with PMA. Opsonized, Alexa 488-labeled E. coli is used to measure PMN phagocytosis. Fc OxyBurst allows us to measure Fc receptor kinetics and phagovacuole oxidative burst activity. Incubation of leukocytes with viable bacteria and subsequent determination of viable versus dead bacteria will allow us to quantify the bacterial killing process. The PMN oxidative burst assay has been used extensively in prior work from this lab while the protocols for the OxyBurst and phagocytosis assays are being optimized for use in the canine. Initial results from critical care patients suggest that these assays can identify differences in PMN behavior between dogs. Once characterized, a hypoinflammatory state respresents a part of the innate immune response that maybe amenable to pharmacological manipulation and therefore, clinical intervention.

Comparison of Random and Cohort Sampling to Evaluate the Effect of Antimicrobial Use on Resistance

Author List: A Villarroel, DA Dargatz, MD Salman, SR Ladely, PJ Fedorka-Cray.

Abstract:
Antimicrobial use in food-animals is currently under great scrutiny for allegedly being a major source for antimicrobial resistance in human pathogens. Few studies exist on the effect of antimicrobial use on antimicrobial resistance of isolates collected from treated animals. Different sampling strategies need to be evaluated to more effectively study the effect of antimicrobial use on antimicrobial resistance. Fecal samples were obtained from 100 cows and 100 calves repeatedly over a period of 12 months at intervals of 8 weeks. Animals were included in one of 2 groups; treated with an antimicrobial within the 30 days prior to the first sampling occasion (n=50) and controls (not treated within 30 days, n=50). At each sampling occasion, another 50 animals were randomly selected to obtain fecal samples. Among isolates obtained from calves, random sampling yielded similar results in prevalence of resistance to samples from the cohort groups, both for *Salmonella* spp. and *E.coli*. However, in cows, random sampling yielded higher prevalence of *Salmonella* spp. isolates than cohort sampling (7.8% in random vs. 4.5% in controls and 0.8% in treated). Distribution of resistance patterns was very similar for isolates obtained from cohorts and random samples, both in calves and cows. As for the effect of antimicrobial use on antimicrobial resistance, in general, with fewer days between last antimicrobial treatment and sampling, isolates showed resistance to more antimicrobials.

Longitudinal Study on Isolation and Resistance Patterns of Salmonella spp. and E. coli obtained from Dairy Cattle

Author List: A Villarroel, DA Dargatz, MD Salman, SR Ladely, PJ Fedorka-Cray.

Abstract:
Antimicrobial resistance in bacterial species, pathogen and commensal, derived from food production animals is of concern. The objective of this study was to describe *Salmonella* spp. and non-type specific *Escherichia coli* isolated from the same animals and environmental areas over time and their resistance patterns. Sampling occurred at a local dairy over a 12 month period, at 8 week intervals. Samples were collected from animal feces (cows and calves), feed, drinking water, hospital milk, colostrum and flush water. *Salmonella* serotypes recovered from environmental and fecal samples collected from the same groups of animals differed and the resistance patterns of both *Salmonella* and *E. coli* were also different. *Salmonella* serotypes were different in calves and cows, and so were resistance patterns both for *Salmonella* and *E. coli*. There was also limited similarity between resistance patterns found in *E. coli* and *Salmonella* isolates. Out of 24 animals with a positive *Salmonella* culture in feces at any occasion over the 12-month study period, only 2 adult cows had 2 positive cultures with different serotypes in each culture. All other animals with a positive *Salmonella* culture had only one positive sample during the whole study period. Based on our data, we conclude that in field conditions *Salmonella* and *E. coli* may not exchange genetic material as frequently as previously suggested. These results may be partially due to effective biosecurity procedures on this particular dairy, avoiding cross-contamination between cow and calf-areas.

Superovulation of Mares with Equine FSH

Author List: NL Logan, PM McCue, EL Squires

Abstract:
The use of superovulation in the equine embryo transfer industry is increasing as many breed registries now allow multiple foals to be registered out of the same mare in a single year. The goals of this experiment were to: 1) evaluate the effect of short term eFSH therapy during the divergence phase of the estrous cycle, 2) investigate the efficacy of using eFSH over multiple consecutive cycles. In Experiment 1, 35 light horse mares were randomly assigned to one of three groups. Group 1 (control) mares were given prostaglandin 5-7 days post ovulation and inseminated and given hCG when the largest follicle was ≥ 35mm in diameter. Embryo recovery was attempted 8 days after hCG. Group 2 (divergence) mares received 12.5 mg eFSH twice daily for three days beginning when the first ovarian follicle reached 20-25 mm in size. Mares were administered hCG, inseminated and embryo recovery was performed as previously described. Group 3 (standard protocol) mares received eFSH twice daily until ≥ 50% of the cohort of follicles was≥ 35mm in diameter. Mares were administered hCG, inseminated and flushed as described. In Experiment 2, 30 mares were randomly assigned to one of three groups. Group 1 mares served as untreated controls. Group 2 mares received eFSH twice daily for three consecutive cycles. Group 3 mares received eFSH for three cycles, with a cycle of rest between each superovulated cycle. Insemination and embryo recovery for all groups were performed as described in Experiment 1. Mares in the standard superovulation protocol group had a higher ovulation (5.4 ± 2.2) and embryos recovery (2.6 ± 2.6) rate than did mares in either the control or divergence groups ($p<.05$). The number ovulations was significantly higher for divergence (3.8 ± 2.8) than for control (1.2 ± 0.4) mares ($p<.05$). In the second experiment, the number of days of treatment were significantly greater for mares treated during consecutive cycles (8.2 ± 2.2 days) ($p<.0001$) than for mares treated every other cycle (4.1 ± 1.2 days). In summary, treating mares for a limited number of days with eFSH at the time of follicle divergence resulted in superovulation and decreased the cost of therapy by over 35%. Mares can be superovulated over consecutive cycles without an adverse effect on ovarian function, although the number of treatments may be increased.

INTERACTION OF DI-TRI-OCTAHEDRAL (DTO) SMECTITE WITH EQUINE COLOSTRAL ANTIBODIES IN VITRO

Author List: J Boggs Lawler, DM Hassel, JL Traub-Dargatz, C Hirota, DR Hyatt, PM McCue

Abstract:
Di-tri-octahedral (DTO) smectite has prophylactically been administered to foals in the early neonatal period for life-threatening Clostridiosis; however, its effect on colostral immunoglobulins has not been evaluated. Colostrum was harvested from 9 healthy broodmares at foaling and stored at -70˚C. Baseline IgG levels were evaluated by single radial immunodiffusion (SRID). DTO smectite was added to colostral samples in serial concentrations ranging from 1:4 to 1:32. Colostral samples were incubated with DTO smectite at 37˚C for 1 hour and evaluated by SRID. This range of concentrations mimics the estimated ratio of DTO smectite to colostrum and gastric contents in a foal's GI tract following administration of the recommended dosage of DTO smectite (3TB in 30mls water). The 1:4 concentrations represent approximately 1 hour of colostral ingestion, while the 1:32 concentrations mimic 6-12 hours of ingestion of good quality colostrum. Results of this *in vitro* study revealed that co-incubation of equine colostrum with DTO smectite decreases SRID detection of IgG in a dose dependent manner. While the effect was negligible in samples representing 6-12 hours of colostral ingestion, the 1:4 concentrations each had a consistent decrease in SRID. It is hypothesized that DTO smectite is absorbing IgG by nonspecific binding and may decrease the bioavailability of colostral antibodies. Based on extrapolation from these *in vitro* results, caution should be exercised during prophylactic administration of DTO smectite to healthy foals prior to consumption of an adequate quantity of good quality colostrum until *in vivo* studies can be conducted.

Activation of Pulmonary Immunity by Liposome-DNA Complexes

Author List: A. Goodyear, C. Bosio, and S. Dow.

Abstract:
Background. Liposome-DNA complexes (LDC) are potent activators of innate immunity. Following parenteral administration, LDC elicit potent antitumor activity mediated by NK cells and IFN-g release. Treatment with LDC is also capable of eliciting protective immunity against aerosol with certain viral and bacterial pathogens. We therefore investigated local pulmonary immune responses to inhalation of LDC as well as the ability of LDC to elicit protective immunity against several bacterial pathogens. **Methods**. The uptake of LDC by target cells in the airways and lungs was investigated using flow cytometry and microscopy and labeled LDC. The effects of LDC treatment on cell numbers and inflammatory cell and cytokine responses were also assessed. Mice were treated with LDC and then subjected to inhalational challenge with certain bacterial pathogens and the effects on protective immunity were assessed. **Results**. Early after administration, LDC were taken up primarily by airway dendritic cells (DC). LDC uptake triggered activation of DC, along with an influx of neutrophils and monocytes into the airways. Intrapulmonary administration of LDC also elicited local production of pro-inflammatory cytokines in the lungs and airways. Pre-treatment with LDC elicited protective immunity to inhalational challenge with *Francisella tularensis* and the protective effects on bacterial challenge are currently being investigated. **Conclusions**. Inhalation of LDC led to local activation of pulmonary innate immune responses. Airway DC were the primary early target cells for uptake and activation by LDC. Following uptake of LDC, the DC were activated and then many disappeared from the airways. LDC may therefore be useful for generating protective local pulmonary immunity against certain inhaled pathogens.

IMPDH drug resistance gene to select for HIV-1 resistant macrophages from lentiviral vector transduced CD34 cells

Author List: J Thompson, JK Yee, J Zaia, R Akkina

Abstract:
Current AIDS therapeutic regimens are expensive and invariably lead to the emergence of drug resistant strains. Additional approaches to treatment that employ anti-HIV siRNAs in a gene therapy setting offer considerable promise. To achieve sustained efficacy, siRNAs need to be gene transduced into hematopoietic stem cells for long term engraftment in the recipient. Post-transplantation, chances for higher engraftment rates are increased if the transplanted stem cells have a selective advantage. Based on this consideration, a gene coding for a mutated version of the enzyme inosine monophosphate dehydrogenase (IMPDH) that confers resistance to the drug mycophenolic acid (MPA) was engineered into a lentiviral vector containing a siRNA targeting HIV-1 p24. The reporter gene GFP was expressed as a fusion protein with that of IMPDH. Our goal in these studies is to determine if this vector confers drug resistance to macrophages derived from gene transduced CD34 progenitor cells as a first step toward its clinical use. Our results have shown that MPA resistant macrophages can be selected when transduced CD34 cells are cultured with appropriate cytokines in the presence of MPA at 12µM concentration whereas non-transduced CD34 cells did not survive this selection. Selected macrophages expressed the transgenes as determined by FACS for GFP and exhibited normal levels of CCR5, CXCR4, CD4, CD14, and MHCII. Up regulation of the costimulatory molecule B7.1 after LPS stimulation was also normal when compared to controls. Thus, drug selected siRNA transgenic macrophages appeared to be phenotypically and functionally normal. Viral challenge experiments with R5 tropic Bal1 strain of HIV-1 showed that the transgenic macrophages resist infection. These data demonstrate the utility of incorporating a drug resistance gene for selection of vector transduced cells and pave the way for *in vivo* selection experiments.

Clinical Usefulness of Echocardiography for Detection of Atrial Masses in Dogs with Primary Splenic or Subcutaneous Hemangiosarcoma

Author List: AS Skope, D Thamm, D Vail

Abstract:
Canine hemangiosarcoma is a malignant tumor of endothelial cells that occurs more commonly in the dog than any other species. The most common sites for primary hemangiosarcoma in the dog are the spleen, the right atrium and subcutaneous tissues. Previous studies have shown that at the time of necropsy, 25 percent of dogs with primary splenic hemangiosarcoma also have right atrial masses. Consequently, echocardiogram is a routinely performed as a staging test in canine hemangiosarcoma. However, echocardiogram is not a very sensitive method for detecting right trial masses. It is our clinical impression that atrial masses are diagnosed via echocardiography in only a small percentage of dogs with primary splenic or subcutaneous hemangiosarcoma. Medical records of dogs diagnosed with primary splenic or subcutaneous hemangiosarcoma and who had an echocardiogram at the time of initial diagnosis over a 10 year period were obtained from the Colorado State University Veterinary Medical Center. Information obtained from the records included breed, age, sex, initial complaint, physical examination findings, primary tumor site, size of primary tumor, presence of metastasis, median survival time, median time to progression, and echocardiogram results. A total of 184 cases that met the initial search criteria were identified. To date, 53 cases have been reviewed. Our findings so far, suggest that echocardiogram detects atrial masses in less than five percent of cases. More data and statistical analysis will be available at the time of presentation.

Vaccination with rhVEGF for Inhibition of Angiogenesis in Dogs with Soft Tissue Sarcoma

Author List: D Kamstock, R Elmslie, D Thamm, S Dow

Abstract:
Background: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor secreted by neoplastic cells which induces tumor-associated vasculature. Inhibition of VEGF activity is viewed as an important therapeutic goal for inhibiting tumor angiogenesis and controlling tumor growth. Active immunotherapy against VEGF is one strategy for inhibiting tumor angiogenesis. **Aims:** 1) Determine whether vaccination with rhVEGF can elicit the production of anti-rhVEGF antibodies in dogs with cancer. 2) Determine whether induction of anti-VEGF antibodies is associated with inhibition of tumor angiogenesis. **Methods:** Eight dogs with soft tissue sarcoma were enrolled in a tumor vaccine clinical trial. Dogs were vaccinated every 2 weeks for 6 weeks then every 4 weeks for 12 weeks with 50 and 25ug/ml of rhVEGF respectively. Tumor volume was monitored throughout treatment. Tumor biopsies were obtained pre-treatment, at 6 weeks, and 16 weeks to assess microvessel density and tumor infiltrating leukocytes. Plasma and serum were obtained at each vaccination time point to evaluate circulating levels of anti-rhVEGF antibodies and canine VEGF. **Results:** Of the 8 dogs initially enrolled, 4 were dropped from the study; 3 due to progressive disease, and 1 due to lameness. Of the remaining 4 dogs, 2 demonstrated a humoral response against huVEGF. Of these 2 dogs, one demonstrated a concurrent decrease in circulating VEGF concentrations, suggestive of cross-reactivity, along with a significant decrease in microvessel density and a partial tumor response. Of the remaining 3 dogs that completed the study, 2 had a partial response, and one had stable disease. **Conclusions:** Vaccination with rhVEGF in dogs with soft tissue sarcoma can elicit a humoral response against VEGF in some dogs. In some dogs anti-VEGF antibodies are associated with a reduction in tumor angiogenesis. Additional studies to assess the ability of anti-huVEGF antibodies to neutralize canine VEGF are underway.

Poster Presentations

Faculty

COMPUTED TOMOGRAPHY IMAGING OF OVONE PULMONARY ADENOCARCINOMA DISEASE PROGRESSION

Author List: S Hudachek†, A DeLille†, J DeMartini‡, S Kraft†, W Dernell†

Abstract:Introduction: Ovine pulmonary adenocarcinoma (OPA) induced by Jaagsiekte sheep retrovirus (JSRV) shares both symptomatic and histopathologic characteristics with human bronchioloalveolar carcinoma (BAC). To date, there has been no work done to monitor the progression of OPA to further validate this model. The aim of this study was to follow the development of JSRV-induced neoplastic lesions in the lungs of infected sheep using Computed Tomography (CT) technology as a noninvasive imaging modality.

Methods: Seven neonatal lambs were inoculated intratracheally with JSRV isolated from the lung tumor tissue of previously infected animals. Lung images were acquired post inoculation (PI) on a monthly basis thru 4 months (1 animal), 5 months (2 animals), and 6 months (4 animals).

Results: By 4 months PI, all 7 lambs had developed disseminated miliary nodules. In the 3 lambs imaged at 5 months PI, that pattern had progressed to severe coalescing nodules and lung consolidations. The nodular pattern seen in all 4 lambs that were carried out to 6 months had partially or completely regressed.

Conclusions: Overall, all seven animals inoculated with the virus manifested tumor nodules, a disease induction rate of 100%. Post mortem histopathology confirmed that the nodules visualized via CT were neoplastic lesions, specifically adenocarcinomas. Future studies aim to compare the gene expression profile of OPA with the human BAC profile. If a molecular similarity between OPA and BAC is apparent, we can then validate the OPA sheep as a suitable animal model for human lung cancer studies

Proteomic profiling using SELDI-TOF mass spectrometry in Canine Mast Cell Tumors

Author List: SE Lana1, PJ Gaines2, TD Powell2, SJ Walmsley2, KL Estredge2, N Wisnewski2, SJ Withrow1

Abstract:
Proteomics is the study of proteins in biologic specimens. Surface enhanced laser desorption ionization time of flight (SELDI-TOF) mass spectrometry is a powerful new tool for proteomic profiling. Proteins in biological samples are selectively bound to chip surfaces of various chemistries and subjected to mass spectrometry. Bound proteins are separated by mass and the relative quantities of each protein compared among samples. Multiple biomarkers or unique protein signatures can be compared in a single assay to improve sensitivity and specificity. The objective of this study is to investigate the utility of this technology for serum proteomic profiling of canine mast cell tumors. **Methods**: Serum samples were collected from 20 dogs: 10 grade 2 and 10 grade 3 tumors. Each sample was separated into 4 fractions using a strong anion exchange resin from a commercially available kit. Each fraction was selectively bound to 4 different chips, having either strong anion exchange, weak cation exchange, nickel chelating, or hydrophobic interaction surface chemistries. The chips were subjected to SELDI-TOF mass spectrometry, and the resulting protein profiles compared between the two groups using the Biomarker Wizard and Biomarker Patterns software, Ciphergen Biosystems, Inc (Fremont, CA). **Results:** To date several putative biomarkers have been identified. These proteins have been used in combination to build classification tree models that differentiate grade 2 from grade 3 tumors. Preliminary results using cross-validation indicate that trees have greater than 90% sensitivity and specificity. **Conclusions:** Proteomic profiling using SELDI-TOF technology in canine serum is feasible. Further testing is warranted.

Impact of antimicrobial use on susceptibility of commensal enteric bacteria in horses.

Author List: M. Dunowska, P. Morley, D. Hyatt, D. Dargatz, J. Traub-Dargatz

Abstract:
Hypothesis: Horses in a veterinary hospital are likely to have more antimicrobial resistant fecal flora than healthy horses from a general population. In addition, horses treated with antimicrobial drugs (AMD) are likely to have more resistant fecal flora than those not treated with AMD. **M and M**: G1 were horses treated with AMD at a veterinary hospital (CSU-VTH) for the previous 3 days and on day of fecal collection, G2 were horses that were patients at CSU-VTH for at least the previous 4 days but not treated with AMD and G3 were healthy horses housed outside CSU-VTH that had not been treated with AMD in at least the previous 5 days. Standard bacteriologic methods were used to recover *E. coli* from fecal samples. When available, 3 *E. coli* colonies from each sample were tested. The National Antimicrobial Resistance Monitoring System (NARMS) Antimicrobial susceptibility testing (AST) methods for 15 AMD were used. **Results:** For G1, 256 *E. coli* isolates were tested from 87 horses; for G2, 221 *E. coli* isolates were tested from 85 horses and for G3, 249 *E. coli* isolates were tested from 78 horses. *E. coli* isolates from G1 were more likely to have MIC above the cutoff point for 12 of 15 AMD tested in comparison with those from G 2 or G3 ($p<0.05$). *E. coli* isolates from G2 were more likely to have MIC above the cutoff point for 6 of 15 AMD tested in comparison with those from G3 ($p<0.05$). **Conclusion:** Based on this study it would appear that the use of antimicrobial drugs increases the antimicrobial resistance of commensal bacteria not only for the horses treated but for other hospitalized equids.

Rampant Testicular Dysgenesis in Sitka Black-Tailed Deer on Kodiak Island, Alaska

Author List: DNR Veeramachaneni, RP Amann, JP Jacobson, D Paetkau, MS Bornman

Abstract:
Cryptorchidism occurs in humans and animals, predisposes for testicular cancer, and might result from mutations in Insl3 or Great genes [1]. Recently we established [2] that on the low-lying Aliluik Peninsula (AP; ~14x50 km) of Kodiak Island, 61 of 94 Sitka Black-Tailed Deer (SBTD) hunted were bilateral cryptorchid (BCO) and 7 were unilateral cryptorchid; 70% of BCOs had abnormal antlers. All 11 abdominal testes studied contained various dysplastic and neoplastic cells, as did 2 of 10 scrotal testes. Elsewhere on Kodiak Archipelago, only 5 of 65 deer were BCO. We are unaware of any other non-experimental population containing >70% cryptorchid animals. We assumed there were 3 plausible "causes" for 72% cryptorchid SBTD on the AP. First, a classic mutation in a gene(s) essential for testes descent with marked concentration via inbreeding. This was discounted by: (a) incidence of transformed cells [see 3]; (b) lack of geographic barrier isolating the population; and (c) analyses of microsatellite DNA for 12 loci leading to the conclusion that SBTD on the AP and elsewhere in the Kodiak Archipelago represented one population. Second, a residual epigenetic effect altering or blocking expression of Insl3, Great, and/or other genes, consequent to historic exposure of a founder(s) to an estrogenic endocrine disruptor agent (EDA); epigenetic effect concentrated via inbreeding and transmitted via gametes [see 4]. This is not supported by 1(b) & 1(c) and needs a direct study. Third, exposure of fetuses to an estrogenic EDA ingested by their dam concurrent with pregnancy or earlier might impact fetal development at 25-35% of gestation; analyses of contaminant residues in tissues, browse, and water are in progress. Supported by CVMBS-CRC. Further investigation is being sought via a NIEHS-R21. [1] Kaleva & Toppari. Cell Tissue Res. Epub, 2005. [2] Veeramachaneni et al. Env. Health Perspect. Epub, 2005. [3] Veeramachaneni. Inter. J. Androl. Epub, 2005. [4] Anway et al. Science 308:1466, 2005.

"Does the Gender of the Client and the Veterinarian Influence Communication in Veterinary Visits?"

Author List: Shaw, J.R1; Bonnett, B.N2; Adams, C.L.2; Roter, D.L3.

Abstract:
Research reports indicate that male and female physicians demonstrate different communication styles and that the gender of the patient influences physician communication. Although there is substantial literature on physician-patient communication, few studies have investigated the relationship between gender-specific communication and clinical outcomes. There is minimal communication research in veterinary medicine, and none pertaining to veterinarian, client or patient factors that might impact how practitioners communicate with clients. The potential for gender to affect client communication is starting to be considered practically in response to the increasing number of female veterinary students across North America. 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 (pet) 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 preventative and three acute appointments. The Roter Interaction Analysis System (RIAS), a quantitative communication assessment tool, was utilized for the first time in veterinary medicine to describe visit communication. Communication variables, verbal dominance, relationship-centered care, communication patterns, and length of visit will be used as response (outcome) variables. Predictor variables of primary interest (veterinarian and client gender) as well as, other possible covariates and confounders (including practice, veterinarian, client and pet factors) will be analyzed for their relationship with the outcomes. Preliminary analysis revealed a complex relationship between client and veterinarian gender and communication pattern. Results of this study will inform the development of future outcomes-based studies and communication curricula. Understanding gender-related communication is important, since concordant or discordant communication may impact quality of care, which is applicable to human medicine.

Epidermal Growth Factor Promotes the Malignant Phenotype in Canine Mammary Carcinoma

Author List: DH Thamm, B Rose S Dreitz, DM Vail

Abstract:
Mammary gland carcinoma (MGC) is one of the most common neoplastic causes of mortality encountered in dogs worldwide. There is little information available regarding nonsurgical therapies for this common disease. The receptor tyrosine kinases EGFR and HER2 (erbb1 and erbb2), receptors for epidermal growth factor (EGF) and other related growth factors, mediate a variety of oncogenic functions in human MGC, and strategies targeting these receptors are showing great promise in the clinic. While previous studies have demonstrated the presence of EGFR and HER2 in canine MGC tissues, the functional role played by signaling through these receptors has not been studied. The goal of this study was to determine the in vitro effects of EGF on the proliferation, invasion, survival, and chemosensitivity in canine MGC cells. The canine MGC cell lines CMT12 and CMT27 were provided by Dr. Lauren Wolfe. Anchorage-dependent and independent growth and alterations in sensitivity to the antineoplastic drug doxorubicin (DOX) in response to EGF were assessed by means of a bioreductive fluorometric assay (Alamar Blue). Protection from serum starvation and DOX-induced apoptosis was assessed using annexin V-FITC / propidium iodide staining and flow cytometry. Stimulation of cell invasion through a synthetic basement membrane (Matrigel) was assessed using standard Boyden chamber assays. Under low serum conditions, both cell lines proliferated in response to recombinant human EGF. Anchorage-independent growth was similarly enhanced. EGF also enhanced the resistance of both cell lines to DOX. EGF was capable of inhibiting serum starvation and DOX-induced apoptosis in CMT12 but not CMT27. Both cell lines showed enhanced Matrigel invasion in response to EGF. In conclusion, EGF was shown to stimulate a wide array of features promoting the malignant phenotype in canine MGC. Strategies targeting EGFR/HER2 signaling may hold promise as novel treatments for this common canine cancer.

The Association Of Bartonella Spp. Infection With Chronic Stomatitis In Cats

Author List: KL Dowers, MR Lappin.

Abstract:
Stomatitis is a debilitating disease in cats that leads to oral pain, anorexia, weight loss, and occasionally euthanasia. The syndrome is thought to have multiple causes, but recent work suggests that *Bartonella* spp. may play a role. The objective of this clinical study was to determine the prevalence of *Bartonella* spp. DNA in blood and *B. henselae* serum antibodies in client-owned cats with stomatitis and in age- and geographically-matched healthy control cats. Blood and serum samples from 34 affected cats and 34 age-matched healthy control cats were submitted by veterinarians from around the United States. Polymerase chain reaction (PCR) was used to amplify *Bartonella* spp DNA and serum antibody titers against *B. henselae* were determined by ELISA. FeLV antigen and FIV antibodies were also determined. For cases where oral biopsy samples were provided, the PCR assay was also performed on tissue samples. Survey information regarding housing status (multiple or single cat households), previous FeLV/FIV status, flea exposure, vaccination history and history of upper respiratory infections (URI) were collected for both affected and control cats. No significant differences in the prevalence rates for PCR-positive cats between affected (8.89%) and control cats (8.89%) or for antibody-positive cats between the affected group (67.6%) and the control group (58.8%) were found. The only survey factor with significant correlation with stomatitis was history of URI [$p<0.05$]. Of the 18 oral tissue samples submitted, only 1 was PCR-positive. This study underscores the difficulty of correlating *Bartonella* spp. test results with clinical disease in individual cats because of the high prevalence rates of antibody-positive animals within the healthy population. A larger scale epidemiologic study should be conducted to further assess the usefulness of routine *Bartonella* spp. antibody and PCR testing of cats with chronic stomatitis.

West Nile Virus Surveillance in Northern Colorado 2004

Author List: BG Bolling, CG Moore, SL Anderson, KB Krug, AM Barker, CD Blair, BJ Beaty

Abstract:
Adult mosquitoes were collected from April through November 2004 in riparian areas located in Larimer and Weld Counties. Collections were made weekly using CDC light traps and gravid traps. Over 17,000 mosquitoes were collected, pooled, and analyzed by RT-PCR for the detection of West Nile virus. Data will be presented on the species composition of collections as well as the spatial and temporal distribution of WNV isolates. A comparison of mosquito collections and WNV activity for 2003 and 2004 will be discussed.

Methods for Strain typing of Mycobacterium leprae

Author List: M Kimura, NA Groathouse, X Weng, JC Beltran, HC Kim, WC Black IV, PJ Brennan and VD Vissa

Abstract:
The availability of the genome sequence of an isolate of *M. leprae* has allowed for the exploration of multiple locus variable number of tandem repeats analysis (MLVA) as a method for strain typing and identification of chains of transmission of leprosy. To date, we and other researchers have demonstrated that 32 loci are polymorphic in *M. leprae* clinical isolates. Three single nucleotide polymorphisms (SNPs) have also been reported. Ten polymorphic loci including six minisatellites (repeat units ≥ 6 bases) and four microsatellites (repeat units < 6 bases) were mapped using DNA extracts from a collection of human clinical isolates from three countries (China, Colombia, Philippines). The overall allelic and genetic diversity of isolates from each country were found to be different. Ideally, for increasing the discrimination between isolates, multiple VNTR loci should be mapped for each sample. Since *M. leprae* cannot be grown in any media, the amount of genetic material is limited, and thus PCR based methods are required. As strain typing methods are expected to be implemented in the near future for a large number of samples collected from patients and their contacts, high throughput, standardized and automated methods are required. We demonstrate the feasibility of multiplex PCR for the amplification of 12 VNTR loci. Furthermore, the inclusion of fluorescent dye labeled sense primers, followed by fragment length analyses using denaturing gel electrophoresis allowed for the rapid determination of the copy number at each locus.

Association between local clinical signs and important outcomes of clinical mastitis episodes in dairy cattle

Author List: J.R. Wenz, K. Whitman, F.B. Garry

Abstract:
Clinical mastitis (CM) is the most common and costly infectious disease of dairy cattle. There are many local clinical signs associated with inflammatory changes of the mammary gland. Treatment and prognosis of CM is often decided based on these signs, however, there have been no studies evaluating the association of important outcomes of CM and these signs in cows with mild systemic disease. Cows with CM exhibiting mild systemic disease signs (N=240) from a 1500 cow dairy were enrolled in the study. Cows were examined for firmness and swelling of the affected mammary gland and character of the secretion (thin, thick or serum). Milk culture results and intramammary (IMM) treatment were recorded. Outcomes assessed were re-treatment (RTX), recurrent CM episode in the same quarter 15-60 later (RECUR), dried quarter, death or culling and sick pen days (SPD). Data was evaluated using PROC GENMOD and GLM in SAS. RTX occurred in 27% (63/231) and RECUR in 25% (51/206) of CM episodes. Dried quarter and culling occurred in ~5% of CM episodes and no deaths occurred. RTX was 3.23 (1.12-9.31) times more likely in cows with serum secretion. RTX was 4.30 (1.77-10.3) and 6.33 (2.11-19.0) times more likely in cows with swelling and those receiving pirlymicin IMM, respectively. Cows with serum had significantly greater SPD (11.6) than those with thick (6.9) or thin (7.4) secretions (P<0.001). RECUR was 4.05 (1.11-14.8) times more likely in cows with a mixed infection and 2.79 (1.02-7.64) times more likely in cows with no growth culture than cows with a Gram Pos. culture result. RECUR was 3.6 (1.30-10.1) and 4.24 (1.60-11.2) times more likely in cows with swelling and those **without** firmness, respectively. Firmness and swelling were seen in ~70% of cows and therefore would likely have little discriminating ability. Serum secretion was only seen in 17% of cows and was associated with more re-treatment, however, it was not associated with cow removal or loss of quarter.

Re-examination of the Etiology of Fatal Undifferentiated Fever / Bovine Respiratory Disease of Feedlot Cattle

Author List: CW Booker, PS Morley, ED Janzen, PT Guichon, GK Jim, OC Schunicht, BK Wildman, TJ Pittman, T Perrett

Abstract:
Feedlot calves were examined to determine the microbiological agents involved in fatal BRD and to investigate the relationships between microbiological agents and pulmonary pathology. The study included 99 calves that died with peracute, acute, subacute, and chronic bovine respiratory disease (BRD) or control animals with no BRD. Pathology was assessed using digital imaging, gross postmortem examinations, and histopathology. Immunohistochemistry (IHC) was performed on three lung samples from each animal to detect the presence of *M. haemolytica* (MH), *M. bovis* (MB), *H. somni* (HS), bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), bovine respiratory syncytial virus (BRSV), and parainfluenza-3 virus (PI3V). Using IHC, MH and MB were the most commonly identified pathogens in all stages of BRD, with MH detected in 85-100% of early stage disease and MB detected in 94% of chronic disease. Across all stages of BRD, BVDV was detected in 8-40% of cases, HS was detected in 0-39% of cases, and the other pathogens studied were detected in <20% of cases. There were significant ($P < 0.05$) positive associations between BVDV and MH and HS and MB and significant ($P < 0.05$) negative associations between HS and MH and HS and BVDV. There were strong positive ($P <0.05$) associations between IHC staining for MH and the occurrence of fibrino-necrotizing pneumonia and IHC staining for MB and the occurrence of "mycoplasma-like" necrosis. Additional analyses are currently underway to further characterize the histopathology and IHC findings and to investigate relationships between pathogens and between pathogens and pathologic findings.

Strain Typing of North American M.bovis Isolates

Author List: LR Martinez, *BN Harris, *JN Payeur, PJ Brennan, VD Vissa, and RL Jones

Abstract:
Mycobacterium bovis, the etiologic agent of tuberculosis in animals, has a wide host range infecting many domestic and wild animal species. Economic losses to agriculture due to *M. bovis* infection are significant. Although most cases of human tuberculosis are caused by the organism *M. tuberculosis*, *M. bovis* is a zoonotic pathogen that can cause human tuberculosis and remains a public health concern in developing countries. The recent publication of the entire genomic sequence of the *M. bovis* clinical isolate AF2122/97 has enabled the identification of tandem repeat sequences within the genome. Some of these loci have been shown to be hyper-variable in their repeat numbers, and are referred to as Variable Number Tandem Repeats (VNTRs). These markers have been utilized to type isolates primarily in Europe. To date, there are no published studies on VNTR typing of North American isolates. Therefore, in collaboration with the USDA/APHIS/NVSL, we have embarked on a molecular epidemiological study to develop protocols and select markers to differentiate North American *M. bovis* isolates. With an initial sample of 10 isolates, we were able to differentiate 8 separate strains with VNTR typing based on 27 known loci. We will continue to type isolates from the U.S. provided to us by the USDA, as well as type isolates from Mexico in collaboration with the National Institute for Forestry, Agriculture and Animal Research (INIFAP). With a greater sample size, we will be able to determine which VNTR markers, and which combination of these and other molecular markers, will be most useful in the epidemiology of *M. bovis* in North America.

Melioidosis: Novel Therapies for an Emerging Disease

Author List: A Kumar, H Blair, H Schweizer

Abstract:
Burkholderia pseudomallei is the causative agent of melioidosis, a rare but severe and often fatal tropical disease that is endemic in Southeast Asia and Northern Australia, but has also been documented in South America and is suspected to be pandemic. The related bacterium *Burkholderia mallei* causes glanders, a serious zoonotic disease of equine species, which can also infect humans. Because *B. mallei* has previously been used as a biowarfare agent and both organisms have high weaponization potential, they are listed as National Institute of Allergy and Infectious Diseases Category B Priority Pathogens. The highly infectious nature of these hardy, saprophytic bacteria, together with increased world travel, make them likely causes of emerging infectious diseases as well. Treatment of melioidosis is greatly complicated due to *B. pseudomallei*'s intrinsic resistance to most classes of antibiotics. Current therapies are expensive and include 10 day intravenous administration of cephalosporins, followed by oral eradication therapy with a regimen of two to four drugs for 3-5 months. Infections of a large number of people would therefore be disastrous in terms of financial and human costs. Preliminary data indicate that multidrug efflux pumps of the nodulation and cell division family are responsible for *B. pseudomallei*'s intrinsic and acquired antibiotic resistance. Using powerful molecular and genetic tools, we are currently characterizing and assessing the clinical significance of these pumps and are constructing surrogate strains that each express a single efflux pump as a tool for efflux pump inhibitor discovery. Future work will include screening of synthetic and natural compound libraries for efflux pump inhibitor candidates. It is hoped that these efforts will ultimately provide new therapeutic approaches for melioidosis by increasing the effectiveness of existing drug therapies, and by enabling the use of cheaper and readily available drugs.

Expression and Pharmacologic Inhibition of Anti-Apoptotic Bcl2 Family Members in Canine Lymphoma

Author List: DM Vail, B Rose, S Dreitz, DH Thamm

Abstract:
Non-Hodgkin's lymphoma (NHL) is a common human and canine cancer. While remission can often be achieved using chemotherapy, drug-resistant relapse is common. Bcl-2 family proteins are central regulators of programmed cell death, and anti-apoptotic members such as Bcl-2 and Bcl-xL are overexpressed in many cancers. The Bcl-2 family small molecule inhibitor ABT-737 has shown efficacy against human NHL cells, but its efficacy against canine cancer is unknown. The goal of this study was to determine the expression of Bcl-2 in canine NHL tissues, and to determine the effect of ABT-737 on canine NHL cell proliferation and chemosensitivity in vitro.

The canine B-cell NHL cell line 1771 was provided by Dr. Ann Jeglum. ABT-737 was provided by Abbott Laboratories. The effect of ABT-737 on cell growth under various serum conditions was assessed using a bioreductive fluorometric assay (Alamar Blue). The effect of ABT-737 on chemosensitivity was assessed by incubating the cells with varying concentrations of the antineoplastic drug doxorubicin (DOX), with or without ABT-737, followed by determination of relative viable cell number by Alamar Blue. Bcl-2 expression in paraffin-embedded canine NHL tissues was assessed using standard immunohistochemical techniques.

The majority of canine NHL tissues evaluated to date have expressed Bcl-2 protein by immunohistochemistry. ABT-737 inhibited 1771 growth in a dose-dependent fashion. This effect was more profound under conditions of reduced serum, e.g. when cells were under an apoptotic stress. ABT-737 was also capable of potently enhancing the antiproliferative effect of DOX on 1771 cells.

In conclusion, ABT-737 appears to be an active drug against canine NHL. We are currently are evaluating the expression of other Bcl-2 members in canine NHL tissues, assessing the effect of ABT-737 on cell apoptosis, and evaluating the efficacy of combined DOX and ABT-737 treatment on tumor growth in a canine NHL xenograft.

Effects of fetal calf serum or phenazine ethosulphate (PES) and fructose or glucose on embryonic development and lipid accumulation of bovine embryos

Author List: M. Barceló-Fimbres, and G. E. Seidel Jr.

Abstract:
Slaughterhouse oocytes (n=6222) were maturated in a medium (CDM) similar to SOF plus 0.5% fatty acid-free BSA (FAF-BSA) and hormones (M-CDM) for 23 h at 38.5ºC in 5% CO_2 in air. Oocytes and frozen-thawed sperm, centrifuged through a Percoll gradient, were co-cultured for 18h in F-CDM (CDM+heparin). Zygotes were cultured at 38.5ºC in 5%CO_2/5%O_2/90%N_2 in CDM-1 (CDM+ nonessential amino acids, 10µM EDTA, 0.5% FAF-BSA, and 0.5mM fructose or glucose in expt 1 and glucose in expt 2). In both experiments, after 48h 8-cell embryos were cultured 135h in CDM-2 (CDM-1+essential amino acids, no EDTA, and 2 mM fructose or glucose). A factorial design with 2 hexoses and 3 additives in CDM-2 (Control; 10% FCS; and 0.3µM PES, an electron acceptor that oxidizes NADPH) was used for both experiments, each replicated 8x. For expt 1, day 7.5 blastocysts were fixed and stained with Sudan Black B to quantify cytoplasmic lipid droplets. A digital photo at 600x of the equatorial part of the embryo was evaluated by classifying lipophilic droplet diameters as small (S, <2 µm), medium (M, 2 to 6 µm), or large (L, >6 µm), reported as number of lipid droplets per 1,000 μm^2. Data were analyzed by ANOVA. For expt 1, 8-cell embryo production per oocyte matured was not affected by fructose or glucose (P>0.1) (70 *vs.* 68%, respectively); however, blastocyst rates per oocyte matured (B/O), and per 8-cell embryo (B/E) were higher (P<0.01) for fructose than glucose. There were no differences between control, PES, and FCS (P>0.1) for B/O, or B/E. For expt 2, B/O and B/E were higher (P<0.01) for fructose than glucose. No differences were found for additives (P>0.1) control, FCS or PES for B/O or B/E respectively. There was an interaction (P<0.05) between additives and hexoses for blastocyst production, because the benefit of fructose compared to glucose was greater for controls than FCS or PES (means no presented). Accumulations of each size of lipid droplets were less for PES (P<0.05) than control and FCS. Control and PES were lower than FCS (P<0.05) for S, M, and L droplets. There was no effect of fructose or glucose (P>0.1) on numbers of S, M, or L droplets (Table 1). In conclusion, fructose produced more blastocysts than glucose after 8-cell development, but there was no hexose effect either before this stage or in lipid accumulation. PES reduced, and FCS increased lipid accumulation relative to controls.

A Prospective Study Evaluating Whole Vody MRI For Staging Lymphoma in Dogs

Author List: S Kraft, EK Randall, S Lana, A Avery, C Olver.

Abstract:
Introduction: The purpose of this study was to evaluate whether whole body magnetic resonance imaging (WB-MRI) could represent a single non-invasive procedure, giving equivalent information as routine diagnostic cancer staging. Canine lymphoma was selected as the model due to its high incidence, multicentricity and potential for bone marrow involvement. **Methods:** WB- MRI studies were done in a 1.5 Tesla GE Signa MRI on 11 dogs with suspected stage 4 or 5 lymphoma. Dogs were imaged via 3 overlapping fields of view using STIR and TI weighted pulse sequences and a post contrast T1 sequence of the abdomen after IV injection of gadolinium DTPA (0.1mmol/kg). Diagnostic tests included CBC, chemistry panel, thoracic radiographs, abdominal US including hepatic/splenic fine needle aspirates (FNA), FNA of peripheral lymph nodes and bone marrow, bone marrow biopsy and polymerase chain reaction (PCR). Organs, lymph nodes and bone marrow were assessed on WB-MR images as being + or - for lymphoma and compared to routine diagnostic results. **Results:** WB-MRI studies took approximately 1 hr; the procedure was well tolerated in 9/11 dogs although 2 dogs became hyperthermic. WB-MRI was sensitive to lymph node and lung involvement, but only moderately sensitive for diffuse liver and spleen infiltrate compared to FNA. Sensitivity of WB-MRI of bone marrow varied depending upon the standard of comparison. Results were + for lymphoma in 3/11 dogs by aspirate/biopsy, in 9/11 dogs by PCR, and in 6/11 dogs by WB-MRI. **Conclusions:** WB- MRI is practical and convenient for staging cancer. WB-MRI provided information equivalent to routine diagnostic procedures when evaluating lymph nodes and lungs. However, WB-MRI was less sensitive than FNA/cytology to diffuse involvement of the liver or spleen. WB-MRI could potentially increase detection of bone marrow infiltrate over aspirates or biopsies, but image quality of the appendicular skeleton during WB-MRI needs further improvement.