Comparative & Experimental Medicine and Public Health Research Symposium

May 21 & 22, 2012

Program & Schedule

Sponsored by the College of Veterinary Medicine, Center for Health Policy and Services Research, Tennessee AgResearch, UT Graduate School, and the UTK Office of Research

Welcome

Once again, the University of Tennessee (UT) Agricultural Campus is hosting a symposium for UT investigators with animal and human health interests. This symposium is growing explosively and is rapidly becoming a calendar event for the Knoxville campuses of UT. Comparative and Experimental Medicine (CEM), a graduate program that is shared by the College of Veterinary Medicine and the Graduate School of Medicine, initiated this symposium in 2007 as an event to showcase the research of students and new investigators in their program. In 2008, the symposium was opened to participants throughout the Knoxville campuses, and there was a four-fold increase in presentations with representation from 19 different UT departments and programs. For the fourth consecutive year, the Center for Health Policy and Services Research has teamed with CEM to produce a joint *Comparative & Experimental Medicine and Public Health Research Symposium* hosting an even larger group of scientists including 76 presenters representing 21 different UT departments and programs.

The *Comparative & Experimental Medicine and Public Health Research Symposium* has gained both a reputation and recognition for providing an excellent venue for students and new investigators to gain experience showcasing their work as oral presentations. In addition, the gathering of UT investigators with related and varying interests provides opportunities for the creation of new ideas, collaborations, and networking that will enhance health-related research at the UT Knoxville campuses. The joint sponsorship of the symposium by the College of Veterinary Medicine, the UT Center for Health Policy and Services Research, Tennessee AgResearch, the UT Graduate School, and the UT Knoxville Office of Research is unprecedented and signifies both a shared recognition of the need for such a symposium and a cooperative spirit in bringing this exciting event to reality.

We are happy to welcome all participants and attendees and hope the experience will be as positive as it is promising.

Larry Arrington, Chancellor
University of Tennessee
Institute of Agriculture

Landon Mathers

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We wish to acknowledge the following university programs and individuals, without whom this event would not be possible:

College of Veterinary Medicine

Center for Health Policy and Services Research

Tennessee AgResearch

UTK Office of Research

UT Graduate School

Misty Bailey
Tammy Berry
Debra L Butenko
Michael Cunningham
Paul Campbell Erwin
Michael McEntee
Carole Myers
Keith Roberts
Kim Rutherford
Kendall Stokes
Anik Vasington

We appreciate the contributions of session moderators and judges.

Thanks also to the UTCVM chapter of Phi Zeta, the UTIA chapter of Gamma Sigma Delta, 2012 Center of Excellence Summer Student Research Program participants, and our sponsors and exhibitors.

Jimmy Cheek, *Chancellor*
UT Knoxville

Larry Arrington, *Chancellor*
UTIA

James Thompson, *Dean*
College of Veterinary Medicine

Lee Riedinger, *Interim Vice Chancellor for Research*
UT Knoxville Office of Research

William F. Brown, *Dean*
Tennessee AgResearch

Robert Rider, *Dean*
College of Education, Health & Human Sciences

Carolyn Hodges, *Dean*
UT Graduate School

Schedule at a Glance

Monday, May 21

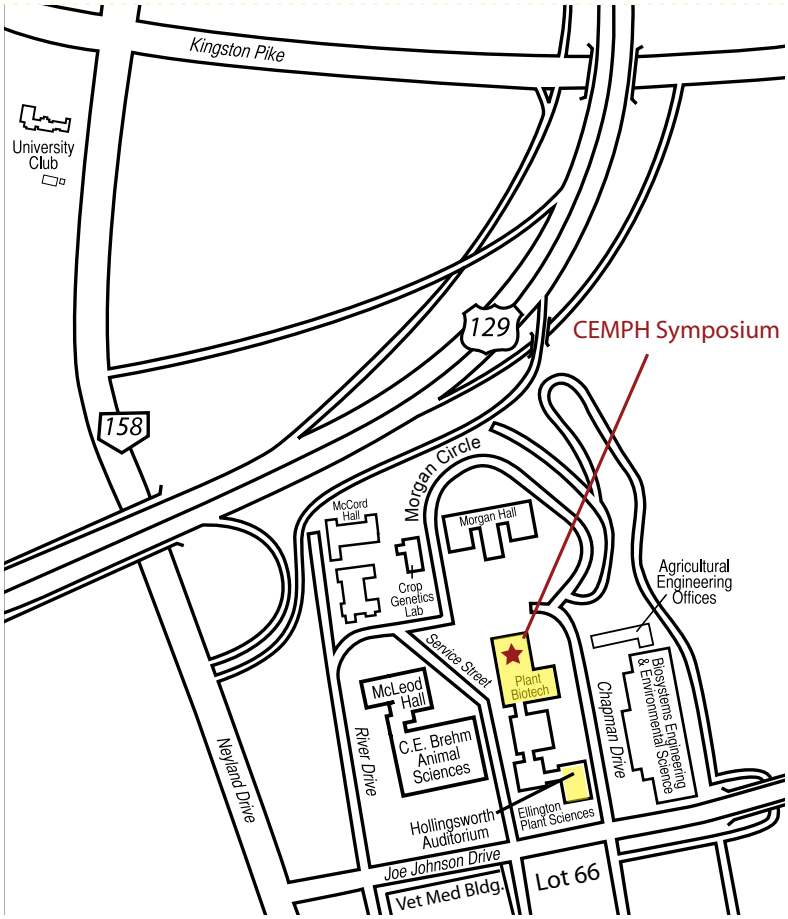
	Room	Event
8:30-9:00	PBB	Morning refreshments
9:00-10:00	156/157 PBB	Keynote address: Sten H. Vermund, MD, PhD, "AIDS/TB/STIs in Africa: How Did We Get Here and Where are We Going?"
10:30-12:00	See session matrix (p. 6)	New investigator presentations
12:00-1:00	160 PBB	Featured speaker: Jay Whelan, MPH, PhD, "Diet: A Treatment for Cancer?"
1:30-3:00	See session matrix (p. 7)	New investigator presentations

Tuesday, May 22

	Room	Event
8:30-9:00	PBB	Morning refreshments
9:00-10:00	156/157 PBB	Plenary address: David Allison, PhD, "Open-Mindedness and Skepticism about Causes and Alleviators of the Obesity Epidemic"
10:30-12:00	See session matrix (p. 8)	New investigator presentations
12:00-1:00	160 PBB	Featured speaker: Lee Riedinger, PhD, "Interdisciplinary Research and Education–A Growing Emphasis in Academia"
1:15-4:15	See session matrix (p. 9)	New investigator presentations
6:00	Hollingsworth Auditorium	Awards banquet & after-dinner address: Dr. Gary McCracken, "White Nose Syndrome – An Emerging Infectious Disease of Bats in North America"

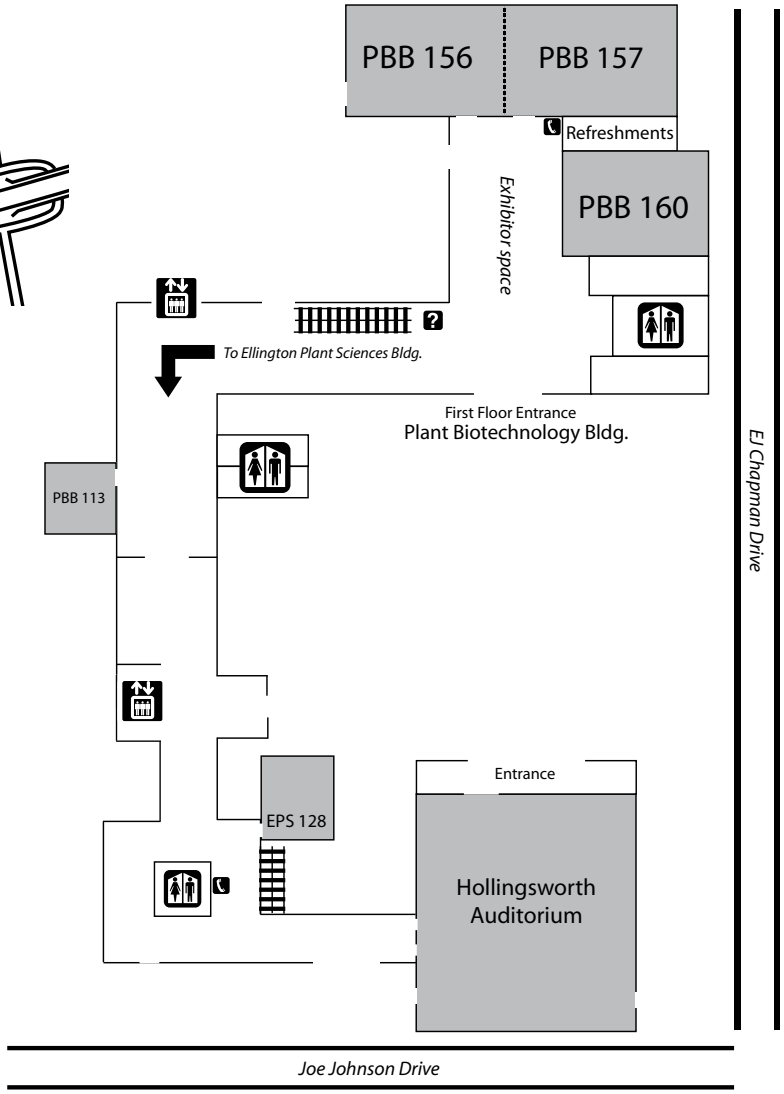
PBB, Plant Biotechnology Building (*see map on p. 5*)

Location Information



University of Tennessee Agricultural Campus

Plant Biotechnology Building



Parking

All parking in campus lots is by permit only. Faculty, staff, and students may ride the "T" (free for all UT faculty, staff, & students with ID). The T: East to West circles every 15 minutes between 7:00 am and 6:00 pm. Students with valid permits should park in designated student parking areas.

__Notice__: Lot 66 may no longer be used by those without permits for that specific lot (violators may be ticketed or towed). All visitors will need a temporary parking permit.

Session Matrix (Abstracts on pp. 14-44)

Monday, May 21

	Oncology & Cancer Cell Biology	Clinical Sciences	Bacterial Virulence & Transmission
	Rm. PBB 156/157	**Rm. PBB 113**	**Rm. PBB 160**
10:30	1. Effects of Stimulatory and Inhibitory Neurotransmitters on Pancreatic Cancer Cells and Their Normal Cells of Origin (**Al-Wadei**)	11. Logic: A Pain in the Anterior Cingulate! (**Phillips**)	23. The Transcriptome Expression Profiles of *S. uberis* UT888 after Co-culture with Primary Bovine Mammary Epithelial Cells (**Kerro Dego**)
10:45	2. Intervention of Human Breast Cell Carcinogenesis Chronically Induced by 3, 4, 4'-trichlorocarbanilide (**Sood**)	12. State-Dependent Neurophysiology II: Depression (**Callaway**)	24. Pursuit of in silico Discovery of Novel Gene Features Associated with Virulence/Pathogenic Interactions from *Streptococcus uberis* Genomic Data (**Luther**)
11:00	3. Premalignant and Malignant Breast Cell Carcinogenesis Induced by Dietary Carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (**Choudhary**)	13. Self-Regulation as a Cure for Anxiety (**Di Loreto**)	25. *Streptococcus uberis*, Biofilms, and Fluorescent Bioreporters (**Prado**)
11:15	4. Cyclooxygenase-2 Induction by Masitinib (AB1010) in Head and Neck Squamous Cell Carcinomas through Activation of MAPK Signaling Pathway (**Rathore**)	14. Depression, Neurocognition, and Cingulate Cortex Lesions in Multiple Sclerosis (**Shaw**)	26. Antimicrobial Susceptibility and Genetic Characterization of *Staphylococcus pseudintermedius* Isolates from 206 Dogs (**Videla**)
11:30	5. Role of Nicotine-Derived Nitrosamine Ketone and Benzo[a] pyrene on 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-Induced Breast Cell Carcinogenesis (**Pluchino**)	15. Effects of a Home-Based Exercise Program on Perception of Illness and Adaptation in Heart Failure Patients (**Harris**)	27. Immune Response to *Staphylococcus pseudintermedius* (**Solyman**)
11:45	6. Initial Experience with VSV-hIFNβ-NIS in Dogs: A Novel Oncolytic Virus (**LeBlanc**)	16. Does Increasing Step Width Alter Knee Biomechanics in Medial Compartment Knee Osteoarthritis Patients During Stair Descent? (**Paquette**)	28. In vitro Characterization of Cee, A Novel Periplasmic Ferric Enterobactin Esterase in *Campylobacter* (**Mo**)
12:00	**BREAK for Lunch – See schedule on p. 4**		

*PBB, Plant Biotechnology Building

Monday, May 21

	Oncology & Cancer Cell Biology	Clinical Sciences	Bacterial Virulence & Transmission
	Rm. PBB 156/157	**Rm. PBB 113**	**Rm. PBB 160**
1:30	7. Epithelial to Mesenchymal Transition and Stem-Like Cell Properties Targeted by Dietary Compounds in Suppression of Chronic Breast Cell Carcinogenesis (**Rathore**)	17. Incisional Wound Healing in Ball Pythons (*Python regius*): A Comparison of Carbon Dioxide Laser, 4.0 MHz Radiosurgery, and Scalpel (**Hodshon**)	29. Identification of Cj0843c, a Putative Lytic Transglycosylase Involved in Beta-Lactam Resistance in *Campylobacter jejuni* (**Ximin**)
1:45	8. Characterization of PPARγ Ligand MCC-555 on AOM-Induced Colorectal Tumorigenesis (**Imchen**)	18. Laparoscopic-Assisted Ovariectomy of Tigers (*Panthera tigris*) using the Ligasure Device (**Steeil**)	30. The Stress of *Enterococcus faecalis* (**Saito**)
2:00	9. Characterization of a Novel Truncated EpCAM Induced by Sulindac Sulfide (**Liggett**)	19. Amino Acid, Iodine, Selenium, and Coat Color Status among Hyperthyroid, Siamese, and Age-Matched Control Cats (**Sabatino**)	31. Regulation of the Toxic, Small Zor Proteins in *Escherichia coli* O157: H7 (**Wen**)
2:15	10. NAG-1, a Secreted TGF-β Superfamily Cytokine, Localizes Both in the Cytoplasm and Nucleus (**Kyung-Won**)	20. Magnetic Resonance Imaging of the Canine Cranial Abdomen: Quantitative and Qualitative Evaluation of Various Imaging Sequences (**Manley**)	
2:30		21. Effects of Intravenously-Administered Esomeprazole Sodium on Gastric Juice pH in Adult Female Horses (**Videla**)	
2:45		22. Evaluation of Fluorocoxib A, a Novel COX-2-Targeted Optical Imaging Agent (**Cekanova**)	

Tuesday, May 22

	Innovative Biomedical Technologies	Pharmacology & Pharmacokinetics	Population Health & Epidemiology
	Rm. PBB 113	**Rm. PBB 156/157**	**Rm. PBB 160**
10:30	32. Assessing Medical Fellows' Baseline Knowledge in Research Methods and Statistics (**Barlow**)	48. The Effect of Fentanyl on Sevoflurane Minimum Alveolar Concentration Preventing Motor Movement (MACNM) in Dogs (**Reilly**)	65. Exploring the Role of Social Capital in Resettlement, Health, and Well-being among Burundian Refugees in Knoxville: Implications for Health Interventions and Policy (**Njororai**)
10:45	33. A Neural Network Model for Mortality Prediction in ICU (**Xia**)	49. Pharmacokinetics of Terbinafine after Oral Administration of Single Doses in Hispaniolan Amazon Parrots (*Amazona ventralis*) (**Evans**)	66. Continuous Monitoring of Hormonally-Active Compounds in Effluents from Hallsdale-Powell Wastewater Treatment Facility at Knoxville, TN (**Wang**)
11:00	34. Diagnostics for Mild Cognitive Impairment and Early Alzheimer's Disease (**McBride**)	50. The Effect of Topical 2% Delta-9-Tetrahydrocannabinol Suspension on Aqueous Humor Flow Rate and Intraocular Pressure in Normal Dogs (**Fischer**)	67. How a PAH-Degrading Microbial Community is Affected by Temperature Change (**Smartt**)
11:15	35. Revealing Putative Gene Networks Perturbed by Low-Dose Ionizing Radiation using a Differentially-Correlated Graph Generated by a Two-Stage Statistical Filter (**Naswa**)	51. Effect of Siam Weed Extracts on Anti-Inflammatory Activity (**Pandith**)	68. *Amblyomma americanum* L. at Ames Plantation: Distribution, Seasonality, Habitat Associations, and Pathogens (**Hendricks**)
		Endocrinology	
11:30	36. Rapid and Stable Binding of Peptide p5 to Visceral Amyloid Deposits In Vivo Yields a Potential Radiotracer for PET/CT Imaging (**Martin**)	52. Do Plastic Bottles Leach Substances that Interfere with Human Hormones? (**Eldridge**)	69. Transmission of *Toxoplasma gondii* in Wildlife in the Southeastern United States (**Gerhold**)
11:45	37. Improving Bacterial Bioluminescence in Human Cells for Novel Imaging Applications (**Xu**)	54. Estrogenic Activity Produced by *Talinum paniculata* (Jacq.) Gaertn's Leaf Extract in Ovariectomized Rats (**Thanamool**)	
12:00	**BREAK for Lunch–See schedule on p. 4**		

Tuesday, May 22

	Innovative Biomedical Technologies	Endocrinology	Viral Pathology & Immunity
	Rm. PBB 113	**Rm. PBB 156/157**	**Rm. PBB 160**
1:15	38. 18FLT-PET/CT for Non-invasive Functional Imaging of Canine Bone Marrow (**Rowe**)	55. Testicular Steroidogenesis in Pre-pubertal Rats Exposed to Aroclor-1242 (**Sashi Papu John**)	70. Comparison of RT-PCR and RT-LAMP Assays for Human Norovirus GII Detection (**Cao**)
1:30	39. Effects of Environmental Carcinogens on Canine Mesenchymal Stem Cells (MSC) Isolated from Adipose Tissue (**Tanco**)	56. Juvenile Hormone Interaction with Insulin Signaling is Critical for Lipid Metabolism during Tsetse Pregnancy (**Baumann**)	71. Community Composition Matters: Amplification Hosts Facilitate Ranavirus Outbreaks in Larval Amphibian Communities (**Brenes**)
		Nutrition & Metabolism	
1:45	40. Bone Marrow-Derived Mesenchymal Stem Cells (BMMSC) for the Treatment of Canine Osteoarthritis (**Westling**)	57. Influence of Fruit Variety and Course Sequence on Fruit Intake during a Snack in Preschool-Aged Children (**Cardoso**)	72. Chemical Disinfection of Human Norovirus Surrogates for the Prevention of Nosocomial Human Norovirus Outbreaks (**Cao**)
2:00	41. Comparison of In Vitro Adherence, Proliferation, and Differentiation of Equine Bone Marrow-Derived Mesenchymal Stem Cells to Identify an Allogenic Donor and to Determine the Effect on Proliferation with Platelet-Rich Plasma (**Carter-Arnold**)	58. Active Video Gaming Compared to Unstructured, Outdoor Play in Children: Measurements of Estimated Energy Expenditure and Percent Time in Moderate-to-Vigorous-Intensity Physical Activity (**MacArthur**)	73. Size-Structured Model for Tissue Cyst Growth of *Toxoplasma gondii* (**Sullivan**)
2:15	42. Bacterial Cellulose and Equine-Derived Bone Marrow Mesenchymal Stem Cells as a Potential Biomaterial Construct for Tissue Engineering of Cartilage and Bone (**Favi**)	59. Do Weight Status and the Level of Dietary Restraint Moderate the Relationship between Package Unit Size and Food Intake? (**Haire**)	74. MicroRNA-155 Regulates Brain Inflammation but Promotes Ocular Immunopathology after Herpes Simplex Virus Infection (**Mulik**)
2:30	43. Polymeric Transfection of Human Aortic Smooth Muscle Cells (**Stephens**)	61. Seasonal Changes in Leptin and White Adipose Tissue in American Black Bears (**Hill**)	75. Regulatory T Cells Control the Clinical Expression of Viral Immunopathology during the Clinical Phase of Herpetic Stromal Keratitis (**Veiga-Parga**)
2:45	**BREAK**		
3:00	44. Automatic Detection of ECG Electrode Misplacement: A Tale of Two Algorithms (**Xia**)	62. Pathway Profiling Identifies Mechanisms of Adipose Deposition in Domestic Chickens (**Ji**)	76. TNFR25SF and Galectin-9 Combination Therapy Reduces Herpes Simplex Virus-1-Induced Stromal Keratitis by Expanding Regulatory T Cells and Reducing Pathogenic T Effectors (**Jagadeesh Reddy**)
3:15	45. Rapid Detection of Bovine and Human Tuberculosis by AC Electrokinetics-Enhanced Impedimetric Method (**Cui**)	53. Identification of Bile Salt Hydrolase Inhibitors, a Promising Alternative to Antibiotic Growth Promoters (**Smith**)	77. Controlling Herpes Simplex Virus-Induced Ocular Inflammatory Lesions with the Lipid-Derived Mediator Resolvin E1 (**Rajasagi**)
3:30	46. Short-Term Direct Electric Current Exposure Decreases Caspace-3 Activity in Colon Cancer Cells (**Barham**)	63. Angiotensinogen Gene Silencing in 3T3-L1 Adipocytes Reduces Lipid Accumulation and Adipose Inflammation (**Xin**)	78. Use of the M3 Chemokine Binding Protein to Understand Cytomegalovirus Dissemination (**Robinson**)
3:45	47. Monitoring the Metabolic Dynamics of Human Cells Grown in 2-D vs. 3-D Culture Environments (**Webb**)	64. Cytokine-Mediated Regulation of the Monocyte Chemoattractant Protein -1 (MCP-1) Gene by NF-kB (**Burke**)	79. Effectiveness of Small Interfering RNA (siRNA) to Inhibit Feline Coronavirus Replication (**Anis**)
4:00		60. Metabolomic Analyses of the Effects of Obesity and Genetic Variation on Ovarian Energy Metabolism (**Ernest**)	

Featured Speakers

Sten H. Vermund, MD, PhD

Vanderbilt Institute for Global Health
Vanderbilt University

"AIDS/TB/STIs in Africa: How Did We Get Here and Where are We Going?"
Monday Keynote Address

Sten Vermund, MD, PhD, is Amos Christie Chair in Global Health, professor of pediatrics, and director of the university-wide Vanderbilt Institute for Global Health. He has founded two organizations to spearhead HIV prevention, care, and treatment in Africa: the Centre for Infectious Disease Research in Zambia and Friends in Global Health in Mozambique and Nigeria. He leads the HIV Prevention Trials Network for the NIH and holds research and training grants in HIV/AIDS, African medical education, and US global research. Dr. Vermund has received the Superior Service Award from the US Public Health Service and the Albert Einstein College of Medicine lifetime alumni achievement award.

Jay Whelan, MPH, PhD

Professor & Head
Department of Nutrition
University of Tennessee

"Diet: A Treatment for Cancer?"
Monday Featured Speaker

Jay Whelan, PhD, is a lipid biochemist by training, with an expertise in lipid metabolism as it relates to acute and chronic diseases. Specifically, he is interested in how omega-3 and omega-6 polyunsaturated fats mediate health and disease. Of particular interest are those lipids from fish products (the omega-3 fats) and arachidonic acid, the major dietary antagonist of omega-3 fats. His earlier work focused on how omega-3 fats impact bioactive lipids associated with cardiovascular disease and inflammation. Currently, the research in his laboratory centers on the cellular and biomolecular effects of dietary fats as they relate to a variety of cancers. Specifically, he investigates the effects of omega-3 and omega-6 fats (primarily arachidonic acid) on the growth and development of tumors as they progress from benign forms to metastatic cancers. He has discovered that dietary arachidonic acid can negate the beneficial effects of omaga-3 fats. His research focuses on colorectal cancer, pancreatic cancer, and prostate cancer, but he believes his research can apply to other cancers. He is also interested in establishing human equivalent dosing in rodent diets for polyunsaturated fats to improve translational research in this area, and establishing better data for determining daily intakes of polyunsaturated fats. Dr. Whelan has more than 70 peer-reviewed publications and is the administrative director of both the UT Affymetrix Microarray Core Facility and the Animal Research Facility at the UT Jessie Harris Building. He obtained his PhD in Nutritional Sciences from Penn State in 1988 and his MPH from the University of North Carolina at Chapel Hill in 1982.

Featured Speakers

David B. Allison, PhD

Distinguished Professor & Associate Dean for Science
Director, Office of Energetics & Nutrition Obesity Research Center
University of Alabama at Birmingham

"Open-Mindedness and Skepticism about Causes and Alleviators of the Obesity Epidemic"
Tuesday Plenary Address

David B. Allison received his PhD from Hofstra University in 1990. He then completed post-doctoral fellowships at the Johns Hopkins University School of Medicine and the NIH-funded New York Obesity Research Center at St. Luke's/ Roosevelt Hospital Center. Before joining the faculty of the University of Alabama at Birmingham (UAB), he was a research scientist at the NY Obesity Research Center and Associate Professor of Medical Psychology at Columbia University College of Physicians and Surgeons until 2001. At UAB, he is currently also director of the Office of Energetics. He has authored over 450 scientific publications and edited five books. In addition, Dr. Allison has won several awards, including the 2002 Lilly Scientific Achievement Award from The Obesity Society, the 2002 Andre Mayer Award from the International Association for the Study of Obesity, and the National Science Foundation-administered 2006 Presidential Award for Excellence in Science, Mathematics, and Engineering Mentoring. In 2009, he was also awarded the Centrum Award from the American Society of Nutrition and the TOPS research achievement award from the Obesity Society. He holds several NIH and NSF grants, and is currently vice chair of the Board of Trustees for the International Life Science Institute, North America. Dr. Allison's research interests include obesity, quantitative genetics, clinical trials, and statistical and research methodology.

Lee Riedinger, PhD

Professor of Physics
Director, CIRE
Interim Vice Chancellor for Research and Engagement
Office of Research, University of Tennessee

"Interdisciplinary Research and Education – A Growing Emphasis in Academia"
Tuesday Featured Speaker

Dr. Lee Riedinger has been on the UT faculty since 1971. His research is focused on searching for the occurrence of tetrahedral nuclear shapes and includes experiments at the ATLAS accelerator at Argonne National Laboratory. Dr. Riedinger's research has been funded by the Department of Energy since 1976, and various sabbatical leaves have been spent at the Niels Bohr Institute in Denmark. From 1983–84, Dr. Riedinger was the science advisor to Tennessee Senator Howard Baker, who was the majority leader of the U.S. Senate. He has received the Francis G. Slack Award from the Southeastern Section of the APS (2005), the Macebearer award at UT (2009), and fellowship in the American Association for the Advancement of Science (2011). From 1993 to 1996, he was the first chair of the Tennessee Science and Technology Advisory Council, which advised the governor and the legislature on technical priorities. From 2004 to 2006, he served as the ORNL Associate Laboratory Director for University Partnerships, to extend the capabilities of the laboratory through joint programs with universities, including joint faculty hires and joint institutes. Since 2006, he has led various efforts to bring a greater focus on energy at UT, including an Energy Working Group to map directions in energy-related research and education, and helped initiate a center devoted to sustainable energy. In September 2010, he was appointed as the first director of the UT/ORNL Bredesen Center for Interdisciplinary Research and Graduate Education (CIRE), which is the home of a new doctoral program in energy science and engineering.

Featured Speakers

Dr. Gary F. McCracken

Professor & Head
Ecology & Evolutionary Biology
James R. Cox Professor
University of Tennessee

"White Nose Syndrome – An Emerging Infectious Disease of Bats in North America"
Featured After-dinner Address

Dr. Gary F. McCracken received a bachelor's degree in biology, with honors, from the University of Notre Dame and a doctorate degree in ecology and evolutionary biology from Cornell University. After completing a postdoctoral fellowship with the University of California and the University of Rochester, McCracken joined the UT Knoxville faculty in 1979. He attained rank of full professor in 1990 and was appointed department head in 2008.

His research concerns the distribution of organisms—particularly bats, insects, and viral pathogens—in space, their behavior and interactions, and resulting impacts on genetic population structure. His expertise in conservation biology, ecology of infectious diseases, and animal behavior has gained worldwide recognition. He co-authored the book *Functional and Evolutionary Ecology of Bats* in 2006, and has published more than 130 peer-reviewed research articles in journals such as *Science*, *Animal Behavior,* and *Conservation Biology*. His work also has appeared in many popular magazines including *National Geographic* and *Natural History*.

McCracken has given more than 200 presentations on his research at locations around the world. He has taught courses at academic institutions in Trinidad, China, Ecuador, Nepal, Malaysia, and Taiwan. He has also served on boards and directorships of 13 professional organizations during the course of his career. Currently he is on the Board of Trustees of Bat Conservation International, a member of the Assembly of Delegates for the Organization for Tropical Studies, and a member of the Scientific Advisory Boards of the Lubee Foundation and the Selah Nature Conservancy. Throughout his career, McCracken's research has attracted more than $7 million in federal and private funding. He has received grants from the National Geographic Society, Great Smoky Mountains National Park, National Science Foundation, and different departments within the U.S. government. Grants from the U.S. Fish and Wildlife Service and the U.S. Geological Service are currently funding his research on the spread, behavior, and contacts of the White-Nose Syndrome in North American bats.

In 2009, McCracken was elected a Fellow of the American Association for the Advancement of Science, a prestigious honor recognizing the nation's top scientists. He has also been recipient of the Gerrit S. Miller, Jr. Award, the UT Research and Creative Achievement Award, and the College of Arts and Sciences' Faculty Public Service Award.

Abstracts

THE UNIVERSITY of TENNESSEE

Awards Descriptions

- **Graduate Student Category**: Travel awards for the top 3 presentations. 1st Place – $1,000; 2nd Place – $750; 3rd Place – $500
- **Intern/Resident Category**: Travel award for the top presentation. $1,000
- **Research Associate Category**: Travel award for the top presentation. $1,000
- **Assistant Professor Category**: Travel award for the top presentation. $1,000
- **Gamma Sigma Delta Award for Excellence in Agricultural & Related Sciences**: Top graduate student presentation representing Gamma Sigma Delta's high standards of scholarship in agricultural and related sciences. $250
- **Phi Zeta Award for Excellence in Animal Health Research**: Top presentation representing Phi Zeta's goal to excel in scholarship and research in matters pertaining to the welfare and diseases of animals. $250

1. Effects of Stimulatory and Inhibitory Neurotransmitters on Pancreatic Cancer Cells and Their Normal Cells of Origin

Mohammed Al-Wadei, Hussein Al-Wadei, Hildegard Schuller

Experimental Oncology Laboratory, Biomedical and Diagnostic Sciences (Al-Wadei, Al-Wadei, Schuller), Comparative & Experimental Medicine (M. Al-Wadei)

Pancreatic cancer is the fourth-leading cause of cancer deaths in developed countries. Smoking is an established risk factor for this malignancy, but the underlying mechanisms are poorly understood. Previous reports have provided evidence that nicotinic acetylcholine receptors (nAChRs) and beta-adrenergic receptors (β-ARs) stimulate the growth and migration of pancreatic cancer cells. But a potential cooperation of these two receptor families in the regulation of pancreatic cancer has not been studied to date. Using two pancreatic cancer cell lines and immortalized pancreatic duct epithelia in vitro, our current data show that all three cell lines synthesized and released the catecholamine neurotransmitters noradrenaline and adrenaline upon exposure to nicotine and that this activity was regulated by the α3, 5 & 7-nAChRs. Gene knockdown of these receptors showed a significant reduction in nicotine-induced catecholamine production in these cells. Moreover, our results show that all three cell lines produced the inhibitory neurotransmitter γ-aminobutyric acid (GABA), an activity inhibited by gene knockdown of the α4β2nAChR and suppressed by chronic nicotine via receptor desensitization. All of the observed adverse effects of chronic nicotine were reversed by treatment of the cells with GABA, suggesting the potential usefulness of this agent for the improvement of PDAC intervention strategies in smokers. Our findings identify this hitherto unknown autocrine catecholamine loop as an important regulatory cascade in pancreatic cancer that may prove a promising new target for cancer intervention. Supported by grants R01CA042829 and R01CA130888 (National Cancer Institute to HS).

2. Intervention of Human Breast Cell Carcinogenesis Chronically Induced by 3, 4, 4'-trichlorocarbanilide

Shilpa Sood, Shambhunath Choudhary, Hwa-Chain Robert Wang

Biomedical and Diagnostic Sciences (Sood, Choudhary, Wang), Comparative and Experimental Medicine (Sood)

Trichlocarban, or 3, 4, 4'-trichlorocarbanilide (TCC), is widely used as an antibacterial in personal care products such as bath soaps, detergents, and cleansing lotions. Recent studies suggest that TCC acts as an endocrine disruptor to enhance the ability of steroid hormones (estrogen or testosterone) to induce estrogen and androgen receptor-mediated gene expression. Due to its environmental persistence and widespread use, there are concerns about its possible impact on human health. To determine whether TCC exposure may contribute to the development of breast cancer, we used our chronic carcinogenesis model to investigate whether cumulative exposures to TCC may induce progressive carcinogenesis of human breast epithelial MCF10A cells. Our results indicate that cumulative exposures of MCF10A cells to TCC at physiologically-achievable nanomolar levels result in cellular acquisition of cancer-associated properties of reduced dependence on growth factors, anchorage independent growth, and increased cell proliferation. These cellular changes were accompanied by upregulation of the ERK pathway and Nox1-induced ROS production. Our study also showed that curcumin, a component of turmeric extracted from rhizomes of the Indian herb *Curcuma longa*, was able to suppress TCC-induced cellular carcinogenesis. Our study indicates that chronic exposure to TCC can contribute to the development of breast cell carcinogenesis, and TCC-induced cellular carcinogenesis is preventable by curcumin.

3. Premalignant and Malignant Breast Cell Carcinogenesis Induced by Dietary Carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

Shambhunath Choudhary, Shilpa Sood, Robert L. Donnell, Hwa-Chain R. Wang

Biomedical and Diagnostic Sciences (Choudhary, Sood, Donnell, Wang), Comparative and Experimental Medicine (Sood)

More than 85% of breast cancers are sporadic and attributable to long-term exposure to environmental carcinogens, such as those in the diet, through a multi-step disease process progressing from non-cancerous to premalignant and malignant stages. The dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP) is one of the most abundant heterocyclic amines found in high-temperature cooked meats and is recognized as a mammary carcinogen. However, PhIP's mechanism of action in breast cell carcinogenesis is not clear. Here, we demonstrated, for the first time, that cumulative exposures to PhIP at physiologically-achievable nano-molar concentrations effectively induced progressive carcinogenesis of human breast epithelial MCF10A cells from a non-cancerous stage to premalignant and malignant stages in an exposure-

dependent manner. Progressive carcinogenesis was measured by increasingly-acquired, cancer-associated properties of reduced dependence on growth factors, anchorage-independent growth, proliferation, migration, invasion, tumorigenicity with metastasis, and increased stem-like cell populations. These cellular changes were accompanied by biochemical and molecular changes, including upregulated H-Ras gene expression, ERK pathway activation, Nox-1 expression, reactive oxygen species (ROS) elevation, increased HIF-1α, Sp1, TNF-α, MMP-2, MMP-9, VEGF, and ALDH activity. Our results indicated that the Ras-ERK-Nox-ROS pathway played an important role in not only initiation but also maintenance of cellular carcinogenesis induced by PhIP.

4. Cyclooxygenase-2 Induction by Masitinib (AB1010) in Head and Neck Squamous Cell Carcinomas through Activation of MAPK Signaling Pathway

Kusum Rathore, Maria Cekanova

Small Animal Clinical Sciences

Masitinib is a novel tyrosine kinase inhibitor that selectively targets the c-Kit cytokine receptor, the platelet-derived growth factor receptors α and β (PDGFR-α/β), and the Src-family kinases. Head and neck cancer refers to a group of biologically-similar cancers, which include squamous cell carcinomas and adenocarcinomas, 90% of which originate from epithelial cells. Oral squamous cell carcinoma (OSCC) is the second most common oral tumor in dogs. In this report, we investigated the effects of masitinib on canine and human squamous cell carcinomas by MTS proliferation assay, Western blotting (WB), and immunofluorescence. We established and characterized primary canine OSCC (K9OSCC). COX-2 expression in primary tumor and isolated cells was confirmed by immunohistochemistry (IHC) and WB analysis. Doubling time of K9OSCC was calculated by cell counting. The 5 μM masitinib inhibited cell proliferation of human and canine SCC by 30–40% after 48 hr (MTS assay). Novel non-steroidal anti-inflammatory drugs (NSAIDs) enhanced masitinib-induced inhibition of cell proliferation of human and K9SCC. Interestingly, masitinib induced expression of cyclooxygenase-2 (COX-2) in K9OSCC and human squamous cell carcinoma SCC-25 in a dose- and time-dependent manner (WB analysis). Masitinib-induced COX-2 expression was initiated through the mitogen-activated protein kinase signaling pathway. COX-2 induction by masitinib in SCC is one of the unintended drug-resistant effects of similar tyrosine kinase inhibitor, imatinib, described in patients as published previously. Therefore, treatment using masitinib may need to be combined with COX-2 inhibitors (NSAIDs) to improve outcomes in human and canine patients.

5. Role of Nicotine-Derived Nitrosamine Ketone and Benzo[a]pyrene on 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-Induced Breast Cell Carcinogenesis

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Breast cancer is the most common type of cancer affecting women in North America and Europe. More than 85% of breast cancers are attributable to long-term exposure to low doses of environmental carcinogens, such as those in tobacco and diet. We have been developing a chronically-induced breast cell carcinogenesis model wherein we repeatedly expose non-cancerous, human breast epithelial MCF10A cells to bioachievable concentrations of environmental carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[a]pyrene (B[a]P), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) to progressively induce breast cell carcinogenesis. Our studies showed that MCF10A cells exposed to combined NNK and B[a]P (NB) for 20 cycles increasingly acquired cancer-associated properties, such as reduced dependence on growth factors (RDGF), anchorage-independent growth (AIG), and increased cell proliferation, but did not induce tumorigenicity. Repeated exposure to PhIP for 20 cycles caused MCF10A cells to acquire not only the above-mentioned cancer-associated properties, but also tumorigenicity with metastatic capabilities. In this study, we aim to understand whether exposure to NB can enhance PhIP-induced carcinogenesis. MCF10A cells were exposed to NB, PhIP, or NB+PhIP (NBP) for 5–20 cycles. We then used RDGF and AIG assays to address whether there is a difference in carcinogenesis between cells exposed to NB vs NBP. Our results showed that cells exposed to NBP were more carcinogenic than their NB-exposed counterparts. Cells exposed simultaneously to NNK, B[a]P, and PhIP had more colonies in RDGF and AIG assays and also showed higher rates of cellular proliferation and increased extracellular signal-regulated kinase (ERK) pathway activation. Based on these findings, it appears that NNK and B[a]P are capable of enhancing PhIP-induced breast cell carcinogenesis.

6. Initial Experience with VSV-hIFNβ-NIS in Dogs: A Novel Oncolytic Virus

Amy K. LeBlanc, Gina D. Galyon, Robert Donnell, Casey J. LeBlanc, Shruthi Naik, Kah-Whye Peng, Stephen J. Russell

Small Animal Clinical Sciences (A LeBlanc, Galyon), Biomedical and Diagnostic Sciences (C LeBlanc, Donnell) and Department of Molecular Medicine, Mayo Clinic College of Medicine (Naik, Peng, Russell)

B cell malignancies affect thousands of pet dogs yearly. Current chemotherapy strategies carry significant risk of toxicity, are protracted in length and expense, and do not provide durable long-term remission. Herein we describe the first exploration of oncolytic virotherapy in dogs, a novel approach to cancer therapy employing viruses with natural or engineered cancer cell-specific tropisms to mediate tumor destruction. The focus of our work, VSV-hIFNβ-NIS, selectively replicates in and destroys tumor cells inducing rapid tumor debulking. The IFNβ gene insert allows for immune-mediated elimination of residual disease by generation of tumor-specific cytotoxic T cells, resulting in long-term tumor control. IFNβ also exerts a protective effect on normal cells by virtue of VSV's susceptibility to the antiviral effects of Type I IFNs. NIS expression allows imaging of tumor cells infected with VSV-hIFNβ-NIS via concentration of isotopes such as 18F-tetrafluoroborate (18F-TFB). Two normal research beagle dogs received a single dose of 1011 TCID50 of VSV-hIFNβ-NIS and were studied for 30 days. Hepatotoxicity, bone marrow suppression, and gastrointestinal toxicity were noted but spontaneously normalized without intervention within 5–7 days. Virus was detected via qRT-PCR within blood, urine, and buccal swabs after administration of VSV-hIFNβ-NIS, with sharp declines by days 21–30. Dogs' immune response to VSV-hIFNβ-NIS is delineated by anti- VSV-hIFNβ-NIS antibody production and serum IFNβ levels. Future studies are planned to identify the maximum tolerated dose (MTD) of VSV-hIFNβ-NIS. Successful back-translation of VSV-hIFNβ-NIS will be a major step forward in canine cancer therapy.

7. Epithelial to Mesenchymal Transition and Stem-Like Cell Properties Targeted by Dietary Compounds in Suppression of Chronic Breast Cell Carcinogenesis

Kusum Rathore, Hwa-Chain Robert Wang

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Cancer stem-like cells and the epithelial-to-mesenchymal transition (EMT) are postulated to play roles in various stages of cancer development, but their roles in chronic breast cell carcinogenesis remain unclear. Here, we investigated the roles of properties and markers associated with stem-like cells and the EMT in carcinogenesis chronically induced by chemical carcinogens, as well their roles in intervention of carcinogenesis by dietary components. We repeatedly treated immortalized, non-cancerous, human breast epithelial MCF10A cells with pico-molar concentrations of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (B[a]P) in culture to progressively induce acquisition of cancer-associated properties and stem-like cell- and EMT-associated properties and markers. We used dietary green tea catechins (GTC) and grape seed proanthocyanidin extract (GSPE), at non-cytotoxic concentrations, in intervention of these associated properties and markers. We detected that cumulative exposures to low doses of NNK and B[a]P resulted in cellular acquisition of stem-like cell- and EMT-associated properties and markers in addition to cancer-associated properties. The stem-like cell-associated properties and markers included increases in mammosphere formation, and in aldehyde dehydrogenase-positive and CD44+/CD24- cell populations. The EMT-associated properties and markers included mesenchymal cell morphology; increased cell migration, invasion, and mobility; and changed expression of E-Cadherin, EpCAM, Vimentin, and MMP-9. We also detected that non-cytotoxic GTC and GSPE were effective at intervening in cellular acquisition of stem-like cell- and EMT-associated properties and markers induced by NNK and B[a]P. Stem-like cell- and EMT-associated properties and markers could be considered as new targets to use in identifying induction and intervention of breast cell carcinogenesis.

8. Characterization of PPARγ Ligand MCC-555 on AOM-Induced Colorectal Tumorigenesis

Temjenmongla Imchen, Kyung-Won Min, Jorden Manasse, Seung Joon Baek

Biomedical & Diagnostic Sciences (Imchen, Kyung-Won, Manasse, Baek), Comparative and Experimental Medicine (Kyung-Won)

PPARγ agonists affect cell proliferation, differentiation, and apoptosis in a PPARγ-dependent and/or-independent manner, and thereby represent a potentially important family of chemopreventive/therapeutic compounds for cancer treatment. Dual ligands for PPARα and PPARγ, such as MCC-555, have been developed to improve treatment of metabolic syndrome, including hyperglycemia and hyperlipidemia. Interestingly, these dual ligands also possess anti-proliferative activities against a variety of cancer cell lines with greater potency than conventional PPARγ specific ligands. In this study, chemopreventive properties of MCC-555 in colorectal tumorigenesis were evaluated using azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) in A/J mice. Thirty female mice were randomly assigned to three groups: (1) positive control treated with carboxymethylcellulose (CMC) plus AOM; (2) treated with 30 mg/kg MCC-555 plus AOM; or (3) treated with 60 mg/kg MCC-555 plus AOM. At 6 weeks of age, all animals were gavaged with CMC or MCC-555 for 4 weeks starting 1 week before initiation of AOM. AOM was injected i.p. once a week for 4 weeks at 10 mg/kg for all groups. Animals were sacrificed and colonic ACF enumerated after staining with methylene blue. The colons were also stained with H&E for assessing mitosis. Apoptosis was evaluated by TUNEL assay. We found that MCC-555 suppressed AOM-induced ACF in A/J mice, compared to the control group. Administration of MCC-555 resulted in a decrease in mitotic cells and increased apoptotic cells in the colon. Our data strongly suggest that MCC-555 has an effect on the early events of colon carcinogenesis, thus providing evidence that it is a potential preventive compound for CRC.

9. Characterization of a Novel Truncated EpCAM Induced by Sulindac Sulfide

Jason Lee Liggett, Seung Joon Baek

Biomedical and Diagnostic Sciences (Liggett, Baek), Comparative and Experimental Medicine (Liggett)

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used over the counter to treat headaches and inflammation, as well as clinically to prevent cancer among high-risk groups. The inhibition of cyclooxygenase (COX) activity by NSAIDs plays a role in their anti-tumorigenic properties. NSAIDs also have COX-independent activity that is not fully understood. Our lab has previously shown that NSAIDs induce other anti-tumorigenic genes including NSAID-activated gene (NAG-1) and activating transcription factor 3 (ATF3). In this study, we report that sulindac sulfide (SS), a conventional NSAID, facilitates novel cleavage of epithelial cell adhesion molecule (EpCAM) protein in a COX-independent manner. EpCAM is a type I transmembrane glycoprotein that has been implemented as an over-expressed oncogene in many cancers including colon, breast, pancreas, and prostate. EpCAM was recently described to undergo two cleavage events, an extracellular cleavage that frees the potentially oncogenic outer portion of the protein, and the release of the intracellular region of EpCAM, which complexes with beta-catenin and, once freed, translocates to the nucleus to initiate transcription of beta-catenin target genes. We found EpCAM to be down-regulated by SS in a manner that is independent of transcription, de novo protein synthesis, kinase activity, and proteasomal degradation. Our findings have led us to believe that SS cleaves a novel site on the extracellular portion of EpCAM, closer to the N-terminus than the previously-reported cleavage. The generation of this novel truncated fragment of EpCAM by NSAIDs may provide a novel mechanism by which NSAIDs affect anti-tumorigenesis in a COX-independent manner.

10. NAG-1, a Secreted TGF-β Superfamily Cytokine, Localizes Both in the Cytoplasm and Nucleus

Kyung-Won Min, Seung Joon Baek

Biomedical and Diagnostic Sciences (Min, Baek), Comparative and Experimental Medicine (Kyung-Won)

NSAID-activated gene (NAG-1), a member of the TGF-β superfamily, is involved in tumor progression and development. The over-expression of NAG-1 in cancer cells results in growth arrest and increases in apoptosis, although some reports of NAG-1 expression in normal and transformed tissue have been inconsistent. It has been reported that NAG-1 is induced by many anti-cancer compounds at the transcription and translational level; however, the biological function of NAG-1 has not been elucidated in detail. One problem in studying NAG-1 signaling pathways results from the lack of knowledge of its receptor/or binding proteins. Like other TGF-β superfamily members, NAG-1 also seems to

have dual functions dependent on cellular context. We found that NAG-1 is highly expressed in the nucleus as a pro-NAG-1 form. The pattern of accumulation of NAG-1 in the nucleus is different between cell types. NAG-1 is more highly accumulated in the nucleus in HCT-116 and U20S cancer cells; however, NAG-1 is more highly expressed in cytoplasm in human embryonic kidney 293 cells. Subsequently, we found that 16 amino acids of the N-terminal region of NAG-1 contribute to translocation of NAG-1 into the nucleus. Absence of this region elicits a high ratio of NAG-1 accumulation in the nucleus regardless of cell type. In addition to the secreted form of NAG-1, the nuclear form of NAG-1 may have a different function. Immunoprecipitation experiments show that NAG-1 binds to smad2, implying that NAG-1 may modulate the smad pathway by acting as co-activator/or co-repressor. This is the first finding that NAG-1 also localizes in the nucleus; a novel nuclear function of NAG-1 may open a new avenue in cancer research.

11. Logic: A Pain in the Anterior Cingulate!

Sherman M. Phillips, Rex L. Cannon, Debora R. Baldwin, Dominic Di Loreto, Brittany M. Thompson, Sara M. Mosteller

Psychology

The current study used quantitative EEG and LORETA source localization to facilitate real-time inquiry into active, cortical regions of interest (ROI) supporting logic and deductive inference. To date, neural correlates and functional connectivity of deductive reasoning remain unclear. Six undergraduate students (three female); mean age = 26.167; SD = 9.38, participated in this study. Subjects underwent continuous EEG recording in four conditions (eyes closed and eyes open baselines, learning [priming], and syllogism validation). Subject responses were marked within the EEG record, extrapolated, and compared for significance using standardized low-resolution electromagnetic tomography for 6,329 5-mm^3 voxels. Statistical analyses revealed current source density supporting evaluation processes in deduction were specific to the left hemisphere, BA 30 parahippocampal gyrus, anterior cingulate and activity in the right frontal lobe regarding beta frequency. Decisions compared to instruction (learning) produced increases in all frequency domains in various cortical regions. Delta frequency showed increase in BA 10, and a distributed pattern in the cingulate gyrus. Theta showed maximal increases at BA 10 and AC (BA 32), and right BA 18, 19, 37, and 40. Alpha frequency showed an increase in left temporal and posterior cingulate (i.e., may reflect language processing and semantics). Beta showed an increase in BA 19 (precuneus) and decrease in anterior regions. Plausible interpretation of the data may denote the importance of low frequency bands in information retrieval and network integration of syntax, semantics, and other executive processes as a function of deductive inference decision making in the AC, PFC, and PCC.

12. State-Dependent Neurophysiology II: Depression

Kelley G. Callaway, Rex L. Cannon, Debora R. Baldwin, Gregory L. Stuart, Deborah P. Welsh, Ken Phillips, Dominic Diloreto, Sherman T. Phillips, Samantha A. Sprague

Psychology (Callaway, Cannon, Baldwin, Stuart, Welsh, Diloreto, S. Phillips, Sprague), College of Nursing (K. Phillips)

Major Depressive Disorder (MDD) is characterized by excessive periods of sadness, hopelessness and worthlessness, anhedonia, and increased suicidality. The neurobiologic features of MDD are still unclear. This study utilizes a novel method to examine MDD and to address the indications of DSM-IV-TR which propose that MDD abnormalities in the electroencephalogram (EEG) and other experimental methods are state-dependent (i.e., are present only when depressive symptoms are present). This study was conducted with 20 participants (12 female), with a mean age of 20.68, SD = 4.0. Ten participants had a prior/current diagnosis of MDD, and 10 participants had no prior MDD diagnosis. EEG was recorded while participants completed 4-min EEG eyes-closed and eyes-opened baselines and the Beck Depression Inventory (BDI). The participant BDI responses were marked within the EEG record. These segments were extrapolated and contrasted within and between groups using Neuroguide Software (Applied Neuroscience Laboratories). Mean BDI scores between groups was significant: MDD = 14.33, SD = 9.5; Control = 3.6, SD =2.92. The topographic EEG amplitude showed less power in frontal regions in both baseline conditions. The BDI condition showed increased activity in right parietotemporal areas in depressed individuals compared to controls. Further, depressed individuals showed higher activity in current source density in the hippocampal and amygdaloid complex during the BDI. Specific differences were noted during the BDI between controls and depressed individuals. State-dependent neurophysiology appears to provide a method to evaluate MDD as compared to normative samples. Clinical implications, diagnostic implications, and future research directions will be discussed.

13. Self-Regulation as a Cure for Anxiety

Dominic Di Loreto, Rex Cannon, Deborah Baldwin, Sherman Phillips, Samantha Sprauge

Clinical Neuroscience, Self-Regulation, and Biological Sciences Laboratory; Psychology

Anxiety is characterized as a negative emotional state that is mediated by neuronal mechanisms also involved in panic disorder, specifically in the form of anticipatory anxiety. Biological models thus far have hypothesized dysregulation in serotonergic, dopaminergic, and GABAergic systems. These hypotheses are based primarily on the effects of pharmaceuticals and have been inadequate in elucidating etiology. We propose an alternative hypothesis that implicates disruptions in functional integration of neural networks important to self-regulation. For the current study, six (four male) participants with a prior diagnosis of anxiety or anxiety with a comorbid syndrome completed between 15 and 20 sessions of spatial-specific EEG operant conditioning (LORETA Neurofeedback) to improve self-regulation. All participants were able to produce significant learning effects across sessions, which include network convergent learning. Post-training assessment revealed significant reductions in anxiety according to the Personality Assessment Inventory (PAI) and significant increases in executive functions as measured with subtests from the Delis-Kaplan Executive Function System (DKEFS). Functional correlations between neurological and behavioral data demonstrate specific network involvement in these symptom reductions and provide data to develop a potential intervention for anxiety disorders in 20 days or less.

14. Depression, Neurocognition, and Cingulate Cortex Lesions in Multiple Sclerosis

Tiffany L. Shaw, Randall G. Trudell, Rex L. Cannon, Alex M. Khaddouma

Cole Neuroscience Center: Multiple Sclerosis Clinic (Trudell), Applied Psychology (Shaw), Clinical Neuroscience, Self-Regulation, and Biological Psychology Laboratory, Psychology (Shaw, Cannon, Khaddouma)

Depression can be characterized as a process involving negative automatic thoughts (or self-talk), and biases in attention, interpretation, and memory. There is an inverse relationship between depressive symptoms and cognitive efficiency or executive processing. Cognitive impairment is demonstrated in 40–65% of multiple sclerosis patients, including those in the earliest stages. Generally, neurocognitive deficits found in MS are ascribed to white matter and subcortical lesions. This study examines the relationship between lesions in the cingulate cortex, depression, and neurocognition in MS. This study was conducted with 18 patients (12 female) diagnosed with MS. Patients completed the color-word interference tasks from the Delis-Kaplan Executive Function System (DKEFS), Beck Depression Inventory (BDI) and the computerized self-test (CST) (3). We ranked MRI data on the basis of lesions present in the cingulate gyrus (anterior/posterior) given its role in depressive disorders and cognitive impairment. The depressed patients scored higher on the BDI and lower on the CST than non-depressed patients. The posterior cingulate showed significant positive associations with the BDI and inverse associations with the CST. Interestingly, the PC showed positive associations with the overall slower performance on the DKEFS task. Current data implicate the posterior cingulate's role in depression, neurocognition, and executive processing. Typically, research suggests the anterior rather than posterior regions of cingulate cortex play an important role in depressive features. In this sample of MS patients the inverse is found. This has broad implications for the study of depression and cognition in MS.

15. Effects of a Home-Based Exercise Program on Perception of Illness and Adaptation in Heart Failure Patients

Robin Harris

College of Nursing

Heart failure patients experience decreased functional capacity from chronic symptoms associated with this condition. Exercise has been shown to increase activity tolerance and improve quality of life in heart failure patients. While physiologic responses to exercise in heart failure patients have been well-documented, the influence of exercise on an individual's perception of degree of disability due to chronic illness and adaptive responses based on adaptation to chronic illness theory has not been studied. A randomized controlled trial is underway to examine the effects of a 12-week home-based combined aerobic and resistance training exercise intervention on an individual's perception of degree of disability and adaptive responses to chronic illness in patients with systolic heart failure (EF < 40%). Study aims include evaluation of effects of 12-week home-based exercise intervention on: 1) perception of degree of functional limitation due to heart failure, 2) physiologic adaptation to chronic illness, 3) psychosocial adaptation to chronic illness, and 4) number of hospitalizations for heart failure for participants during the 12-week study. Findings from

this quantitative study will increase knowledge of effects of exercise on adaptation to chronic illness in heart failure patients with implications to improve patient care and reduce health care costs. Methods to improve care delivery and health outcomes that are patient-centered and evidence-based have important policy implications at the local and national level.

16. Does Increasing Step Width Alter Knee Biomechanics in Medial Compartment Knee Osteoarthritis Patients during Stair Descent?

Max Paquette, Songning Zhang

Kinesiology, Recreation and Sport Studies

Stair negotiation is one of the most challenging locomotor tasks of daily living for older adults, and one of the first complaints from older adults suffering knee osteoarthritis (OA) is difficulty in stair walking. This study investigates the effects of increased step width (SW) on knee biomechanics and knee pain in medial compartment knee OA patients during stair descent. Thirteen medial compartment knee OA patients were recruited for the study. A motion analysis system was used to obtain three-dimensional lower limb kinematics during stair descent. An instrumented three-step staircase with two additional customized wooden steps was used to collect GRF. Participants performed five stair descent trials at their self-selected speed at preferred, wide, and wider SW. Participants rated knee function before the experiment and knee pain after each SW condition. Increased step width showed no difference in subjective knee pain in knee OA patients. Second peak knee adduction angle was reduced, but peak knee abduction moments were unchanged with increasing step width in knee OA patients. It appears that differences in timing of 2nd peak knee adduction angle between step width conditions may be related to the unchanged 2nd peak knee abduction moment between SW conditions. Further studies investigating compartmentalized joint contact forces during stair walking are warranted.

17. Incisional Wound Healing in Ball Pythons (*Python regius*): A Comparison of Carbon Dioxide Laser, 4.0 MHz Radiosurgery, and Scalpel

Rebecca Hodshon, Patricia Sura, Juergen Schumacher, Agricola Odoi, James Steeil, Kim Newkirk

Small Animal Clinical Sciences (Hodshon, Sura, Schumacher, Steeil), Biomedical and Diagnostic Sciences (Newkirk, Odoi)

We evaluated carbon dioxide laser (laser), radiowave radiosurgery (RWRS), and scalpel for skin incisions in six adult ball pythons (*Python regius*) and compared histologic reactions and wound healing. In each snake, twelve 2-cm skin incisions (four incisions per modality) were made and closed with surgical staples, and incision sites were assigned a daily gross score (0 to 3). From each snake, one skin biopsy representing each modality was taken on days 2, 7, 14, and 30. Degree of necrosis and fibrosis was measured; sections were assessed for presence of granulomas and bacteria, and were assigned subjective scores for necrosis, fibrosis, inflammation, and degree of healing. Histologic wound score was calculated, and frequency distribution of healing was determined. Macroscopically, laser incisions had significantly higher scores of dehiscence than RWRS and scalpel. Compared to scalpel, laser and RWRS caused significantly more necrosis on days 2 and 14 and days 2 and 7, respectively. On day 30, fibrosis was significantly greater for laser than for scalpel. On all days, inflammation scores were highest for laser incisions. Histologic wound scores for scalpel were significantly lower than laser and RWRS at all time points. By day 30, 100% of scalpel, 95% of laser, and 83% of RWRS incisions were healed. In snakes, scalpel skin incisions result in less necrosis and granuloma formation, and heal more rapidly than laser and RWRS incisions. Further studies should investigate whether delayed wound healing is clinically significant.

18. Laparoscopic-Assisted Ovariectomy of Tigers (*Panthera tigris*) Using the Ligasure Device

James Steeil, Patricia A. Sura, Edward C. Ramsay, Sabrina Reilly, Reza Seddighi, Jacqueline Whittemore

Small Animal Clinical Sciences (Steeil, Sura, Ramsay, Reilly, Whittemore), Large Animal Clinical Sciences (Seddighi)

Laparoscopic ovariectomy using a vessel-sealing device and a three-port technique was performed in seven tigers. A comparison group of seven tigers that underwent traditional ovariohysterectomy was assembled using a medical records search. Mean operative times for laparoscopic ovariectomy were compared to standard ovariohysterectomy, and mean combined laparoscopic incision length compared to standard ovariohysterectomy incision lengths. Significance was set at $P \leq .05$. Mean surgical time for laparoscopic ovariectomy (82 min, range 71–126 min) was significantly shorter than standard ovariohysterectomy surgical time (129 min, range 80–165

min, *P* = .007). Mean combined laparoscopic incision length (8.07 cm, range 3.80–9.50 cm) was significantly shorter than the mean incision length for standard ovariohysterectomy (13.57 cm, range 12.00–20.00 cm, *P* = .009). There were no clinically important complications observed in either group. Laparoscopic ovariectomy has a significantly shorter surgical time and combined incision length compared to standard ovariohysterectomy in tigers, and appears to be a safe and rapid sterilization method for tigers. Equipment cost and the necessity for advanced training may limit its use in some institutions. Further prospective evaluation is warranted to determine whether it is associated with decreased morbidity, mortality, or cost.

19. Amino Acid, Iodine, Selenium, and Coat Color Status among Hyperthyroid, Siamese, and Age-Matched Control Cats

Bethany Sabatino, Claudia Kirk, Barton Rohrbach, Jane Armstrong

Small Animal Clinical Sciences (Sabatino, Kirk, Armstrong), Biomedical and Diagnostic Sciences (Rohrbach)

Hyperthyroidism is common among older cats, but its pathogenesis remains poorly understood. Siamese and Himalayan cats have a reduced risk of hyperthyroidism compared to other cat breeds. Tyrosine is the amino acid precursor for thyroxine and melanin. Earlier studies reported tyrosine as a limiting amino acid in some cat foods, resulting in poor coat melanin production in dark-coated cats. Because Siamese and Himalayan cats have unique tyrosine metabolism responsible for a pointed coat color, this study evaluated the relationship between tyrosine status and coat color in hyperthyroid cats. The objective of this study was to determine if tyrosine or phenylalanine are altered in hyperthyroid cats compared to normal cats and if light or pointed coat color is protective with greater tyrosine availability due to lowered use for melanin production. Twenty-seven client-owned cats with (n = 12) and without (n = 15) hyperthyroidism were studied. Coat color (nine white or pointed; 18 dark), breed, and diet history were recorded. Whole blood was collected for complete blood count, serum chemistry, total thyroxine, serum iodine, serum selenium, and plasma amino acid determination in fasted cats. A mixed-model ANOVA with cat and group included as class variables was used to evaluate the relationship between group study factors (significance = $P \leq .05$). Tyrosine and phenylalanine levels were not significantly different among light or dark cats or cats with or without hyperthyroidism. Altered tyrosine metabolism associated with coat color does not explain the reduced risk of hyperthyroidism in pointed or light coat colored cats.

20. Magnetic Resonance Imaging of the Canine Cranial Abdomen: Quantitative and Qualitative Evaluation of Various Imaging Sequences

Rebecca Manley, Andrea Matthews, Federica Morandi, George Henry, Gordon Conklin, Katherine DeAnna, Ann Reed

Small Animal Clinical Sciences (Manley, Morandi, Henry, Conklin, deAnna), Office of Information Technology (Reed), Atlantic Veterinary College, Department of Companion Animals, University of Prince Edward Island (Matthews)

Motion artifact is a major limitation of abdominal magnetic resonance imaging (MRI). Technical advances and the implementation of faster sequences have allowed the acquisition of MR images that reduce artifact secondary to motion, while concurrently providing excellent anatomic detail. The study objective was to quantitatively and qualitatively compare selected MR sequences for imaging of the canine cranial abdomen. Sixteen MR sequences were evaluated in 10 normal dogs including sequences designed to minimize artifacts, such as breath-holding and respiratory navigation. Subjective evaluation of images for diagnostic quality and artifacts was performed by four observers independently. For a quantitative comparison, signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) were calculated for each sequence. The sequence with the overall highest mean diagnostic quality score was the dorsal T2 turbo spin echo (TSE) with breath-holding and fat saturation. The sequence with the lowest mean diagnostic quality score was the dorsal T2 fast spin echo (FSE). The sequence with the highest SNR for all evaluated organs was the sagittal T1 spin echo. The SNR/CNR did not correlate with the subjective assessment of overall diagnostic quality for the majority of the sequences evaluated. The four sequences considered to have the highest diagnostic quality were the dorsal T2 TSE with FS and BH, transverse T1 gradient echo (GRE) with BH, dorsal T2 half-Fourier single shot TSE (HASTE) with respiratory navigation (RN), and the transverse T1 SE of the caudal abdomen. The SNR and CNR did not correlate with the observed diagnostic quality.

21. Effects of Intravenously-Administered Esomeprazole Sodium on Gastric Juice pH in Adult Female Horses

Ricardo Videla, Carla S. Sommardahl, Sarah B. Elliott, Angelis Vasili, Frank M. Andrews

Comparative and Experimental Medicine (Videla), Large Animal Clinical Sciences (Sommardahl), Biomedical and Diagnostic Sciences (Elliot), Randlab Australia Pty Ltd, Australia (Vasili), Department of Veterinary Clinical Sciences, Louisiana State University (Andrews)

Gastric ulcers are common in horses, and treatment of horses that cannot be administered oral medication can be problematic. Our objective was to evaluate the efficacy of esomeprazole sodium (administered intravenously) on gastric juice pH and gastric ulcer scores in horses. Esomeprazole sodium (0.5mg/kgIV) was administered once daily to eight horses (treatment group), and saline (5mL IV) was administered to four horses (control group) for 13 consecutive days. Gastroscopy was performed, and gastric juice pH and gastric ulcer score were recorded before and 1 hr after the administration of esomeprazole sodium or saline on days 1 and 5, then on day 14, 23 hr after the 13th daily dose of esomeprazole sodium or saline. When compared with values before treatment, gastric juice pH was higher in esomeprazole sodium-treated horses (4.25 +/- 2.39 versus 6.43 +/- 1.18; = .002). Also, gastric juice pH was higher (*P* = .001) in esomeprazole sodium-treated horses compared with saline-treated control horses on day 5 and on day 14 values. Gastric ulcers were seen in 5/12 (43%) horses in the study. Esomeprazole sodium shows promise for treatment of gastric ulcers in horses with signs of dysphagia, gastric reflux, or other conditions that restrict oral intake of the current Federal Drug Administration-approved omeprazole paste.

22. Evaluation of Fluorocoxib A, a Novel COX-2-Targeted Optical Imaging Agent

Maria Cekanova, Md. Jashim Uddin, Joseph W. Bartges, Amanda Callens, Alfred M. Legendre, Gina Galyon, Kusum Rathore, Lawrence J. Marnett

Small Animal Clinical Sciences (Cekanova, Bartges, Callens, Legendre, Galyon, Rathore), Department of Biochemistry, School of Medicine, Vanderbilt University (Uddin, Marnett)

The sensitivity and specificity of cystoscopy imaging can be significantly improved by fluorescent signals from imaging agents that specifically accumulate in tumors. In this study, we evaluated an optical imaging agent, Fluorocoxib A for detection of spontaneously-occurring canine tumors that express the cyclooxygenase-2 (COX-2) enzyme. The newly synthesized 5-carboxy-X-rhodamine-conjugated derivative of indomethacin is a non-steroidal anti-inflammatory drug that selectively targets COX-2 in tumors. In this study, we evaluated the single-dose safety and pharmacokinetic properties of Fluorocoxib A in research dogs. Single-dose study in dogs supports the safe administration of 1 mg/kg per intravenous injection determined in human studies, based on no adverse effects detected by physical examination and in blood and urine samples. Pharmacokinetic parameters in beagles (n = 6) were assessed from collected plasma at 0, 0.5, 1, 2, 4, 8, and 24 hr after administration of 1 mg/kg Fluorocoxib by i.v. over 20 min using high-performance liquid chromatography (HPLC) analysis. Fluorocoxib A was well-tolerated by dogs after i.v. administration and no adverse effects were observed. The pharmacokinetic studies using 1mg/kg showed a high peak of Fluorocoxib A (98.4 ± 13.5 nM) in the plasma collected at 0.5 h with gradual clearance from the blood until 24 h by HPLC analysis. In our study we evaluated the specificity of Fluorocoxib A to selectively bind to COX-2-expressed bladder, colorectal, and oral carcinomas in dogs using endoscopy in vivo. IHC and WB analysis confirmed COX-2 expression in tested carcinomas biopsies. Spontaneous cancers in companion dogs offer a unique model for human cancer biology and help to validate novel cancer therapeutics and imaging agents.

23. The Transcriptome Expression Profiles of *S. uberis* UT888 after Co-culture with Primary Bovine Mammary Epithelial Cells

Oudessa Kerro Dego, Douglas A. Luther, Raul A. Almeida

Animal Science

In spite of the increasing prevalence of *S. uberis* mastitis, its pathogenesis and associated virulence factors are not clearly defined. Most times, host-pathogen interactions lead to specific changes in the pathogen proteome by inducing transcription of genes required to successfully infect the host. The various virulence molecules produced at different phases of bacterial growth allow bacteria to sense and adapt to their immediate environment and very rapidly overcome host defense mechanisms to achieve colonization and subsequent infection. We hypothesize that *S. uberis* senses the presence of specific host factors in the mammary gland and expresses specific ligands thus enhancing bacterial attachment to and internalization into host cells and promoting the progression of *S. uberis* intramammary infections. The aim of this study was to identify and characterize virulence factors of *S. uberis*

differentially expressed during bacterial interaction with bovine primary mammary epithelial cells. Mid-log phase *S. uberis* cultures were co-incubated with primary bovine mammary epithelial cells for 2 and 4 hr. At each incubation point, mRNA of *S. uberis* was purified from co-cultures and sequenced using high throughput mRNA sequencing (RNA-Seq). Results of this investigation provided a list of up- and down-regulated genes with specific roles in bacterial adaptation to the environment, pathogenicity to the host, and attachment to and internalization into a host cells. Detailed evaluation of genes of interest will enhance our understanding about the pathogenesis of *S. uberis* and provide potential immunological tools to control this economically important disease of dairy cows.

24. Pursuit of in silico Discovery of Novel Gene Features Associated with Virulence/Pathogenic Interactions from *Streptococcus uberis* Genomic Data

Douglas A. Luther, Oudessa Kerro-Dego, Stephen A. Kania, Loren J. Hauser, Arnold M. Saxton, Raul A. Almeida

Animal Science (Luther, Kerro-Dego, Saxton, Almeida); Biomedical and Diagnostic Sciences (Kania), Computational Biology and Bioinformatics Group ORNL, Microbiology (Hauser)

Bovine mastitis is the most expensive disease of the dairy industry, and *Streptococcus uberis* is one of the most prevalent causes. Lack of knowledge about virulence factors and pathogenesis of *S. uberis* intramammary infection hampers the development of specific preventive measures. In this project, high throughput sequencing of the genome of the clinical strain *S. uberis* UT888 was used to 1) generate a reference genome sequence for evaluating RNA-Seq data from *S. uberis* UT888 cultured in in vitro and in vivo conditions and 2) to make in silico comparisons with the reference strain *S. uberis* 0140J. Preliminary results showed differences between these strains. For example, genome sizes were 2,143 and 1,852 kbp for *S. uberis* UT888 and 140J, respectively; number of functional subsystems was 333 and 258, while CDSs were 2123 and 1824 for strains UT88 and O140J, respectively. Differences were also detected in the size of specific subsystems. For example, in the strain UT888 the virulence subsystem included 53 CDSs, while in O140J there were 44 CDSs. Other important differences were noticed in the membrane transport, regulation/cell signaling, and stress response functional subsystems. Comparison of the genome of *S. uberis* UT888 with that corresponding for strain 0140J will begin to establish a pan genome and identify variations between strains. These variations will be used to define suitable targets for vaccine development and build a foundation upon which gene products and strain phenotypes can be associated with virulence, thus enhancing our understanding of the pathogenesis of this economically important disease of dairy cows.

25. *Streptococcus uberis*, Biofilms, and Fluorescent Bioreporters

Maria E. Prado, Dan Close, Oudessa Kerro-Dego, Steve Ripp

Animal Science (Prado, Kerro-Dego), Center for Environmental Biotechnology (Close, Ripp)

Streptococcus uberis is an important environmental pathogen that causes mastitis in dairy cows. These infections can be clinical or subclinical in nature, and sometimes become chronic, which has been associated with the ability to form biofilms in other bacterial pathogens. The objectives of this study were to: 1) compare the ability of different *S. uberis* mastitis isolates to form biofilms in vitro and 2) develop an *S. uberis* fluorescent bioreporter to further characterize biofilm formation using microfluidics. *S. uberis* strains (n = 38) were screened by qualitative and quantitative assays (microtiter plate [MP], Congo Red agar [CRA]), and for presence of genes associated with biofilm formation (comEA, comEC, comX, agrC, fasA and luxS) and capsule synthesis (hasABC) by PCR. An *S. uberis* bioreporter consisting of the mCherry gene linked to the luxS promoter was constructed. All strains produced biofilms when tested by the CRA method, whereas most strains were positive by MP (30 of 38). Strains were further classified as negative (21%), weak (13%), and strong (66%) biofilm formers based on the MP assay. Competence and quorum sensing genes were amplified by PCR in 97% (37/38) of strains evaluated. Capsule formation did not appear to influence *S. uberis's* ability to produce biofilms (32/38 capsule-positive). We successfully created an *S. uberis* fluorescent bioreporter and monitored biofilm formation under flow conditions. Results suggest that *S. uberis* is capable of forming biofilms, and genes associated with biofilms appear to be highly conserved. In addition, use of an *S. uberis* bioreporter is an effective means for assessing biofilm formation under flow conditions.

26. Antimicrobial Susceptibility and Genetic Characterization of *Staphylococcus pseudintermedius* Isolates from 206 Dogs

Ricardo Videla, Cristina Lanzas, Samar M. Solyman, Amy M. Worron, David A. Bemis, Stephen A. Kania

Comparative and Experimental Medicine (Videla, Solyman), Molecular Biology and Enteric Bacteriology, Tennessee Department of Health (Worron), Biomedical and Diagnostic Sciences (Lanzas, Bemis, Kania)

Staphylococcus pseudintermedius, the primary cause of canine pyoderma, is therapeutically challenging due to a high, and increasing, rate of resistance to antibiotics. The objectives of this study are to investigate the genetic diversity within canine *Staphylococcus pseudintermedius* isolates in the United States, to characterize the antimicrobial susceptibility profile of these isolates, and to explore possible associations based on geographical region, genetic relatedness, and susceptibility patterns. Samples from 233 dogs were obtained from veterinary diagnostic facilities located in different regions of the United States. Species identification was confirmed on 206 isolates by phosphate acetyltransferase (pta) gene PCR followed by MboI restriction analysis. Pulse-field gel electrophoresis (PFGE) allowed differentiation of 22 clusters with one major clonal group. Conventional or real-time PCR for MecA gene was performed to determine susceptibility to methicillin, and the Kirby-Bauer disk diffusion method was used to test the susceptibility to 13 antimicrobials. A total of 24 different susceptibility profile groups were identified. A positive correlation between the largest clonal population and the most commonly encountered susceptibility pattern was found. These findings represent a significant epidemiological contribution and emphasize the need for the development of innovative therapeutics to address the increasing number of *S. pseudintermedius* isolates resistant to the most clinically useful antimicrobials that are available to veterinarians.

27. Immune Response to *Staphylococcus pseudintermedius*

Samar Solyman, Stephen Kania, Christine Cain, Derek Adrian, David Bemis

Biomedical and Diagnostic Sciences (Solyman, Kania, Cain, Bemis), Small Animal Clinical Sciences (Adrian)

Little is known about the immune response to *S. pseudintermedius*, an important canine pathogen that has developed a high prevalence of methicillin resistance. Understanding the nature of the protective immune response would facilitate the design of new therapeutics. The aim of this study was to characterize the nature of the immunoglobulin G (IgG) response in 14 dogs with pyoderma and 14 healthy dogs with no history of pyoderma to antigens from five genetically distinct *S. pseudintermedius* isolates and five different staphylococcal species. Blood samples were collected at the time of patient admission, and a second set of blood samples was collected after 3 to 8 weeks. The results indicated significant differences in the immune reactivity of the five isolates with the dog sera in both groups. All 28 dogs showed IgG reactivity to multiple *S. pseudintermedius* antigens. Antibodies to some antigens were seen only in samples from infected dogs. The highest reactivity was seen in ST 96, which is a methicillin-susceptible isolate involved in *S. pseudintermedius* infections, and ST 71, which is a known methicillin-resistant clone widely distributed in Europe. *S. delphini* was the lowest reactive staphylococcal species.

28. In vitro Characterization of Cee, A Novel Periplasmic Ferric Enterobactin Esterase in *Campylobacter*

Yiming Mo, Ximin Zeng, Jun Lin

Animal Science

Campylobacter jejuni is a leading bacterial cause of human enteritis in the United States, and the ferric enterobactin (FeEnt) acquisition system plays a critical role in *Campylobacter* pathogenesis. Recently we identified a novel periplasmic enterobactin (Ent) esterase Cee that plays a critical role in Ent-mediated iron acquisition in *Campylobacter*. However, catalytic efficiency and kinetics of Cee are still largely unknown. Here we report the enzymatic analysis of purified Cee by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Both Ent and salmochelins (the C-glucosylated Ent analogues) were used as substrates for analysis. IroE, IroD, and Fes, the *E. coli* homologs of Cee, were also purified and used in conjunction with Cee for comparative enzyme analysis. Both TLC and HPLC analyses showed that Cee displayed exceptionally high efficiency to hydrolyze Ent. Unlike the periplasmic IroE that tends to hydrolyze Ent just once to produce linearized trimers, Cee could efficiently catalyze the hydrolysis of Ent, generating linear trimer, dimer, and monomer products. Cee also displayed exceptionally higher catalytic efficiency for Ent than its homolog IroD that is located in cytoplasm. Moreover, HPLC showed that Cee could hydrolyze samochelins including both DGE

(di-C-glucosylation) and TGE (tri-C-glucosylation) with a higher efficiency on DGE than TGE. This study reveals unique features of Cee when compared to its homologs in other bacteria, and strongly supports an uncharacterized and fascinating process for Ent-mediated iron acquisition in Gram-negative bacteria.

29. Identification of Cj0843c, a Putative Lytic Transglycosylase Involved in Beta-Lactam Resistance in *Campylobacter jejuni*

Ximin Zeng, Barbara Gillespie, Samantha Brown, Jun Lin

Animal Science

Beta-lactams are an important class of antibiotics for treating bacterial infections. Emergence of beta-lactam resistance has greatly compromised clinical effectiveness of this group of antibiotics. Despite prevalent beta-lactam resistance in *Campylobacter jejuni*, the leading bacterial cause of human diarrhea in the United States, the molecular basis of beta-lactam resistance in *C. jejuni* is still largely unknown. In this study, in vivo random transposon mutagenesis was performed to identify genes required for beta-lactam resistance in *C. jejuni* 81–176. Screening of a 2,800-mutant library identified 22 mutants with increased susceptibility to ampicillin. Direct sequencing indicated that 20 mutants have transposons inserted in the genes encoding the CmeABC drug efflux pump while the other two have insertions in Cj0843c (a putative lytic transglycosylase gene) and its upstream gene Cj0844c. Further complementation and molecular manipulation in a different strain background demonstrated that Cj0843c contributes to both intrinsic and acquired beta-lactam resistance in *C. jejuni*. In addition, the CmeABC efflux pump and Cj0843c played synergistic roles in beta-lactam resistance. Genomic examination and PCR analysis also demonstrated that Cj0843c is widely distributed in *C. jejuni*. In summary, we have identified a novel mechanism of β-lactam resistance in *C. jejuni*, which will help us better understand the development and regulation of β-lactam resistance, a significant issue in many bacterial pathogens.

30. The Stress of *Enterococcus faecalis*

Holly Saito, Elizabeth Fozo

Microbiology

Enterococcus faecalis is a commensal organism of the mammalian intestine, as well as an opportunistic pathogen. *E. faecalis* can colonize and survive in multiple locations in the human host, which allows it to cause a wide array of complications such as wound infections, cystitis, and bacterial endocarditis. The adaptations that allow *E. faecalis* to survive stressful or changing conditions may provide insight into this organism's ability to cause disease. We are specifically interested in the physiological and genetic changes that occur when *E. faecalis* is grown under the membrane-stressing agent, bile, which is found in mammalian intestines. When grown at a physiological level of bile (0.2%), *E. faecalis* OG1RF alters its membrane fatty acid content and cell morphology. Gas chromatography results show that the membrane contains longer fatty acids and a modestly higher ratio of unsaturated fatty acids compared to cells grown in media alone. Ongoing work will determine if these changes are due to fatty acid uptake and not de novo fatty acid synthesis. Cells grown in the presence of 0.2% bile generally have a higher quantity of cocci in chains than pairs. Although this low level of bile does not impact growth significantly, these cells do withstand severe membrane stress better than those grown without bile. Together, this suggests that the ability to withstand the damaging effects of bile can protect *Enterococcus* from additional stress, which may contribute to its ability to cause disease.

31. Regulation of the Toxic, Small Zor Proteins in *Escherichia coli* O157: H7

Jia Wen, Bethany Miracle, Pranay Dogra, Elizabeth Fozo

Microbiology

As an emerging class of gene expression regulators, small non-coding RNAs (sRNAs) have been found in all domains of life. In bacteria, a subset of sRNAs can repress the expression of small hydrophobic proteins. These small proteins are usually under 60 amino acids in size and are toxic when overproduced. The sRNA base pairs with the toxin mRNA and affects either the translation or the stability of the toxin mRNA. These gene pairs are referred to as type I toxin-antitoxin systems. A previous bioinformatics search predicted that the highly homologous zorO-orzO and zorP-orzP loci in *Escherichia coli* O157: H7 (EHEC) were type I loci, with zorO/zorP encoding the toxins and orzO/orzP encoding the antitoxin sRNAs. Overexpression of either zor gene is toxic to *E. coli*; however, co-expression of the cognate orz gene can neutralize this toxicity, indicating zor-orz are true type I toxin-antitoxin loci. Despite the extensive similarities between the gene pairs, only OrzO, but not OrzP, can repress the zorO expression. We have utilized mutagenesis studies and the inherent toxicity of Zor production to determine how the Orz sRNAs

recognize and discriminate between the two very similar zor targets. This knowledge could aid investigators to design better synthetic, antisense RNAs as therapeutics and biotechnological tools. Ongoing studies are aimed at examining regulation of zor beyond the sRNAs and why EHEC possesses these loci.

32. Assessing Medical Fellows' Baseline Knowledge in Research Methods and Statistics

Patrick Barlow

Educational Psychology and Counseling, Graduate School of Medicine

The present study details the development and initial pilot testing of a novel course assessment instrument targeting first-year medical fellows' baseline research methods and statistics knowledge. Medical professionals have increasingly embraced the use of evidence-based medicine (EBM) in their clinical practices where the utmost importance is placed on using a critical appraisal of medical literature in the clinical decision-making process. Alarmingly, the rate of errors in reporting and interpreting the statistical procedures that drive EBM decisions is estimated at between 30–90%. To address this issue, the Office of Medical Education, Research, and Development at the University of Tennessee Graduate School of Medicine (GSM) has developed a five-module educational intervention, which specifically targets teaching statistics and research methods to medical residents and fellows. Careful reflection of key course learning objectives led to a 14-item assessment instrument, which asked the fellows a series of questions on basic epidemiologic research designs as well as common statistics seen in medical literature. Fellows completed it on the first and last day of class to assess their responsiveness to specific course material. The fellows demonstrated a statistically significant increase in assessment score from pretest to posttest, which reflected an overall increase in content mastery following the modules. Feedback from this small pilot analysis has since been used to refine both the modules and the instrument in order to better identify the specific educational needs of these busy medical professionals.

33. A Neural Network Model for Mortality Prediction in ICU

Henian Xia, Xiaopeng Zhao

Mechanical, Aerospace and Biomedical Engineering

Many types of acuity scoring systems have been developed for the intensive care unit (ICU). This study aimed to develop an artificial neural network model for patient-specific prediction of in-hospital mortality. Data from the PhysioNet/Computing in Cardiology Challenge 2012 is used in this study. It consists of 12,000 ICU records. The records were collected during the first 48 hr after admission to the ICU and include up to 41 variables. The problem was tackled as a supervised learning problem. Characteristics of each patient based on the ICU data and his/her outcome were fitted to a neural network model. Different feature selection methods were also applied. Two events were defined to evaluate the performance of the proposed method by PhysioNet/Computing in Cardiology Challenge 2012. The first event defined a score that is the minimum of the sensitivity (the fraction of in-hospital deaths that are predicted) and the positive predictivity (the fraction of correct predictions of in-hospital deaths). The second event defined a score that is a modification of the Hosmer-Lemeshow H statistic; the lower this score, the better the algorithm. Our models based on the neural network achieved a score of 0.46 for event 1 and a score of 28.3 for event 2 for the testing dataset, significantly better than the benchmarking scores, which are 0.31 for event 1 and 66.55 for event 2.

34. Diagnostics for Mild Cognitive Impairment and Early Alzheimer's Disease

Joseph McBride, X. Zhao, N. Munro, Charles Smith, Gregory Jicha, Lee Hively, Lucas Broster, Y. Jiang

Mechanical, Aerospace, and Biomedical Engineering (McBride, Zhao), Oak Ridge National Laboratory (Munro, Smith, Jicha, Hively, Broster), Department of Behavioral Science, University of Kentucky College of Medicine (Jiang)

Mild cognitive impairment (MCI) and early stages of Alzheimer's disease (AD) cause similar cognitive degeneration including progressive memory loss, shrinking vocabulary, and lower ability to execute precise motor movements. MCI is currently detected based on self/family-reported behavioral histories and some more objective measures, but typically not in the primary care setting. By the time primary care providers detect significant changes, the patient has often progressed to AD and it is too late for effective treatment. Currently, the

most definitive means for diagnosing AD is determination of cerebrospinal fluid (CSF) biomarker proteins; this, however, requires patients to undergo a lumbar puncture, a painful and potentially dangerous procedure. This study explores a convenient, noninvasive alternative to current methods using scalp EEG for detection of MCI and AD at early stages. Resting EEG records from 46 age-matched subjects (mean age 75.6)—15 normal controls (NC), 16 MCI, and 15 with early stage AD—were examined. Dynamic spectral and entropy features were computed and used as features in a support vector machine (SVM) model to discriminate between the three groups. Analyses demonstrate discrimination accuracies of 93.55% for MCI vs. NC, 90% for AD vs. NC, and 87.10% for AD vs. MCI. These results suggest the potential of scalp EEG as an efficacious method for noninvasive diagnosis of MCI and early AD.

35. Revealing Putative Gene Networks Perturbed by Low-Dose Ionizing Radiation using a Differentially-Correlated Graph Generated by a Two-Stage Statistical Filter

Sudhir Naswa, Michael A. Langston, Bo Ji, Suchita Das, Arnold Saxton, Brynn H.Voy

Genome Science and Technology (Naswa), Electrical Engineering and Computer Science (Langston), Animal Science (Ji, Suchita, Saxton, Voy)

Strong stimuli elicit changes in gene expression that can be detected with statistical methods for differential expression like ANOVA. In contrast, more modest stimuli induce changes in gene expression that may confer significant biological impact but are difficult to detect by ANOVA, especially in the presence of background genetic variation. We hypothesized that capturing mutual relationships of genes by their differential co-expression (correlation) would uncover effects of modest biological stimuli that would be missed by traditional differential expression approaches. To test this hypothesis we developed a two-stage statistical filter to generate a differential correlation graph (DCG) that identifies differences in gene networks between control and treatment groups. We applied this approach to microarray data produced from five strains of inbred mice exposed to a single low dose of ionizing radiation. The exposure level (10 cGy) is consistent with exposures obtained during increasingly common diagnostic CT scans, which may increase cancer risk. RNA was extracted from bone marrow of control and irradiated mice 24 hr after exposure and profiled using the Illumina microarray platform. After producing the DCG, permutation testing was used to identify statistically significant network hub genes with higher connectivity than would be expected by chance. Whereas differential expression methods identified few differences between control and irradiated mice, differential co-expression revealed gene networks highly enriched with radiation sensitive genes, with hub membership enriched in members of the BRCA complex. These findings illustrate the value of differential correlation for extracting the biological response to subtle environmental stimuli.

36. Rapid and Stable Binding of Peptide p5 to Visceral Amyloid Deposits In Vivo Yields a Potential Radiotracer for PET/CT Imaging

Emily B. Martin, Stephen J. Kennel, Tina Richey, Alan Stuckey, Dustin Osborne, Jonathan S. Wall

Department of Medicine (Martin, Kennel, Richey, Wall), Department of Radiology (Kennel, Stuckey, Osborne, Wall), Comparative and Experimental Medicine (Martin)

Amyloid deposition contributes to the pathology of numerous disorders including type-2 diabetes, chronic inflammation, and myeloma. No method exists in the United States to detect these deposits in patients; consequently, there is a need for specific, quantitative imaging radiotracers to assist in diagnosis, disease staging, and monitoring response to therapy. The purpose was to examine amyloid binding dynamics of peptide p5 – a potential radiotracer for PET/CT imaging with 124I. We used dynamic PET/CT imaging to study 124I-p5 in mice with reactive (AA) amyloidosis or healthy WT controls. Additionally, we performed serial SPECT imaging with 125I-p5 up to 72 hr pi. Specific amyloid reactivity was confirmed via micro-autoradiography and quantified by image analysis and biodistribution measurements. In healthy mice, 124I-p5 peptide was rapidly cleared by the kidneys with a peak uptake time of ~7 min where it underwent dehalogenation. Liberated 124I-iodide then appeared in the stomach with additional uptake in the thyroid. In AA mice, however, 124I-p5 peptide bound rapidly in the liver. Longer-term studies demonstrated that 125I-p5 persisted in amyloid-laden organs for >72 hr, achieving tissue-to-muscle ratios of 168:1 and 234:1 for liver and spleen, respectively. Radioiodinated p5 peptide rapidly accumulated in visceral AA amyloid deposits and persisted in these organs for >72 hr pi, but the peptide was dehalogenated and quickly catabolized in healthy mice. Sustained binding in diseased mice renders this peptide suitable for amyloid detection in patients using PET/CT imaging. This work was supported by PHS grant R01DK079984 from the NIDDK.

37. Improving Bacterial Bioluminescence in Human Cells for Novel Imaging Applications

Tingting Xu, Dan Close, Steven Ripp, Gary Sayler

Microbiology (Xu, Ripp, Sayler), Center for Environmental Biotechnology (Xu, Ripp, Sayler), Joint Institute for Biological Sciences (Close, Sayler), 490 Biotech (Close, Ripp, Sayler)

The recent expression of bacterial bioluminescence (*lux*) system in human cells has the potential to contribute to high throughput screening and non-invasive *in vivo* imaging due to its lack of substrate requirement; however, its signal intensity is lower than that of other bioluminescent reporters. To identify points for optimization of bioluminescence, *lux* genes were assembled into vectors containing either the luciferase encoding *luxAB*, substrate processing *luxCDEfrp*, or the complete set (*luxCDABEfrp*) of *lux* genes. All possible combinations of these vectors were introduced into both wild type and previously characterized bioluminescent human (HEK293) cells. While neither expression of the *luxAB* or *luxCDEfrp* genes alone was capable of producing bioluminescence, expression of the genes together produced 10^5 photons/sec (p/s) in wild type cells. In previously characterized bioluminescent HEK293 cells, gene copy number was determined to be the limiting factor for signal intensity with the co-transfection of additional *luxAB* and *luxCDEfrp* genes increasing output by 2- and 5-fold, respectively. The largest output gain (9.9×10^6 p/s) was realized by co-transfection of the full *lux* cassette. Reporter function was demonstrated following co-transfection of the *luxCDABEfrp* vector into previously transfected cells harboring TET-On promoter regulated *luxCDEfrp* genes. This strategy allowed for statistically significant ($P < .05$) increases in bioluminescence at 100 ng/ml doxycycline and permitted real-time monitoring over 24 hr. These results demonstrate that increases in the copy number of the *lux* genes can enhance signal output and incorporation with regulated copies can provide a means for the creation *lux*-based human cell bioreporters.

38. 18FLT-PET/CT for Non-invasive Functional Imaging of Canine Bone Marrow

Josh Rowe, Casey LeBlanc, Stephen Kania, Shelley Newman, Murthy Akula, Gina Galyon, Misty Long, Stephen Kennel, George Kabalka, Amy LeBlanc

College of Veterinary Medicine (Rowe, C LeBlanc, Kania, Newman, Galyon, A LeBlanc), Graduate School of Medicine, Molecular Imaging and Translational Research Program (Akula, Long, Kennel, Kabalka, A LeBlanc), Comparative and Experimental Medicine (Rowe)

Non-invasive techniques to evaluate bone marrow function are needed to optimize care of dogs with hematopoietic disorders. Positron emission tomography/computed tomography (PET/CT) using 18F-fluoro-l-deoxythymidine (18FLT), a PET tracer that physiologically concentrates in bone marrow and acts as a marker of proliferation, offers a non-invasive imaging technique for whole-body assessment of marrow proliferation. Three clinically normal dogs were imaged using 18FLT for assessment of bone marrow proliferation at baseline and at 7 and 14 days post administration of a single dose of cyclophosphamide (225 mg/m2 PO) to non-selectively suppress hematopoiesis. Regions of interest were drawn over sites of clinical relevance including common marrow sampling sites. Standardized uptake values were calculated. Bone marrow aspirates from the proximal humerus and wing of the ilium were assessed within 24 hr of each imaging time point by cytology and flow cytometric cell cycle analysis for comparison with PET imaging findings. Despite profound circulating neutropenia (median neutrophil nadir 580 cells/uL, 8 days post-cyclophosphamide administration), no significant changes in overall marrow cellularity were detected. Significant changes between sampling time points were detected in the granulocytic index (ratio of early to late granulocytic precursors) in wing of ilium aspirates ($P = .007$), and flow cytometric cell cycle analysis was indicative of DNA insult at all sites sampled. Strong physiologic 18FLT uptake remained present in axial and proximal appendicular marrow sites. Changes over time in SUVmax were significant ($P = .003$) in proximal humerus but not wing of ilium. Changes observed in marrow aspirates in this study preclude correlation between 18FLT signal and marrow function.

39. Effects of Environmental Carcinogens on Canine Mesenchymal Stem Cells (MSC) Isolated from Adipose Tissue

Valeria Tanco, Kusum Rathore, Laura Wright, Amanda Carters, Maria Cekanova

Small Animal Clinical Sciences (Tanco, Rathore, Wright, Carters, Cekanova), Comparative and Experimental Medicine (Tanco)

One in eight women in the United States will develop breast cancer at some point in their lives. Recent studies provide clear evidence that exposure to certain environmental substances during early stages in life play an important role in the development of cancer. The dog model offers a unique opportunity to study human breast cancer given the spontaneous development of the

disease, their similarities in physiological development, and the similarities in environmental exposure to carcinogens. The interactions between stromal and epithelial cells as well as growth factors and cytokines produced by stromal cells play an important role in breast cancer development and progression. In this study, we evaluated the effects of two carcinogens, benzo-a-pyrene (BaP) and bisphenol A (BPA), on canine MSC isolated from adipose tissue (ADMSC). We assessed the effects of these carcinogens on canine ADMSC proliferation and differentiation. Effects of these carcinogens on adipogenic differentiation of canine ADMSC were assessed after 21 days by Oil Red O staining. The effects of BaP and BPA on cell proliferation of canine ADMCS were assessed by MTS proliferation assay. BaP inhibited adipogenic differentiation of canine ADMSC in a dose-dependent manner compared to untreated ADMSC after 21 days. Conversely, BPA did not affect adipogenic differentiation of canine ADMC at any tested concentration after 21 days. Proliferation of canine ADMSC was not affected by BaP or BPA after 48 hr. The influence of environmental chemicals on ADMSC and its relationship to breast cancer is still poorly understood and further studies are required.

40. Bone Marrow-Derived Mesenchymal Stem Cells (BMMSC) for the Treatment of Canine Osteoarthritis

Meredith Westling, Valerie Tanco, Kusum Rathore, Anna Sonntag, Gina Galyon, Laura Wright, Amanda Carter, Alfred Legendre, Darryl Millis, Maria Cekanova

Small Animal Clinical Sciences (Westling, Tanco, Rathore, Sonntag, Galyon, Wright, Carter, Legendre, Millis, Cekanova), Comparative and Experimental Medicine (Tanco)

Osteoarthritis (OA) is a common condition that affects approximately 20% of the canine population. This disease is caused by loss of articular cartilage and development of inflammation within a joint resulting in decreased range of motion and loss of function. Currently, non-steroidal anti-inflammatory and adjunctive analgesic drugs are used for chronic pain relief in combination with joint supplements to prevent further loss of cartilage. The use of mesenchymal stem cells (MSC) as a new therapy for OA is being investigated. MSC have the capacity to regenerate and repair damaged tissues in addition to anti-inflammatory effects in joints. Currently, we are investigating the use of autologous bone marrow-derived MSC (BMMSC) isolated in our laboratory for the treatment of OA in both clinical patients and research dogs. The isolated canine BMMSC cells were evaluated to confirm stem cell origin by differentiation and cell marker expression profiles. A clinical case for treatment of elbow osteoarthritis has had increased use of the limb 5 weeks after intra-articular injection of autologous BMMSC. Six research dogs with experimentally-induced OA of the stifle had an intra-articular injection of autologous BMMSC and evaluation for objective improvement of lameness using kinetic gait analysis at 1, 3, and 5 weeks post-injection. The first week evaluation did not show any significant changes due to the chronicity and severity of OA. Later time points are currently under investigation. More than a single injection of BMMSC and further follow-up may be required to fully evaluate positive response in chronic and severe OA.

41. Comparison of In Vitro Adherence, Proliferation, and Differentiation of Equine Bone Marrow-Derived Mesenchymal Stem Cells to Identify an Allogenic Donor and to Determine the Effect on Proliferation with Platelet-Rich Plasma

Jessi Carter-Arnold, Nancy Neilsen, Lisa Amelse, Rosalie Mize, Madhu Dhar

Large Animal Clinical Sciences (Carter-Arnold, Amelse, Mize, Dhar), Biomedical and Diagnostic Sciences (Neilsen)

Our objective was to compare in vitro adherence, proliferation, and potential for differentiation of equine mesenchymal stem cells (eMSCs) from various donors and to determine the effect on proliferation of autogenous eMSCs by adding platelet-rich plasma (PRP). eMSCs from six horses were cultured in vitro and evaluated for viability, proliferation, osteogenesis, chondrogenesis, adipogenesis, and gene expression. Varying concentrations of PRP were added to eMSCs, and their proliferation was measured using MTS assay at days 2 and 6. One horse demonstrated a 1.0–1.8-fold higher rate of proliferation and a better potential for chondrogenesis, osteogenesis, and adipogenesis. This same horse demonstrated a relatively higher expression of Sox2 mRNA. PRP at a 30% concentration resulted in a 1.8- to 2.0-fold increase in cell proliferation over the controls. The ability of eMSCs to proliferate and undergo osteogenesis and chondrogenesis varied between horses. Some donors supply eMSCs that are far superior to the others in viability, proliferation, differentiation, and gene expression. Data supports the rationale of identifying allogenic eMSC donors in hopes of improving treatment outcome. PRP augments proliferation of eMSCs, and therefore, a combination of the two could enhance the therapeutic effect in culture and during local injection.

42. Bacterial Cellulose and Equine-derived Bone Marrow Mesenchymal Stem Cells as a Potential Biomaterial Construct for Tissue Engineering of Cartilage and Bone

Pelagie Favi, Nancy Neilsen, Roberto Benson, Ryan Hammonds, Christopher Stephens, Madhu Dhar

Materials Science and Engineering (Favi, Benson, Hammonds), Biomedical and Diagnostic Sciences (Neilsen), Surgery, Graduate School of Medicine (Stephens), Center for Materials Processing (Stephens), Large Animal Clinical Sciences (Dhar)

The purpose of this study was to evaluate cell proliferation and viability, and osteocyte and chondrocyte differentiation of equine-derived bone marrow mesenchymal stem cells (EqMSCs) when seeded on bacterial cellulose (BC) scaffolds as a potential scaffold for the tissue engineering of cartilage and bone. BC is a biocompatible natural polymer. BC was synthesized using the bacterium *Gluconacetobacter sucrofermentans*. An equine model was chosen due to similarities in size, load, and types of joint injuries suffered by horses and humans. Isolated, expanded, and characterized EqMSCs were seeded on the BC scaffolds, and fluorescence microscopy confirmed adherence and expansion. EqMSCs positively expressed the undifferentiated pluripotent mesenchymal stem cell surface markers CD44 and CD90. MTS assay demonstrated increasing proliferation and viability with time (days 2, 7, and14). Cell-scaffold constructs were cultured for 14 days under osteogenic and chondrogenic conditions. Osteocytes were positive for alizarin red, and chondrogenesis was confirmed by alcian blue staining of the chondrocytes. In summary, biocompatible BC scaffolds support proliferation and viability, and osteogenic and chondrogenic differentiation of Eq-MSCs cultured onto its surface in vitro, allowing for future potential use for tissue engineering therapies.

43. Polymeric Transfection of Human Aortic Smooth Muscle Cells

Chris Stephens, Deidra Mountain, Trey Fisher, Faith Creekmore, Stacy Kirkpatrick, Oscar Grandas

Department of Surgery, Graduate School of Medicine (Stephens, Mountain, Fisher, Creekmore, Kirkpatrick, Grandas), Center for Materials Processing, College of Engineering (Stephens)

Polymeric transfection using degradable cationic polymers possesses a high translational potential due to its high efficiency and low toxicity. Additionally, the use of a polymeric vector eleviates the safety concerns associated with viral vectors. For these studies, human aortic smooth muscle cells were transfected with nanoparticles composed of poly(beta-amino ester), PBAE, and siRNA. Transfection was conducted in buffer and serum containing cell culture media to study the effect of absorbed serum proteins on transfection efficiency. The particle characteristics associated with transfection efficiency, namely particle diameter and zeta potential, were characterized using dynamic light scattering and electrophoretic mobility.

44. Automatic Detection of ECG Electrode Misplacement: A Tale of Two Algorithms

Henian Xia, Gabriel A. Garcia, Xiaopeng Zhao

Mechanical, Aerospace, and Biomedical Engineering

Artifacts in electrocardiograms (ECG) due to electrode misplacement can lead to wrong diagnoses. Various computer methods have been developed for automatic detection of electrode misplacement. Here, we reviewed and compared the performance of two algorithms with the highest accuracies on several databases from Physionet. These algorithms were implemented into four models. For clean ECG records with clearly distinguishable waves, the best model produces excellent accuracies (≥ 0.984) for all misplacements except the LA/LL interchange (0.874). However, the accuracies are significantly lower for records with noises and arrhythmias. Moreover, when the algorithms are tested on a database that is independent from the training database, the accuracies may be poor. For the worst scenario, the best accuracies for different types of misplacements range from 0.361 to 0.784. A large number of ECGs of various qualities and pathological conditions are collected every day. To improve the quality of health care requires more robust and accurate algorithms for automatic detection of electrode misplacement, which shall be developed and tested using a database of extensive ECG records.

45. Rapid Detection of Bovine and Human Tuberculosis by AC Electrokinetics-Enhanced Impedimetric Method

Haochen Cui

Electrical Engineering and Computer Science

Here we show a rapid and simple way of detecting bovine and human tuberculosis through impedance measurement. The detection principle lies in antigen-antibody binding, which takes place only when an antigen

and disease-positive antibody meet in the serum; such binding could be greatly enhanced by AC electrokinetics. Double layer capacitance would change accordingly, either increase or decrease, depending on the change of the double layer's thickness and surface area. After loading certain samples into our off-the-shelf SAW chip, disease positivity or negativity could be determined within 2 min by calculating the impedance change rate of the chip (especially the capacitance change rate, i.e. dC/dt). Compared with traditional biomedical methods, i.e. ELISA, accuracy of impedance measurement reached 100% for human TB (11/11 samples) and 85% for bovine TB (17/20 samples). Furthermore, the limit of detection shows the lowest detectable concentration reaches 10 ng/ml or even less.

46. Short-Term Direct Electric Current Exposure Decreases Caspace-3 Activity in Colon Cancer Cells

Lee Barham, Seung Joon Baek, James Fleming, Gary Sayler

Microbiology (Barham), Biomedical and Diagnostic Sciences (Baek), Center for Environmental Biotechnology (Fleming, Sayler)

Successful clinical electrochemical treatment of solid tumors has been demonstrated in skin, lung, liver, pancreas, and breast tissue. Recently, treatment of tumor cells with direct current (DC) has been shown to induce apoptosis in human leukemic and oral mucosa cancer cells. We set out to investigate the anti-proliferative and apoptotic effects of direct electric current exposure on SW480 colon cancer cells using a sophisticated electrochemical approach. Our experimental system uses precisely microfabricated platinum electrodes on silica chips in a three-electrode configuration interfaced with an electrochemical potentiostat. Cells were grown on electrode chips using RPMI media contained within rubber gasket frames. Indium tin oxide (ITO) transparent slide placed on the surface of the frames functioned as the counter electrode. Cells were exposed for 300 sec in triplicate to a DC field strength of 1.6–2.3 V/cm with current densities ranging from 10–1000 μA/cm2. After 24 hr the cells were tested for: 1) cell viability using a tetrazolium/formazan assay and 2) apoptosis using a caspase 3/7 assay. Contrary to previous reports we found that caspase activities decreased 24 hr after electric current exposure, and these effects occur at levels below those that adversely affect cell viability. In addition, we conclude that the observed decrease in cell viability with increasing current is due to necrosis instead of apoptosis. While a reduction in caspase activity may not be useful from a cancer therapy perspective, it suggests a treatment modality for cells that undergo premature or unwanted apoptosis such as neural cells in neurodegenerative disorders.

47. Monitoring the Metabolic Dynamics of Human Cells Grown in 2-D vs. 3-D Culture Environments

James D. Webb, Daniel Close, Gary Sayler

Center for Environmental Biotechnology (Webb, Sayler), Joint Institute for Biological Sciences (Close, Sayler)

Cultures grown on plates create sheets of cells called monolayers that result in unnatural arrangements of cellular populations. With a shift toward the use of 3-D tissue-culture scaffolds allowing cells to be grown in environments mimicking their natural growth conditions, there is a need to evaluate how these techniques affect cellular metabolism. We hypothesized that since in vivo cells participate in intercellular interactions through the extra-cellular matrix, and since 3-D culture conditions provide a similar setting for growth, cultures grown under 3-D conditions would express higher rates of growth and metabolism compared to traditional methods. In these experiments, the metabolic dynamics of a human kidney cell line (HEK293) were assayed under multiple conditions: cultures of cells grown in suspension, a monolayer on traditional cell culture-treated plastic-ware, and a 3-D colony on tissue culture scaffolds. Measuring bioluminescent production from the bacterial bioluminescence (lux) gene cassette, equal numbers of cells were evaluated for metabolic efficiency during growth under each culture condition. The lux-cassette, composed of five genes derived from the bacterial insect pathogen, Photorhabdus luminescens, was chosen to monitor metabolic rate because the availability of its intracellular substrates (and therefore light output) is tied directly to the metabolic rate and health of the cell. Light output from HEK293 cells expressing lux genes correlated tightly with metabolic function as measured under varying amounts of cellular stress and, over 24-hr growth periods, the metabolic efficiency of cells subjected to each of the growth conditions was evaluated continuously, compared, and demonstrated to be significantly different.

48. The Effect of Fentanyl on Sevoflurane Minimum Alveolar Concentration Preventing Motor Movement (MACNM) in Dogs

Sabrina Reilly, Reza Seddighi, Christine M. Egger, Barton W. Rohrbach, Thomas J Doherty, Wen Qu, James R. Johnson

Small Animal Clinical Sciences (Reilly, Egger); Large Animal Clinical Sciences (Seddighi, Doherty); Biomedical and Diagnostic Sciences (Rohrbach); Pharmaceutical Sciences (Qu, Johnson)

Fentanyl decreases the minimum alveolar concentration (MAC) of volatile anesthetics; however, tolerance to the MAC-reducing effects of opioids has been reported. There is no information on fentanyl's effects on sevoflurane MACNM in dogs. The objectives of this study were to determine the effects of fentanyl on sevoflurane MACNM, and to evaluate if acute tolerance develops. Baseline sevoflurane MACNM (MACNM-B) was determined in six dogs on three occasions. Dogs were randomly assigned to fentanyl treatments (T) as a loading dose (Ld) and CRI: T1–7.5 µg/kg and 3.0 µg/kg/hr; T2–15.0 µg/kg and 6.0 µg/kg/hr; T3–30.0 µg/kg and 12.0 µg/kg/hr. A first post-treatment MACNM (MACNM-I) determination was initiated 90 min after the start of the CRI, and a second MACNM (MACNM-II) determination was initiated three hr after MACNM-I was established. Data were analyzed using a mixed-model ANOVA. The overall median MACNM-B for all groups was 2.55%. All treatments decreased ($P < .05$) MACNM-B, and the decrease from baseline was 21.6%, 35.0%, and 40.6% for T1, T2, and T3, respectively. Percentage change in T1 differed ($P < .05$) from T2 and T3; however, T2 did not differ from T3. MACNM-I was not significantly different from MACNM-II within treatment groups. Fentanyl doses in the range of 3–12 µg/kg/hr significantly decreased the sevoflurane MACNM. Tolerance to fentanyl did not occur under the study conditions.

49. Pharmacokinetics of Terbinafine after Oral Administration of Single Doses in Hispaniolan Amazon Parrots (*Amazona ventralis*)

Erika E. Evans, Lee C. Emery, Sharon K. Cox, Marcy J. Souza

Small Animal Clinical Sciences (Evans), College of Veterinary Medicine Class of 2013 (Emery), Biomedical and Diagnostic Sciences (Cox, Souza)

Terbinafine hydrochloride has been useful in the treatment of avian aspergillosis. Results of pharmacokinetic studies and dose recommendations for terbinafine have been published for red-tailed hawks and penguins. Oral doses of 15 mg/kg were reportedly being used clinically for treatment of aspergillosis in psittacine birds with favorable results, but preliminary pharmacokinetic trials using that dosage failed to reach measurable plasma levels in African greys. In this study, single doses of terbinafine (30 mg/kg or 60 mg/kg) were administered orally to four and eight birds, respectively. All four birds administered the 30 mg/kg dose were fasted. Of the eight birds administered the 60 mg/kg dose, three were fasted and five were fed. Plasma terbinafine concentrations were determined at intervals via high-pressure liquid chromatography. After administration of 30 mg/kg terbinafine, mean (± SD) plasma concentration peaked in approximately 2.3 hr at 90 ± 58 ng/mL, whereas a 60 mg/kg dose in fed birds resulted in peak mean (± SD) plasma concentration of 304 + 232 ng/mL in 5 hr, and a 60 mg/kg dose in fasted birds resulted in a peak mean (± SD) plasma concentration of 673 ± 399 ng/mL in 1.7 hr. Plasma terbinafine concentrations reached potential therapeutic levels based on the minimum inhibitory concentration range against *A. fumigatus* in humans (0.02 – 5 µg/mL) at both the 30 mg/kg and 60 mg/kg doses, but failed to reach the calculated pharmacokinetic concentration that could be achieved with a 22 mg/kg dose in red-tailed hawks (0.8–1.6 µ/mL).

50. The Effect of Topical 2% Delta-9-Tetrahydrocannabinol Suspension on Aqueous Humor Flow Rate and Intraocular Pressure in Normal Dogs

Kristin M. Fischer, Daniel A. Ward, Diane V.H. Hendrix

Small Animal Clinical Sciences

The objective of this study was to determine the effect of 2% delta-9-tetrahydrocannabinol (THC) on the aqueous humor flow rate (AHFR) and intraocular pressure (IOP) in normal dogs. Twenty-one ophthalmologically normal dogs were used in a randomized, longitudinal crossover design. Following acquisition of baseline IOP and AHFR data, dogs were randomly divided into two treatment groups and were administered one drop of either THC or THC vehicle to one randomly-assigned eye every 12 hr for nine doses. The untreated eye did not receive any medications throughout the study. IOPs and AHFRs were reassessed after the final dose of medication. A washout period of at least 7 days was allowed before the treatments were crossed over. The dogs were then treated in the same designated eye with the opposite drug, and the fluorophotometry and tonometry evaluations

were repeated. Mean ± SD IOPs in the morning were 15.86 ± 2.48 mmHg at baseline, 12.54 ± 3.18 after THC, and 13.88 ± 3.28 after THC vehicle (P = .027). Mean ± SD IOPs in the evening were 13.69 ± 3.36 at baseline, 11.69 ± 3.94 after THC, and 12.13 ± 2.99 after THC vehicle (P = .039). Mean ± SD AHFRs were 5.59 ± 2.22 µl/min at baseline, 4.63 ± 1.22 after THC treatment, and 4.93 ± 2.30 after THC vehicle (P = .667). Topical application of 2% THC had a minor reducing effect on IOP in normal dogs. Our data do not support the notion that this change in IOP is due to reduction in the production of aqueous humor, but low statistical power of the AHFR comparisons could have hidden such an effect.

51. Effect of Siam Weed Extracts on Anti-Inflammatory Activity

Hataichanok Pandith, Xiaobo Zhang, Wandee Gritsanapan, Seung Joon Baek

Biomedical and Diagnostic Sciences

Siam weed (*Chromolaena odorata* (L.) King and Robinson) is a medicinal herb used for a variety of ailments, especially bleeding, wound healing, and anti-inflammation. It has been reported that there are many bioactive compounds in this plant. Among them, scutellarein tetramethyl ether (4',5,6,7-tetramethoxy-flavone) may be an active compound for blood coagulation and may be involved in anti-inflammatory activity of Siam weed. However, the molecular mechanism by which this plant affects anti-inflammatory activity has not been studied. In this study, we examined the expression of several inflammatory proteins and performed biochemical assays including measuring anti-oxidant activity and nitro-oxide releasing with macrophage cells. Cyclooxygenase (COX)-2 and inducible nitro oxide synthase (iNOS) are critical enzymes that play important roles in inflammation. The level of protein and mRNA expression of these enzymes induced by lipopolysaccharide (LPS) was evaluated in the presence or absence of Siam weed extracts or scutellarein tetramethyl ether using RAW 264.7 macrophage cells. LPS increases COX-2 and iNOS protein and mRNA level in RAW cells; however, the treatment of Siam weed extracts and scutellarein tetramethyl ether dramatically suppressed these enzymes in a dose-dependent manner. We also found that Siam weed extracts increase anti-oxidant activity and decrease NO releasing. Thus, our results will provide a potential use of Siam weed extract and/or scutellarein tetramethyl ether, its active compound, in the treatment of inflammatory-related diseases.

52. Do Plastic Bottles Leach Substances that Interfere with Human Hormones?

Melanie Eldridge, Fu-Min Menn, Gary Sayler

Center for Environmental Biotechnology (Eldridge, Menn, Sayler), Joint Institute for Biological Sciences-ORNL (Menn, Sayler)

Eastman's Tritan copolyester is a novel plastic manufactured using three monomers, di-methylterephthalate (DMT), 1,4-cyclohexanedimethanol (CHDM), and 2,2,4,4-tetramethyl-1,3-cyclobutanediol (TMCD), in various ratios. Tritan polymers are used for the manufacture of various types of food, water, and beverage containers (e.g. Camelback bottles) as well as in medical devices. In light of recent public attention over the presence of endocrine active compounds that may be able to leach out of plastics, studies were conducted to understand whether such a concern exists for Tritan copolyester. Accordingly, studies were conducted on extracts from finished pellets and molded bars of Tritan to assess estrogenic or androgenic potential. The extraction procedures simulated those recommended by FDA in their safety assessment of plastics used for food packaging and exaggerate actual end-use conditions. Specifically, incubation conditions consisted of incubating the various products in water, 10%/90% and 50%/50% ethanol/water solutions (240 hr at 40°C), and in a 50%/50% solution for 2 hr at 70°C. To simulate harsh dishwasher conditions, extracts were obtained after plastics' incubation in Cascade (10 g/L) for 240 hr at 70°C, then 240 hr at 40°C in the three extraction solutions. Extracts obtained from these solutions were concentrated 2000X using Hydrophilic-Lipophilic-Balance SPE disks as outlined in EPA Method 1694. Assessment of EA and AA activity was determined using bioluminescent bioreporters as outlined by Sanseverino et al. (2009). Results showed no evidence of either EA or AA for any Tritan product under any of the extraction conditions. Accordingly, these results further reaffirm the safety of Tritan copolyester.

53. Identification of Bile Salt Hydrolase Inhibitors, a Promising Alternative to Antibiotic Growth Promoters

Katie Smith, Zhong Wang, Ximin Zeng, Yiming Mo, Jun Lin

Animal Science

Antibiotic growth promoters (AGP) have been used as feed additives to improve average body weight gain and feed efficiency in food animals for more than 5 decades.

However, there is a worldwide trend to limit AGP use to protect food safety and public health, raising an urgent need to discover effective alternatives to AGP. Previous studies have shown that the growth-promoting effect of AGP is highly correlated with the decreased activity of intestinal bile salt hydrolase (BSH), an enzyme that is produced by various gut microflora and is actively involved in host lipid metabolism. Thus, BSH inhibitors are likely promising feed additives to replace AGP for improving animal growth performance. In this study, the genome of *Lactobacillus salivarius* NRRL B-30514, a BSH-producing strain isolated from chickens, was sequenced by 454 GS FLX sequencer. Sequence analysis identified two putative bsh genes. His-tagged recombinant BSH of one bsh was produced for enzymatic analyses. The BSH displayed hydrolysis activity for both glycoconjugated and tauroconjugated bile salts. The optimal pH and temperature for the BSH activity were 5.5 and 41°C, respectively. Screening of a panel of dietary compounds identified some potent BSH inhibitors, such as copper, which has been recently demonstrated to promote feed digestion and body weight gain of different food animals. Together, this study identified and characterized a BSH with broad substrate specificity from a chicken *L. salivarius* strain, and strongly supported our hypothesis that BSH inhibitors are promising alternatives to AGP for enhancing the productivity and sustainability of food animals.

54. Estrogenic Activity Produced by *Talinum paniculata* (Jacq.) Gaertn's Leaf Extract in Ovariectomized Rats

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Institute of Science, Suranaree University of Technology (Thanamool, Kupittayanant), Animal Science (Moustaid-Moussa)

The purpose of this study was to evaluate the potential estrogenic bioactivity of *Talinum paniculata* (Jacq.) Gaertn (TP) methanolic extract. Female Wistar rats (250–300 g) were bilateral ovariectomized (OVX) and randomly treated for 42 days post-OVX with leaf extract at two doses (1,000 and 100 mg/kg/day) with a positive control of 17ß-estradiol (10 µg/kg/day) in sesame oil as vehicle. The body weight of the OVX rats significantly increased compare to the non-OVX rats. In contrast, their relative uterus weight to body weight significantly decreased. The serum estradiol level was elevated in plant extract-treated rats (1,000 mg/kg) when compared with vehicle control group. In addition, the high density lipoprotein (HDL) and low density lipoprotein (LDL) level were also measured. The result showed that in both doses of plant extract-treated groups significantly raised HDL but lowered LDL levels ($P < .05$). All these data suggest that the leaf extract of TP possess estrogenic activity in OVX rats, which can reduce unexpected menopausal symptoms and be used as an alternative for hormone replacement therapy. Funded by Office of the Higher Education Commission, Thailand.

55. Testicular Steroidogenesis in Pre-pubertal Rats Exposed to Aroclor-1242

Arokya M. Sashi Papu John, In-Shik Kim, S. M. L. Chamindrani Mendis-Handagama

Biomedical and Diagnostic Sciences

The present study examined the testicular steroidogenesis in pre-pubertal offspring of rat dams treated with either 80 µg (H) or 8 µg (L) of Aroclor-1242. Three groups of lactating mothers received daily SC injections of either 200 µl of corn oil (controls) or H and L doses of Aroclor-1242 in corn oil. Pups were euthanized at 7, 14, and 21 days (n = 8). Body weight, testis weight, serum testosterone, and testicular testosterone secretion in vitro were not different ($P > .05$) among the age-matched (AM) controls and Aroclor-treated rats. Serum androstenedione in 7 and 14 day H and L rats were not different from their AM controls. However, serum androstenedione was lower in 21-day H rats than in their AM controls and L rats. Moreover, luteinizing hormone (LH)-stimulated testicular androstenedione secretory capacity in vitro was reduced in 7 and 14 days for H rats and 21 days for H and L rats compared to their AM controls. Stereology revealed that newly formed adult type LC (NFLC) in Aroclor-treated rats were sparse. Androstenedione is a major androgen secreted by the NFLC, and decrease in androstenedione indicates reduced NFLC in those rats. No changes observed in serum testosterone and LH-stimulated testicular testosterone secretion in vitro in Aroclor-treated rats and AM-controls are due to fetal LC, which are still present in pre-pubertal testes; they secrete an abundance of testosterone. In conclusion, lactational Aroclor-1242 exposure of neonatal pre-pubertal male rats causes arrest in LC differentiation and results in low testicular androstenedione secretion in pre-pubertal rats.

56. Juvenile Hormone Interaction with Insulin Signaling is Critical for Lipid Metabolism during Tsetse Pregnancy

Aaron Baumann, Joshua B. Benoit, Geoffrey M. Attardo, John K. Moulton, Thomas G. Wilson, Serap Aksoy

Entomology & Plant Pathology (Baumann, Moulton), School of Public Health, Yale University (Benoit, Attardo, Aksoy), Evolution, Ecology, and Organismal Biology, Ohio State University (Wilson)

Tsetse flies are the sole vector of African trypanosomiasis, which causes sleeping sickness in humans and nagana in cattle. These flies employ a unique, viviparous reproductive strategy: females undergo pregnancy, nourishing intrauterine larvae through a process remarkably similar to lactation in mammals, which occurs through a heavily modified accessory gland called the milk gland (MG). The hormonal control of tsetse pregnancy remains largely uncharacterized. Juvenile hormone (JH) signaling is fundamental to insect development and reproduction, but the molecular mechanisms of JH action are poorly understood. Several recent studies indicate that the JH pathway interacts with insulin/IGF signaling (IIS) to control diverse physiological processes in insects, including reproduction. Application of JH analog (JHA) insecticides disrupts reproduction in tsetse flies, specifically by interfering with MG physiology. In this study, we examined the molecular interaction of JH and IIS during pregnancy in the tsetse fly (*Glossina morsitans morsitans)*. Our results indicate that JH and IIS pathways are critical for lipid utilization during pregnancy. SiRNA suppression of the paralogous JH receptor candidates Met and gce decreased total body lipid content and fecundity and induced larval abortion. Temporal expression profiles for key genes in JH and IIS pathways showed tight coordination with the pregnancy cycle. Furthermore, insulin injection phenocopied application of the JHA methoprene, modulating the expression of the IIS genes HDAC4 and Bmm, and a group of novel, tsetse-specific proteins expressed exclusively in the MG. From our results we provide a model for JH/IIS interaction during pregnancy in tsetse flies.

57. Influence of Fruit Variety and Course Sequence on Fruit Intake during a Snack in Preschool-Aged Children

Chelsi Cardoso, Shannon Looney, Hollie Raynor

Nutrition

Variety and course sequence are factors known to impact consumption, but these factors have not been examined regarding fruit intake. This study investigated the effect of fruit variety and course sequence on fruit intake during an afternoon snack in preschool-aged children. A 2 x 2 crossover design (within-subject conditions of fruit variety and course) was conducted on Wednesday afternoons in a preschool setting. Sixteen children (4.1 ± 0.7 yrs; 56.3% female, 75.0% white, 6.3% Hispanic, 60.7 ± 27.0 body-mass-index [BMI] percentile; 0.50 ± 1.28 BMI z-score) completed four, 20-minute snack sessions. Foods served included 50 g applesauce, 50 g peaches, 100 g cheese (fruit variety) or 100 g applesauce, 100 g cheese (fruit non-variety) over one 20-minute course (one course) or one, 10-minute fruit course and one 10-minute, cheese course (two course). Repeated measures analyses of covariance found a significant (P = .011) interaction of fruit variety and course, indicating that in the fruit non-variety conditions, more fruit was consumed in the two course as compared to one course condition (75.3+ 25.5 g vs. 61.3 + 30.4 g, P < .05). Additionally, a significant main effect of course was found, with more fruit consumed in the two course as compared to one course condition (75.0 + 27.4 vs. 64.3 + 32.5 g, P < .01). When amount and energy of all foods consumed were examined, no differences were found between conditions. In preschool-aged children, serving fruit as a first course in a snack may increase consumption of fruit.

58. Active Video Gaming Compared to Unstructured, Outdoor Play in Children: Measurements of Estimated Energy Expenditure and Percent Time in Moderate-to-Vigorous-Intensity Physical Activity

Susan B. MacArthur, Hollie A. Raynor

Nutrition

Active video games (AVGs) may be a source of physical activity (PA) for young children. This study compared AVGs to unstructured outdoor play (OP) via accelerometery and direct observation (DO) in estimated

energy expenditure (EE) and percent time engaged in moderate-to-vigorous-intensity physical activity (% MVPA) in two, 15-min sessions. Sixteen, normal-weight, school-aged children (6.4 + 0.8 yr, 62.5% male, 100% white, standardized body mass index [zBMI] = -0.18 + 0.66) completed two 15-min sessions of either AVG (Xbox 360 Kinect, Kinect Adventures! River Rush! video game), or OP, where children engaged in unstructured outdoor play in a playground with at least one other child. Sessions were counterbalanced and conducted in the morning. A 5-min "warm-up" period was provided, followed by 15 min of measured activity. An Actical accelerometer on the left hip measured estimated EE and %MVPA. DO was conducted using the Children's Activity and Rating Scale (CARS) from which %MVPA was coded. Controlling for session order and percent relative humidity, no difference was found in estimated EE between OP (15.5 + 7.8 kcals) and AVG (17.0 + 7.1 kcals). For %MVPA, AVG was significantly greater than OP as assessed by accelerometer (74.6 + 31.1% vs. 67.5 + 32.2%, $P < .05$) and DO (23.8 + 12.4% vs. 13.2 + 13.0%, $P < .05$). These findings suggest that AVGs and OP are comparable physical activities, and that AVGs may be a source of PA for children.

59. Do Weight Status and the Level of Dietary Restraint Moderate the Relationship between Package Unit Size and Food Intake?

Chrystal Haire, Hollie Raynor

Nutrition

Single-serving packages (SSPs), as compared to standard packages (STPs), may assist in reducing consumption. Those concerned about the amount they consume, such as overweight (OW) and dietary restrained individuals, may be more influenced by SSPs. This study examined if weight and restraint status moderate the influence of package size on consumption. It was hypothesized that SSPs would help to reduce intake in OW and/or restrained individuals. Using a 2 x 2 x 2 (unrestrained/restrained x normal weight[NW]/OW x SSP/STP) between-subjects factorial design, 64 participants (23.7+/-3.3 yrs.; 81.3% white; 96.9% non-Hispanic) were randomly assigned to receive 20 oz. of pretzels packaged in either SSPs or STPs to eat ad libitum for 4 days. Each condition contained eight participants. Total grams of pretzels consumed was determined by subtracting pre- and post-consumption weight of packages provided. Using an analysis of variance for total grams of pretzels consumed, there was a significant interaction ($P = .01$) between package size and weight status. OW participants in the STP condition ate more compared to OW participants in the SSP condition (204.4+/-144.9g vs. 107.0+/-101.9g, $P < .05$). For participants in the STP condition, OW participants ate more than NW participants (204.4+/-144.9g vs. 112.7+/-58.9g, $P < .05$). No significant difference was found between OW and NW participants in the SSP condition for amount consumed. For OW individuals, STPs increased consumption compared to SSPs, which did not occur for NW individuals. While SSPs may not influence OW individuals to eat less in comparison to NW individuals, replacing STPs with SSPs may assist with reducing overconsumption.

60. Metabolomic Analyses of the Effects of Obesity and Genetic Variation on Ovarian Energy Metabolism

Ben Ernest, Shawn Campagna, Lannett Edwards, Arnold Saxton, Brynn Voy

Animal Science (Ernest, Edwards, Saxton, Voy), Chemistry (Campagna), Graduate School of Genome Science and Technology (Ernest, Saxton, Voy)

Over one-third of women of reproductive age in the United States are obese, making them three times more likely than lean women to experience infertility. Fertility and the production of developmentally-competent oocytes rely on precisely-coordinated metabolic interactions between germinal and somatic compartments of ovarian follicles. Systemic metabolism is perturbed in obesity, but it is currently unknown how this impacts metabolism in the ovary and if the potential metabolic disruption contributes to obesity-associated infertility. We hypothesize that obesity alters glucose and lipid metabolism in the cumulus-oocyte complex in a manner that compromises fertility. Metabolomics offers a powerful approach to characterizing cellular metabolic responses to experimental conditions by simultaneously quantifying hundreds of small molecules ("metabolites"). We use liquid chromatography-mass spectrometry-based metabolomics to quantify effects of the ovarian follicular environment on energy metabolism in mouse oocytes and the somatic cells that support them. Recombinant inbred lines of mice are used as a population-based model representing a spectrum of variation in fatness, fertility, and insulin sensitivity. We have found that genetic variation strongly influences ovarian energy metabolism, fat accumulation lining the reproductive tract, and reproductive function across a panel of recombinant inbred mice. Ongoing efforts are dedicated to defining the metabolic alterations in both oocytes and supporting somatic cells that are consistently associated with maternal fatness, independent of genetic background.

Our long term goal is to determine if obesity-associated changes in the follicular environment influence metabolic processes critical for producing healthy oocytes.

61. Seasonal Changes in Leptin and White Adipose Tissue in American Black Bears

Elizabeth Hill, Brynn Voy, Edward Ramsay

Animal Science (Hill, Voy), Small Animal Clinical Sciences (Ramsay)

Black bears have an intricate seasonal physiology, eating an entire year's worth of food in 7–9 months and losing 15–20% of fat during hibernation with almost no activity. The black bear thus represents a novel model in which to study seasonal regulation of food intake and metabolism. What controls the seasonal changes in fat deposition and metabolism in bears is unknown. We hypothesized that expression of adipokines, such as leptin, that regulate food intake and metabolism, vary seasonally in American black bear adipose tissue in a manner that correlates with fat storage. The study population consisted of wild nuisance bears from the Great Smoky Mountains National Park, wild bears from New Jersey, and captive bears from zoos in Tennessee and North Carolina. Blood and subcutaneous fat were collected from all bears, and abdominal fat and liver samples were collected from nuisance bears that were euthanized. Body length, girth circumference, and body weight were measured for each bear. Quantitative PCR (QPCR) was used to observe changes in mRNA expression of leptin and other adipokines in the fat samples collected across seasons. Circulating levels of non-esterified fatty acids (NEFA), triglycerides, beta-hydroxybutyrate, insulin, and glucose were measured to assess lipid and glucose metabolism. Adipocyte size was measured from H&E-stained sections of adipose tissue as an additional index of adiposity. A leptin radioimmunoassay (RIA) will be validated for use in bears to measure serum leptin concentrations. Initial analysis suggests a relationship between BMI, leptin expression, and season. Complete results will be presented.

62. Pathway Profiling Identifies Mechanisms of Adipose Deposition in Domestic Chickens

Bo Ji, Ben Ernest, Jessica R. Gooding, Susan J. Lamont, Jean Simon, Shawn R. Campagna, Arnold M. Saxton, Brynn H. Voy

Animal Science (Ji, Saxton, Voy), Animal Science, Iowa State University (Lamont), Institut National de la Recherche Agronomique (Simon), Chemistry (Campagna, Gooding), Genome Science and Technology (Ernest)

Excessive fat deposition in domestic broiler chickens is associated with reduced feed efficiency and coincides with diminished immune and reproductive function in broiler breeders, creating an economic concern for the broiler industry. We reasoned that understanding the adipose tissue response to food restriction, a method used commercially to attenuate fat deposition, would highlight pathways that might be manipulated to reduce fatness, thereby improving broiler health and reducing feed costs. Molecular and metabolic pathways that were altered by acute (5-hr) feed restriction in abdominal adipose tissue of broilers were identified by integrating transcriptomic (Affymetrix microarray) and metabolomic (liquid chromatography-mass spectrometry) data. We hypothesized that some of the same pathways differentially regulated by fasting also differed between genetically lean and fatty chickens in a manner consistent with fat storage. Parallel differences in these pathways in adipose tissue from naturally lean chickens (Fayoumi and leghorn) vs. broilers were tested using microarray profiling. Mixed linear model and multivariate clustering analysis confirmed our hypothesis that adipose tissue expression profiles of fasted broilers more closely resembled profiles from genetically distinct, lean lines of chickens than profiles from fed broilers. Gene ontology and KEGG pathway analyses revealed that the set of genes altered in common by fasting and leanness is enriched in pathways related to lipid and fatty acid biosynthesis and metabolism, signaling, and adipogenesis. Collectively, these results identify potential pathways through which fat accretion may be attenuated through genetic selection or management practices.

63. Angiotensinogen Gene Silencing in 3T3-L1 Adipocytes Reduces Lipid Accumulation and Adipose Inflammation

Wenting Xin, Nishan S. Kalupahana, Suzanne L. Booker, Nalin Siriwardhana, Arnold M. Saxton, Naima Moustaid-Moussa

Animal Science, Obesity Research Center

Angiotensinogen (Agt), the only known precursor for the hypertensive hormone Angiotensin II (Ang II), is expressed in adipose tissue. Studies from our laboratory and others have linked adipocyte-derived Agt to obesity, inflammation, and insulin resistance. To directly determine the contribution of adipose Agt to these disorders, we generated 3T3-L1 cells stably transfected with Agt-shRNA

or a scrambled sequence (Sc-shRNA) to study the changes in metabolic and inflammatory markers as well as global transcriptional alterations that were induced by Agt silencing. 3T3-L1 adipocytes lacking Agt exhibited significantly lower levels of Ang II and triglycerides. Microarray study further revealed several candidate genes involved in lipid metabolism and inflammatory pathways. These included Gpd1, Ces3, Retsat, and novel pattern recognition receptor Nod1, acute phase protein Saa3, transcription factor Stat1, and chemokine Cxcl12, all of which were down-regulated by Agt silencing. Knockdown of the Agt gene also reduced the production of pro-inflammatory adipokines including IL6, TNF-α, and MCP-1, as well as the gene expression levels of major lipo/adipogenic markers. In conclusion, our findings suggest that adipose tissue-derived Agt plays a critical role in adiposity and the associated inflammatory and metabolic complications.

64. Cytokine-Mediated Regulation of the Monocyte Chemoattractant Protein -1 (MCP-1) Gene by NF-kB

Susan J. Burke, Jason J. Collier

Nutrition

Autoimmune-mediated destruction of islet beta cells occurs when immune cells infiltrate the islet and secrete pro-inflammatory cytokines, such as interleukin-1beta (IL-1beta) and gamma-interferon (gamma-IFN). One protein responsible for immune cell recruitment is MCP-1; MCP-1 overexpression in mice leads to islet immune cell invasion, insulitis, and diabetes. The cytokines IL-1beta and gamma-IFN induce the expression of the MCP-1 gene in both rat and human islets and beta-cell lines. Here we investigated the transcriptional regulation of the MCP-1 gene in response to the pro-inflammatory cytokines IL-1beta and gamma-IFN in 832/13 rat insulinoma cells. In 832/13 cells, a 6-hr exposure to 1ng/mL IL-1beta and 100U/mL gamma-IFN increases MCP-1 mRNA levels 400-fold. siRNA-mediated suppression of the p65 subunit of NF-kB (77%) blocked the cytokine-mediated induction of MCP-1 mRNA levels by 89.5% during 6hr incubation with IL-1beta and gamma-IFN. Also, a promoter luciferase construct containing 3.6kb of the MCP-1 gene relative to the transcriptional start site was induced 50-fold by 6-hr exposure to IL-1beta + gamma-IFN. Furthermore, mutation of either the NF-kB sites within the proximal promoter region impaired the ability of cytokines to activate this luciferase construct by 96.7%. p65 overexpression induced the endogenous MCP-1 gene to a level similar to that seen with IL-1beta + gamma-IFN and also potentiated the cytokine-mediated induction an additional 373% relative to control. By contrast, overexpression of a mutant p65 (S276A) could not induce the MCP-1 gene. We conclude that p65 is required for cytokine-mediated induction of the MCP-1 gene.

65. Exploring the Role of Social Capital in Resettlement, Health, and Well-being among Burundian Refugees in Knoxville: Implications for Health Interventions and Policy

Fletcher Njororai

Public Health

This is an exploratory study on the role of social capital in refugee resettlement in relation to health and well-being among Burundian refugees in Knoxville. The study aims at informing health interventions and making policy recommendations. The Burundian refugees who arrived in Knoxville in 2007 faced systems unprepared for their arrival and transition. With limited resources and a very small staff, the local refugee resettlement agency struggles to resettle the incoming refugees with unique needs–having little or no exposure to schooling and education, and illiterate in Kirundi, their national language. The hypothesis underlying this study is that availability and use of various social ties and networks makes a difference to individual well-being, and access to and utilization of resources. In this study, a mixed-method research design was used for data collection. A convenient sample of 50 adults participated in the study. A structured interview was administered with question items for quantitative data and open-ended questions for qualitative data. The items elicited information on size and content of refugee social networks; strengths of network ties; functions of the social networks; and how social connections have impacted accessing resources, resettlement, and well-being. Preliminary data analysis revealed a correlation between age and overall connectedness. As age increased, overall connectedness decreased; and for individuals, as age increased, connectedness with neighbors and other immigrants decreased while connectedness with church increased. More data analyses are being run as well as thematic analysis of qualitative data for triangulation of the results for making recommendations.

66. Continuous Monitoring of Hormonally-Active Compounds in Effluents from Hallsdale-Powell Wastewater Treatment Facility at Knoxville, TN

Jun Wang, Melanie Eldridge, Fu-min Menn, Gary Sayler

Microbiology, Center for Environmental Biotechnology

Endocrine disruptive chemicals (EDCs) have drawn great public concern due to their harmful health impact on both human beings and animals. The wastewater treatment process is the major barrier in preventing EDCs' release into an aquatic environment. However, there is no established standard method for evaluating the performance of wastewater treatment in EDC removal. Traditional wastewater treatment uses concentrated microorganisms (activated sludge) for organic compound removal and secondary clarification for water-solid separation, while an emerging technology called membrane bioreactor (MBR) uses a filter for water-solid separation, allowing higher density of biomass and greater loading capacity. Hallsdale-Powell Utility District (HPUD) at Knoxville, TN, recently adopted the state-of-the-art MBR, running parallel with its traditional activated sludge process. This setup provides great opportunity for comparing the performance of MBR and conventional activated sludge in EDC removal. In this study, we performed a 6-month continuous monitoring on the effluent from HPUD using a high throughput yeast bioluminescent assay. Effluent from both traditional activated sludge and MBR in HPUD were sampled each week and extracted using the established solid phase extraction method. Extraction efficiency was evaluated using GC-MS. Standardized bioluminescent yeast-based bioassay was used to evaluate the estrogenic/androgenic equivalency of each sample. Our results showed that androgenic compounds are attenuated in HPUD to below-detection-limit level. Estrogenic compounds can be detected in effluents from both MBR and traditional activated sludge. The membrane bioreactor produced effluent with, on average, ~10 fold less estrogenic equivalency than that of the traditional activated sludge.

67. How a PAH-Degrading Microbial Community is Affected by Temperature Change

Abby Smartt, Alice Layton, Elizabeth Fozo, Steve Ripp, Archana Chauhan, Gary Sayler

Microbiology (Smartt, Layton, Fozo, Ripp, Sayler), Center for Environmental Biotechnology (Smartt, Layton, Ripp, Chauhan, Sayler), Joint Institute for Biological Sciences, Oak Ridge National Laboratory (Sayler)

Polycyclic aromatic hydrocarbons (PAHs) are soil contaminants found in a variety of areas. PAHs are implicated in causing human health problems, such as lung, liver, and kidney damage, and increasing risks for cancers. In efforts to reduce PAHs in soil, bioremediation has been employed, using microbes indigenous to contaminated soil or engineered, to breakdown PAHs into less harmful metabolic intermediates. To develop a better understanding of bioremediation in situ, environmental factors that influence the microbial population and their ability to metabolize PAHs need to be taken into consideration. New genomic information allows us to identify genes that are affected by temperature change that can be investigated in conjunction with PAH metabolism within a microbial community. An example is represented by a long-term lysimeter field release study started in 1996 containing recombinant *Pseudomonas fluorescens* HK44. High throughput sequencing was used to generate phylogenetic and metagenomic sequences to compare soils before and after long-term storage at 4°C, and analyzed using RAST servers. HK44 was grown at 4°C and 28°C; RNA was harvested, and the expression of cold shock genes was analyzed using Northern blot assays. Phylogenetic analyses of lysimeter soils stored at 4°C indicated a shift in microbial population with an increased *Pseudomonas* population after one year. Genes for cold shock proteins were detected in both the metagenome and HK44 genome and analyzed to determine expression over a range of temperatures. Northern blot analysis shows that while there is expression of cspA at both 28°C and 4°C, expression is greater at 4°C.

68. *Amblyomma americanum* L. at Ames Plantation: Distribution, Seasonality, Habitat Associations, and Pathogens

Brian Hendricks, Graham Hickling, Kevin Moulton, Rebecca Trout-Fryxell

Entomology and Plant Pathology (Hendricks, Moulton, Trout-Fryxell), Forestry, Wildlife, and Fisheries (Hickling)

The current status of tick-borne disease in southeastern United States is uncertain due to emerging and resurging pathogens. We are assessing tick-borne disease prevalence in west Tennessee by identifying tick species present at Ames Plantation and Hardeman and Fayette counties, screening the ticks collected for pathogens, and determining habitat associations of the most common tick species, *Amblyomma americanum*. Ticks will be collected biweekly from April 2012 until October 2012, using vegetation drags and CO_2 traps. All ticks collected will be identified to species level. *A. americanum* will be screened for *Ehrlichia* spp., *Rickettsia* spp., and *Borrelia burgdorferi* senso lato. *A. americanum* habitat associations will be displayed in ArcGis 9.3.1 and identified from land cover, soil , and climate data. Preliminary sampling indicates that *A. americanum* is the most abundant tick species in Tennessee. *A. americanum* presence was positively correlated with deciduous forests incorporating good drainage, sandy or medium texture soil, moderate ground temperature, and high relative humidity. The habitat profile produced for *A. americanum* in this study will help facilitate future control of tick-borne diseases in the southeastern United States. The pathogen status of sampled ticks has not yet been assessed, but it is known from the literature that *A. americanum* vectors *Ehrlichia* spp., but is unlikely to spread the etiological agent of Lyme disease.

69. Transmission of *Toxoplasma gondii* in Wildlife in the Southeastern United States

Richard Gerhold, Graham Hickling, Sharon Patton, Aly Chapman, Chunlei Su

Center for Wildlife Health, Forestry, Wildlife, and Fisheries (Gerhold, Hickling), Biomedical and Diagnostic Sciences (Patton, Chapman), Microbiology (Su)

Toxoplasmosis, caused by *Toxoplasma gondii*, is one of the most common infections of humans and animals worldwide. Infections occur by ingestion of oocysts in contaminated food or water or by ingestion of tissue cysts in undercooked meat. Genotyping has disclosed that the majority of *T. gondii* isolates in North America belong to one of three clonal lineages (types II, III, and 12) that vary in virulence. *Toxoplasma gondii* infection occurs in many wild birds and mammals; however, genotype data for isolates from these hosts are lacking. Previous studies of isolates from U.S. wildlife suggest that wild species maintain a greater diversity of *T. gondii* genotypes than is found in agricultural animals, suggesting a wild/feral animal diversity model. To further understand the diversity of *T. gondii* in southeastern U.S. wildlife, we screened sera from multiple wild bird and mammal species for *T. gondii* exposure via the modified agglutination test. Heart or tongue from select seropositive animals was digested and inoculated into mice to propagate *T. gondii* tachyzoites. Tachyzoites were genotyped by the multiplex multilocus nested PCR-RFLP method employing 10 genetic markers. Of the 161 sampled wild animals, 60 (37.3%) were seropositive. Genotyping results of nine white-tailed deer and one mink disclosed five distinct strains, including the type 12 and type III lineages common in U.S. wildlife, two other previously identified genotypes, and one novel genotype. We conclude that *T. gondii* is prevalent in wildlife from the southeastern United States, and further research is needed to understand the *T. gondii* diversity and transmission dynamics.

70. Comparison of RT-PCR and RT-LAMP Assays for Human Norovirus GII Detection

Cong Cao, Doris H. D'Souza

Food Science and Technology

Loop-mediated isothermal amplification is a novel, simple method increasingly researched for foodborne-pathogen testing. Previous studies have shown the ability of the RT-LAMP assay to detect human noroviruses (hNoVs) in clinical samples. The RT-LAMP method does not require sophisticated equipment needed by PCR assays and can be carried out in a simple waterbath. To date, the detection sensitivity obtained by RT-PCR and RT-LAMP assays has not been compared. This study compared the detection sensitivity of previously described real-time RT-PCR to RT-LAMP assays using clinical outbreak samples. Human norovirus GII clinical isolates (total of nine) were assayed using serial dilutions of RNA extracts obtained by heat-release or the TRIzol method and real-time SYBR Green I based RT-PCR kits and RT-LAMP assays in triplicate. The detection limit study indicated that viral RNA extracted by the TRIzol method was consistently detected at one log RT-PCR unit (-1 dilution) using SYBR Green I based-RT-PCR. The detection limit was improved by one-log using the RT-LAMP assay compared to RT-PCR assay. Viral RNA recovered from heat-released RNA was detected at one-log higher than RNA extracts by the RT-

PCR assay (two log RT-PCR units), but a lower detection limit was obtained by the RT-LAMP assay. Our results show that the RT-LAMP assay can be completed within 1.5 hr followed by agarose gel electrophoresis, which is ~ 2 hr faster compared to the RT-PCR assay. The RT-LAMP assay is more sensitive than RT-PCR assay when using Trizol RNA extracts. Furthermore, the RT-LAMP assay bypasses the need for expensive PCR machines.

71. Community Composition Matters: Amplification Hosts Facilitate Ranavirus Outbreaks in Larval Amphibian Communities

Roberto Brenes, Matthew J. Gray, Debra L. Miller

Center for Wildlife Health, Forestry, Wildlife and Fisheries

Declines in amphibian populations from disease outbreaks could be mediated by host susceptibility and have a direct effect on trophic-level interactions in aquatic ecosystems. Our objective was to determine if the outcome of a ranaviral disease outbreak in an amphibian community was dependent on which species was initially exposed to the virus, and if trophic shifts in an experimental aquatic ecosystem occurred as mortality progressed. Our communities were composed of larval *Ambystoma maculatum*, *Pseudacris feriarum,* and *Lithobates sylvaticus*, which are known to have low, moderate, and high susceptibility to ranavirus. The experiment was conducted outdoors in 320-L mesocosms, and treatments consisted of one, all, or none of the species initially exposed to frog virus 3. Initial exposure occurred under controlled laboratory conditions in 1-L water baths (103 PFU/mL) for 3 days prior to distribution of the larvae to the mesocosms. Mortality rates after 60 days depended on which species was initially exposed to the pathogen. Community-wide mortality rates from ranaviral disease were greatest ($\bar{x}$ = 50%) when *L. sylvaticus* tadpoles were initially exposed and lowest ($\bar{x}$ = 5%) when *A. maculatum* larvae were exposed. Initial exposure of *P. feriarum* larvae to ranavirus resulted in 40% and 15% mortality to *L. sylvaticus* and *A. maculatum*, respectively. Changes in phytoplankton and periphyton biomass and zooplankton abundance were associated with ranavirus-induced mortality. Our results demonstrate that amphibian community composition can affect ranaviral disease outcomes, and ranavirus outbreaks can induce trophic cascades. Due to the high susceptibility of *L. sylvaticus*, this species may function as a superspreader of ranavirus and be instrumental in causing community-wide outbreaks.

72. Chemical Disinfection of Human Norovirus Surrogates for the Prevention of Nosocomial Human Norovirus Outbreaks

Cong Cao, Doris H. D'Souza

Food Science and Technology

Benzalkonium chloride (BAC), potassium peroxymonosulfate (KPMS), and n-alkyl dimethyl benzyl ammonium chloride (ADBAC) are currently used to decontaminate surfaces. They are known to be effective against a wide range of pathogenic bacteria and also viruses. However, their effects on human noroviruses have not been studied. The goal of this study was to determine the effect of these three chemicals over 1 hr at room temperature against human norovirus surrogates, feline calicivirus (FCV-F9), and murine norovirus (MNV-1) in vitro. Our results revealed that treatment of FCV-F9 (at low titers of 4 log PFU/ml) with 0.32 mg/ml BAC for 5, 10, and 15 min resulted in a <1 log PFU/ml reduction, with ~1 log PFU/ml reduction after 30 min, and ~2 log PFU/ml after 1 hr. FCV-F9 showed no significant titer reduction by 1:128 ADBAC after 5, 10, 15, or 30 min, with only ~1 log PFU/ml reduction after 1 hr. No obvious MNV-1 (at 4 log PFU/ml) reduction was obtained after treatment with 0.32 mg/ml BAC and 1:128 ADBAC for 5 or 10 min, but was inactivated by at least 3 log10 PFU/ml (based on countable plaques) after 1 hr by 0.32 mg/ml BAC, with ~1 log PFU/ml reduction using 1:128 ADBAC after 30 min and 1 hr. Low titers of FCV-F9 and MNV-1 were reduced to undetectable levels by KPMS for all tested times at 0.5%.
Our results indicate that KPMS appears to be the most suitable of the three tested chemicals for HNoV surrogate inactivation in the environment.

73. Size-Structured Model for Tissue Cyst Growth of *Toxoplasma gondii*

Adam Sullivan, Xiaopeng Zhao, Yasuhiro Suzuki, Michael Gilchrist

Mechanical, Aerospace and Biomedical Engineering (Sullivan, Zhao), Ecology and Evolutionary Biology (Gilchrist), Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky (Suzuki)

Intracellular parasite replication within a target cell is typically modeled using cell population dynamics to show replication and suppression of replication during acute infection of a parasitic invasion. The dynamics of chronic infection are investigated to study the steady-state dynamics of chronic *Toxomplasma gondii* infection.

The distribution of various cyst sizes within brain tissue is modeled as a function of both time and size. By modeling both the growth rate and the bursting rate of cysts (which introduces many new parasitic infections in newly exposed target cells), the system can be analyzed to determine characteristics of chronic infection. A steady-state analysis is performed to analyze the long-term chronic infection of the parasite. Various functional forms of the growth rate and bursting rate are considered to match available data of cyst-distributions. Parameter fitting is performed on all test cases for growth and bursting rates, with the best cases for fitting the data chosen by examining the AIC.

74. MicroRNA-155 Regulates Brain Inflammation but Promotes Ocular Immunopathology after Herpes Simplex Virus Infection

Sachin Mulik, Sid Bhela, Leon Richardson, Fernanda Gimenez, Pradeep Reddy, Naveen K Rajasagi, Tamara Veiga-Parga, John Xu, Patrick Y. Lu, Barry T. Rouse

Biomedical and Diagnostic Sciences (Mulik, Bhela, Richardson, Gimenez, Reddy, Rajasagi, Veiga-Parga, Rouse), Sirnaomics, Gaithersburg, MD (Xu, Lu), Comparative and Experimental Medicine (Mulik)

Herpes simplex virus (HSV) infection of humans can lead to life threatening herpes simplex encephalitis (HSE) and sometimes blinding ocular lesion stromal keratitis (SK). Here, we show that mice with a deficiency of miR-155 are highly susceptible to HSE, and a majority of mice (70%) die after ocular infection with HSV. Acyclovir treatment (a day after the virus reaches the brain) protected miR-155KO mice from HSE. Of note, miR-155KO survivors developed attenuated SK lesions and revealed significant reduction in pathogenic CD4 T cells in corneas and lymphoid organs. Local inhibition of miR-155 did not increase incidence of HSE but led to diminished SK lesions. Building on our previous observations, blockade of miR-132 and miR-155 resulted in severe reduction in SK lesions and diminished ocular angiogenesis. In conclusion, we have discovered a dual role for miR-155, a regulator of brain inflammation while a promoter of ocular immunopathology after HSV infection.

75. Regulatory T Cells Control the Clinical Expression of Viral Immunopathology during the Clinical Phase of Herpetic Stromal Keratitis

Tamara Veiga-Parga, Barry T. Rouse

Biomedical and Diagnostic Sciences (Veiga-Parga, Rouse), Comparative and Experimental Medicine (Veiga-Parga)

Ocular herpes simplex virus infection can cause a blinding CD4+ T cell orchestrated immunoinflammatory lesion in the cornea called stromal keratitis (SK). Lesion severity can be influenced by the balance of CD4+ T effectors and Foxp3+ regulatory T cells (Treg) at the onset of infection. We have evaluated the role of Treg during the ongoing clinical phase of the disease by taking advantage of transgenic mice, which express a diphtheria toxin receptor-enhanced GFP fusion protein under the control of the Foxp3 locus, permitting Treg depletion. Depleting Treg during the clinical phase of the disease resulted in significantly more severe lesions. This outcome was explained both by Treg influencing the activity of inflammatory T cells at the lesion site as well as effects in the lymphoid tissue that reduced trafficking of T cells and neutrophils to the eye, as well as the activation state of T effectors. Our results demonstrate that Treg can beneficially influence the impact of ongoing tissue damaging responses to a virus infection and imply that therapies that boost Treg function hold promise as a modality to control a lesion that is an important cause of human blindness.

76. TNFR25SF and Galectin-9 Combination Therapy Reduces Herpes Simplex Virus-1-Induced Stromal Keratitis by Expanding Regulatory T Cells and Reducing Pathogenic T Effectors

Pradeep BJ Reddy, Amol Suryawanshi, Taylor H. Schreiber, Naveen K Rajasagi, Sachin Mulik, Mitsuomi Hirashima, Eckhard R. Podack, Barry T. Rouse

Biomedical & Diagnostic Sciences (Reddy, Rajasagi, Mulik, Rouse); Comparative and Experimental Medicine (Mulik), Emory Vaccine Center, Emory University (Suryawanshi); Microbiology and Immunology, University of Miami Miller School of Medicine (Schreiber, Podack); Immunology and Immunopathology, Faculty of Medicine, Kagawa, Japan (Hirashima)

Ocular infection with HSV-1 results in a chronic immunoinflammatory reaction in the cornea, which is primarily orchestrated by CD4 T cells. Hence, targeting proinflammatory CD4 T cells or increasing the representation of cells that regulate their function is a relevant therapeutic strategy. In the present report, we use an agonistic Mab (4C12) to TNF receptor 25 (TNFR25) that selectively expands numbers of regulatory

T cells (Tregs). Treatment early after ocular HSV infection with 4C12 caused a three-fold increase in the Treg numbers in the cornea along with a consequent reduction in SK lesion severity. However, 4C12 treatment was less effective if given 6 days after infection since it expanded proinflammatory T effectors that also express TNFR25. To suppress the latter population, galectin-9 (Gal-9) was used which causes apoptosis of Th1 cells. When therapy with both 4C12 and gal-9 was used in combination, the recipients showed significantly reduced angiogenesis and SK lesions over single treatment controls. The beneficial outcome of combination therapy was attributed to the expansion of the Treg population that expressed CD103 needed for trafficking to inflammatory sites along with a marked reduction in the infiltration of pathogenic CD4 T cells. Levels of several proinflammatory cytokines and chemokines were also reduced. Our results demonstrate that combination therapy may be a promising approach to control HSV-induced SK lesions, a common cause of human blindness.

77. Controlling Herpes Simplex Virus-Induced Ocular Inflammatory Lesions with the Lipid-Derived Mediator Resolvin E1

Naveen K. Rajasagi, Pradeep B. J. Reddy, Sachin Mulik, Barry T. Rouse

Biomedical & Diagnostic Sciences (Rajasagi, Reddy, Mulik, Rouse), Comparative and Experimental Medicine (Mulik)

Stromal keratitis (SK) is a chronic immunopathological lesion of the eye caused by herpes simplex virus-1 (HSV-1) infection and is a common cause of blindness in humans. The inflammatory lesions are primarily perpetuated by neutrophils with the active participation of CD4+ T cells. Therefore, targeting these immune cell types represents a potentially valuable form of therapy to reduce the severity of disease. Resolvin E1 (RvE1), an endogenous lipid mediator, was shown to promote resolution in several inflammatory disease models. In the present report, we determined if RvE1 administration started at different times after ocular infection of mice with HSV could influence the severity of SK lesions. Treatment with RvE1 significantly reduced the extent of angiogenesis and SK lesions. RvE1-treated mice had fewer numbers of inflammatory cells that included Th1 and Th17 cells as well as neutrophils in the cornea. The mechanisms by which RvE1 acts appear to be multiple. These include reducing the influx of neutrophils and pathogenic CD4+ T cells, increasing production of the anti-inflammatory cytokine IL-10, and inhibitory effects on the production of pro-inflammatory mediators and molecules such as IL-6, IFN-γ, IL-17, KC, VEGF-A, MMP-2, and MMP-9, that are involved in corneal neovascularization and SK pathogenesis. These findings are the first to show that RvE1 treatment could represent a novel approach to control lesion severity in a virally-induced immunopathological disease.

78. Use of the M3 Chemokine Binding Protein to Understand Cytomegalovirus Dissemination

Wilson Robinson, Tom Masi, Pranay Dogra, Courtney Copeland, Mandy Miller-Kittrel, Timothy Sparer

Microbiology

Human cytomegalovirus (CMV) is a main cause of infectious congenital defects as well as morbidity and mortality in immunocompromised individuals. Part of the reason CMV is able to successfully persist in its host is due to its ability to manipulate the body's immune response. Although the virus often uses various methods to avoid immune detection, evidence suggests that it is also able to disseminate from the initial site of infection using chemokine-based recruitment of leukocytes. In this study we investigated the role of chemokine-induced leukocyte recruitment in vivo using recombinant murine CMV overexpressing a broad-based viral chemokine binding protein. Using the murine CMV bacterial artificial chromosome (pARK25), we generated a series of recombinants that express the M3 protein, a chemokine binding protein from the gamma herpesvirus MHV-68. We will use this protein to "inactivate" secreted chemokines that will potentially influence leukocyte migration to the site of infection. If leukocytes are largely responsible for disseminating CMV to other organs, we expect to find a decrease in leukocyte recruitment, which could affect the levels of viremia and subsequent dissemination to multiple organs. Understanding the immunological components necessary for CMV dissemination will allow the development of novel anti-CMV therapies.

79. Effectiveness of Small Interfering RNA (siRNA) to Inhibit Feline Coronavirus Replication

Eman Anis, Rebecca Penrose-Wilkes, Stephen Kania, Alfred M. Legendre, Melissa Kennedy

Biomedical and Diagnostic Sciences (Anis, Wilkes, Kania, Kennedy), Small Animal Clinical Sciences (Legendre); Dept. of Virology, Faculty of Veterinary Medicine, University of Minufiya, Sadat City, Egypt (Anis)

Feline infectious peritonitis (FIP) continues to be a significant cause of mortality in cats. Feline coronavirus

(FCoV), the agent of FIP, primarily targets intestinal epithelial cells but in certain cats, virus mutation may occur that allows the virus to replicate efficiently in monocytes and macrophages. In cats with an inadequate cell-mediated immune response, systemic virus proliferation continues, and the humoral response that is mounted against the virus precipitates immune-mediated pathology. Once afflicted, there is no treatment to prevent progression to death. In this study, we evaluated the ability of siRNA to inhibit the in vitro viral replication and gene expression of FCoV. Three synthetic siRNA targeting three different regions of the FCoV genome were tested for their antiviral effects against two different strains of FCoV: FIPV WSU 79-1146 and FECV WSU 79-1683. Efficacy was determined by real-time RT-PCR of intracellular viral genomic RNA and flow cytometry for viral protein expression. siRNAL, which targets the leader gene, resulted in more than 90% and 80% reduction in FIPV WSU 79-1146 and FECV WSU 79-1683 protein expression, respectively. siRNAM, which targets the membrane gene, resulted in more than 40% reduction in protein expression of the two strains. These preliminary findings show that FCoV translation and replication can be specifically inhibited using siRNA targeting coding and noncoding region of viral genome, suggesting a potential therapeutic application of RNAi in treating FIP.

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