FIRST Annual

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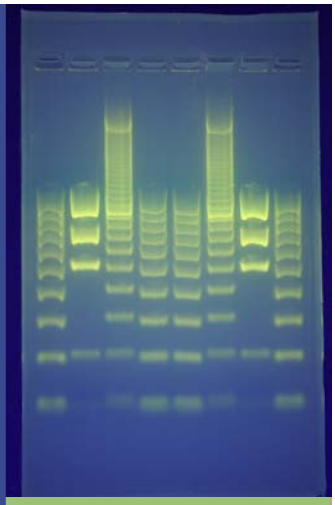

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Comparative & Experimental Medicine Graduate Student Research Symposium

PROGRAM

첫 째 로

PRATHAM

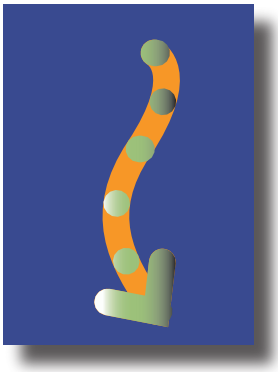
June 22, 2007

THE UNIVERSITY of TENNESSEE
Comparative and Experimental Medicine

About the Symposium

Welcome to the First Annual Comparative and Experimental Medicine Graduate Student Research Symposium. This research conference was initiated to acknowledge and show appreciation for the scholarly contributions of graduate students to the university. The goal of the symposium is to foster networks and collaborations within our program by giving us an opportunity to share research results, exchange ideas, promote collaboration, and network with scientists in a variety of disciplines.

Graduate students have been invited to give oral and poster presentations based on research done in any of the broad discipline areas within the Comparative and Experimental Medicine Program. This year's event has 15 student participants, representing 6 departments. In addition, the students presenting today represent 5 different countries and 4 different states. The international diversity of the program is celebrated on the program's cover with the inclusion of the word "first" in 5 different languages, all of which are spoken by one or more of our presenters.



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Schedule of Events

Morning

Continental Breakfast & Poster Set-up (Brick foyer*) — 8:00 am - 9:00 am

Opening Remarks (A118) — 9:00 am - 10:00 am
Dr. Michael Blackwell
Dr. Michael Zemel – The Metabolic Shift: Calcium, Calcitriol and Energy Metabolism

Break (Brick foyer) — 10:00 am - 10:15 am

Poster Presentations (1st floor hallway) — 10:15 am - 12:00 pm

Break for Lunch — 12:00 pm - 1:00 pm
(*Presenters and RSVPs only:* Sequoyah Room, Dr. Carl Jackson presents "How to Find a Post-Doc Position")

Afternoon

Oral Presentations — 1:00 pm - 2:00 pm

A117 *(moderator Dr. T.W. Schultz)*

Angela Lusby, *Validation of an ELISA Assay for Measuring Serum Adiponectin in Felids*; **Beth Wilson**, *The Effect of Calcium and Dairy in Weight Loss and Body Composition;* **Robin L. Cissell**, *Detection of Malignant Catarrhal Fever Virus White-Tail Deer Variant in Tennessee using Real-Time Polymerase Chain Reaction*; **Karissa Dawn Laughter,** *A Descriptive Study of Campylobacteriosis Cases Reported in East Tennessee*

A118 *(moderator Dr. Robert N. Moore)*

Jonathan Phipps, In Vitro *Silencing of Amyloidogenic λ6 Light Chain Production;* **Amanda Peretich**, *Hormonal Regulation of* Atp10c*, a Type 4 P-type ATPase, in Mouse Adipocytes;* **Ferenc Tóth,** *Effects of an Intravenous Endotoxin Challenge on Glucose and Insulin Dynamics in Mares;* **Dongwei Wu,** *Lysophosphatidic Acid Induces the Expression of Tissue Factor and Early Growth Response Gene-1* In Vivo

Break (Brick foyer) — 2:00 pm - 2:15 pm

Oral Presentations — 2:15 pm - 3:15 pm

A117 *(moderator Dr. T.W. Schultz)*

Hye Mee Joo, *Quantitative Aspects of Influenza Virus-Specific Memory B Cells Generated by Infection and Vaccination;* **Sharvan Sehrawat**, In Vitro *Generated Antigen Specific CD4+CD25+Foxp3+ Regulatory T Cells Control the Severity of Virus Induced Immunoinflammatory Lesion;* **Pranita Sarangi**, *IL-10 and Natural Regulatory T Cells: Two Independent Anti-Inflammatory Mechanisms in HSV-Induced Ocular Immonopathology*

A118 *(moderator Dr. Robert N. Moore)*

Jason Liggett, *NUANCE, a Potential Novel Oncogene is Inhibited by Nonsteroidal Anti-Inflammatory Drugs in Human Colorectal Cancer Cells;* **Nichelle Whitlock,** *Nonsteroidal Anti-Inflammatory Drug Activated Gene (NAG-1) as a Chemopreventive Target in Canine Tumorigenesis;* **Shambhunath Choudhary**, *Oncogenic H-Ras Facilitates Apoptosis Induced by Histone Deacetylase Inhibitors in Human Urinary Bladder Cancer Cells;* **Mugdha Sukhthankar**, *Green Tea Catechins Suppress Basic Fibroblast Growth Factor In Vitro And In Vivo*

Closing Remarks & Awards (A118) — 3:45 pm - 4:30 pm
Dr. Karla Matteson

* All events will take place in the Veterinary Teaching Hospital

Comparative and Experimental Medicine Program Office

The Comparative and Experimental Medicine Program Office established the Graduate Student Research Symposium as one of the many professional and academic development opportunities it provides for students.

College of Veterinary Medicine

Dr. Robert N. Moore
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Associate Professor, Department of Medical Genetics & program liaison
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Lucy Simpson
Medical Administrative Assistant
R308 UT Medical Center
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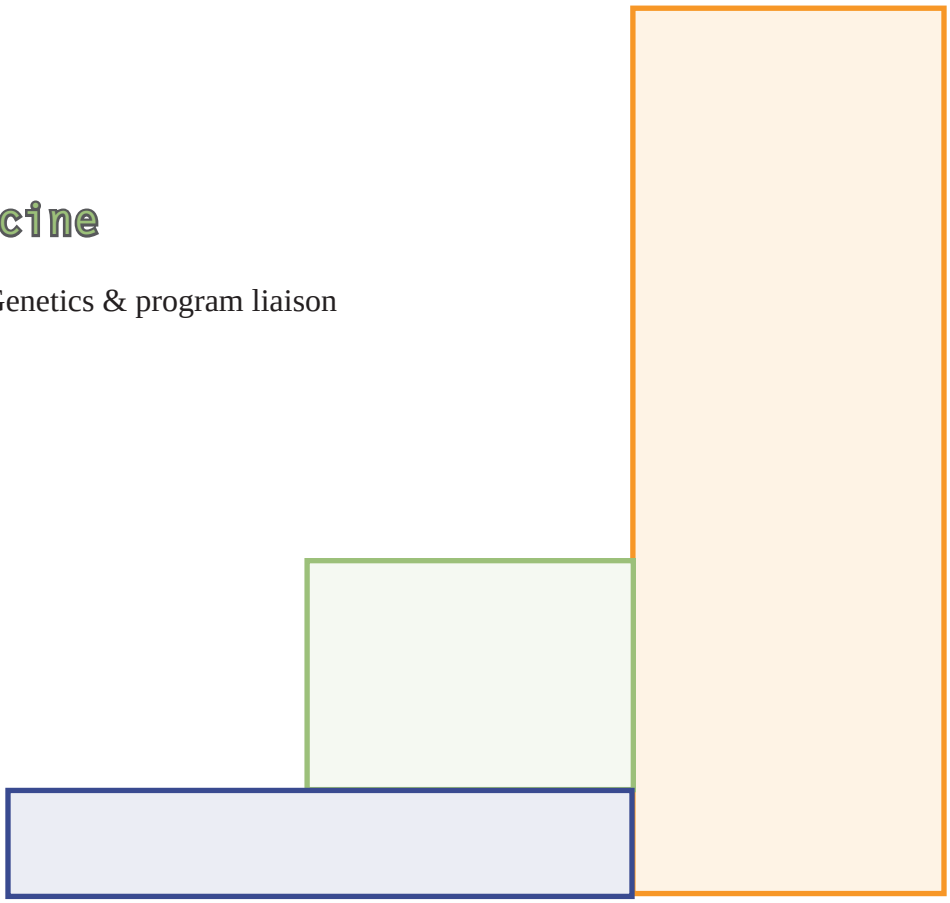

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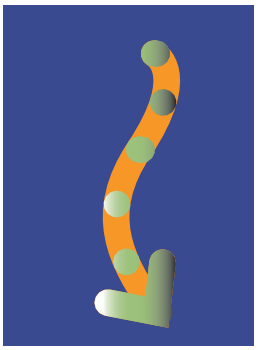

Drs. Robert N. Moore and T.W. Schultz, moderators
Dr. Michael Blackwell, opening speaker
Dr. Michael Zemel, keynote speaker
Dr. Karla Matteson, closing speaker
Ms. Linda Sangster, volunteer extraordinaire
Dr. Carl Jackson, St. Jude Children's Research Hospital
Organizers and staffers of the symposium

Breakfast provided by UT Federal Credit Union
Display frames provided by Exhibitor Source
Breaks provided by Fisher Scientific and Millipore
Lunch for presenters provided by St. Jude Children's Research Hospital
Prizes for "How Well Do You Know Your Colleagues' Research?" contest provided by ThermoFisher and Comparative & Experimental Medicine program

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Speakers

Dr. Michael Blackwell was appointed Dean of the University of Tennessee College of Veterinary Medicine in August 2000 following an illustrious career in the US Public Health Service (USPHS) in which he attained the rank of Assistant Surgeon General (Rear Admiral). Dr. Blackwell earned his BS (1973) and DVM (1975) from Tuskegee University, his Master of Public Health (1981) from Loma Linda University, and Certification in Epidemiology from the US Public Health Service. He operated a private practice in Oklahoma prior to joining the FDA's Center for Veterinary Medicine (CVM) in September 1977. He was appointed Chief Veterinarian of the USPHS and Deputy Director of the CVM in June 1994. In February 1999, he was named Chief of Staff, Office of the Surgeon General.

Dr. Michael B. Zemel is a Professor of Nutrition and Medicine and directs the Nutrition Institute at the University of Tennessee. He received his graduate training in nutritional sciences and physiology at the University of Wisconsin-Madison. From 1980 until assuming his present position in 1990, Dr. Zemel served as assistant and then associate professor in the Department of Nutrition and in the Endocrinology Division of the Department of Medicine at Wayne State University (WSU). He was also Research Endocrinologist at the VA Medical Center, affiliated with the WSU School of Medicine, from 1987 to 1990. Dr. Zemel is author of over 150 refereed publications primarily describing investigations of the role of cell calcium regulation in obesity, insulin resistance and hypertension. His current work focuses on obesity genetics, the regulation of human adipocyte lipogenesis and lipolysis via calcium-linked mechanisms, and novel modulation of obesity by dietary calcium and dairy products.

Dr. Carl Jackson is a member of the Hematology-Oncology Department faculty at St. Jude Children's Research Hospital and is the Associate Director of Academic Programs. He earned his PhD from the University of Tennessee-Knoxville in 1971. His research interests are megakaryocyte differentiation, hemopoiesis, hemopoietic regulation, platelet function, and radiation hematology. One of Dr. Jackson's most recent publications, "Acute myeloid leukemia-associated Mkl1 (Mrtf-a) is a key regulator of mammary gland function," appeared in the journal *Molecular and Cellular Biology* (2006;26:5809-26). Recently, Dr. Jackson has initiated a nonprofit research institute devoted to the development of prevention and treatment for chronic health problems of mature adults. The Olympic Peninsula Seniors' Health Research Institute will be located in Sequim, Washington.

Dr. Karla Matteson received her PhD in biochemistry from the Medical College of Wisconsin in 1981. She began her training in genetics during her post-doctoral appointment in the Department of Genetics at Baylor College of Medicine in Houston and three years as a staff scientist in the Department of Pediatrics at the University of California-San Francisco. Dr. Matteson came to the University of Tennessee Medical Center in 1986, where she set up a research laboratory in the molecular biological investigation of adrenal steroid producing genes, established the clinical molecular genetic diagnostic laboratory, and assumed the leadership of the biochemical genetics laboratory within the Genetics Center. In the more than 20 years she has been with the Medical Center, Dr. Matteson has worked with dozens of students within the CEM program as a committee member and taught a variety of courses.

Abstracts

THE UNIVERSITY of TENNESSEE
Comparative and Experimental Medicine

1:00-2:00 pm
A117

Angela L. Lusby,[1] Claudia A. Kirk,[1] Stephen A. Kania,[2] Mohamed Abd-Eldaim,[2] Joseph W. Bartges[1]

1. Small Animal Clinical Sciences, 2. Comparative Medicine
Major Professor: Dr. Claudia Kirk

Validation of an ELISA Assay for Measuring Serum Adiponectin in Felids

Adiponectin is a hormone secreted from adipose tissue that closely associates with insulin resistance. Unlike other adipokines, adiponectin decreases as fat mass increases. Measuring adiponectin in cats will allow researchers to assess hormone function and may lead to improved screening tests and treatments for Type 2 diabetes. A commercial ELISA kit is available for adiponectin quantification in mice and rats (B-Bridge International). This study validated the assay's ability to accurately measure serum adiponectin in felids. The ELISA kit's antibody binding to feline adiponectin was confirmed using Western blot analysis. Intra-assay variation was determined using six duplicates of three serum samples measured in three separate assays. Inter-assay variation was tested using three serum samples across five assays. Linearity of the assay was determined by diluting three serum samples to 1:500, 1:1000, 1:2000, and 1:4000 over three separate assays. Assay recovery was measured by spiking two feline serum samples with 4 ng/ml, 2ng/ml, 1ng/ml, or 0.5ng/ml of mouse adiponectin over three separate assays. The parameters for validation of the ELISA assay were acceptable and support the use of this assay for detection of feline adiponectin in serum. Intra-assay and inter-assay coefficients of variation were 3.7% and 6.5%, respectively. Samples showed acceptable linearity with an average R^2 of 0.96. The average recovery of mouse adiponectin spiked into samples was 83%. In conclusion, the ELISA kit tested in this study appears to be a valid method for measuring serum adiponectin levels in cats.

Beth Wilson, Michael B. Zemel
Nutrition
Major Professor: Dr. Michael B. Zemel

The Effect of Calcium and Dairy in Weight Loss and Body Composition

Researchers have disagreed on the impact of high dairy and high calcium diets on weight and fat loss. Of the six published clinical trials, four concluded that dairy and calcium augment weight and fat loss during energy restriction and two concluded no effect. Statistical outcomes are impacted by small sample sizes due to reduced statistical power. Meta-analyses allow for a grand analysis of study endpoints with a sufficiently large sample size. In order to evaluate the effects of dietary calcium and dairy products on weight loss and body composition, a combined analysis of data from all studies was conducted. The combined analysis included all trials of similar design. In each study adult subjects were placed on energy-restricted diets. Three treatments were evaluated: diet only; diet plus high calcium; and diet plus high dairy. Four outcome variables (changes from baseline to endpoint in weight [n=305], total fat [n=303], trunk fat [n=259], and lean mass [n=260]) were analyzed. The high dairy treatment resulted in significant decreases in weight, total fat, and trunk fat beyond those in the other treatments. Differences in study had an effect in each of the outcome variables, demonstrating that the differences in these six studies impacted average changes from baseline. Changes in trunk fat and lean mass were influenced by both treatment group and study, with a significant interaction between these effects. Lean mass was equally preserved across treatment groups, with no significant difference attributable to treatment. Increasing dairy intake augmented weight and fat loss secondary to energy restriction.

Robin L. Cissell,[1] Shahira Abdel Wahab,[2] Stephen A. Kania,[2] Robert L. Donnell[1]

1. Pathobiology, 2. Comparative Medicine
Major Professor: Dr. Robert L. Donnell

Detection of Malignant Catarrhal Fever Virus White-Tailed Deer Variant in Tennessee using Real-Time Polymerase Chain Reaction

Malignant catarrhal fever (MCF) is a lymphoproliferative and inflammatory syndrome that affects many ruminant species. This syndrome is caused by several gamma herpesviruses within the rhadinovirus subgroup, the most prevalent of which is believed to be ovine herpesvirus-2 (OvHV-2). This virus is endemic in sheep in Tennessee, and clinical cases are known to occur historically in other ruminant species across the state. In 2000, a novel variant associated with the white-tailed deer was described, designated white-tailed deer variant malignant catarrhal fever virus (MCFV-WTD). We hypothesize that MCFV-WTD is present in Tennessee wild deer populations and can be spread to domestic livestock. Convenience samples of formalin-fixed lymph nodes from wild deer populations in east and middle Tennessee harvested in the fall of 2005, as well as blood samples and frozen lymph nodes obtained in 2006, will be used to test this hypothesis. Real-time polymerase chain reaction methodology will be developed and validated for testing formalin-fixed and fresh frozen lymph node samples, as well as blood samples, to determine the presence of MCFV-WTD. Enzyme-linked immunosorbent assay will be performed using sera from 2006 (year) blood samples. We hope to demonstrate the previously undocumented presence of MCFV-WTD virus in the wild population of white-tailed deer in Tennessee and use this methodology to detect evidence of MCFV-WTD virus infection in domestic livestock such as cattle.

Karissa Dawn Laughter, Barton Rohrbach, Agricola Odoi
Comparative Medicine
Major Professor: Dr. Agricola Odoi

A Descriptive Study of Campylobacteriosis Cases Reported in East Tennessee

*Campylobacte*r infections are a leading cause of gastrointestinal illness worldwide. Up to 2.5 million cases occur each year in the United States, resulting in an annual cost of approximately $8.0 billion. The reported incidence of campylobacteriosis in the United States during 2006 was 12.71 per 100,000 population, while in Tennessee it was only 7.4 per 100,000. In spite of this lower incidence in Tennessee, some counties in East Tennessee have experienced incidence rates higher than the national average. This suggests that localized pockets of high rates may exist and may be associated with specific risk factors that, if identified, would provide information for improving control strategies. This study will evaluate data reported through the Foodborne Disease Active Surveillance Network (Foodnet) between 2003 and 2006 in East Tennessee. The purpose of the study is to identify high risk communities to assist health officials in planning control programs. We will assess spatial and temporal patterns in the distribution of reported cases of campylobacteriosis, identify risk factors, and describe the demographic characteristics of cases. Geographic Information Systems will be employed to investigate the association of disease risk with environmental factors. Data analyses are underway, and preliminary results suggest there is an increase in reported cases during the summer months and a higher incidence in some communities.

1:00-2:00
A118

Jonathan Phipps, James S. Foster, Daniel P. Kestler, Steve Kennel, Alan Solomon, Jonathan S. Wall
Medicine, Human Immunology/Cancer Program
Major Professor: Dr. Jonathan S. Wall

In Vitro Silencing of Amyloidogenic λ6 Light Chain Production

A main contributing factor to the morbidity and mortality of patients with multiple myeloma results from the pathologic deposition of monoclonal Ig light chains, i.e., Bence-Jones proteins (BJP), as casts within renal tubules. As an alternative to plasma cell chemotherapy to reduce BJP synthesis and thus prevent myeloma (cast) nephropathy, we have investigated another means to achieve this objective, namely, RNA interference (RNAi). Experimentally, we have stably transfected the SP2/O mouse myeloma cells with a construct containing the V, J, and C region of a λ 6 BJP (Wil) under the control of a CMV promoter and have shown that they constitutively express measurable quantities of mRNA and protein Wil. Treatment of these cells with synthetic, small double stranded small interfering RNAs (siRNA) based on the Wil protein sequence reduced target mRNA levels by 13-fold at 48 h and protein production by 30% at 72 h, as compared with controls. These data provide conclusive evidence that RNAi can effectively reduce BJP production *in vitro* and provide the base for testing the clinical potential of this strategy using a relevant *in vivo* murine model of myeloma (cast) nephropathy.

Amanda Peretich,[1] Guozhang Mao,[2] Nancy Neilsen,[2] Xuemin Xu,[2] Carla Sommardahl,[1] Madhu Dhar[1]
1. Large Animal Clinical Sciences, 2. Pathobiology
Major Professor: Dr. Madhu Dhar

Hormonal Regulation of *Atp10c*, a Type 4 P-type ATPase, in Mouse Adipocytes

Mice heterozygous for a novel type 4 ATPase, *Atp10c*, exhibit typical hallmarks of insulin resistance syndrome including obesity, hyperinsulinemia, and insulin resistance. Type 4 ATPases are aminophospholipid translocases and have been associated with human disorders of intramembranous transport. Although there is a consensus on their physiological function in intracellular signaling and protein trafficking, their precise biological roles are poorly understood. *Atp10c* is highly expressed in adipose tissue, but its function in adipocytes to modulate glucose and lipid metabolism is unclear. In the present study, we have established an adipocyte cell system to investigate the role of *Atp10c* in insulin signaling and adipocyte differentiation. 3T3-L1 cells are used as an *in vitro* model, and primary cultures are used as an *in vivo* model. Adipocyte differentiation was assessed by Oil Red O staining and mRNA expression of the adipocyte markers resistin and peroxisome proliferator-activated receptor-γ (PPARγ). We demonstrated for the first time that *Atp10c* is expressed in undifferentiated and differentiated cells *in vitro* and *in vivo*, suggesting a direct link between *Atp10c* and adipocyte development. Our data unequivocally show that *Atp10c* mRNA and its cognate protein are significantly down-regulated during adipocyte differentiation beginning at day 2. In 3T3-L1 cells, this decrease coincides with the transcriptional and morphological changes that are characteristic of adipocytes. Furthermore, *Atp10c* expression is up-regulated 2-fold by insulin and dexamethasone in 3T3-L1 adipocytes, suggesting regulation by hormonal inducers. Currently, experiments are underway to investigate the effects of suppression and overexpression of ATP10C on other adipogenic markers.

Ferenc Tóth,[1] Nicholas Frank,[1] Sarah B. Elliott,[1] Raymond J. Geor,[2] Raymond C. Boston[3]
1. Large Animal Clinical Sciences, 2. Middleburg Agricultural Research and Extension Center, Virginia Polytechnic and State University, Middleburg, VA 20117, 3. Department of Clinical Studies, University of Pennsylvania, Kennett Square, PA 19348.
Major Professor: Dr. Nicholas Frank

Effects of an Intravenous Endotoxin Challenge on Glucose and Insulin Dynamics in Mares

Our objective is to evaluate the effects of exogenous endotoxin on glucose and insulin dynamics in mares. Sixteen adult mares were evaluated, with each mare receiving one treatment per week for 2 weeks according to a randomized crossover study design. Treatments consisted of 20 ng/kg body weight of *Escherichia coli* O55:B5 lipopolysaccharide (LPS) administered intravenously over a 30-minute period in 60 mL sterile saline (treatment) or sterile saline alone (control). Frequently-sampled intravenous glucose tolerance test (FSIGT) procedures were performed at -24h, 24h, and 48h relative to endotoxin administration, and data were assessed by minimal model analysis. Only 13 of 16 mares exhibited a clinical response to LPS, characterized by mild colic and leukopenia. Injection of LPS significantly lowered (P = 0.041) insulin sensitivity (SI), and raised (P = 0.006) the acute insulin response to glucose (AIRg) over time. Mean ± SD SI significantly decreased from 2.9 ± 1.9 prior to treatment to 0.9 ± 0.9 after 24h (a 69% reduction), before returning to 1.5 ± 0.9 $\times 10^{-4}$ L/min/mU by 48h. Mean ± SD AIRg significantly increased from 520 ± 196 prior to treatment to 938 ± 620 (80% increase) and 755 ± 400 (45% increase) mU•min/L at 24 and 48h post-injection, respectively. Mean glucose effectiveness (Sg) was not significantly altered by LPS administration. Insulin sensitivity was lowered for 24h after intravenous injection of LPS, and affected mares exhibited a compensatory pancreatic response. These disturbances in glucose and insulin dynamics may contribute to the development of laminitis in horses.

Dongwei Wu, Longsheng Sun, Mingqi Tan, Feng Hao, Xuemin Xu, Robert Donnell, Mei-Zhen Cui
Pathobiology
Major Professor: Dr. Mei-Zhen Cui

Lysophosphatidic Acid Induces the Expression of Tissue Factor and Early Growth Response Gene-1 *in vivo*

Tissue factor, the prime initiator of the blood coagulation cascade, is the key mediator of thrombosis. Early growth response gene (Egr-1) is an important transcription factor that regulates expression of an array of genes that are involved in the development of atherosclerosis and thrombosis. We previously reported that lysophosphatidic acid (LPA), the potent bioactive component of oxidized low density lipoprotein-induced Egr-1 transcription, is regulated by CREB and SRF transcription factors in vascular smooth muscle cells *in vitro*. The present study examines whether and how LPA affects tissue factor and Egr-1 expression in vascular walls and in other tissues *in vivo*. Our Northern blotting results demonstrate that LPA markedly induces the expression of messenger RNA of Egr-1 and tissue factor in aorta, heart, lung, kidney, and liver in living mice. *In situ* hybridization analysis demonstrates that strong Egr-1 mRNA signal has been detected in these tissues in response to LPA. The expression of Egr-1 protein is also markedly induced by LPA in these tissues. Our data also demonstrate that activation of mitogen-activated protein kinase (MAPK) is rapidly induced by LPA in these tissues. Pretreatment with MAPK kinase inhibitor U0126 completely blocked LPA-induced expression of Egr-1 and tissue factor in these tissues. These data indicate that LPA induces expression of Egr-1 and tissue factor via the MAPK signaling pathway *in vivo*. Our results suggest that LPA, through activation of Egr-1 that in turn regulates tissue factor expression, contributes to the development of atherosclerosis and thrombosis.

2:15-3:15
A117

Hye Mee Joo, Yuxia He, Mark Y. Sangster
Microbiology
Major Professor: Dr. Mark Y. Sangster

Quantitative Aspects of Influenza Virus-Specific Memory B Cells Generated by Infection and Vaccination

Memory B cells (MBCs) generated by infection or vaccination contribute significantly to resistance to infection. However, quantitative aspects of MBC generation have received little attention. Our objective was a quantitative analysis of the virus-specific MBC pool induced by influenza infection of the respiratory tract in mice. Virus-specific IgG MBC frequencies in different anatomical compartments were determined by limiting dilution assay (LDA) 8-12 weeks after infection. The LDA was based on in vitro stimulation of influenza-specific MBCs to divide and differentiate into antibody-secreting cells, which were detected by ELISPOT assay. Influenza-specific IgG MBCs were identified in a broad range of anatomical locations, a result consistent with the concept of MBC distribution to secondary lymphoid tissues throughout the body. However, MBCs were not equally distributed. In addition, a kinetic analysis demonstrated early circulation of MBCs in the blood and rapid trafficking into the lung. MBCs in the lung may play an important role in protection and could serve as a goal of vaccination regimens.

Sharvan Sehrawat, Barry T. Rouse
Pathobiology
Major Professor: Dr. Barry T. Rouse

***In Vitro* Generated Antigen Specific CD4+CD25+Foxp3+ Regulatory T Cells Control the Severity Of Virus Induced Immunoinflammatory Lesion**

The purpose of this study is to discover novel means of controlling an immunoinflammatory ocular disease caused by Herpes simplex virus infection (HSV). The focus will be on immunotherapy using adoptive transfers of T lymphocytes that are induced to generate regulatory activity *in vitro*. We have defined the optimal conditions for inducing regulatory T cells (Tregs) that are specific to a peptide derived from ovalbumin (OVA). More than 70% of the cells were shown to express the signature phenotype Foxp3+ and were shown to have regulatory activity *in vitro*. Two models were used to determine if the *in vitro* generated, OVA specific Treg could influence the expression of ocular disease following infection of mice with HSV. One model was immunocompetent BALB/c mice and another was T cell transgenic mice, which develop stromal keratitis after HSV infection even though their T cells recognize only the OVA peptide. The adoptive transfer of cells before or early after infection was shown to significantly reduce the severity of lesions in comparison to control animals not given Tregs. We were also able to demonstrate the presence of adoptively transferred cells in the ocular lesion as well as in several other tissues. The effect of cell transfer on viral clearance and the immune response of the recipient will also be reported. These results demonstrate that immunotherapy is a valuable means of controlling viral immune inflammatory disease. The fact that the ova specific Tregs were effective in the BALB/c model has important implications in understanding mechanisms by which the T cells function *in vivo*.

Pranita P. Sarangi, Barry T. Rouse
Pathobiology
Major Professor: Dr. Barry T. Rouse

IL-10 and Natural Regulatory T Cells: Two Independent Anti-Inflammatory Mechanisms in HSV-Induced Ocular Immunopathology

Controlling the extent of the immunoinflammatory disease caused by HSV-1 in the corneal stroma represents an important therapeutic objective. One prominent mechanism involves the production of the cytokine IL-10 and another the activity of natural regulatory T cells (nTregs). Although we have shown that blocking IL-10 could neutralize the activity of nTregs, it is not known whether under *in vivo* conditions, IL-10 and nTreg influence the corneal pathology independently or in concert. Interestingly, IL-$10^{-/-}$ animals depleted of nTregs prior to ocular infection showed more severe SK lesions as compared to undepleted IL-$10^{-/-}$ animals. These results show the presence of two independent

anti-inflammatory mechanisms in stromal keratitis (SK). Along this line, non regulatory cells were the major source of IL-10 in the spleen and draining lymph nodes (DLNs) of infected mice, although animals depleted of Tregs showed higher levels of IL-10 in the DLNs. In addition, we failed to find evidence for inducible regulatory cells in the infected wild-type mice. Furthermore, by using wild-type, IL-$10^{-/-}$ and Treg depleted wild-type and IL-$10^{-/-}$ animals, we have compared the relative contribution of IL-10 and nTregs in controlling SK pathology. Significantly higher lesion severity was noted in nTreg-depleted IL-$10^{-/-}$ followed by IL-$10^{-/-}$, nTreg-depleted wild-type and undepleted wild-type animals. Intracellular cytokine staining with UV-inactivated HSV and polyclonal stimulation of the cells isolated from infected corneas, cervical DLNs and spleens revealed that the IL-10-mediated mechanisms more profoundly influenced the immunopathogenesis of SK as compared to nTregs and that the latter likely acted in an IL-10 independent manner.

This work was supported by a National Institute of Health Grant EY05093.

2:15-3:15
A118

Jason L. Liggett, Seung Joon Baek
Pathobiology
Major Professor: Dr. Seung Joon Baek

NUANCE, a Potential Novel Oncogene is Inhibited by Nonsteroidal Anti-Inflammatory Drugs in Human Colorectal Cancer Cells

The discovery and characterization of new oncogenes can lead to improved targets for chemotherapeutic drugs. Sulindac sulfide (SS) is a well known drug for treating inflammatory disease and cancer. However, its mechanisms are not fully understood. Our lab has previously shown it to be a strong inducer of non-steroidal anti-inflammatory drug activated gene one (NAG-1.) This makes SS a prime candidate for further study. Affymetrix microarray was performed using HCT-116 cells treated with SS, and NUANCE (nucleus and actin connecting element) was selected from microarray data based on its novelty in relation to colorectal cancer. This gene was recently described as a novel connection between the actin skeleton and the nuclear envelope, containing an N-terminal actin binding domain and a C-terminal transmembrane domain. RT-PCR was performed in at least three replicates, and data was normalized to GAPDH. SS diminished NUANCE mRNA expression to about one third of the levels observed in the vehicle treated HCT-116 cells. The inhibitory action of SS on NUANCE mRNA was shown to be time and dose dependant. Various other NSAIDs were selected (tolfenamic acid, diclofenac, SC-560, and DFU) and demonstrated that inhibition of NUANCE mRNA was not unique to SS. Additionally, HT-29 showed a slight, albeit significant, reduction in NUANCE mRNA, while SW480 cells did not significantly change. We hypothesize that NUANCE is a potential novel oncogene in human colorectal cancer cells. Our results suggest that this action is cyclooxygenase-independent. Furthermore, expression of NUANCE protein in cells and tissues will be explored using immunofluorescence.

Nichelle Whitlock,[1] Kiyoshi Yamaguchi,[2] and Seung Joon Baek[1]
1. Pathobiology, 2. Current address: Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
Major Professor: Dr. Seung Joon Baek

Nonsteroidal Anti-Inflammatory Drug Activated Gene (NAG-1) as a Chemopreventive Target in Canine Tumorigenesis

Nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1), a divergent member of the transforming growth factor-β (TGF-β) superfamily, was previously identified as a gene induced by several anti-tumorigenic compounds including NSAIDs and peroxisome proliferator-activated receptor γ (PPARγ) ligands. NAG-1 was also found to have anti-tumorigenic and/or pro-apoptotic activities in several types of human cancer cells. However, canine NAG-1 has yet to be characterized. In this report, we show that the properties of NAG-1 expression exist in canine osteosarcoma. The predicted NAG-1 cDNA sequence encodes nine conserved cysteine residues and a RXXR site in the middle of the pro-peptide. Phylogenetic analysis indicated that the canine NAG-1 was more homologous with mouse and rat than with human. The NAG-1 protein was detected in canine liver, lung, and kidney tissues. We also found that, as reported previously for human NAG-1, expression of canine NAG-1 is increased by treatment with some NSAIDs, including piroxicam and SC-560. In addition, the PPARγ ligand rosiglitazone also increased

NAG-1 expression, as assessed by reverse transcription-PCR. This study demonstrates that, like human NAG-1, canine NAG-1 is up-regulated by NSAIDs as well as anti-tumorigenic compounds in osteosarcoma and may provide an important role of NAG-1 in prevention of canine cancers.

Shambhunath Choudhary, Hwa-Chain Robert Wang
Pathobiology
Major Professor: Dr. Hwa-Chain Robert Wang

Oncogenic H-Ras Facilitates Apoptosis Induced by Histone Deacetylase Inhibitors in Human Urinary Bladder Cancer Cells

We sought to understand the connection between the selectivity of histone deacetylase inhibitors (HDACIs), a new class of anticancer agents shown to induce apoptosis in transformed cancer cells, and the pro-apoptotic ability of oncogenic H-Ras to facilitate that apoptosis. More than 35% of human urinary bladder cancers involve oncogenic H-Ras activation. In addition to tumorigenic ability, oncogenic H-Ras possesses a novel pro-apoptotic potential to facilitate the induction of apoptosis by HDACIs, which are highly toxic to transformed cells. To understand this connection, we introduced oncogenic H-Ras into urinary bladder J82 cancer cells to mimic an acquisition of H-ras gene activation in tumor development. Expression of oncogenic H-Ras promoted J82 cells to acquire tumorigenic ability. Meanwhile, oncogenic H-Ras increased susceptibility and reduced resistance of J82 cells to HDACIs, including FR901228 and Trichostatin A (TSA), for inducing apoptosis. The extrinsic and intrinsic caspase pathways, the B-Raf and extracellular signal regulatory kinase (ERK) pathway, the stress-activated protein kinase (SAPK) pathway, the cell cycle dependent kinase inhibitors p21Cip1 and p27Kip1, and core histone contents are regulated differently by FR901228 in oncogenic H-Ras-expressed J82 cells than their counterparts in parental J82 cells, contributing to the increased susceptibility to the induction of selective apoptosis. Our results lead us to a suggestion that HDACIs activate the pro-apoptotic potential of oncogenic H-Ras, indicating a potential therapeutic value of this new class of anticancer agents in the control of human urinary bladder cancer that has acquired oncogenic H-Ras.

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Green Tea Catechins Suppress Basic Fibroblast Growth Factor In Vitro And In Vivo

Epigallocatechin gallate (*EGCG*) is the most abundant catechin compound found in green tea and has been shown to act as anti-angiogenic agent, suppressing the pro-angiogenic factors, but the precise mechanism is not yet known. On the basis of previous reports, which showed suppression of bFGF by green tea catechins, we found that ECG and EGCG suppressed bFGF expression at the translation level. Western blot analysis using catechin-treated cells showed that EGCG/ECG suppressed bFGF more significantly than other catechins. To investigate further mechanisms, we examined several signaling pathways using known inhibitors; interestingly, only lactacystin treatment affected EGCG-induced bFGF suppression in human colorectal cancer cells. This result suggests that EGCG may facilitate protein degradation of bFGF. Finally, animal experiments were performed using Min mice. The tumor load and tumor number were significantly less in EGCG treated mice. The ELISA assay carried out using the small intestinal tissue samples from these Min mice showed a significant decrease in mice fed with EGCG. All the data were supportive to prove the hypothesis of down regulation of bFGF by EGCG.


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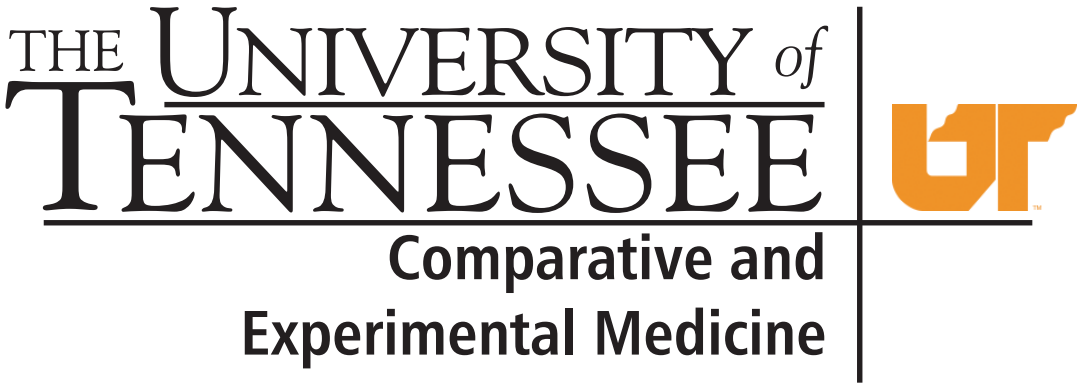
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