Comparative & Experimental Medicine and Public Health Research Symposium

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June 20 & 21, 2011

Program & Schedule

Sponsored by the College of Veterinary Medicine, Center for Public Health, Tennessee AgResearch, 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 third consecutive year, the Center for Public Health has teamed with CEM to produce a joint *Comparative & Experimental Medicine and Public Health Research Symposium* hosting an even larger group of scientists including 88 presenters representing 22 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 Public Health, Tennessee AgResearch, 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.

Lastly, it is important to mention that the speakers to be presenting their research are an incredibly diverse group, and this international diversity is celebrated on the program's cover with the inclusion of the word *discover* in eight different languages, which are spoken by one or more of the presenters.

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

Buddy Mitchell, Interim Chancellor
University of Tennessee
Institute of Agriculture


Greg Hirshoren

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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 Public Health

Tennessee AgResearch

UTK Office of Research

Misty Bailey	William Kelch
Tammy Berry	Michael McEntee
Debra L Butenko	Andrew Patten
Michael Cunningham	Kim Rutherford
Paul Campbell Erwin	Kendall Stokes
Margot Emery	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, 2011 Center of Excellence Summer Student Research Program participants, and our sponsors and exhibitors.

Jimmy Cheek, *Chancellor*
UT Knoxville

Buddy Mitchell, *Interim Chancellor*
UTIA

James Thompson, *Dean*
College of Veterinary Medicine

Wes Hines, *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

Schedule at a Glance

Monday, June 20

	Room	Event
8:00-9:00	Brick foyer, VMB	Welcome reception with refreshments
9:00-10:15	A118 VMB	Keynote address: Dr. Franziska Grieder, "One Health, One Medicine: Veterinary Research Scientists for the 21st Century"
10:30-12:15	See session matrix (p. 6)	New investigator presentations
12:30-1:30	A118 VMB	Featured speaker: Dr. Jeffrey M. Becker, "Post-doctoral Opportunities"
1:45-4:45	See session matrix (p. 7)	New investigator presentations

Tuesday, June 21

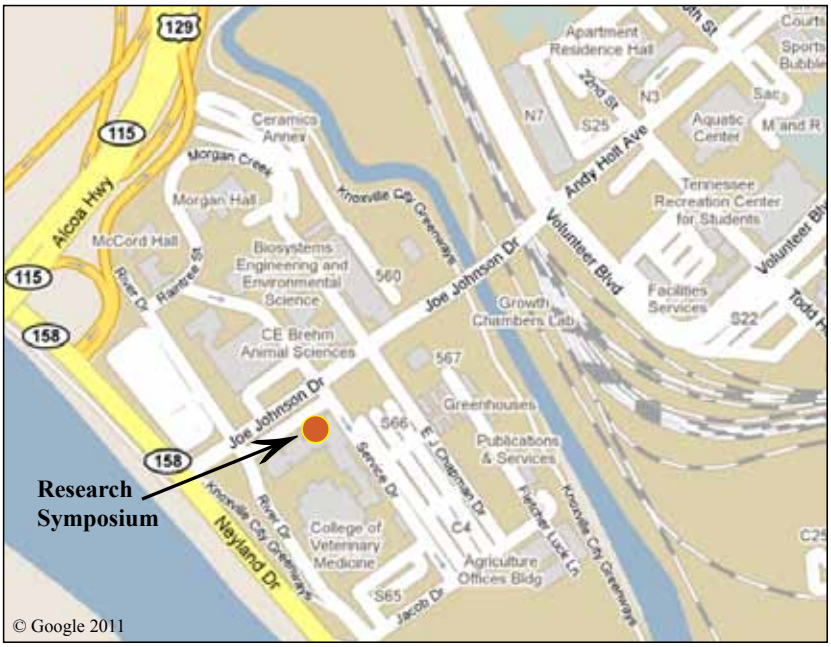
	Room	Event
8:00-9:00	Brick foyer, VMB	Refreshments
9:00-10:00	A118 VMB	Plenary address: Dr. Gary Foster, "Prevention and Treatment of Obesity: From the Schools to the Clinic"
10:30-12:15	See session matrix (p. 8)	New investigator presentations
12:30-1:30	A118 VMB	Featured speaker: Dr. Louis Gross, "Interdisciplinary Modeling Approaches to Public Health: Examples from NIMBioS"
2:00-3:45	See session matrix (p. 9)	New investigator presentations
6:00	UT Visitor's Center (Neyland Drive)	Awards banquet & after-dinner address: Dr. William Bass, "Beyond a Dog-eat-dog World" ***(Ticket required)***

VMB, Veterinary Medicine Building (*see map on p. 5*)

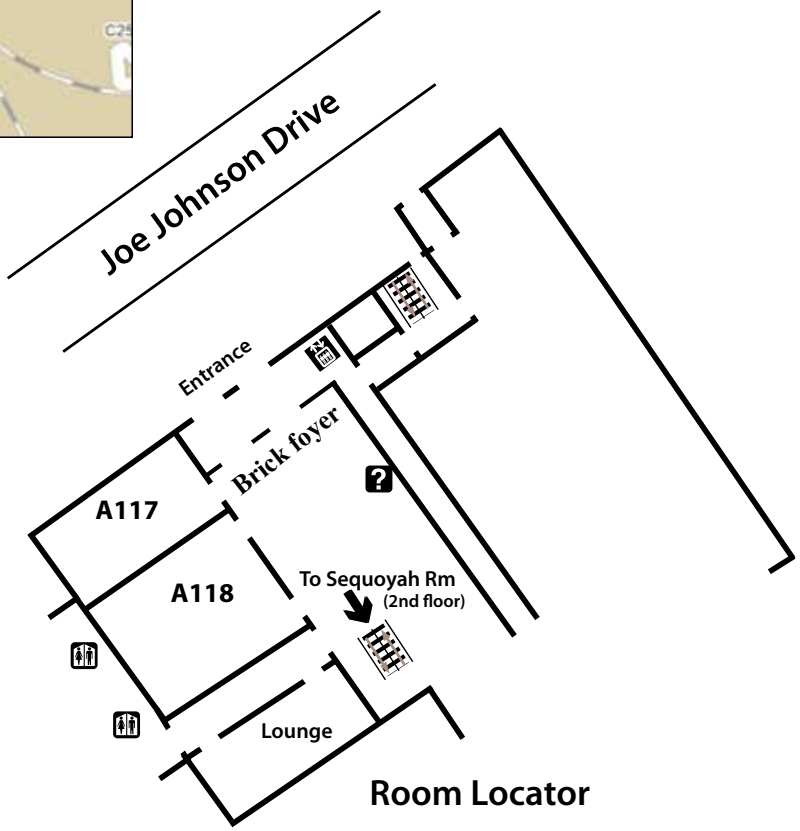
THE UNIVERSITY of TENNESSEE
INSTITUTE of AGRICULTURE
COLLEGE of VETERINARY MEDICINE

UNIVERSITY of TENNESSEE
Center for Public Health
THE FUTURE OF PUBLIC HEALTH

Location Information



University of Tennessee Agricultural Campus



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. ***During the banquet, parking at the Visitor's Center is free, and no permit is needed.***

__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)

	Bacterial & Fungal Virulence and Transmission	Public Health	Clinical Sciences
	Rm. VMB Sequoyah	**Rm. VMB A117**	**Rm. VMB A118**
10:30	1. Mathematical Modeling of Within-host Transmission Dynamics of *Toxoplasma gondii* **(Sullivan)**	18. Curricular Competencies Related to Cultural Competence for the Education and Training of Registered Dietitians (**Medico**)	35. Early Prediction of Response to Chemotherapy in Non-small Cell Lung Cancer Patients Using 18F-FDG PET/CT (**Laine**)
10:45	2. Multilocus Sequence Typing (MLST) for Characterization of Methicillin-resistant and Methicillin-susceptible Clones of *Staphylococcus pseudintermedius* (**Solyman**)	19. The Psychosocial Effects of Chronic Beryllium Disease (**Miller**)	36. Transient Ischemic Dilation in Adenosine PET-CT Myocardial Perfusion Scintigraphy (**Mojtahedi**)
11:00	3. Characterization of Small RNAs and Small Proteins in EHEC (**Wen**)	20. The Relationship Between Greenway Siting, Active Transport Access, and Types of Users (**Wolff**)	37. The Functionality of Microchips in Post-Magnetic Resonance Imaging (**Haifley**)
11:15	4. What Did the Tick Eat for Lunch Before it Had You for Dinner? (**Scott**)	21. Use of Bioluminescent Yeast Bioreporters to Detect Endocrine Disruptors in Brazilian Waterways (**Eldridge**)	38. COX-2-expressing Canine Transitional Cell Carcinoma Detected by Novel Imaging Tracer Fluorocoxib (**Cekanova**)
11:30	5. Involvement of *Streptococcus uberis* Adhesion Molecule (SUAM) in Adherence to and Internalization of *Streptococcus uberis* into Bovine Mammary Epithelial Cells (**Almeida**)	22. Effect of Two Selective Serotonin Reuptake Inhibitors (SSRIs) on Global Gene Expression Changes in Larval Zebrafish (*Danio rerio*) (**Park**)	39. Magnetic Resonance Imaging of Canine Mast Cell Tumors (**Pokorny**)
11:45	6. Virulence Gene Expression Patterns in *E. coli* Isolates from Bovine Mastitis after Co-culture with Primary Bovine Mammary Epithelial Cells (**Kerro-Dego**)	23. Assessment of Exposure to Environmental Estrogens in Male Largemouth Bass (*Micropterus salmoides*) (**Wilson**)	40. Measurement of Immunologic Response in Horses and Dogs Vaccinated with Xenogenic Plasmid DNA Encoding Human Tyrosinase (**Lembcke**)
12:00	7. Association between Presence of Urovirulence Factors and Antimicrobial Resistance Patterns in 221 Uropathogenic *Escherichia coli* Samples Isolated from Dogs (**Wells**)	24. Application of Bioluminescent Yeast-based High Throughput Bioassay for Monitoring Endocrine Active Compounds in Wastewater (**Jun**)	41. Inflammatory Responses to Intravenous Lipopolysaccharide Infusion in Horses with Equine Metabolic Syndrome (**Tadros**)
12:15	**BREAK for Lunch – See schedule on p. 4**		

*VMB, Veterinary Medicine Building

Monday, June 20

	Bacterial & Fungal Virulence and Transmission	Oncology & Cancer Cell Biology	Clinical Sciences
	Rm. VMB Sequoyah	**Rm. VMB A117**	**Rm. VMB A118**
1:45	8. Abundance and Prevalance of Bacterial and Viral Pathogens in Bangladesh Surface and Ground Water During Wet and Dry Seasons (**Smartt**)	25. Effect of Fibrin Inhibitory Peptides on Tumor Growth, Metastasis, and Angiogenesis (**Brown**)	42. Characterization of Bone Marrow-derived Adult Equine Mesenchymal Stem Cells (EMSCs) and their Potential for Therapeutic Application in an In Vitro Model of Corneal Healing (**Breickner**)
2:00	9. Propidium Monoazide for the Rapid Molecular Detection of Viable *Salmonella enterica* (**Techathuvanan**)	26. Precancerous Carcinogenesis of Human Breast Epithelial Cells by Chronic Exposure to 3, 4, 4'-Trichlorocarbanilide (**Sood**)	43. Effects of Intravenously-administered Esomeprazole Sodium on Gastric Juice pH in Adult Female Horses (**Videla**)
2:15	10. The Role of Phosphatidylethanolamine in *C. albicans* Virulence and GPI Anchor Synthesis (**Davis**)	27. Social Stress Stimulates and GABA Inhibits the Progression of Pancreatic Cancer Xenografts in Nude Mice (**Ullah**)	44. Effect of Electroejaculation on Plasma Concentrations of Cortisol and Substance P in Beef Bulls (**B. Whitlock**)
2:30	11. Fatty Acid Synthase 1: The Role of Fatty Acid Biosynthesis in Membrane Stress and Virulence in *C. albicans* (**Rodrigues**)	28. PPARγ Ligand-induced Apoptosis in Pancreatic Cancer Cells (**Min**)	45. Treatment Outcome of Dogs with Methicillin-resistant and Methicillin-susceptible *Staphylococcus pseudintermedius* Pyoderma (**Bryan**)
2:45	12. Phosphatidylserine Synthase and Phosphatidylserine Decarboxylase are Required for Cell Wall Integrity and Virulence in *Candida albicans* (**Montedonico**)	29. Proliferation of Cell Lines from Pancreatic Ductal Adenocarcinomas and their Normal Ductal Epithelia: Stimulation by Acute and Chronic Exposure to Nicotine and Ethanol via Modulation in Neurotransmitter Production (**Al-Wadei**)	46. Agreement Between The T2 and HASTE (Half-fourier-acquisition Single-shot Turbo Spin-echo) Sequences in the Evaluation of Intervertebral Disc Disease in Dogs (**Mankin**)
3:00	13. Vacuolar Protein Sorting Components Regulate Biofilm Formation in Yeast by Flo11p-dependent and Flo11p-independent Mechanisms (**Sarode**)	30. Green Tea Catechins at Non-cytotoxic Levels Suppress Cellular Carcinogenesis Induced by Environmental Carcinogens (**Rathore**)	47. The Effect of Nitrous Oxide on Isoflurane Minimum Alveolar Concentration (MAC) and MAC Derivatives in Dogs (**Voulgaris**)
3:15			48. Effect of Local Anesthetic on Microorganisms in a Murine Model of Surgical Site Infection (**Sams**)
3:30	**Exercise Science & Kinesiology**	**BREAK**	
3:45	14. Does Physical Activity Explain Ethnic Differences in Cardiorespiratory Fitness Among U.S. Adults? (**Ceaser**)	31. Sulindac Sulfide Facilitates Novel Cleavage of Epithelial Cell Adhesion Molecule (EpCAM) in Colorectal Cancer Cells (**Liggett**)	
4:00	15. A Compendium of Energy Costs of Physical Activities for Individuals Who use Manual Wheelchairs (**Conger**)	32. Post-translational Modification of Egr-1 by Resveratrol (**N. Whitlock**)	
4:15	16. Reduced Peak Cycle Exercise Heart Rate in Obese, African American Children (**Flynn**)	33. Effects of Branched-chain Amino Acids on Mitochondrial Metabolism and Cell Cycle in Cancer Cells (**Filhiol & Johnstone**)	
4:30	17. Why is There So Much Confusion about the VO2 Plateau? A Re-examination of the Work of Hill et al. (1923) (**Castle**)	34. Effect of Adiposity and Inflammatory Mediators on Breast Cancer (**Siriwardhana**)	

	Nutrition & Metabolism	Viral Pathology & Immunity	Innovative Technologies
	Rm. VMB A118	**Rm. VMB A117**	**Rm. VMB Sequoyah**
10:30	49. Modulation of Tumor Formation by Chemopreventive Compounds in Animal Models of Colorectal Cancer (**Richardson**)	63. Controlling Viral Immunoinflammatory Lesions by Modulating Aryl Hydrocarbon Receptor Signaling (**Veiga Parga**)	77. For Driving and Not Drinking: Brewer's Yeast as a Biofuel Production Tool (**Close**)
10:45	50. (-)-Epigallocatechin Gallate Facilitates the Proteasomal Degradation of Cyclin D1 in Colon Cancer Cells (**Zhang**)	64. Role of MicroRNA-132 in Angiogenesis After Ocular Infection With Herpes Simplex Virus (**Mulik**)	78. A Pilot Assessment of a Novel Technique to Evaluate Intrapulmonary Concentrations of Drugs in Pigs (**Villarino**)
11:00	51. Characterization of Nudix Type Hydrolase 6 (NUDT6) and Its Potential Role in DNA Repair (**Smolensky**)	65. IL-17A Disrupts Physiological Balance Between VEGF-A and sVEGFR-1 and Promotes Corneal Neovascularization Post-HSV Infection Through Multiple Mechanisms (**Suryawanshi**)	79. Sensitivity and Specificity of Tissue Impedance Determination for Correct Veress Needle Placement in Cadaverous Cats (**Hyink)**
11:15	52. *Heteropterys aphrodisiaca* Tea Therapy Causes Reduction in Prostate Weight in the Absence of Reducing Circulating Testosterone Levels and Testicular Testosterone Secretory Capacity In Vitro (**Sashi Papu John**)	66. The Influence of Galectin-9/ Tim-3 Interaction on HSV-1 Latency (**Jagadeesh Reddy**)	80. Polymer-mediated Therapeutic Delivery for Neural Interface Applications (**Yu**)
11:30	53. The Contribution of Regional Adiposity to Obesity-associated Female Infertility via Differential Protein Hormone Secretion (**Ernest**)	67. Augmentation of Immunity to Influenza A Virus by Manipulating a Host Counter Inflammatory Pathway (**Sharma**)	81. Parallel FEM Simulation of Electromechanics in the Heart (**Xia**)
11:45	54. Regulation of the Inducible Nitric Oxide Synthase Gene (iNOS) by Cytokines Requires Phosphorylation of the p65 Subunit of NF-kappaB at Ser 276 and Ser536 and Signal Transducer and Activator of Transcription 1 (STAT1) at Tyr701 (**Burke**)	68. The Application of Chitosan for the Reduction of Foodborne Viral Surrogates on Produce (**Ganapathy**)	82. Detection of Traumatic Brain Injury from Scalp EEG Using Event-related Tsallis Entropy Functionals (**McBride**)
12:00	55. Regulation of Monocyte Chemotactic Protein 1 Gene Expression by Activation of Nucleotide Oligomerization Domain Containing Protein 1 in Adipose Tissue Inflammation and Obesity (**Hu**)	69. Mathematical Modeling of the Spread of Mouse Cytomegalovirus from the Foot Pad to Salivary Gland (**Dogra**)	83. Kinetic Assessment of Myocardial 14(R,S)-[18F]Fluoro-6-Thia-Heptadecanoic Acid and 18F-Fluorodeoxyglucose Uptake with Positron Emission Tomography/ Computed Tomography (PET/CT) in Domestic Cats (**LeBlanc**)
12:15	**BREAK for Lunch–See schedule on p. 4**		

Tuesday, June 21

	Nutrition & Metabolism	Neurology	Innovative Technologies
	Rm. VMB A118	**Rm. VMB A117**	**Rm. VMB Sequoyah**
2:00	56. The Role of Cytomegalovirus Infection on Adipose Tissue Development (**Copeland**)	70. Molecular Mechanism of PSAP-induced Apoptosis (**T. Li**)	84. Normal Biodistribution of 3'-Deoxy-3'-[18F]Fluorothymidine (18FLT) in Adult Cats (**Rowe**)
2:15	57. Role of mTOR and β-hydroxy-β-methylbutyrate (HMB) in Leucine Stimulation of Muscle Mitochondrial Biogenesis and Fatty Acid Oxidation (**Stancliffe**)	71. LPA May Contribute to Alzheimer's Disease (**Shi**)	85. Generation of Three-dimensional Tissue Using Stem Cell Seeding of Polymeric Scaffolds (**Stephens**)
2:30	58. Characterization of ATP10C and Multiple Signaling Pathway Proteins in C2C12 Myotubes (**Hurst**)	72. Molecular Mechanism of Death Receptor-6 (DR-6)-induced Apoptosis (**Zeng**)	86. Development and Commercialization of a Conformal Ultrawideband Multilayer Lens Applicator (CUMLA) for Therapeutic Hyperthermia (**Phillips**)
2:45	59. Ex Vivo Effects of Dairy Products on Monocyte-vascular Endothelial Cell Adhesion (**Curry**)	73. Glia-derived TGF-beta Signaling Mediates Metamorphosis-associated Programmed Neuronal Cell Death in the *Drosophila* Central Nervous System (**Wang**)	87. Toxicity Screening of Damnacanthal Nanoparticle Using Bioluminescent Yeast-Reporter (**Chaisutatip**) CANCELED
3:00	60. In Vivo and In Vitro Studies of Fat Deposition in Chickens (**Ji**)	74. EcR and Usp-mediated Programmed Cell Death of Corazonergic Neurons in *Drosophila melanogaster* (**Sehgal**)	88. Development of a Novel High Throughput Screening Method for Estrogenic Compounds (**Xu**)
3:15	61. Silencing of the Angiotensinogen Gene in Adipocytes Decreases Lipid Accumulation And Secretion of Pro-inflammatory Markers (**Xin**)	75. Analysis of Gene Structure and Expression of the Neuropeptide Corazonin and Corazonin Receptor in the House Fly, *Musca domestica* (**Sha**)	
3:30	62. Histamine Induces Egr-1 Expression in Human Aortic Endothelial Cells via the H1 Receptor-mediated Protein Kinase Cdelta-dependent ERK Activation Pathway (**Hao**)	76. Mechanisms Underlying Cell-specific Expression of Pigment Dispersing Factor in *Drosophila melanogaster* (**Nair**)	

Featured Speakers

Franziska B. Grieder, DVM, PhD

Director, Division of Comparative Medicine, NCRR
NIH

"One Health, One Medicine: Veterinary Research Scientists for the 21st Century"

Monday Keynote Address

Dr. Franziska Grieder is the Director of the Division of Comparative Medicine, National Center for Research Resources (NCRR) at the National Institutes of Health (NIH). Her responsibilities include the management of the eight national primate research centers; development of mammalian and non-mammalian animal model resources, including knockout mice; pre- and post-doctoral training for veterinarians, and other research projects. Together with her staff of nine professionals, she provides leadership, fiscal and managerial oversight, and scientific direction for research grants and contracts with a budget in excess of $190 million. Dr. Grieder attended veterinary school at the University of Zurich in Switzerland and subsequently earned both an MS and a PhD degree in viral pathogenesis from the University of Wisconsin-Madison. As a faculty member of the Medical School of the Uniformed Service University (USU) in Bethesda, Maryland, she led a laboratory conducting research studies evaluating early immune and neuroimmunological responses to Venezuelan equine encephalitis virus. Her teaching responsibilities covered areas of virology, immunology, and infectious disease for both medical and graduate students. She holds an adjunct appointment at the associate professor level at USU, consulting on ongoing research projects in viral pathogenesis, teaching lectures in two courses, and participating in graduate student committees.

Dr. Jeffrey M. Becker

Chancellor's Professor, Head, Department of Microbiology
University of Tennessee

"Post-doctoral Opportunities"

Monday Featured Speaker

Dr. Jeffrey M. Becker's research involves the study of peptide hormones and their receptors, membrane transporters, and fungal virulence. He has trained more than 30 doctoral students who hold faculty or staff positions at many major institutions, has published more than 240 peer-reviewed articles, and has been awarded grants for research from many national agencies. He currently holds a National Institutes of Health (NIH) grant in its 33rd year of continuous funding. Becker is an elected Fellow of the American Academy of Microbiology and the American Association for the Advancement of Science. He serves on the NIH Drug Discovery and Mechanisms of Antimicrobial Resistance Study Section, on the editorial board of *Antimicrobial Agents and Chemotherapy*, and as associate editor of the journal *Microbiology*. Becker has been a consultant to the pharmaceutical companies Eli Lilly, Merck, and SmithKline-Beecham.

Featured Speakers

Gary Foster, PhD

Director, Center for Obesity Research and Education
Professor, Medicine and Public Health
Temple University School of Medicine

"Prevention and Treatment of Obesity: From the Schools to the Clinic"

Tuesday Plenary Address

Dr. Gary Foster's research interests include the prevention and treatment of obesity, and he studies a variety of treatment approaches including behavior therapy, pharmacotherapy, and surgery. He evaluates obesity prevention strategies in schools and communities. His current research studies include the effects of weight loss on sleep apnea, the safety and efficacy of low- and high-carbohydrate diets, and the prevention of obesity in school and community settings. He has authored or coauthored more than 125 scientific publications and three books on the etiology and treatment of obesity. Dr. Foster has been a frequent presenter at national and international scientific meetings and has also had considerable clinical experience treating overweight patients in individual and group settings for over 20 years. In addition, Dr. Foster has received numerous awards and honors including Outstanding Contributions to Health Psychology from the American Psychological Association and an Honorary Membership from the American Dietetic Association; he served as President of the Obesity Society in 2008. His research on the school-based prevention of obesity (Pediatrics, 2008) was cited by the American Heart Association as one of the top 10 advances in cardiovascular research in 2008.

Dr. Louis J. Gross

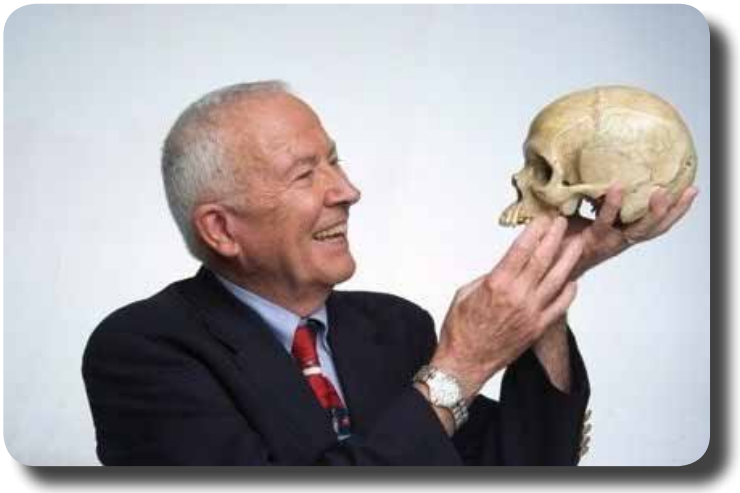
James R. Cox and Alvin and Sally Beaman Distinguished Professor of Ecology and Evolutionary Biology and Mathematics
Director, NIMBioS
Director, The Institute for Environmental Modeling
University of Tennessee

"Interdisciplinary Modeling Approaches to Public Health: Examples from NIMBioS"

Tuesday Featured Speaker

The latest endeavour for Dr. Louis J. Gross is the National Institute for Mathematical and Biological Synthesis (NIMBioS), a National Science Foundation-funded center to foster research and education at the interface between math and biology. His research focuses on applications of mathematics and computational methods in many areas of ecology, including disease ecology, landscape ecology, spatial control for natural resource management, photosynthetic dynamics, and the development of quantitative curricula for life science undergraduates. He has led the effort at UT to develop an across trophic level modeling framework to assess the biotic impacts of alternative water planning for the Everglades of Florida. In addition, he has co-directed several courses and workshops in mathematical ecology at the International Centre for Theoretical Physics in Trieste, Italy. In 2006, he became a Distinguished Scientist awardee of the American Institute of Biological Sciences and is a Fellow of the American Association for the Advancement of Science. He completed a PhD in applied mathematics at Cornell University and has been a faculty member at UTK since 1979. He is a long-time volunteer for Jubilee Community Arts and Community Shares, has hosted and produced folk music programs for WUOT-FM, performs with the Lark in the Morn English Country Dancers, and serves as house sound engineer for concerts at the Laurel Theatre in Knoxville.

Featured Speakers



Dr. William Bass

Professor Emeritus

"Beyond a Dog-eat-dog World"

Featured After-dinner Address

Dr. William M. Bass is a professor emeritus in the Anthropology Department at the University of Tennessee. Crucial to the development of the forensic anthropology specialty shortly after his arrival at the university in 1971, Dr. Bass soon found worldwide fame when he established the Anthropological Research Facility in 1980. This facility, also known as the "Body Farm," is an outdoor field laboratory wherein scientists explore postmortem changes in the human body in order to better estimate time of death. Dr. Bass's human identification and skeletal analysis services have proven invaluable for various state and local law enforcement agencies, as well as for Tennessee medical examiners and district attorney offices.

Dr. Bass holds a Ph.D. in Anthropology from the University of Pennsylvania and is a diplomate of the American Board of Forensic Anthropology. He has been involved in anthropological research and teaching for over 50 years and has received grants from the National Science Foundation, the National Geographic Society, and the National Park Service, among others. His excellence has been honored many times over, but notably, he has been named the National Professor of the Year by The Council for Advancement and Support of Education (1985-86), and received the Distinguished Fellow Award from the American Academy of Forensic Sciences. He was also the seventh recipient of the Adelaide Medal from the International Association of Forensic Sciences for his amazing career in forensic sciences.

More recently, Dr. Bass has embarked on a popular crime mystery writing career, with five completed fiction novels in his Bones series (with author and documentary film-maker Jon Jefferson under the name "Jefferson Bass"); the latest novel, *The Bone Yard*, had a 2011 release. He also has two non-fiction works and has consulted with author Patricia Cornwell on her best-selling crime novels. Dr. Bass will have books available for sale at the awards banquet.

Abstracts

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THE UNIVERSITY of TENNESSEE

1. Mathematical Modeling of Within-host Transmission Dynamics of *Toxoplasma gondii*

Adam Sullivan, Chunlei Su, Xiaopeng Zhao

Mechanical, Aerospace and Biomedical Engineering (Sullivan, Zhao); Microbiology (Su)

Toxoplasma gondii is a protozoan capable of replicating sexually in cats and asexually in other warm-blooded animals. By using a three dimensional mesh of both the brain and spleen, it is possible to simulate using a computational model to demonstrate the entire life-cycle within an intermediate host. A cellular automata model is developed to demonstrate the dynamics of the parasite, where each cell follows the same set of rules for each discrete time-step. This cellular automata model allows for data simulations to be run of the parasite within a mouse to determine dominant phenomenon and display graphical images and animations.

2. Multilocus Sequence Typing (MLST) for Characterization of Methicillin-resistant and Methicillin-susceptible Clones of *Staphylococcus pseudintermedius*

Samar M. Solyman, Chad C. Black, J.R. Fitzgerald, B. Duim, E. van Duijkeren, J.A. Wagenaar, Laura C. Eberlein, David A. Bemis, Stephen A. Kania

Comparative Medicine (Solyman, Black, Eberlein, Bemis, Kania), Comparative and Experimental Medicine (Solyman), Laboratory of Bacterial Evolution and Pathogenesis, The Roslin institute and Centre for Infectious Diseases, New Royal Infirmary, University of Edinburg, Edinburg (Fitzgerald, Wagenaar), Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands (Duijkeren, Duim)

Staphylococcus pseudintermedius, the bacterium most commonly associated with canine pyoderma, has become a newly emerging methicillin-resistant pathogen. There is limited information about the population genetic structure within this species. Foundational studies of the *Staphylococcus intermedius* group examined allelic polymorphisms in four genes. In this study, four additional slowly evolving genes with allelic polymorphisms were identified from numerous candidates and were included in a multilocus sequence typing (MLST) scheme. Application of the new, eight-gene MLST method to a total of 179 diverse *S. pseudintermedius* isolates identified 101 different sequence types (STs). This represents an additional 23 sequence types beyond those identified previously. Most methicillin-resistant *S. pseudintermedius* isolates were further subdivided to new STs. The expanded MLST scheme enables an unambiguous method for assigning *S. pseudintermedius* to clones that will give new insight into the population and the evolution of the species, and provide a better understanding of the spread of antibiotic resistance.

3. Characterization of Small RNAs and Small Proteins in EHEC

Jia Wen, Bethany Miracle, Pranay Dogra, Elizabeth Fozo

Microbiology

Many RNAs function not to encode protein, but to regulate gene expression by binding to a target mRNA and inhibiting its translation. In bacteria, these regulatory RNAs are called small RNAs (sRNAs) because their sizes vary from 50 to 200 nucleotides. A subset of bacterial sRNAs represses the expression of hydrophobic proteins under 50 amino acids in length. These proteins are highly toxic when overproduced. Gene pairs consisting of a toxic protein and its regulatory sRNA are referred to as type I toxin-antitoxin loci. Although their functions are not known, we hypothesize that they are stress response loci. A bioinformatics-based search identified two highly homologous toxin-antitoxin loci named zorO-orzO and zorP-orzP in enterohemorrhagic *Escherichia coli* (EHEC). Here, zor encodes the toxic protein and orz encodes the repressive sRNA. Northern analysis shows that the expression of the zor transcripts and Orz sRNAs are altered in response to growth condition, indicating that the genes may be subjected to transcriptional regulation. Overproduction of the Zor small protein is toxic; however, this toxicity can be neutralized by co-expression of the corresponding antitoxin sRNA. Despite the extensive sequence similarity between zorO-orzO and zorP-orzP pairs, only OrzO, not OrzP, can repress ZorO expression. Our cumulative data suggest that zor gene expression is regulated at multiple levels, and the Orz sRNAs are important for posttranscriptional control of their cognate zor genes. Ongoing and future studies are aimed at understanding how zor gene expression is regulated and why EHEC possesses these potentially toxic genes.

4. What Did the Tick Eat for Lunch Before it had You for Dinner?

Cathy Scott, Jessica Harmon, Carl Jones, Graham Hickling

Forestry, Wildlife and Fisheries (Scott, Hickling), Entomology and Plant Pathology (Harmon, Jones)

Determining which host species feed ticks is vital to understanding the population dynamics and pathogen transmission of tick species implicated in diseases of medical and veterinary importance. Assessing host preference by sampling attached ticks from captured hosts is problematic because of marked variation in

trapping efficiency of different host species. Molecular assays provide an alternative approach, by allowing host selection to be determined from minute traces of host blood that persist in recently-molted ticks. We designed 12S rDNA mitochondrial gene probes suited for use in reverse line blot assays of ticks feeding on common host species in the Eastern United States. The assay combines conventional PCR with a biotin label primer and reverse line blots which can be stripped and re-hybridized up to 20 times, making the method significantly less expensive and requiring less interpretation than previous methods of tick blood meal identification. Probes were designed that encompass species, genus, genus groups, and/or families of 8 reptile, 11 aves, and 34 mammal hosts. Blood meals from feeding and questing nymphal and adult ticks were successfully identified in *Amblyomma americanum* and eight other Ixodid tick species; blood meals were detected at a higher rate for *A. americanum* adults (48.3%) than for nymphs (30.4%; $P = 0.018$). Development of this probe library, suitable for southeastern U.S. ecosystems, opens the way for experimental validation studies and, thereafter, eco-epidemiological investigations of numerous tick/host systems.

5. Involvement of *Streptococcus uberis* Adhesion Molecule (SUAM) in Adherence to and Internalization of *Streptococcus uberis* into Bovine Mammary Epithelial Cells

Raul A. Almeida, Xueyan Chen, Maria E. Prado, Stephen P. Oliver

Animal Science

The development of effective control procedures for *Streptococcus uberis* mastitis pathogen have been hampered by limited knowledge on virulence factors used by this important environmental mastitis pathogen to cause intramammary infections (IMI) in dairy cows. A potential virulence factor referred to as *Streptococcus uberis* Adhesion Molecule (SUAM), which uses bovine lactoferrin (LF) as a molecular bridge to enhance adherence to and internalization into host cells, was recently reported by our group. We conducted a comparative adherence and internalization study using a natural SUAM mutant (*S. uberis* UT754), a SUAM deletion mutant (-sua *S. uberis* UT888), and the wild type strain *S. uberis* UT888 using two mammary epithelial cell lines (MAC-T and UV-BME cells). UT754 adhered to and internalized into mammary epithelial cells significantly less than UT888 (adherence 10.0% vs. 100%; internalization 23.9% vs. 100%), but higher than - sua UT888 (adherence 10% vs. 6.7%; internalization 23.9% vs. 0%). The presence of LF significantly enhanced adherence and internalization of UT888 but had no effect on - sua UT888 or UT754. No differences in adherence or internalization were observed between UV-BME and MAC-T mammary epithelial cells. Results from this investigation corroborates previous observations regarding the involvement of SUAM in adherence to and internalization of *S. uberis* into bovine mammary epithelial cells confirming its potential role in the pathogenesis of *S. uberis* mastitis.

6. Virulence Gene Expression Patterns in *E. coli* Isolates from Bovine Mastitis after Co-culture with Primary Bovine Mammary Epithelial Cells

Oudessa Kerro Dego, Stephen P. Oliver, Raul A. Almeida

Animal Science

Current bovine mastitis control programs effectively reduce contagious mastitis pathogens but have a marginal effect on environmental pathogens such as *Escherichia coli*. Intramammary infections (IMI) caused by *E. coli* are often acute, with local and systemic clinical manifestations that typically are cleared within 7 days. In some cases, however, if not diagnosed early and treated, it could result in serious systemic reaction and death. In the last few years, persistent *E. coli* IMI characterized by mild clinical manifestations that last a few days, followed by subsequent episodes of clinical mastitis during lactation, have been increasingly reported. Factors responsible for pathogenesis of *E. coli* IMI and variation in clinical manifestations are not known. There are reports indicating that the outcome of *E. coli* IMI is mainly determined by cow factors. However, recent research demonstrated that virulence attributes of *E. coli* strains have significant impact on the outcome of *E. coli* IMI. The goal of this study was to determine the presence of known virulence genes of pathogenic *E. coli* in strains isolated from bovine acute and persistent IMI and evaluate their expression pattern after co-culture with bovine mammary epithelial cells. We evaluated the expression patterns of virulence associated genes of *E. coli* after co-culture with PBMEC using qRT-PCR. Our results showed significant up-regulation of ler, eae, flic, and iutA genes mainly in the strains of *E. coli* associated with persistent IMI. The pathogenesis and clinical severity of *E. coli* IMI may be determined by combined effects of host-pathogen factors.

7. Association between Presence of Urovirulence Factors and Antimicrobial Resistance Patterns in 221 Uropathogenic *Escherichia coli* Samples Isolated from Dogs

Jennifer Wells, Joe Bartges, Steven A. Kania, David Bemis

Small Animal Clinical Sciences (Wells, Bartges), Comparative Medicine (Kania, Bemis)

Escherichia coli is the most prevalent pathogen associated with cystitis and pyelonephritis in dogs. Presence of urovirulence factor genes (UVFs) in *E. coli* may increase its antimicrobial resistance. Our objective was to compare four UVFs with resistance to antimicrobial drug classes in 221 canine uropathogenic *E. coli* (UPEC) isolates collected from 184 different canine patients and submitted to the University of Tennessee Microbiology Laboratory in 2007. Multiplex polymerase chain reaction was used to detect cytotoxic necrotizing factor (cnf), hemolysin (hlyD), S-fimbrial adhesion (sfa), and pilus associated with pyelonephritis gene G allele III (papGIII). In vitro susceptibility to antibiotic classes was evaluated, and resistance was compared to UVF presence. UVFs per isolate were 0 UVFs = 127 (57%), 1 = 27 (12%), 2 = 4 (2%), 3 = 22 (10%), and 4 UVFs = 41 (19%). Frequency of UVF presence was sfa (33%), hly (24%), cnf (24%), and pap (19%). Presence of four UVFs; hly, cnf, and sfa together; and sfa alone were associated with less antimicrobial resistance ($P < .001$). Mean resistance to antimicrobial class by number of UVFs expressed was 0 UVFs = 4.1 ± 3.3 classes, 1 = 1.2 ± 1.1, 2 = 0.0 ± 0.0, 3 = 0.8 ± 1.6, and 4 UVFs = 0.5 ± 1.2 classes. UVFs were present in 43% of UPEC and were negatively correlated with antibiotic resistance.

8. Abundance and Prevalence of Bacterial and Viral Pathogens in Bangladesh Surface and Ground Water During Wet and Dry Seasons

Abby E. Smartt, Alice C. Layton, Daniel E. Williams, Gary S. Sayler

Center for Environmental Biotechnology

In Bangladesh, an estimated 69,000 people die from diarrhea yearly due to widespread fecal contamination of ground and surface waters. The goal of this study was to identify diarrheal pathogens in surface and ground water, in order to determine which contribute to the transmission of diarrheal disease through drinking water. The commonly-isolated diarrheal pathogens from patients treated in the region's health clinics are *Vibrio cholerae*, rotavirus, Shigella, and pathogenic *E. coli*, so quantitative PCR assays were identified or designed to test for these pathogens. For this study, ground water from 50 wells and surface water from 10 ponds were collected and filtered in both wet and dry seasons. The filters were shipped in dry ice to the United States for qPCR analyses. Cloning and sequencing was also performed to verify the presence of pathogenic genes. During the wet season, rotavirus was found in 40%, Vibrio and Shigella each in 10%, and pathogenic *E. coli* in 8% of the wells. Vibrio, Shigella, and pathogenic *E. coli* were found in 7%, 14%, and 14% of the surface water samples, respectively. The elt gene from ETEC and the ipaH gene from Shigella were cloned from surface water samples, and the rotavirus VP6 gene was cloned from multiple well water samples. Dry weather samples are currently being analyzed for both viral and bacterial pathogens. This research suggests that a better understanding of how pathogens enter and are transported in ground water is needed to improve risk assessment of exposure to waterborne pathogens in Bangladesh.

9. Propidium Monoazide for the Rapid Molecular Detection of Viable *Salmonella enterica*

Chayapa Techathuvanan, Doris H. D'Souza

Food Science & Technology

Rapid detection of viable *Salmonella enterica* in food environments is important to curb foodborne gastroenteritis outbreaks. This study aimed to optimize propidium monoazide (PMA)-DNA-based PCR and DNA-based, loop-mediated isothermal amplification (LAMP) for selective, viable *S. enterica* detection for comparison with traditional cultural, PCR, and LAMP methods. Overnight, *S.* Enteritidis cultures at 107–108 CFU/ml were either autoclaved (121°C, 15 min) or heat (60°C, 3.5 min) or ultraviolet (UV) pasteurized (5-cm distance, 5 min) in duplicates, replicated twice, and treated with or without PMA. Serially-diluted control and treated samples were enumerated on XLT4 agar, and DNA was extracted from 1-ml samples using DNAzol. PCR and LAMP assays were carried out using invA primers and detected by agarose gel electrophoresis. Culture-based assays showed *S.* Enteritidis detection of 107–108 for overnight culture, 104–105 for heat pasteurized, 101 CFU/ml for UV pasteurized, and none for autoclaved cells. Detection of autoclaved cells dropped by only 2 to 3 logs using PCR and LAMP, with no detection by PMA-PCR and PMA-LAMP. Heat-pasteurized *Salmonella* showed detection at 105 CFU/ml by both PCR and LAMP, and 3- to 4-log lower detection by both PMA-PCR and PMA-LAMP. For UV inactivated cells, both PMA- and non-PMA-based PCR and LAMP assays showed the same detection limit at 104CFU/ml. Our results demonstrate that PMA-PCR and PMA-LAMP assays show comparable results to traditional cultural methods for viable cell detection (except UV

treatment) but is rapid within 24 hr. PMA-PCR or PMA-LAMP shows potential for viable cell detection in the food industry.

10. The Role of Phosphatidylethanolamine in *C. albicans* Virulence and GPI Anchor Synthesis

Sarah E. Davis, Anthony Montedonico, Todd B. Reynolds

Microbiology

Systemic fungal infections caused by the yeast *Candida albicans* have a mortality rate of approximately 30%. In order to develop better methods of treating systemic fungal infections, it is important to understand the virulence mechanisms of *C. albicans*. Loss of key enzymes in the phospholipid de novo pathway results in an attenuation in virulence. The phosphatidylserine synthase (Cho1p) of *C. albicans* as well as the phosphatidylserine decarboxylases (Psd1p and Psd2p) are required for virulence. Loss of virulence in mutants lacking these genes correlates with a drop in cellular phosphatidylethanolamine (PE). PE is a precursor in generating glycosylphosphatidylinositol (GPI) anchors, and we hypothesize that the decrease in PE is causing attenuation in virulence due to a drop in GPI anchored protein (GPI-AP) synthesis. GPI anchors are responsible for anchoring proteins to the fungal cell wall and serve two major functions in virulence: 1) Many GPI-APs are known virulence factors and some (e. g. Als3p) allow adhesion and invasion into host cells, resulting in damage. 2) GPI-APs decorate the outer cell wall with mannan and conceal the pathogen from the immune system by “cloaking” an underlying pathogen-associated molecular pattern called ß1,3-glucan. When mannan is lost, the β1,3-glucan is exposed and recognized by the innate immune system. Our mutants that are reduced in PE have increased β1,3-glucan exposure and reduced in Als3p. Altogether, these data indicate that our hypothesis is correct that there is a reduction in GPI-APs on the cell surface of these mutants, which results in attenuated virulence.

11. Fatty Acid Synthase 1: The Role of Fatty Acid Biosynthesis in Membrane Stress and Virulence in *C. albicans*

Marissa M. Rodrigues, Todd B. Reynolds

Microbiology

The interaction between *Candida albicans* and the host is a key determinant towards disease progression. Nutrient limitation is a significant stress that pathogens have to encounter within the host, and *C. albicans* has developed a battery of nutrient acquisition strategies to assist in its ability to cause disease. Deletion of key enzymes in biosynthetic pathways, however, can have adverse effects on virulence properties of this organism and are therefore good antifungal targets. In this study, we have demonstrated that deletion of the FAS1 (fatty acid synthase 1) gene, which is essential for de novo biosynthesis of fatty acids, is important for *C. albicans'* virulence. A *C. albicans* fas1-/- mutant cannot grow in vitro in the absence of an exogenous supply of fatty acids. Therefore, the virulence defect of the mutant is presumed to be due to an inability in acquiring essential fatty acids from the host. Surprisingly, the mutant can grow and form hyphae in mouse serum and at least initiate hyphal formation in the host 6 hr post-infection; yet 24 hr post-infection, the fungal load of the mutant is severely reduced in the kidneys of mice. Fatty acid profiling of the fas1-/- mutant grown in mouse serum revealed an altered fatty acid composition in its cell membrane during growth in serum, which may contribute to its reduced virulence. The membranes of the fas1-/- mutant may be more susceptible to various stresses within the host. Consistent with this, the mutant is sensitive to osmotic stress and may be more readily eliminated by the host.

12. Phosphatidylserine Synthase and Phosphatidylserine Decarboxylase are Required for Cell Wall Integrity and Virulence in *Candida albicans*

Anthony E. Montedonico, Ying Lien Chen, Sarah Kauffman, John R. Dunlap, Fu-Min Menn, Todd B. Reynolds

Microbiology (Montedonico, Chen, Reynolds), Advanced Microscopy and Imaging Center (Dunlap), Center for Environmental Biotechnology (Menn)

The fungal pathogen *Candida albicans* is a leading cause of fungal infections in humans. The immune suppressed population is especially at risk for developing *C.albicans* infections. Interestingly, enzymes involved in phospholipid biosynthesis in fungi have been proposed as potential new drug targets. The phosphatidylserine (PS) synthase (Cho1p) of the fungal pathogen *Candida albicans* is necessary for virulence in a mouse model of systemic infection. The *C. albicans* PS synthase mutant (cho1Δ/Δ) has cell wall defects, and we hypothesize that these defects cause loss of virulence. PS is a precursor for the synthesis of phosphatidylethanolamine (PE) by PS decarboxylase enzymes Psd1p and Psd2p, and the complete loss of PS in the cho1Δ/Δ mutant leads to a subsequent drop in PE. We have discovered that the decrease in PE alone in a psd1Δ/Δ psd2Δ/Δ mutant clearly contributes to the loss of virulence and likely acts by affecting adhesin presentation. However, the loss of PS in addition to the drop in PE observed in the cho1Δ/Δ causes a much stronger defect in the cell wall

and much poorer masking from the immune system. Therefore, we hypothesize that PS has a role independent of PE in mediating the maintenance of the cell wall. We have found that loss of PS in mutants deleted for CHO1 homologs in *Saccharomyces cerevisiae* and *C. albicans* share common cell wall phenotypes. In addition, we have elucidated several genes that may play a role in rescuing cell wall defects associated with PS loss in *S. cerevisiae*.

13. Vacuolar Protein Sorting Components Regulate Biofilm Formation in Yeast by Flo11p-dependent and Flo11p-independent Mechanisms

Neha Sarode, Bethany Miracle, Todd Reynolds

Genome Science and Technology (Sarode), Microbiology (Miracle, Reynolds)

Saccharomyces cerevisiae haploid cells (Σ1278b) grow in a unipolar manner on rich (YPD) medium agar plates, staying connected to one another and forming filaments invading the agar, referred to as invasive growth. When inoculated on a YPD plate containing 0.3% agar, they generate elaborate “floral” patterns on the surface, referred to as mats. Both mat formation and invasive growth are dependent on Flo11p (cell-wall glycoprotein adhesin), but these phenotypes are also distinct based on mutations that affect one but not the other. For example, vps27Δ mutant has a defect in mat formation but not invasive growth. VPS27 protein is required for sorting of cargo proteins into endosomal intermediates called multi-vesicular bodies (MVBs). In contrast, another MVB sorting mutant vps25Δ causes defects in both mat formation and invasive growth. VPS25 acts downstream of Vps27p, sorting proteins into MVBs. VPS25, along with the other ESCRT components, is necessary for processing of the Rim101p transcription factor that regulates FLO11 expression. Non-ESCRT MVB sorting proteins like Vps27p are not required for Rim101p processing and appear to affect mat formation in a FLO11-independent manner. These results suggest a model in which the MVB pathway controls mat formation by two overlapping mechanisms. Mechanism 1 requires a functional MVB pathway and affects mat formation in a FLO11-independent manner and does not affect invasive growth (eg. vps27Δ). Mechanism 2 is superimposed on mechanism 1 and affects both mat formation and invasive growth in a FLO11-dependent manner (eg. vps25Δ). Further exploration of mechanism 1 will be presented.

14. Does Physical Activity Explain Ethnic Differences in Cardiorespiratory Fitness among U.S. Adults?

Tyrone Ceaser, Eugene Fitzhugh, Dixie Thompson

Kinesiology

Previous research has shown differences in cardiorespiratory fitness (CRF) levels between racial groups. It is unclear how much of these differences can be explained by physical activity (PA). We sought to determine how much of the racial difference in cardiorespiratory fitness could be explained by PA. 3115 adults (18–49 years) completed a submaximal graded exercise test from the National Health and Nutrition Examination Survey (1999–2004) in order to measure CRF, the dependent measure. Independent measures included demographic variables, including race and PA from three different domains: domestic, transportational, and leisure time. The analysis used SUDDAN statistical software, the primary procedure being regression. VO2max was highest among Mexican Americans (40.9 + 0.5 ml/kg-1/min -1), followed by non-Hispanic whites (40.2 + 0.3 ml/kg-1/min -1), other races (38.8 + 0.7 ml/kg-1/min -1), and non-Hispanic blacks (37.9 + 0.6 ml/kg-1/min -1) (P = 0.0001). Females had a significantly lower VO2max than males (36.0 + 0.3 ml/kg-1/min -1 vs. 43.5 + 0.3 ml/kg-1/min -1, respectively) (P = 0.00). Demographics and race explained 19.7% of the variance in VO2max, with race being significant (P = 0.0001) in the model. When PA was added, the variance in VO2max explained by the model increased by 2.2% (P = 0.004). Race remained as a significant independent predictor of VO2 max. After controlling for physical activity, non-Hispanic blacks and other racial groups still had a significantly lower VO2max than non-Hispanic whites and Mexican Americans. This analysis illustrates that PA does not mediate differences seen in CRF between racial groups.

15. A Compendium of Energy Costs of Physical Activities for Individuals Who Use Manual Wheelchairs

Scott A. Conger, David R. Bassett, Jr.

Kinesiology, Recreation, and Sport Studies

The purpose of this study was to develop a compendium of wheelchair-related physical activities. To accomplish this, we used a systematic review of the literature of the published energy costs of activities performed by individuals who use wheelchairs. A total of 266 studies were identified by a literature search using relevant keywords. Inclusion criteria were studies using individuals who routinely use a manual wheelchair, indirect calorimetry as the criterion measurement, energy

expenditure expressed as METs or VO2, and physical activities typical of wheelchair users. Eleven studies met the inclusion criteria. A total of 63 different wheelchair activities were identified with energy expenditure values ranging from 0.8 to 12.5 kcal/kg/hr. The energy requirements for some activities differed between individuals who use wheelchairs and those who do not. The compendium of wheelchair-related activities can be used to enhance scoring of physical activity surveys and to promote the benefits of activity in this population.

16. Reduced Peak Cycle Exercise Heart Rate in Obese, African American Children

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Kinesiology, Recreation, and Sport Studies (Flynn, Coe), University of Tennessee Health Science Center (Alpert)

Peak heart rate (HR_{peak}) is reduced in obese children compared to normal weight children; however, few studies have assessed relationships between peak oxygen consumption (VO_{2peak}), peak respiratory exchange ratio (RER_{peak}), rating of perceived exertion (RPE), total test time (TT), and HR_{peak}. Our objective was to compare VO_{2peak}, RER_{peak}, RPE, and TT in children with differing HR_{peak}. Subjects were 18 pre-pubertal, African American obese children (age 9.2 ± 1.6 years). Measured height and weight were used to calculate body mass index (BMI), and all subjects were ≥95th percentile based on the Centers for Disease Control BMI for age and sex growth charts. Pubertal status was assessed using Tanner's Criteria. Lean and fat mass were measured using dual X-ray absorptiometry. A cycle test was performed to volitional exhaustion using the McMaster protocol. VO_{2peak} and RER_{peak} were measured using a metabolic system and scaled to lean mass. HR_{peak} was determined using telemetry, and Children's Omni-cycle Scale was used to obtain RPE. HR_{peak} values were categorized into two groups: <190 beats/min (n=12) and ≥190 beats/min (n=6). Independent-samples *t* tests were used to assess differences in variables between groups. There were no significant differences in VO_{2peak} between <190 beats/min and ≥190 beats/min groups (37.5 ± 9.8 vs. 41.7 ± 10.8 ml·kg lean mass^{-1}·min^{-1}). There were no differences in RER_{peak} (1.03 ± 0.08 vs 1.03 ± 0.08), RPE (7.3 ± 1.1 vs. 8.8 ± 2.5), or TT (7.9 ± 2.5 vs 9.2 ± 2.0 min). Results demonstrate that HR_{peak} during cycle exercise may be reduced in some obese children, but this does not appear to be associated with reductions in VO_{2peak}, RER_{peak}, RPE, or TT.

17. Why is There So Much Confusion about the VO2 Plateau? A Re-examination of the Work of Hill et al. (1923)

Richie Castle, David Bassett, Scott Conger

Kinesiology, Recreation, and Sports Studies

Maximal oxygen uptake (VO2max) is regarded as the gold standard for assessing aerobic fitness. In 1923, A.V. Hill et al. proposed that VO2max represents the maximal ability of the body to take in and consume O2 during strenuous exercise. Recently, however, controversy has arisen over the issue of whether a leveling off, or "plateau," in VO2 is needed to verify attainment of VO2max. Our purpose was to test two different VO2max protocols to determine if both protocols show direct evidence of an upper limit on O2 transport capacity. Five adults (18–35 years old) completed a continuous, graded exercise test (CGXT), followed by a discontinuous graded exercise test (DGXT). The CGXT consisted of gradually increasing treadmill running speeds to volitional exhaustion; the peak speed attained was labeled peak treadmill velocity (PTV). Over the next several days, participants ran at 80%, 90%, 100%, 105%, and 110% of PTV for 10 min or until volitional exhaustion. All participants (N=5) achieved a "VO2 ceiling" (or upper limit) on the DGXT, while only 60% (n=3) achieved a "VO2 plateau" on the CGXT. There was no difference between peak oxygen uptake (VO2peak) measured at 90%, 100%, 105%, and 110% of PTV ($P > 0.05$). However, VO2peak at 80% PTV was significantly lower than all other velocities ($P < 0.05$). The VO2 ceiling effect on a DGXT is inherently different than the VO2 plateau effect on a CGXT. In this study, a ceiling was always seen on the DGXT, but a plateau was not always seen on the CGXT.

18. Curricular Competencies Related to Cultural Competence for the Education and Training of Registered Dietitians

Tegan Medico, Betsy Haughton

Nutrition (Medico, Haughton), Public Health (Medico)

Though a multidisciplinary body of literature on developing curricula related to cultural competence for health professionals exists, still lacking from this literature is sufficient input from the dietetic profession. The purpose of this cross-sectional, Internet-based research was to create a model of core curricular competencies related to cultural competence for the education and training of registered dietitians. A random sample of registered dietitians rated 73 proposed curricular competencies for essentiality on a 7-point Likert-like scale (1 = not a priority; 7 = essential). Exploratory principal components analysis

(PCA) with Varimax rotation condensed the proposed competencies with similar variances of responses into factors (model domains) and eliminated competencies that accounted for too little or ambiguous variance. Factors were assigned unique labels based on the prevailing themes of their respective competencies and further interpreted in terms of respondent characteristics via multivariate general analysis of variance (MANOVA). Results based on a 17.9% (n = 1,090) rate of response produced a model with 69 competencies and seven domains: communication and relationships; community collaboration; disparities and diversity in health care; information access, analysis, and use; bias management; food environments; and models and definitions. Significant differences in mean factor ratings were detected between respondents who differed by race and by experience working with diverse individuals and groups. This model is representative of existing research on cultural competence, but it is the first unique to dietetics. It may be used by dietetic education and training programs to systematically plan, implement, and evaluate curricula for cultural competence.

19. The Psychosocial Effects of Chronic Beryllium Disease

Jeffrey R. Miller, Gregory C. Petty, Paul C. Erwin, Charles B. Hamilton, Ernest W. Brewer, Donna L. Cragle

Public Health (Miller, Petty, Erwin, Hamilton), Educational Leadership and Policy Studies (Brewer), Oak Ridge Associated Universities (Cragle)

We have made important gains in our knowledge of chronic beryllium disease (CBD). However, we still do not understand the psychosocial component. Substantial resources are being invested in educational programs, support groups, counseling, compensation programs, etc. to reduce the impact of CBD on workers and their families. These efforts are well-meaning but do not have a theoretical basis supported by empirical data. That makes it difficult, if not impossible, to evaluate the effectiveness of these programs. A mixed-methods research project is underway to develop knowledge that will help health professionals create interventions to mitigate the psychosocial effects of CBD. The study population is workers who have CBD and worked in the Department of Energy complex. Established instruments that measure health status, uncertainty in illness, and psychosocial adjustment to illness will be administered to the participants. A subset of the participants will be interviewed to gain a richer understanding of their disease experience. The results will be analyzed using qualitative and quantitative methods. The results will be used to develop a theoretical model explaining the psychosocial effects of CBD and to validate the use of the instruments in this population. This will fill a void in our understanding of the natural history of CBD and may lead to changes in the psychological, social, financial, and disease management support provided to this population.

20. The Relationship Between Greenway Siting, Active Transport Access, and Types of Users

Dana Wolff, Eugene Fitzhugh, David Bassett, Christopher Cherry

Kinesiology, Recreation, & Sport Studies (Wolff, Fitzhugh, Bassett), Civil and Environmental Engineering (Cherry)

Greenways (GW) are a useful way to promote outdoor physical activity (PA) and can be sited in a variety of settings within the built environment (BE) that allow varying access of the GW through active transport (AT). The purpose of this study was to determine if GWs, with varying potential of AT access (high vs. low) due to siting within the BE, relate to user characteristics and their PA behaviors. A trail intercept survey was administered to 611 adults from Sept. to Nov., 2010, on two GWs in Knoxville, TN, with high (GWlinear) and low AT (GWloop) potential. The survey measured modes of GW access, self-reported GW PA, and demographics. Users (N=216) of GWlinear, compared to GWloop users (N=400), were more likely to be younger, male, and never married. Access of the greenway through AT was significantly greater at GWlinear compared to GWloop (37.0 vs. 4.2%, $P < 0.001$). GWlinear users accumulated significantly more MET/min/wk ($P = 0.012$) and engaged in more vigorous PA (34.7 vs. 22.0%, $P = .000$). Frequency of GW use was lower for GWlinear compared to GWloop (2.62±1.8 vs. 3.24 ±1.9 days/wk, $P < 0.001$). When looking at GW users meeting the 2008 PA Guidelines, however, there was no difference in the proportion of users meeting these guidelines between GWs (57.8 vs. 55.3%, $P = 0.562$). The way in which GWs are sited within the BE are related to how accessible they are to AT. City planners and GW designers should consider GW siting in terms of AT access potential and types of users targeted.

21. Use of Bioluminescent Yeast Bioreporters to Detect Endocrine Disruptors in Brazilian Waterways

Melanie Eldridge

Center for Environmental Biotechnology

New and standard methods are being used to rapidly monitor hormonally-active compound wastewater treatment influent and effluent in drinking water and in surface waters. The long-range goal is to determine whether water treatment strategies are effective for

removing hormonally-active contaminants. Moreover, we seek to determine whether wastewater treatment results in increases or decreases in activity or toxicity. Raw and treated wastewater and drinking water samples were collected from several sources and concentrated by solid-phase extraction. The watersheds where these water utilities are located contain mixed land uses consisting of farmland and urban populations incorporating both septic and municipal sewer systems. Bioluminescent *Saccharomyces cerevisiae* strains BLYES, BLYAS, and BLYR were used to detect the presence of potential environmental estrogenic, androgenic, or toxic compounds, respectively. These strains contain the bioluminescent luxCDABE genes from *Photorhabdus luminescens* and the frp gene from *Vibrio harveyi*, which are expressed on two plasmids either under the control of estrogen response elements (*S. cerevisiae* BLYES), androgen response elements (*S. cerevisiae* BLYAS), or are constitutively expressed for the detection of toxicity (*S. cerevisiae* BLYR). In the presence of estrogenic or androgenic compounds, *S. cerevisiae* BLYES and BLYAS produce bioluminescence, respectively, while *S. cerevisiae* BLYR produces light except in the presence of toxic substances. The bioreporters were exposed to concentrated and diluted wastewater samples to determine whether estrogenic or androgenic activity changed with treatment. In many cases, potential estrogenic and androgenic activity was found in raw wastewater (e.g. in one sample 0.7 ng E2 equivalents/L).

22. Effect of Two Selective Serotonin Reuptake Inhibitors on Global Gene Expression Changes in Larval Zebrafish (*Danio rerio*)

June-Woo Park, Ted Henry

Center for Environmental Biotechnology

Pharmaceuticals can contaminate surface waters after their prescribed medical use and have the potential to negatively affect aquatic organisms. Selective serotonin reuptake inhibitors (SSRIs; e.g., Prozac) are of particular environmental concern because they are biologically active at low concentrations and have been detected in surface waters downstream of wastewater treatment plants. SSRIs are among the most frequently prescribed drugs in human medicine, and patients typically continue treatment over long periods of time. In non-target organisms like fish, SSRIs can act on the neuroendocrine signaling system and potentially influence higher levels of biological organization including reproduction and behavior. We previously found a delayed development of adult sexual morphology in western mosquitofish (*Gambusia affinis*) by the chronic (100d) exposure of SSRI (fluoxetine, 60 ppb), suggesting potential impacts on fish reproduction. For a better understanding of the biochemical pathways underlying SSRIs exposure, we evaluated global gene expression profiles in zebrafish (*Danio rerio*) exposed to two SSRIs (fluoxetine and sertraline) and investigated if expression patterns of specific genes could be used as indicators of SSRI exposure in fish. They were exposed to two concentrations (25 and 250 ppb) of each SSRI for 5 days for microarray analysis (Affymetrix GeneChip Zebrafish Genome Array). No mortality or behavioral abnormalities were observed; however, alterations in global gene expression indicated treatment effects on gene regulation. Many genes were significantly altered (> 1.5 fold) relative to control groups. The observed changes in gene regulation will be exploited to develop biomarkers and thus to monitor the exposure of wild fish to SSRIs.

23. Assessment of Exposure to Environmental Estrogens in Male Largemouth Bass (*Micropterus salmoides*)

Jill K. Wilson, Michelle H. Connolly, Theodore B. Henry

Forestry, Wildlife and Fisheries (Wilson, Henry), Center for Environmental Biotechnology (Connolly, Henry)

Environmental estrogens are substances (both natural and anthropogenic) that mimic the activity of estrogen and can negatively influence the physiology of aquatic organisms. The goal of this research is to determine if male largemouth bass from two East Tennessee reservoirs express elevated levels of vitellogenin-1 gene transcripts (vtg-1), a biomarker for environmental estrogens. Vtg-1 can be upregulated in male fish when environmental estrogens are present in the water and is an indication that other estrogenic effects may be occurring (e.g., alteration of behavior, development, and reproductive physiology), which in turn can lead to population level effects. High levels of vtg-1 are naturally expressed by female fish, as this gene codes for yolk precursor proteins known as vitellogenins. Male and female largemouth bass (*Micropterus salmoides*) liver and gonad samples were collected from two East Tennessee reservoirs, Fort Loudoun and Norris, and a real-time PCR (qRT PCR) assay was developed to assess the expression of vtg-1 in male largemouth bass across the two reservoirs. Preliminary results indicate that Fort Loudoun males expressed 5-fold greater levels of vtg-1 than males collected from Norris, suggesting that fish from Fort Loudoun are responding to the presence of estrogenic compounds. Further studies will be required to determine whether these compounds have led to reproductive effects in these important game fish.

24. Application of Bioluminescent Yeast-based High Throughput Bioassay for Monitoring Endocrine Active Compounds in Wastewater

Jun Wang, M.L. DiClaudio, A.C. Layton, Gary S. Sayler

Microbiology, Center for Environmental Biotechnology

Bioluminescent *Saccharomyces cerevisiae* strains BLYES, BLYAS, and BLYR were developed to detect the presence of potential environmental estrogenic, androgenic, or toxic compounds, respectively. These strains contain the bioluminescent luxCDABE genes from *Photorhabdus luminescens* and the frp gene from *Vibrio harveyi*, which are expressed on two plasmids either under the control of estrogen response elements (*S. cerevisiae* BLYES), androgen response elements (*S. cerevisiae* BLYAS), or are constitutively expressed for the detection of toxicity (*S. cerevisiae* BLYR). In the presence of estrogenic or androgenic compounds, *S. cerevisiae* BLYES and BLYAS produce bioluminescence, respectively, while *S. cerevisiae* BLYR produces light except in the presence of toxic substances. Bioassay using these bioreporter strains has been demonstrated as a high throughput, inexpensive, and rapid screening system for determining potential endocrine disruptive chemicals (EDCs). Recently, this bioassay system has been applied in monitoring the removal of EDCs in the wastewater treatment process. By using lab-scale bioreactors recruiting activated sludge biomass from Hallsdale-Powell Wastewater Treatment Facility in North Knoxville, continuous fill and draw experiments were performed for 7 days, simulating the wastewater treatment process. Samples collected from bioreactors were concentrated by solid phase extraction and tested by bioluminescence yeast reporters to determine whether estrogenic or androgenic activity changed with treatment. Bioluminescence yeast reporters successfully demonstrated a decrease of total endocrine disruptive activity and toxicity in treated wastewater, proving that yeast-based bioluminescent assays can rapidly and effectively gauge whether wastewater treatment methods have modified or removed endocrine-active or toxic compounds.

25. Effect of Fibrin Inhibitory Peptides on Tumor Growth, Metastasis, and Angiogenesis

Patricia Brown, Bill McCartt, Patricia Coan, John Biggerstaff

Nutrition (Brown), Center for Environmental Biotechnology (McCartt, Biggerstaff), Office of Lab Animal Care (Coan)

Fibrin polymer and soluble fibrin inhibit cellular immunity against melanoma cells in vitro via blockade of tumor CD54/Mac-1 binding, which is reversed by specific fibrin inhibitory peptides (FIP). It was hypothesized that FIP treatment in vivo would enhance the anti-tumor immune response in cancer. To test this, we examined the effect of FIP on tumor growth and metastasis in a syngeneic murine primary tumor and metastasis model. C57BL/6 mice were subcutaneously (primary) or intravenously (metastasis) inoculated with GFP expressing B16 melanoma cells followed by subcutaneous FIP or vehicle every 48 hr for 15 days. Mice were euthanized, and primary tumor size was measured in both groups. Primary tumors and lungs (metastasis) were excised, cryosectioned, and microscopically examined for primary tumor microvessel density (von Willebrand's factor), lung metastases (GFP), and leukocyte infiltration (H&E staining). FIP treatment decreased primary tumor size by 69.2% (0.77 + 0.7 cm3) compared to controls (2.51 + 1.46 cm3, $P < 0.01$), microvessel density was inhibited by 75.9 + 15.1% ($P < 0.05$), and lung metastasis by 47.9 + 26.6% ($P < 0.01$). Conversely, leukocyte infiltration was increased by 11.02 + 1.05% ($P < 0.05$). These results show that FIPs reduce tumor growth, metastasis, and angiogenesis in vivo and increased leukocyte infiltration, suggesting their potential use as adjuvant therapeutic agents in cancer immunotherapy.

26. Precancerous Carcinogenesis of Human Breast Epithelial Cells by Chronic Exposure to 3, 4, 4'-Trichlorocarbanilide

Shilpa Sood, Shambhunath Choudhary, Hwa-Chain Robert Wang

Comparative Medicine (Sood, Choudhary, Wang), Comparative and Experimental Medicine (Sood)

Trichlocarban or 3, 4, 4'-trichlorocarbanilide (TCC) is widely used as an antibacterial agent in household and personal care products, such as bath soaps, detergents, and cleansing lotions. Recently published studies suggest that TCC acts as an endocrine disruptor and enhances the ability of steroid hormone (estrogen or testosterone) to induce estrogen receptor- and androgen receptor-mediated gene expression but has undetectable endocrine activity of its own. Due to its environmental persistence and widespread use, there are concerns about its possible impact on human health. To understand whether TCC exposure may contribute to breast cancer, we used our chronic carcinogenesis model to investigate whether cumulative exposures to TCC may induce progressive transformation of human breast epithelial MCF10A cells. Our investigations revealed that cumulative exposures of MCF10A cells to TCC at the micromolar level resulted in cellular acquisition of cancer-related properties of reduced dependence on growth factors, anchorage independent growth, and increased cell

proliferation. The biological changes were accompanied by upregulation of the ERK pathway and Nox1-induced ROS production that could be suppressed by polyphenolic compounds. Our study indicated that chronic exposure to TCC could contribute to development of breast cancer, and TCC-induced cellular carcinogenesis could be preventable by dietary components.

27. Social Stress Stimulates and GABA Inhibits the Progression of Pancreatic Cancer Xenografts in Nude Mice

Mohammed F. Ullah, Hussein A. Al-Wadei, Joel R. Brody, Hildegard M. Schuller

Pathobiology (Ullah, Al-Wadei, Brody, Schuller), Comparative and Experimental Medicine (Brody)

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with poor prognosis due to limited response to currently available therapeutic regimens. Smoking, diabetes, alcoholic consumption, and pancreatitis are risk factors for PDAC. Stress neurotransmitters noradrenaline and adrenaline are the physiological agonists for β-ARs released from nerve endings and the adrenal medulla in response to nicotinic acetylcholine receptor (nAChR) stimulation by acetylcholine. The neurotransmitter γ-aminobutyric acid (GABA) acts as the physiological inhibitor of β-adrenergic signaling by blocking Gαi-mediated activation of adenylyl cyclase. The present study has tested the hypothesis that social stress stimulates the growth of human PDAC xenografts via nAChR-mediated modulation of adrenaline/ noradrenaline and the associated activation of β-adrenergic signaling. Groups of nude mice were exposed to social stress 4 weeks prior to subcutaneous injection with PDAC cell lines and stress continued for another 4 weeks, while mice not exposed to stress served as controls. Our results show a significant stimulation of xenograft growth by social stress accompanied by elevated levels of stress neurotransmitters cortisol and cAMP. An enhanced expression of nAChR subunits α3, α4, α5, and α7 as well as of β-adrenergic pathway proteins, was found in xenografts of stressed animals. By contrast, the inhibitory neurotransmitter GABA and both GAD enzymes were down regulated in animals exposed to stress. These findings suggest that psychological stress reduction strategies as well as pharmacological inhibition of this PDAC stimulating pathway by beta-blockers or GABA-ergic agents should be considered to improve the responsiveness to cancer therapeutics in PDAC patients. (1RC1CA144640)

28. PPARγ Ligand-induced Apoptosis in Pancreatic Cancer Cells

Kyung Won Min, Seung J Baek

Pathobiology, Comparative and Experimental Medicine

Peroxisome proliferator-activated receptor gamma (PPARγ) is a type II nuclear receptor that functions as a transcription factor regulating cell differentiation, metabolism, and tumorigenesis. MCC-555 is a novel peroxisome proliferator-activated receptor-gamma ligand of the thiazolidinedione class that was recently developed as an anti-diabetic drug with unique properties. Some thiazolidinediones have anticancer activity through growth inhibition and apoptosis in a various cancer cell types. The NSAID-activated gene (NAG-1), a member of the TGF-β superfamily, is involved in tumor progression and development. NAG-1 has pro-apototic and anti-tumorigeneic properties in colorectal and lung cancer as assessed by animal studies; however, its roles in pancreatic cancer have not been studied. We found that MCC-555 induces NAG-1 expression in a dose- and time-dependent manner. NAG-1 expression was not blocked by a PPARγ-specific antagonist GW9662, suggesting that MCC-555-induced NAG-1 expression is not dependent on PPARγ activation. The level of NAG-1 transcripts was also reduced by MCC-555. To elucidate the molecular mechanisms by which MCC-555 exerts the expression of NAG-1 in pancreatic cancer cells, several NAG-1 promoter constructs were used and cis- and trans-acting elements responsible for MCC-555 identified. The regulation of NAG-1 is complex, but MCC-555 seems to exert expression of NAG-1 though the GC box located in NAG-1 promoters in an PPARγ-independent manner.

29. Proliferation of Cell Lines from Pancreatic Ductal Adenocarcinomas and Their Normal Ductal Epithelia are Stimulated by Acute and Chronic Exposure to Nicotine and Ethanol via Modulation in Neurotransmitter Production

Mohammed H. Al-Wadei, Hussein A. Al-Wadei, Hildegard M. Schuller

Comparative and Experimental Medicine (M.H. Al-Wadei), Pathobiology (M.H. Al-Wadei, H.A. Al-Wadei, Schuller)

Adenocarcinoma of the pancreas remains a devastating disease and leading cause of mortality in men and women in western countries due to its chemoresistance to innate or acquired therapies. It is well documented that smoking, diabetes, and pancreatitis from chronic alcohol consumption are risk factors for this type of cancer. More than 95% of pancreatic cancers are pancreatic ductal adenocarcinomas (PDACs) that arise from pancreatic

ductal epithelia. Our lab has previously shown that nicotine stimulates proliferation and migration of human small airway epithelial cells and the adenocarcinomas derived from them in vitro due to nicotine-induced changes in the sensitivity of nicotinic acetylcholine receptors. Such changes caused an increase in production and secretion of excitatory neurotransmitters, but decreased production and secretion of inhibitory neurotransmitter γ-aminobutyric acid (GABA). In analogy to these findings, both nicotine and ethanol stimulated cell proliferation in PDAC cell lines Panc-1 and BXPC-3 and in immortalized pancreatic duct epithelial cells HPDE6-C7. Acute and chronic treatment of all cell lines with nicotine or ethanol alone and in combination significantly increased the production of noradrenaline and adrenaline by the cells while significantly reducing GABA production. Western blots were used to assess the potential modulation of nicotinic receptor protein expression as well signaling pathways. Our findings suggest a potential role for these pathways in the development of smoking and alcohol-related pancreatic carcinogenesis and may result in the identification of novel targets for the prevention and therapy of this malignancy. Supported by R01CA042829, National Cancer Institute.

30. Green Tea Catechins at Non-cytotoxic Levels Suppress Cellular Carcinogenesis Induced by Environmental Carcinogens

Kusum Rathore, Hwa-Chain Robert Wang

Comparative Medicine, Genome Science and Technology

Most sporadic breast cancers are attributable to long-term exposure to environmental factors through a multi-year, multi-step, and multi-path disease process involving cumulative genetic and epigenetic alterations in progressive breast cell carcinogenesis. Epidemiologic and experimental studies have shown that green tea catechins (GTCs) may intervene with breast cancer development. However, the mechanisms for GTC prevention of cellular carcinogenesis induced by chronic exposure to environmental carcinogens remain to be elucidated. To study the activity of GTC components in suppression of cellular carcinogenesis, we used our cellular carcinogenesis model that mimics chronic breast cell carcinogenesis induced by cumulative exposures to low doses of the environmental carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (B[a]P). We detected that the four major GTC components epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) at non-cytotoxic levels were able to suppress NNK- and B[a]P-induced cellular carcinogenesis, as measured by suppression of acquired cancer-associated properties of reduced dependence on growth factors, anchorage-independent growth, and increased cell mobility. We also detected that ECG was more effective than EC, EGC, and EGCG in suppression of cellular carcinogenesis. Investigating the mechanisms for GTCs in counteracting carcinogenic activity of NNK and B[a]P, we detected that all the catechins were able to suppress carcinogen-induced reactive oxygen species (ROS), resulting in suppressing ROS-induced ERK pathway activation, cell proliferation, and DNA damage. Our cellular model provides a new platform to identify dietary components, at non-cytotoxic levels, capable of effectively intervening in chronic breast cell carcinogenesis induced by cumulative exposures to low doses of environmental carcinogens.

31. Sulindac Sulfide Facilitates Novel Cleavage of Epithelial Cell Adhesion Molecule (EpCAM) in Colorectal Cancer Cells

Jason Lee Liggett, Seung Joon Baek

Pathobiology, Comparative and Experimental Medicine

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, which 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 which 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 that sulindac sulfide cleaves a novel site, on the extracellular portion of EpCAM, closer to the N-terminus then 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.

32. Post-translational Modification of Egr-1 by Resveratrol

Nichelle C. Whitlock, Seung Joon Baek

Pathobiology (Whitlock, Baek), Comparative and Experimental Medicine (Whitlock)

Resveratrol, a dietary phytoalexin readily available in the diet, is reported to possess anti-tumorigenic properties in several cancers, including colorectal. However, the underlying mechanism(s) involved is not completely understood. Acetylation of non-histone proteins is demonstrated to be associated with increased DNA binding affinity resulting in the transcriptional activation of target genes. Recently, we showed the involvement of the transcription factor early growth response-1 (Egr-1) in resveratrol-mediated activation of activating transcription factor 3 (ATF3). Egr-1 belongs to a family of immediate early/C2H2 zinc-finger genes that is suggested to act as a master regulatory protein involved in cell fate decisions and coordinate the expression of genes associated with cell proliferation, differentiation, and apoptosis. In the present study, we sought to investigate the effect of resveratrol treatment on Egr-1 posttranslational modification and determine the effect of this modification on Egr-1 activity. Here, we demonstrate the increase in Egr-1 activity, as measured by Egr-1 binding site (EBS) promoter activity, after resveratrol treatment; this increase in activity occurred both at endogenous and exogenous levels. Furthermore, Egr-1 is acetylated in the presence of this dietary phytoalexin, suggesting that this posttranslational modification is important to Egr-1 activity. Taken together, these results provide the first evidence that increased Egr-1 activity by resveratrol is mediated, at least partially, through the acetylation of this transcription factor.

33. Effects of Branched-chain Amino Acids on Mitochondrial Metabolism and Cell Cycle in Cancer Cells

Tia Filhiol*, **Megan Johnstone***, Patricia Brown, Antje Bruckbauer, Michael Zemel

*Presenters

Nutrition

The Warburg Effect is a theory that classifies most cancer cells as having a metabolic shift, or a switch from mitochondrial oxidative phosphorylation to glycolysis. Recent data from the Zemel lab demonstrates that the branched-chain amino acid leucine has a unique signaling role for mitochondrial biogenesis, fatty acid oxidation (FAO), and p53 in murine muscle cells and adipocytes. Specifically, leucine acts in the mitochondria as a switch from glycolytic metabolism to oxidative metabolism in these cells. p53 has tumor suppressive and metabolic functions such as promotion of oxidative phosphorylation, FAO, and inhibition of glycolysis. The objective of this study is to determine whether leucine stimulates mitochondrial biogenesis and FAO in the melanoma cancer cell line A375, and to determine the tumor suppressive effects of leucine in A375 cells in vitro. Leucine-treated A375 cells upregulated gene expression of p53 and its downstream target p21 by 60% and 43% ($P < 0.05$) when compared to the control at 2 hr. Subsequent evidence for cell cycle arrest was demonstrated by 30% and 40% ($P < 0.05$) down regulation of cyclin D within 4 and 24 hr of treatments. At 7 hr, flow cytometry showed that leucine caused a 21% decrease in cell proliferation. p53 gene expression was upregulated at 12 hr (48%, $P < 0.05$). These data provide evidence that leucine modulation of energetics may reverse the Warburg Effect. Increased expression of p53 and downstream targets p21 and cyclin D inhibit cell cycle progression and results in an antiproliferative effect in this tumor cell line.

34. Effect of Adiposity and Inflammatory Mediators on Breast Cancer

Nalin Siriwardhana, Rhett Layman, Shiwani Patel, Blair Tage, Ayub Karwandyar, Matthew Clark, Jessica Lampley, Courtney Rhody, Erica Smith, Arnold Saxton, Naima Moustaid-Moussa, Jay Wimalasena

Animal Science (Siriwardhana, Saxton, Moustaid-Moussa), Obstetrics and Gynecology, University of Tennessee Medical Center (Siriwardhana, Layman, Patel, Tage, Karwandyar, Clark, Lampley, Rhody, Smith, Wimalasena)

Our preliminary data suggested that inflammatory cytokines secreted from obese women's adipocytes have profound effects on breast cancer cell growth and invasion. On the other hand, we observed that ω-6 arachidonic acid (AA) increased inflammatory cytokine secretion from adipocytes while ω-3 eicosapentaenoic acid (EPA) either reduced or counteracted this inflammatory cytokine secretion. Thus, our objective was to compare the effect of AA and EPA on breast cancer progression in relation to inflammatory and obesity status. We treated breast cells (non cancerous; MCF10A, cancerous; MCF7 and invasive; MDA231) and adipocyte (adipocytes from lean and obese women) 3D co-cultures with AA, EPA, and AA+EPA to assay the effects on breast cell growth and morphology of acini-like spheroids. Treatment with AA altered the regular acini-like spheroid morphology toward irregular cell structures, but the effect was not comparable to the highly irregular cellular structures formed by MCF7 or MDA MB 231. Further, when AA and EPA pretreated conditioned media

was transferred into growing breast cells, AA treatment showed higher cell growth effects compared to EPA. Further, we observed that pro-inflammatory cytokines significantly alter the mTOR/AMPK-related metabolic cell signaling in cancer cells. Our data suggest fatty acids alter cancer cell metabolism-related signaling cascades via pro-inflammatory cytokines.

35. Early Prediction of Response to Chemotherapy in Non-small Cell Lung Cancer Patients Using 18F-FDG PET/CT

Richard Laine, Karen Wells, Robert Heidel, Josh Schaeferkotter, Misty J. Long, Wahid Hanna, Karl Hubner

Nuclear Medicine (Laine), Radiology (Wells, Hubner), Graduate School of Medicine (Heidel), Medicine (Hanna), Molecular Imaging Translational Research Program (Schaeferkotter, Long)

18F-FDG PET/CT performed 8 and 36 days after initiation of chemotherapy may be predictive of non-small cell lung cancer (NSCLC) tumor metabolic response and length of patient survival. We studied 36 patients with newly-diagnosed NSCLC initiating platinum-based chemotherapy. Each patient was studied at 8, 22, and 36 days after initiation of chemotherapy, and primary lung tumor tissue activity assessed by 18F-FDG PET/CT. All patients exhibited a primary lung tumor with a standard uptake value (SUV) of greater than 2 on 18F-FDG-PET as inclusion criteria. In a prospective analysis, we evaluated the time course of mean metabolic activity in the primary tumor. Metabolic response was defined as a 20% or greater reduction in tumor SUV between days 8 and 36. Kaplan-Meier, independent samples *t* test, and Breslow's analysis were performed to assess differences in survival time between metabolic responders and non-responders. Thirty-five of 36 patients completed all three 18F-FDG PET studies, with one patient completing only days 8 and 36. Median clinical follow-up was 491 days (range, 102–1553 days). Twenty patients were metabolic responders, with a mean survival of 756 days. Sixteen patients were non-responders, with a mean survival of 467 days. Metabolic responders lived significantly longer than metabolic non-responders, $P = 0.05$. Kaplan-Meier analysis demonstrated a significant difference in survival time between responders and non-responders, $P = 0.016$. In patients with NSCLC, tumor metabolic response on 18F-FDG PET/CT performed 8 and 36 days post chemotherapy was a significant predictor of survival time.

36. Transient Ischemic Dilation in Adenosine PET-CT Myocardial Perfusion Scintigraphy

Alireza Mojtahedi, Karen Wells, Myrwood Besozzi, Yitong Fu, George Chacko

Radiology, Nuclear Medicine (Mojtahedi, Wells, Fu, Chacko), Medicine (Wells), Cardiology (Besozzi)

Using 82Rb MPS data, we aim to derive normal values for TID and establish an abnormal TID threshold. Charts reviewed for patients with MPS between Jan 2007–Aug 2010 retrospectively. Normal values were defined by the mean TID from (group 0) 11 patients with normal adenosine 82Rb MPS, age less than 40, and no risk factors for CAD. Mean TID from group 0 was then compared to TID from 46 patients who underwent coronary angiography within 6 months of index MPS (prior coronary stent or CABG excluded). Based on angiography, these patients were further divided into two subgroups: (Group 1) 39 patients with no significant CAD < 70% stenosis, and (Group 2) seven patients with significant CAD > 70% stenosis in at least one vessel. The mean of TID value for group 0 was 0.96 +/- 0.13, versus 1.05 +/-0.14 for patients for group 1 and 1.17 +/-0.24 for group 2. An ANOVA proved significant the overall main effect between the means in different groups (0.031). Tukey analysis demonstrated significant difference between the means in groups 0 and 2 (0.023). Receiver-operating-characteristic curve lacked statistical power to determine a TID threshold for significant CAD. There was an observational upward trend between 82Rb MPS TID values and severity of CAD. Mean 82Rb MPS TID for patients with a low risk of CAD was 0.96 +/-0.13. Comparison to TID values in patients with significant CAD demonstrated an observational upward trend. Our data supports further study of 82Rb MPS TID thresholds predictive of CAD.

37. The Functionality of Microchips in Post-Magnetic Resonance Imaging

Katherine Haifley, Silke Hecht

Small Animal Clinical Sciences – Radiology

Our objective was to determine the functionality of microchips post-magnetic resonance imaging (MRI). This prospective clinical study included 53 client-owned clinical patients implanted with a microchip, presenting for MRI of different areas of the body, for a variety of medical conditions. General anesthesia was induced, and the patient's microchip was scanned using a Home Again universal microchip scanner; the chip number was recorded. The patient was transported to the MRI suite and the MRI was completed. The patient was moved

out of the magnetic environment, and the microchip was scanned again. The patient information and chip number were recorded. The chip numbers pre- and post-MRI were compared. All 53 microchips (26 Home Again chips and 27 AVID microchips) scanned from 53 clinical patients read the same number accurately post MRI of a variety of sites. The data indicates that MRI did not interfere with the functionality of these microchips. This information is valuable for practitioners recommending MRI for their patients, and for clients who have invested in micro-chipping their pets.

38. COX-2-expressing Canine Transitional Cell Carcinoma Detected by Novel Imaging Tracer, Fluorocoxib

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

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

Identification of biomarkers for bladder cancer surveillance and screening is essential for early detection and for improved prognosis and long-term survival. In this study, we evaluated a newly synthesized optical imaging tracer, fluorocoxib, for detection of spontaneously-occurring canine urinary bladder tumors expressing cyclooxygenase-2 (COX-2) enzyme. The newly-synthesized, rhodamine-conjugated analog of indomethacin is a non-steroidal anti-inflammatory drug that selectively targets COX-2 in solid tumors. In this study, we isolated and characterized primary canine transitional cell carcinomas (K9TCC) from biopsy samples obtained during diagnostic (cystoscopy) procedures. Established K9TCC with confirmed COX-2 expression were assessed for tumorigenesis in female nude athymic mice. All tested nude mice (n=9, 100%) developed subcutaneous tumors. Histological analysis revealed that dissected K9TCC xenografts stained with hematoxylin-eosin were partially encapsulated with a solid pattern, partial central necrosis, and with a moderate number of mitotic figures. We assessed the COX-2, E-cadherin staining in K9TCC xenografts using IHC analysis. To evaluate the ability of fluorocoxib to target COX-2 in K9TCC xenografts, four mice were injected with fluorocoxib (1 mg/kg, i.v.) and imaged 2 hr after under anesthesia using the Xenogen IVIS 200 imaging system. Specificity and selective uptake of fluorocoxib by K9TCC was confirmed by comparing the total flux in normal and tumor tissues dissected from mice after euthanasia. The K9TCC tumors had up to 10-fold higher uptake of fluorocoxib compared to normal tissue and confirmed detection of COX-2 positive K9TCC tumors by fluorocoxib. Dogs with naturally-occurring tumors provide important information for preclinical trials in human cancer imaging.

39. Magnetic Resonance Imaging of Canine Mast Cell Tumors

Esteban Pokorny, Silke Hecht, Patricia Sura, Amy LeBlanc, Jeff Phillips

Small Animal Clinical Sciences

Mast cell tumors (MCT) are the most common cutaneous tumors in dogs. The purpose of this study was to describe MR imaging characteristics of (sub)cutaneous MCT and metastatic lymph nodes. Eight dogs were included in the study. MR imaging was performed using a 1T magnet (MAGNETOM Harmony, Siemens). Imaging characteristics of MCT and lymph nodes were described. Metastatic lymph nodes were compared to normal sentinel and contralateral lymph nodes. Lymph node pairs without biopsy of sentinel lymph nodes were excluded from evaluation. Nine MCT were identified in eight dogs. On T2-w sequences, 7/9 MCT were hyperintense, and 2/9 were isointense to adjacent musculature. On T1-w sequences, 5/9 MCT were isointense, and 4/9 were mildly hyperintense. 4/4 MCT were markedly hyperintense on STIR. All MCT were strongly contrast enhancing (5/9 homogeneous and 4/9 heterogeneous). Six lymph node pairs were included in the evaluation. There were five sentinel lymph nodes with metastases and one without. All lymph nodes were T1 isointense. 5/5 metastatic and 1/7 normal lymph nodes were heterogeneously T2 hyperintense. Following contrast administration, all lymph nodes were contrast enhancing. 4/5 metastatic and 1/7 normal lymph nodes had heterogeneous enhancement patterns. Heterogeneous T2 hyperintensity and heterogeneous contrast enhancement are more common in lymph nodes with metastatic MCT than in normal lymph nodes.

40. Measurement of Immunologic Response in Horses and Dogs Vaccinated with Xenogenic Plasmid DNA Encoding Human Tyrosinase

Luis M. Lembcke, Stephen A. Kania, James T. Blackford, Dianne J. Trent, Agricola Odoi, Jeffrey C. Phillips

Comparative Medicine (Kania, Trent, Odoi), Large Animal Clinical Sciences (Blackford), Small Animal Clinical Sciences, Comparative & Experimental Medicine (Lembcke, Phillips)

Xenogenic plasmid DNA constructs have been developed and optimized for immunotherapies targeting cancer in both humans and dogs. Specifically, plasmid vectors containing the tumor antigen tyrosinase have

demonstrated immunoreactivity and clinical benefit in the treatment of melanocytic tumors in these species. Overexpression of the tyrosinase antigen has also been noted in equine melanocytic tumors, supporting its role as a valid tumor antigen in the horse. Vaccination with plasmid constructs containing tyrosinase may thus have translational immunoreactivity in the treatment of equine melanomas. In this study, we evaluated the humoral and cell-mediated response in five horses vaccinated with xenogenic plasmid encoding human tyrosinase. For comparison, the immunologic response of three normal dogs to vaccination was evaluated. Vaccination was effective in generating detectable and long-lasting immunoresponses in all patients. No adverse reactions or signs of autoimmunity were detected. DNA vaccination against proteins preferentially expressed by tumors is a strategy for cancer therapy. Tyrosinase is a protein that has been targeted for the adjunct therapy of melanocytic tumors. Here we describe a methodology that is highly sensitive and specific for the detection of both humoral and cell-mediated immunoreactivity against tyrosinase in equine and canine patients.

41. Inflammatory Responses to Intravenous Lipopolysaccharide Infusion in Horses with Equine Metabolic Syndrome

Lisa Tadros, Nicholas Frank

Large Animal Clinical Sciences, Comparative and Experimental Medicine

Humans that suffer from obesity show exaggerated inflammatory responses, and this may be relevant to the association between increased adiposity and laminitis in horses with Equine Metabolic Syndrome (EMS). This study was performed to test the hypothesis that inflammatory responses to endotoxemia differ between healthy horses and those affected by EMS. Six healthy adult mares and six horses with EMS received an intravenous infusion of lipopolysaccharide (LPS; 20 ng/kg in 60 mL sterile saline) or saline alone. A crossover design was employed with a 7-day washout period. Physical examinations were performed hourly for 9 hr and whole blood was collected at 30, 60, 90, 120, 180, and 240 min for assessment of inflammatory cytokine gene expression. A liver biopsy was performed between 240 and 360 min post-infusion. Data were analyzed using a mixed model ANOVA. Mean rectal temperature, heart rate, and respiratory rate increased following LPS infusion (treatment × time; $P < 0.001$), with higher heart (group × treatment; $P = 0.087$) and respiratory rates (group; $P = 0.017$) detected in EMS horses. Lipopolysaccharide infusion significantly increased whole blood gene expression of tumor necrosis factor α (TNFα), interleukin (IL)-1β ($P < 0.001$), IL-6 ($P < 0.001$), IL-8 ($P < 0.001$), and IL-10 ($P = 0.002$), and hepatic gene expression of IL-6 ($P < 0.001$), IL-8 ($P < 0.001$), and IL-10 ($P = 0.016$). Inflammatory gene expression did not differ significantly between groups, so our hypothesis was not supported. Heart rates tended to be higher when LPS was administered to horses with EMS.

42. Characterization of Bone Marrow-derived Adult Equine Mesenchymal Stem Cells (EMSCs) and their Potential for Therapeutic Application in an In Vitro Model of Corneal Healing

Liesl C. Breickner, Kim M. Newkirk, Diane V.H. Hendrix, Nancy R. Neilsen, Madhu S. Dhar

Large Animal Clinical Sciences (Breickner, Dhar), Comparative and Experimental Medicine (Breickner), Pathobiology (Newkirk, Neilsen), Small Animal Clinical Sciences (Hendrix)

There has been much investigation in the isolation, growth, and potential therapeutic applications of adult equine mesenchymal stem cells (EMSCs). This investigation had two objectives: to characterize bone marrow-derived adult EMSCs and to develop a novel in vitro equine model of corneal wound healing. For the first objective, previously cryopreserved EMSCs from two research horses were culture-expanded. EMSCs stained positive for alkaline phosphatase (AP) and demonstrated expression of embryonic stem cell markers Oct-4, SSEA-4, TRA-60, and TRA-81 via immunofluorescence. Immunophenotyping with flow cytometry revealed surface expression of CD44 and CD90. Multipotency was demonstrated by trilineage differentiation into adipocytes, osteocytes, and chondrocytes. For the corneal model, normal equine corneas were sterilely harvested post-mortem from 18 horses (donation and research horses euthanized for purposes other than this project). Prior to removal, each cornea was divided into four quadrants, and a trephine was used to create wounds. Sections were cultured with and without EMSCs in DMEM-F12 +10% FBS +1% P/S with 0.1 ug/mL cholera toxin in six well plates layered with 1% agarose. On days 2, 4, and 5, samples were fixed and submitted for histopathologic evaluation of wound healing. Data analyses are pending; however, the creation and optimization of this model will permit continued investigation of this EMSC application.

43. 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

Large Animal Clinical Sciences (Videla, Sommardahl, Elliot), Equine Health Studies Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University (Andrews), Randlab Australia Pty Ltd, Australia (Vasili)

Gastric ulcers are common in horses, and treatment of horses that cannot be administered oral medication can be problematic. The objective of this study was to evaluate the efficacy of esomeprazole sodium administered intravenously on gastric juice pH and gastric ulcer scores in horses. Twelve adult female Quarter horses were used. Esomeprazole sodium (0.5 mg/kg IV) was administered once daily to eight horses (treatment group), and saline (5 mL 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. Our results indicated that gastric juice pH was higher in esomeprazole sodium-treated horses after treatment, when compared with values before treatment (6.43 ± 1.18 versus 4.25 ± 2.39, respectively; $P = 0.002$). Also, gastric juice pH was higher ($P = 0.001$) in esomeprazole sodium-treated horses compared with saline-treated control horses on day 5 and on day 14. 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.

44. Effect of Electroejaculation on Plasma Concentrations of Cortisol and Substance P in Beef Bulls

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Large Animal Clinical Sciences (Whitlock), Veterinary Clinical Sciences, The Ohio State University (Coffman), Clinical Sciences, College of Veterinary Medicine, Kansas State University (Coetzee), Animal Science, Berry College, Mt. Berry, GA (Daniel)

Evaluating a semen sample is an integral part of the bull breeding soundness examination. Electroejaculation is a method of obtaining a semen sample but is considered by some to be inhumane on the grounds that it is painful. Numerous techniques have been used to evaluate pain associated with electroejaculation, and all have their strengths and weaknesses. Substance P is involved in the integration of pain, stress, and anxiety. Measurement of plasma concentrations of substance P may enable discrimination between a stressful event and nociception stimuli. We hypothesized that substance P is a more specific measure of pain in bulls following electroejaculation. The objective of this study was to compare plasma concentrations of cortisol and substance P in bulls following electroejaculation. Blood was collected from nine bulls at -60, -30, 0, 2, 10, 20, 30, 45, 60, 75, 90, and 120 min relative to treatment. Treatments (no manipulation, rectal probe insertion, or electroejaculation) were repeated weekly until each bull had received each treatment. Plasma cortisol concentration following electroejaculation (40.64 ± 6.69 nmol/L) was significantly higher than concentrations in probed (25.58 ± 6.69 nmol/L) and control bulls (20.59 ± 6.69 nmol/L). However, plasma substance P concentration following electroejaculation (4.85 ± 0.20 pg/ml) was not different from probed (4.68 ± 0.20 pg/mL) or control bulls (4.95 ± 0.20 pg/mL). An increase in plasma cortisol concentrations in bulls following electroejaculation suggested a likely association with acute stress. Similar plasma concentrations of substance P after electroejaculation suggested a lack of pain associated with nociception.

45. Treatment Outcome of Dogs with Methicillin-resistant and Methicillin-susceptible *Staphylococcus pseudintermedius* Pyoderma

Jacqueline Bryan, Linda Frank, Laura Burgette, Christine Cain

Small Animal Clinical Sciences

Staphylococcus pseudintermedius is the most common causative organism associated with canine pyoderma. Methicillin-resistant (MRSP) strains are increasingly more prevalent, and management of these infections is often challenging. The aim of this study was to investigate the treatment outcome as defined by clinical resolution and treatment duration, and medication side effects of dogs with pyoderma caused by methicillin-susceptible *S. pseudintermedius* (MSSP) and MRSP between January 2008 and April 2010. Medical records were reviewed for antimicrobial therapy, treatment outcome, and side effects. A total of 219 cases were included (125 [57.1%] MSSP, 94 [42.9%] MRSP). In MSSP infections, cephalexin (20.4–39.0 mg/kg BID) and cefpodoxime (6.0–13.2 mg/kg QD) were the most commonly prescribed antimicrobials, accounting for 55 (44.0%) and 44 (35.2%) of cases, respectively. In MRSP

infections, chloramphenicol (30.5–61.9 mg/kg TID) and doxycycline (4.6–11.1 mg/kg BID) were most commonly prescribed, accounting for 53 (56.4%) and 13 (13.8%) of cases, respectively. Adverse effects were reported in 41(18.7%) cases (seven MSSP, 34 MRSP). The most common adverse effects were gastrointestinal, prompting antibiotic discontinuation in 22/41 cases (three MSSP, 19 MRSP). Chloramphenicol was associated with the highest incidence of adverse reactions (29/53 patients). Of 162 cases with follow-up, 46/86 (53.5%) MSSP infections and 28/76 (36.8%) MRSP infections achieved complete clinical resolution at the first recheck examination, 3 to 4 weeks following initial evaluation. All but two cases of MSSP and seven cases of MRSP improved or resolved on subsequent visits. This study demonstrates resistant infections may require additional time to resolve and that chloramphenicol is associated with frequent side effects.

46. Agreement Between The T2 and HASTE (Half-Fourier-acquisition Single-shot Turbo Spin-echo) Sequences in the Evaluation of Intervertebral Disc Disease in Dogs

Joseph M. Mankin, Silke Hecht, William B. Thomas

Small Animal Clinical Sciences

Magnetic resonance imaging (MRI) is commonly used when evaluating patients with intervertebral disc disease (IVDD). Heavily T2 weighted techniques such as HASTE (half-Fourier-acquisition single-shot turbo spin-echo) have proven useful by using the high signal obtained from cerebrospinal fluid. The purpose of this study was to compare HASTE and T2-weighted sequences in dogs with IVDD. MRI studies in 60 dogs (767 individual intervertebral disc spaces) were evaluated. Agreement between T2-W and HASTE sequences was assessed for three criteria: presence of an extradural lesion, severity of compression, and treatment recommendation. There was moderate agreement between T2-W and HASTE sequence as to presence of an extradural lesion ($\kappa = 0.575$). HASTE was in agreement in 96.1% of the cases where no extradural lesion was identified on T2-W images, but only in 58.1% of the cases where extradural lesions were identified on T2-W images. There was fair agreement between T2-W and HASTE sequence as to severity of compression of an extradural lesion ($\kappa = 0.381$). There was moderate agreement between T2-W and HASTE sequence as to treatment recommendations ($\kappa = 0.476$). HASTE was in agreement in 98.4% of the cases where a lesion was considered non-surgical on T2 but only 82.1% of the cases where a lesion was considered surgical on T2. In 1.0% of cases considered not surgical and in 9.8% of cases considered equivocal based on T2, a surgical lesion was identified on HASTE. Acquisition of a HASTE sequence in addition to conventional sequences when evaluating the canine spine is recommended.

47. The Effect of Nitrous Oxide on Isoflurane Minimum Alveolar Concentration (MAC) and MAC Derivatives in Dogs

Debra Voulgaris, Christine M. Egger, Reza Seddighi, Barton W. Rohrbach, Lydia Love, Thomas J. Doherty

Small Animal Clinical Sciences (Voulgaris, Egger), Large Animal Clinical Sciences (Seddighi, Love, Doherty), Comparative Medicine (Rohrbach)

Our objective was to investigate the effects of 70% nitrous oxide (N2O) on the isoflurane (ISO) MAC (I-MAC), MAC-no movement (I-MAC NM), and MAC-blocking autonomic responses (I-MAC BAR) in dogs. The study included six adult, healthy, mixed breed, intact male dogs. Anesthesia was induced with ISO delivered via mask. I-MAC, I-MAC NM, and I-MAC BAR were determined for each dog using a supramaximal electrical stimulus (50 V, 50 Hz, 10 milliseconds). Nitrous oxide (70%) was then administered, and MAC and its derivatives (I-N2O-MAC, I-N2O-MACNM and I-N2O-MACBAR) were determined using the same methodology. Baseline and treatment MAC, MACNM, and MACBAR values were compared. I-MAC, I-MAC NM, and I-MAC BAR values were 1.39±0.14, 1.59±0.10, and 1.72±0.16, respectively. The addition of 70% N2O significantly decreased I-MAC, I-MAC NM, and I-MAC BAR by 32% ($P = 0.0007$), 15% ($P = 0.0026$), and 25% ($P = 0.0053$), respectively. The percent change in I-MAC BAR was not different than the percent change in I-MAC ($P = 0.07$); however, the percent change in I-MAC NM was significantly different than the percent change in I-MAC ($P = 0.0003$) and the percent change in I-MAC BAR ($P = 0.01$). The addition of 70% N2O to ISO-anesthetized dogs resulted in a significant decrease in MAC, MAC NM, and MAC BAR

48. Effect of Local Anesthetic on Microorganisms in a Murine Model of Surgical Site Infection

Valerie Sams, Christy Lawson, Patricia Coan, David Bemis, Kim Newkirk, Michael Karlstad, Jamison Norwood, Brian Daley

Surgery (Sams, Lawson, Karlstad, Norwood, Daley), Office of Laboratory Animal Care (Coan), Comparative Medicine (Bemis), Pathobiology (Newkirk)

Surgical site infections remain a concern for surgeons, with incidence approximately 1.5–5% for all types of surgery. Known factors to contribute to infections include anesthetic use, perioperative antibiotics, and perioperative fluid management. Many in vitro studies

have suggested an antimicrobial effect of local anesthetic. We hypothesized that subcutaneous infiltration of local anesthetic before a surgical incision is made reduces the incidence of postoperative wound infections. We developed a murine model to demonstrate this effect. In a wound infection model using mice, *S. aureus* and *E. coli* were inoculated in clean surgical wounds both infiltrated and non-infiltrated with local anesthetic. Both arms had four groups: non-inoculated, lidocaine and inoculated, lidocaine/marcaine mix and inoculated, and saline and inoculated. Cultures and tissue were taken from all animals and compared based on colony counts, degree of inflammation, and presence of bacteria. Each group inoculated with *S. aureus* consisted of six mice each, and those inoculated with *E. coli* consisted of 6–8 mice each. In the *S. aureus* arm of the study, all inoculated groups had similar findings of colony counts, bacteria, and degree of inflammation regardless of use of infiltrated local anesthetic or saline. In the *E. coli* arm of the study, very little organism was isolated; however, commensal coagulase negative gram-positive cocci did flourish regardless of infiltration of anesthetic or saline without a correlation between degree of inflammation and presence of bacteria. The subcutaneous infiltration of local anesthetic before a surgical incision is made does not reduce the incidence of postoperative wound infection.

49. Modulation of Tumor Formation by Chemopreventive Compounds in Animal Models of Colorectal Cancer

Raphael L. Richardson, Seong-Ho Lee, Agricola Odoi, Seung Joon Baek

Pathobiology (Richardson, Lee, Baek), Comparative and Experimental Medicine (Richardson), Comparative Medicine (Odoi)

Capsaicin is a pungent ingredient in red chili peppers and has been linked to suppression of growth in various cancer cells. We previously showed that capsaicin induces growth arrest and apoptosis of cancer cells by altering β-catenin signaling in human colorectal cancer cells in vitro. 3-3′-Diindolylmethane (DIM) is an ingredient in Brassica vegetables derived from the digestion of indole-3-carbinol. DIM has been shown to have potent anti-cancer properties in various cancers, including colon cancer. In our study, we are investigating the effects of chemopreventive agents in vivo using colon cancer animal models. NAG-1 has been shown to increase with the treatment of chemopreventive/anti-tumorigenic agents including capsaicin. In the carcinogen-induced mice model, NAG-1 knockout mice and wild type mice with a C57BL/6 genetic background are used. The hypothesis was that NAG-1 knockout mice have an increased aberrant crypt foci (ACF) formation compared to the wild type counterpart. Aberrant crypt foci are clusters of apoptotic-resistant, irregular “tube-like” glands in the colon and rectum lining that form before colorectal polyps. The mice treated with chemopreventive agents are expected to have less ACF formation compared to vehicle-treated groups in wild type mice. As an alternative, we are using the genetic model to determine whether chemopreventive agents affect tumor formation in APC KO mice models.

50. (-)-Epigallocatechin Gallate Facilitate the Proteasomal Degradation of Cyclin D1 in Colon Cancer Cells

Xiaobo Zhang, Seung Joon Baek

Pathobiology

Green tea catechins have been well known to have anticarcinogenic effects. Among them, (-)-epigallocatechin gallate (EGCG), the major component of green tea catechins, could play an important role for this effect, based on previous reports. However, the involved mechanism(s) needs further investigation. We previously reported that EGCG can rapidly induce basic fibroblast growth factor (bFGF) degradation in colorectal cancer cells, thereby suppressing tumor formation in APCMin/+ mice. In the present study, we investigated the effect of EGCG on protein expression related to cell cycle in SW480 human colorectal cancer cells. We found that cylin D1 and D3, but not cyclin E, were down-regulated, while p21 was up-regulated in the presence of EGCG. Since the over-expression of cyclin D1 facilitates tumor progression and metastasis, we next studied the mechanism by which EGCG decreased cyclin D1 in dose- and time-dependent manner. Interestingly, EGCG induced down-regulation of cyclin D1 by facilitating proteasomal degradation, not decreasing mRNA expression nor promoter activity of cyclin D1. Furthermore, we found the degradation is independent of cyclin D1 phosphorylation on threonine-286, which was well known for its rapid degradation. Taken together, our data showed a novel mechanism involved in cyclin D1 degradation in the presence of EGCG.

51. Characterization of Nudix Type Hydrolase 6 (NUDT6) and Its Potential Role in DNA Repair

Dmitriy Smolensky, Seung Joon Baek

UT-ORNL Graduate School of Genome Science and Technology (Smolensky), Pathobiology (Baek)

Our lab has reported that Nudix Type Hydrolase 6 (NUDT6) is downregulated at the transcriptional level by green tea catechins. Some data indicate that NUDT6 may

have potential proliferative effects on cancer cells, and thus the exact biological activity need to be determined. While it has a distinct Nudix hydrolase motif, which is known to hydrolyze very specific di- and tri- phosphate substrates, the exact function of NUDT6 has not yet been elucidated. NUDT6 also contains a mitochondrial targeting sequence (MTS), which indicates that it localizes in the mitochondrion and nucleus. We hypothesize that NUDT6 affects the DNA repair mechanism in the mitochondrion. We developed stable over-expression cell lines to study its localization and function. We are investigating the effects of NUDTS on both nuclear and mitochondrial DNA repair using a novel quantitative PCR technique. We also have data to indicate that NUT6 protein levels may be controlled at the post-translational level when cancer cells undergo induced apoptosis by non-steroidal anti-inflammatory drugs (NSAIDS) such as sulindac sulfide. The possible roll of NUDT6 in proliferative pathways and DNA repair provide a new molecular target in cancer and other human diseases, resulting from the DNA repair system.

52. *Heteropterys aphrodisiaca* Tea Therapy Causes Reduction in Prostate Weight in the Absence of Reducing Circulating Testosterone Levels and Testicular Testosterone Secretory Capacity In Vitro

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Comparative Medicine (Sashi Papu John, Gomes, Fecteau, Mendis-Handagama), Animal Science (Grizzle)

Heteropterys aphrodisiaca (HA) is a native Brazilian plant. HA-tea is used to treat nervous and muscular disease. Also, HA-tea infusions in adult rats result in increasing the weights of seminal vesicles, which are testosterone-dependent organs. The prostate is also a testosterone-dependent organ, which increases in size with age and causes prostate disease. We tested the effects of daily HA-tea treatment on prostate weights and testosterone levels in males, using rats, with the hypothesis that the prostate will respond similarly. Sixty-day old control Sprague Dawley rats (n = 6, puberty in rats occurs at 56 days) and tea-treated rats (n = 5) were gavaged daily with either water or tea, respectively for 60 days (duration of rat spermatogenic cycle is 56 days). Blood, testes, and prostates were analyzed. Testis weights, plasma testosterone levels, luteinizing hormone-stimulated testosterone secretion in vitro per testis and per gram of testis were not statistically different ($P < 0.05$). However, the prostate weights were lower in tea-treated rats ($P > 0.01$) than in controls. Findings suggested that daily treatment of HA-tea produced reduction in prostate weights without a decrease in circulating testosterone levels and testicular testosterone producing potential. In conclusion, daily HA-tea treatment may be used as a potential therapy to prevent prostate disease that occurs in males with age advancement.

53. The Contribution of Regional Adiposity to Obesity-associated Female Infertility via Differential Protein Hormone Secretion

Ben Ernest, Brynn Voy

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Nearly 30 million women of reproductive age in the United States are obese, and as such, are three times more likely to suffer from infertility than lean women. Assisted reproduction technology costs $3,000–60,000 from diagnoses and treatment depending on whether in vitro fertilization is required. For these reasons, there is high demand for improved understanding and therapy to counteract obesity-associated infertility. Obesity is associated with changes in the profile of proteins secreted from adipose tissue (adipokines), as well as increased insulin resistance and inflammation peripherally and in the ovaries. Because adipokine profiles vary depending on adipose tissue mass and site of deposition, we are considering the possibility that obesity-induced changes in the adipokine profile significantly compromise fertility. Further, we are considering whether adipose tissue near the ovary more drastically compromises fertility compared to subcutaneous and peri-renal adipose tissue. Using a well-established model of diet-induced obesity—the C57BL/6J mouse line fed a diet high in fat—we are testing the hypothesis that expanded peri-ovarian fat in obesity secretes a unique adipokine profile that, compared to other fat depots, more drastically compromises ovarian function. We present our initial comparison of the gene expression profiles between peri-ovarian fat and other fat depots, as well as our short-term and long-term research aims and the experimental approaches we will use to test our hypothesis.

54. Regulation of the Inducible Nitric Oxide Synthase Gene (iNOS) by Cytokines Requires Phosphorylation of the p65 Subunit of NF-kappaB at Ser 276 and Ser536 and Signal Transducer and Activator of Transcription 1 (STAT1) at Tyr701

Susan Burke, Danhong Lu, Rachel Bellich, Jamison Norwood, Michael Karlstad, Patricia Brown, Steve Minkin, John Biggerstaff, Jason Collier

Nutrition (Burke, Bellich, Collier), Surgery (Norwood, Karlstad), Biological Imaging Unit (Brown, Minkin, Biggerstaff), Stedman Nutrition Center (Lu)

The pro-inflammatory cytokines IL-1beta and gamma-IFN decrease functional islet beta-cell mass by upregulating specific genes that impair function and diminish viability. However, the mechanisms controlling expression of the iNOS gene in response to cytokines are not completely understood. Therefore, we tested the hypothesis that cytokine-mediated control of the iNOS gene requires p65 and STAT1. In 832/13 rat insulinoma cells, 6-hr exposure to 1 ng/mL IL-1beta increased iNOS mRNA levels approximately 100,000-fold, an effect that is potentiated 5-fold by 100 U/mL gamma-IFN. siRNA-mediated suppression of p65 (75%) or STAT1 (91%) blocked the cytokine-mediated induction of iNOS mRNA levels by 71% and eliminated the potentiation by gamma-IFN, respectively. A promoter luciferase construct was induced approximately 100-fold by 6-hr exposure to IL-1beta; this induction is also potentiated 2–3 fold by gamma-IFN, and was blunted 92% and 66% by siRNA duplexes targeting p65 and STAT1, respectively. IL-1beta induced rapid phosphorylation (within 15 min) of p65 at Ser276 and Ser536, which correlated with p65 nuclear translocation (measured via immunofluorescence), while gamma-IFN induced phosphorylation of STAT1 at Tyr701. Overexpression of S276A or S536A p65 mutant constructs, which retain nuclear localization capability, impaired the ability of p65 to drive expression of either a generic NF-kappaB responsive promoter or iNOS gene promoter in both the basal state and in response to IL-1beta. Moreover, overexpression of Y701F STAT1 eliminates the gamma-IFN-mediated potentiation of the iNOS gene. Taken together, we conclude that specific phosphoacceptor sites within p65 and STAT1 are required for maximal induction of the iNOS gene in response to cytokines.

55. Regulation of Monocyte Chemotactic Protein 1 Gene Expression by Activation of Nucleotide Oligomerization Domain Containing Protein 1 in Adipose Tissue Inflammation and Obesity

Pan Hu, Ling Zhao

Nutrition

Obesity is now recognized to be associated with chronic inflammation in adipose tissue, which leads to insulin resistance and a series of metabolic disorders. This heightened state of inflammation is thought to start from expression and secretion of chemokines and adipokines by adipose tissue, leading to infiltration of macrophages and other immune cells, such as T cells, which further contribute to an inflammatory state. However, the mechanisms underlying the adipose inflammation are not well understood. Pattern recognition receptors (PRRs) play critical roles in innate immune response. Here we report a role of nucleotide oligomerization domain containing protein 1(NOD1), a cytosolic PRR, in adipose inflammation. NOD1 activation by its synthetic ligand Tri-DAP significantly upregulates proinflammatory cytokine/chemokine MCP-1 and RANTES mRNA and protein level in differentiated adipocytes. NOD1 activation activates nuclear factor kappa B (NF-kB) and mitogen-activated protein kinase (MAPK) signaling pathways, leading to inflammatory response in a time-dependent manner. Moreover, NOD1 activation leads to attenuated insulin signaling as revealed by decreased tyrosine phosphorylation, but increased inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1), and decreased phosphorylation of downstream target serine/threonine protein kinase Akt, all of which lead to impeded insulin-mediated glucose uptake. Furthermore, NOD1 mRNA level is significantly increased in adipose tissues in a diet-induced obesity mice model, but not in a leptin-deficient obese mice model. Together, our results suggest a role of NOD1 in adipose inflammation in diet-induced obesity.

56. The Role of Cytomegalovirus Infection on Adipose Tissue Development

Courtney Copeland, Nalin Siriwardhana, Naima Moustaid-Moussa, Tim E. Sparer

Microbiology (Copeland, Sparer), Animal Science (Moustaid-Moussa, Siriwardhana)

Obesity affects many aspects of the immune response. With obesity approaching 30% of the total U.S. population, understanding the connection between adipose tissue and immunity to viruses will become increasingly important. Visceral fat is highly inflammatory, exuding many different

chemokines and cytokines that affect immune responses including immunity to viruses. Because cytomegaloviruses (CMVs) contain homologues to host chemokines and cytokines, we hypothesized that CMV infection could affect adipocyte development and inflammatory responses. In order to test this hypothesis, we measured whether CMV could productively infect adipose cells and how CMV infection affects adipokine production. NIH3T3L1 cells were fully or partially differentiated into adipocytes or left untreated. Adipocytes were infected with CMV and the amount of virus produced was measured for 7 days. We show that CMV virus production in adipocytes was equivalent to that of undifferentiated cells. During this time course, supernatants were measured for adipokine production (insulin, MCP-1, leptin, resistin, IL6, and PAI-1) using bead-based Luminex assays. Our results show that CMV infection decreased production of PAI-1 and leptin while increasing MCP-1 and IL6. Our results show that CMV can replicate in adipocytes and subsequently alter its adipokine production. This could have implications not only for CMV dissemination within the host but also the effects of CMV infection on adipocyte development.

57. Role of mTOR and β-hydroxy-β-methylbutyrate (HMB) in Leucine Stimulation of Muscle Mitochondrial Biogenesis and Fatty Acid Oxidation

Renée A. Stancliffe, Meredith Eades, Kenny Smart, Michael B. Zemel

Nutrition

Leucine has been demonstrated to stimulate muscle protein synthesis via an mTOR-mediated mechanism, and we have shown that catabolic systems are also stimulated to fuel this process, resulting in increased mitochondrial biogenesis and fatty acid oxidation (fao). To address the mechanism of this effect, we first determined its mTOR-dependence by assessing leucine stimulation of fao in the presence and absence of 20nM rapamycin; although rapamycin inhibited fao in c2c12 myotubes, the degree of leucine stimulation was preserved (~50%, $P < 0.03$). We next investigated the role of intact leucine (0–0.5 mM) vs. its metabolites, α-ketoisocaproic acid (KIC) (0–0.5 mM) and HMB (0–50 μM). All three compounds induced comparable increases in fao (~60–70%, $P < 0.001$). Both leucine and HMB increased myotube mitochondrial biogenesis (assessed fluorometrically via NAO binding) by ~50%, ($P < 0.005$). Consistent with this, HMB and leucine both stimulated expression of mitochondrial regulatory (PGC-1α and NRF-1) and component (UCP3) genes ($P < 0.01$). These data demonstrate that leucine stimulates mitochondrial biogenesis and fatty acid oxidation independently of mTOR and that these effects appear to be mediated by its metabolite, HMB.

58. Characterization of ATP10C and Multiple Signaling Pathway Proteins in C2C12 Myotubes

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Atp10c is a putative phospholipid translocase that encodes for a type IV P-type ATPase and is a strong candidate for diet-induced obesity and diabetes in both mice and humans. To characterize the ATP10C protein, and to identify its molecular and cellular targets, immunofluorescence and immunoblotting assays were carried out. Using an ATP10C-GFP construct, the molecular weight of the ATP10C-GFP fusion protein was estimated to be about 180 kD, approximate to the expected value. Additionally, ATP10C appears to be localized to the nuclear membrane along with a punctate pattern in and around the nucleus, suggesting an endosomal/trans-golgi organization, strengthening its biological role in protein trafficking in exocytic and endocytic pathways. Using the C2C12 murine skeletal muscle cell line for further analysis, Atp10c expression was silenced (>70%) using transient transfection with ATP10C-specific siRNA sequences, and glucose uptake measured. Results showed a 2.5-fold decrease in glucose uptake, and immunoblotting analysis revealed a significant up-regulation of p38 and p44/42 (P value <0.05) proteins. Comparing the mean fluorescence intensity per cell between wild type and Atp10c-silenced C2C12 myotubes revealed that there was a significant (P value <0.05) down regulation in Akt2 (>6 fold) and a significant (P value <0.05) upregulation of GLUT1 (>25 fold) and IRS-1 (>19 fold). Concluding from these results, ATP10C appears to be a newly-identified protein, affecting glucose uptake via MAPK and/or PI3K signaling. Research support for this project was provided by the Center of Excellence in Livestock Diseases and Human Health, University of Tennessee; and the American Diabetes Association.

59. Ex Vivo Effects of Dairy Products on Monocyte-vascular Endothelial Cell Adhesion

Benjamin Curry, John Biggerstaff, Michael Zemel

Nutrition (Curry, Zemel), Center for Environmental Biotechnology (Biggerstaff)

We previously demonstrated that calcitriol and leucine treatment of adipocytes modulate cytokine production, resulting in a significant effect on monocyte-vascular endothelial cell adhesion in vitro. These adipocyte treatments similarly affected monocyte CD11b and

endothelial cell ICAM-1 expression. Therefore, we have now tested the hypothesis that a dairy-rich diet, by virtue of calcium suppression of calcitriol and of its high leucine content, would decrease monocyte-vascular endothelial cell adhesion, ex vivo. Plasma samples were taken (baseline, day 7, and day 84) from obese clinical trial participants that consumed high or low dairy diets for 12 weeks. Similarly to conditioned medium, plasma was added to fluorescently-labeled monocytes and perfused across a monolayer of fluorescently-labeled vascular endothelial cells. Cell adherence was quantified by microscopy and image analysis. After 7 days, the monocyte to endothelial cell ratio (M:E) markedly decreased from 0.237 ± 0.088 (baseline) to 0.075 ± 0.027 (68% decrease, $P < 0.0002$). The M:E continued to decrease to 0.059 ± 0.016 after 84 days of consuming a high dairy diet (75% decrease compared to baseline, $P < 0.0002$). No trend was observed in those patients consuming a low dairy diet. These data further support the role of dairy products in suppressing vascular inflammation and monocyte adhesion.

60. In Vivo and In Vitro Studies of Fat Deposition in Chickens

Bo Ji, Arnold M. Saxton, Jean Simon, Suchita Das, Shawn R. Campagna, Jessica R. Gooding, Ben Ernest, Brynn H. Voy

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

Excessive fat accumulation in today's commercial broiler chickens due to intensive genetic selection for rapid growth is a problem for both the broiler industry and consumers. Developing approaches to reduce fat deposition requires improved understanding of avian adipose tissue development and physiology. We used microarray profiling to characterize the molecular response to energy manipulation in chicken adipose tissue and to test its sensitivity to insulin. Approximately 3 week-old commercial broiler chickens were fed ad libitum, fasted for 5 hours, or fed but deprived of insulin by injections of anti-insulin serum. Differentially-expressed genes were identified by pair-wise contrasts, and clusters for fasting and insulin neutralization were identified by hierarchical cluster analysis on the differentially-expressed genes. Gene ontology analysis and KEGG were used to identify the metabolic processes associated with each cluster, which identified the common and unique effects of fasting and insulin neutralization on gene expression. In vitro study was performed to test the insulin sensitivity of adipose tissue and expression of inflammatory adipokines in chicken adipose tissue using an adipose tissue explant model. Our results indicated that genes responsive to fasting were involved in lipid biosynthetic and fatty acid metabolism, proteolysis and insulin signaling pathway; insulin neutralization also altered glucose and fatty acid metabolism, but to a lesser extent. The microarray data was confirmed by quantitative RT-PCR, and metabolites that were significantly altered by fasting and insulin neutralization were identified by LC-MS. These data provide a foundation for further study into molecular basis for adipose expansion in poultry.

61. Silencing of the Angiotensinogen Gene in Adipocytes Decreases Lipid Accumulation and Secretion of Pro-inflammatory Markers

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Animal Science (Xin, Booker, Siriwardhana, Moustaid-Moussa), Ut Obesity Research Center (Xin, Kalupahana, Booker, Siriwardhana, Moustaid-Moussa)

Several studies implicated the involvement of Rennin-Angiotensin System (RAS) and its precursor, angiotensinogen (Agt), in metabolic/inflammatory disorders associated with obesity. Indeed, genetic inactivation of Agt reduced adiposity and improved inflammation and insulin resistance, while over-expression of Agt in adipose tissue significantly increased adipocyte hypertrophy, inflammation, and insulin resistance. We propose that these metabolic effects are tightly associated with adipose Agt expression. To test this hypothesis, 3T3-L1 preadipocytes were transfected with shRNA targeting Agt gene. Lipid accumulation and adipogenesis were subsequently assessed following differentiation of transfected cells. As we expected, gene silencing led to low to undetectable levels of Agt expression in mature adipocytes ($P < 0.0001$). Adipocytes lacking Agt accumulated significantly less lipids, and secreted less IL-6 ($P < 0.0001$) and TNF-α ($P < 0.004$). In summary, these studies indicate an important role for Agt in adipose tissue development and immune functions, and further support a potential role for adipose RAS as a therapeutic target in obesity-related metabolic and inflammatory disorders.

62. Histamine Induces Egr-1 Expression in Human Aortic Endothelial Cells via the H1 Receptor-mediated Protein Kinase Cdelta-dependent ERK Activation Pathway

Feng Hao, Mingqi Tan, Xuemin Xu, Mei-Zhen Cui

Pathobiology

Histamine, a potent inflammatory mediator, has multiple effects on the pathogenesis of atherosclerosis. Our study investigates the effect of histamine on the expression of early growth response factor 1 (Egr-1), a master transcription factor that regulates the expression of an array of atherogenic genes in atherosclerotic lesions. Histamine markedly and rapidly induces Egr-1 expression in primary human aortic endothelial cells (HAECs). Histamine-induced Egr-1 expression is dependent on the activation of the H1 receptor. Histamine also rapidly and transiently activates protein kinase C-delta (PKCdelta), extracellular signal-regulated kinase (ERK)1/2, p38 kinase, and c-Jun N-terminal kinase (JNK) prior to Egr-1 induction. Using specific pharmacological inhibitors and small interfering RNA technology, we determined that PKCdelta and ERK, but not p38 and JNK, mediate histamine-induced Egr-1 expression. Importantly, these results suggest a central role of Egr-1 in histamine-induced gene expression and in histamine-induced vascular disease.

63. Controlling Viral Immunoinflammatory Lesions by Modulating Aryl Hydrocarbon Receptor Signaling

Tamara Veiga Parga, Amol Suryawanshi, Barry T. Rouse

Pathobiology, Comparative & Experimental Medicine (Veiga Parga, Suryawanshi, Rouse)

Ocular herpes simplex virus infection can cause a blinding CD4+ T cell orchestrated immunoinflammatory lesion in the cornea called stromal keratitis (SK). Lesion severity is diminished when the balance of proinflammatory effector and regulatory T cells (Treg) emphasizes the latter. In this report, we show that a single administration of TCDD (2, 3, 7, 8- Tetrachlorodibenzo-p-dioxin), a non-physiological ligand for the AhR receptor, was an effective means of reducing the severity of SK lesions. It acted by suppressing the numbers of effector Th1 and Th17 cells and enhanced the representation of Foxp3+ Treg. These effects were the consequence of causing apoptosis of effectors, but not of Tregs. Moreover, in vitro studies revealed that TCDD addition to anti-CD3/CD28 stimulated naïve CD4+ T cells caused a significant induction of Tregs. Since a single TCDD administration given after the disease process had been initiated generated long-lasting, anti-inflammatory effects, the approach holds promise as a therapeutic means of controlling virus-induced inflammatory lesions.

64. Role of MicroRNA-132 in Angiogenesis After Ocular Infection With Herpes Simplex Virus

Sachin Mulik, Shalini Sharma, Barry T. Rouse

Pathobiology, Comparative and Experimental Medicine

MicroRNAs are small regulatory molecules that control diverse biological processes including angiogenesis. Herpes simplex virus (HSV) infection of the eye results in noticeable angiogenesis of the eye, an important step in blinding immunopathology, stromal keratitis (SK). In this study, we report that the expression of microRNA, miR-132 is increased after ocular infection with HSV. Additionally, we provide evidence that miR-132 upregulation was triggered by HSV (at least in vitro) and vascular endothelial growth factor (VEGF) in mice ocularly infected with HSV. The provision of VEGF trap (soluble VEGF receptor 1) resulted in low corneal miR-132 levels during SK. Additionally, neutrophil depletion also diminished miR-132 levels in HSV-infected mice. Provision of anti miR-132 (antagomir-132) nanoparticles to mice resulted in reduction in angiogenesis and diminished immunopathology. The anti angiogenic effect of antagomir-132 was reflected by reduction in angiogenic Ras activity in the corneas. This is the first report to our knowledge about the involvement of any microRNA in the blinding lesion, SK that could be targeted for the control of HSV-induced vision loss.

65. IL-17A Disrupts Physiological Balance Between VEGF-A and sVEGFR-1 and Promotes Corneal Neovascularization Post-HSV Infection Through Multiple Mechanisms

Amol Suryawanshi, Tamara Veiga-Parga, Pradeep Babu Jagdeesh Reddy, Barry T. Rouse

Pathobiology (Suryawanshi, Veiga-Parga, Reddy, Rouse), Comparative and Experimental Medicine (Suryawanshi, Veiga-Parga)

Corneal transparency is a requirement for vision and is achieved mainly through a fine balance between vascular endothelial growth factor A (VEGF-A) and its inhibitory soluble VEGF receptor-1 (sVR-1 or VEGF-A trap). Ocular herpes simplex virus-1 (HSV) perturbs this balance and results in neovascularization of the cornea. Here we show that HSV-induced IL-17A expression in the cornea is a critical factor responsible for an imbalance between VEGF-A and sVR-1. Accordingly, HSV infection of mice lacking IL-17A receptor signaling

(IL-17RA-/-) or neutralization of IL-17A in WT mice showed diminished corneal angiogenesis. IL-17A directly promoted the corneal fibroblast expression of VEGF-A, sVR-1 degrading MMP-7 and -9 as well as pro-angiogenic IL-1B and IL-6. Moreover, IL-17A contributed indirectly to the source of VEGF-A and sVR-1 degrading MMPs through induction of neutrophil chemoattractant, CXCL1/KC. Consequently, increased migration of neutrophils in the HSV-infected cornea diminished the efficacy of the VEGF-A trap by providing an additional source of VEGF-A as well as sVR-1 degrading MMP-7 and -9. Collectively, our results demonstrate the critical role of IL-17A in HSV-induced corneal angiogenesis, and targeting IL-17A-mediated effects could prove a better therapeutic stratagem for management of ocular angiogenesis.

66. The Influence of Galectin-9/Tim-3 Interaction on HSV-1 Latency

Pradeep B.J. Reddy, Sharvan Sehrawat, Amol Suryawanshi, Naveen K Rajasagi, Sachin Mulik, Barry T. Rouse

Pathobiology (Reddy, Suryawanshi, Rajasagi, Mulik, Rouse), Whitehead Institute for Biomedical Research, Cambridge, MA (Sehrawat)

After ocular herpes simplex virus type 1 (HSV-1) infection, CD8+ T cells migrate to and are specifically retained in the trigeminal ganglion (TG), which are considered to play a role in the maintenance of latency. In this report, we show that the majority of CD8+ T cells in the TG of mice ocularly infected with HSV-1 express Tim-3, a molecule that delivers negative signals to CD8+T cells when it engages its ligand, galectin 9 (Gal-9). Since Gal-9 is also up regulated in the TG after HSV-1 infection, it is conceivable that the efficiency of CD8+ T cell function could be influenced by Gal-9/Tim-3 interaction. In support of this, we show that the activity of peptide-stimulated CD8+ T cells from the TG of WT mice produced less IFN-g, TNF-a, and cytolytic molecules such as granzyme B, and were less proliferative in comparison to CD8+ T cells of galectin-9 knockout (Gal-9KO) mice. Ex vivo studies demonstrated that TG cell cultures of WT mice exposed to recombinant Gal-9 caused apoptosis of some CD8+ T cells and induced rapid viral reactivation. Additionally, Gal-9 KO TG cultures showed delayed and reduced viral reactivation compared to WT cultures, demonstrating the greater efficacy of CD8+ T cells to inhibit virus reactivation in the absence of Gal-9. Our results demonstrate that the host homeostatic mechanism mediated by Gal-9/Tim-3 interaction on T cells can influence the outcome of latent HSV-1 infection and that manipulating Gal-9 signals might represent therapeutic means to inhibit HSV-1 reactivation from latency.

67. Augmentation of Immunity to Influenza A Virus by Manipulating a Host Counter Inflammatory Pathway

Shalini Sharma, Barry T. Rouse

Pathobiology, Comparative & Experimental Medicine (Sharma, Rouse)

Reactions to pathogens are usually tuned to effect immunity and limit tissue damage. With chronic infections, where immunity is unsuccessful, tissue damage would be more severe were it not for the operation of numerous host counter-inflammatory events. One such event is the inhibitory effects of some galectins binding to their receptors on effector T cells. A potential downside of counter-inflammatory events is that they may constrain the effectiveness of immunity to acute infections. We present evidence for this situation in mice acutely infected with Influenza A virus (IAV). We show that virus-specific CD8+T cells in both bronchoalveolar lavage (BAL) and spleen upregulate Tim-3 following IAV infection. In an ex-vivo apoptosis assay, addition of Galectin-9 (Gal-9) to splenocytes (d10pi) caused apoptosis of both Tim-3+and DbNPtet+ CD8+T cells. Furthermore, Galectin-9 knockout (G9KO) mice mounted more robust acute phase virus-specific CD8+T cell response and cleared the virus more rapidly than WT mice. G9KO mice also generated higher virus-specific serum IgM, IgG and a more rapid IgA response than WT. When IAV immune mice were challenged with a heterologous IAV, G9KO mice mounted a 4-fold stronger IAV-specific CD8+T cell response than WT mice. Tim-3 fusion protein infusion into infected WT mice enhanced CD8+T cell responses to IAV. Our results could indicate that manipulating galectin signals may represent a convenient approach to improve immune responses to some vaccines.

68. The Application of Chitosan for the Reduction of Foodborne Viral Surrogates on Produce

Radha Ganapathy, Svetlana Zivanovic, Doris H. D'Souza

Food Science and Technology

Produce-related foodborne virus outbreaks are recognized as public health threats worldwide, and novel methods for their control need to be explored. Chitosan is a natural, biodegradable polymer, approved for pre-harvest use as a biopesticide. Its antiviral effects towards foodborne viruses have been recently studied. The objectives of this study were to determine chitosan's suitability for the reduction of foodborne viruses on produce. The effects of three molecular weight chitosans (53, 421, and ~ 1150 kDa) on lettuce and strawberries at 0.7% against human noroviral surrogates, murine norovirus (MNV-1), and feline calicivirus (FCV-F9) were investigated at two storage

temperatures (4°C and room temperature, RT). Our results with chitosan on lettuce and strawberry indicated that increases in contact time and temperature increased FCV-F9 reduction on lettuce, with no observable effects for MNV-1 reduction on either produce. Chitosan of 53kDa decreased FCV-F9 by ~1.6 and ~0.5 log10PFU/ml after 2 hr at RT and 4°C, respectively for lettuce, with negligible decreases of ~0.3 and ~0.1 log10PFU/ml on strawberries after 2 hr at RT and 4°C, respectively. Higher molecular weight chitosans with 421 and 1150 kDa showed lower FCV-F9 reduction than 53kDa with ~0.8 and ~ 0.3 log10PFU/ml reduction, respectively, after 2 hr at RT only for lettuce and no significant reduction at 4°C or on strawberries. Thus, 53kDa chitosan may have application for produce depending on produce type and storage temperature.

69. Mathematical Modeling of the Spread of Mouse Cytomegalovirus from the Foot Pad to Salivary Gland

Pranay Dogra, Yiding Yang, Mindy L. Miller-Kittrell, Tim Sparer, Vitaly V. Ganusov

Microbiology

Human cytomegalovirus (CMV) infects with seroprevalence reaching 100% in some countries. It causes life threatening disease in immune-compromised patients and congenital defects if infection occurs in utero. Mouse CMV (MCMV) is used as a model to understand the spread of the virus in the host to establish persistent infection in salivary glands. MCVM is injected into the foot pad of mice, and viral load is followed over time in organs including the foot pad (FP), spleen (SP), popliteal lymph nodes (LN), and salivary glands (SG). Experiments show decreases of virus in the FP, accumulation then decline of the virus in LN and SP, and delayed increase of virus in SG. We developed mathematical models to understand viral dynamics during the first 3 weeks of MCMV infection of mice. Models excluding virus replication in SG were inconsistent with the data. The minimal model suggests that: i) Virus migrates from FP to LN and SP without death or replication in the FP, ii) Virus half-life T1/2 = 23 hrs in the LN and T1/2 = 2.3 hrs in SP, suggesting an immune system-mediated clearance of virus in these organs, iii) Virus migrates to SG from either LN or SP; however, a definite answer cannot be provided with available data, iv) Delayed appearance of virus in SG is due to extremely low migration rates of virus into SG, v) Increased viral titer in SG is due to viral replication at the rate of 0.9/day. Alternative models for MCMV spread in mice suggest further experiments that will allow for better understanding of how MCMV establishes a chronic infection.

70. Molecular Mechanism of PSAP-induced Apoptosis

Tina T. Li, Lilith L. Zeng, Guozhang Mao, Mei-Zhen Cui, Xuemin Xu

Pathobiology

Presenilin-associated protein (PSAP) was found to be a mitochondrial apoptotic molecule. It could specifically interact with the C terminus of presenilin 1 (PS1), but not presenilin 2 (PS2), both of which play an important role in neurodegenerative disorders like Alzheimer's disease. Overexpression of PSAP induces apoptosis, but the mechanism is largely unknown. In an effort to determine the mechanism by which PSAP induces apoptosis, our recent study revealed that PSAP-induced apoptosis involves cytochrome c release, Bax translocation, and specifically requires apoptosome formation. Thus, our study strongly suggests that PSAP-induces apoptosis through the mitochondrial pathway.

71. LPA May Contribute to Alzheimer's Disease

Jing Shi, Guozhang Mao, Feng Hao, Mei-Zhen Cui, Xuemin Xu

Pathobiology

Vascular factors and inflammatory mechanisms have been implicated in the pathogenesis of Alzheimer's Disease (AD). Specifically, it has been reported that, as a major vascular factor, oxLDL may play a role in neuronal cell death in AD. However, the molecular mechanism by which oxLDL contributes to AD remains unclear. In an effort to study the pathogenetic role of oxLDL in AD, we found that treatment of neuroblastoma N2a cells with the bioactive components of oxLDL, lysophosphatidic acid (LPA), resulted in a marked increase in Abeta formation. Our data established a possible molecular pathogenetic linkage between oxLDL and AD. Our data also demonstrate that LPA induces increased expression of BACE1, the beta-secretase, which is a key enzyme in Abeta production. These novel findings lead to our hypothesis that oxLDL contributes to AD, at least in part, via up-regulation of beta-secretase expression and the resultant increase in Abeta production.

72. Molecular Mechanism of Death Receptor-6 (DR-6)-induced Apoptosis

Lilith L. Zeng, Tina T. Li, Guo Zhang Mao, Mei-Zhen Cui, Xuemin Xu

Pathobiology

Death receptor 6 (DR6) is newly-identified member of the death receptor family. Recently, this new death receptor DR6 has been implicated in the neuronal degeneration observed in the Alzheimer's disease brain. However, the mechanism by which DR6 triggered cell death remains unknown. In the present study, in an effort to determine the mechanism by which DR6 induces apoptosis, our data revealed that DR6-induced apoptosis involves cytochrome c release, Bax translocation, and a series of caspase activation. The finding that caspase inhibitors attenuate DR6-induced apoptosis, but have no effect on DR6-induced cytochrome c release and Bax translocation, strongly suggests that DR6 induces apoptosis through the mitochondrial pathway.

73. Glia-derived TGF-beta Signaling Mediates Metamorphosis-associated Programmed Neuronal Cell Death in the *Drosophila* Central Nervous System

Zixing Wang, Jae Park

Biochemistry, Cellular and Molecular Biology

Program cell death (PCD) is an essential feature for the development of the central nervous system in *Drosophila* as well as in mammals. During metamorphosis, a group of peptidergic neurons (vCrz) are eliminated from the larval central nervous system (CNS) via PCD within 6–7 hr after puparium formation. Using genetic, transgenic, and mosaic analyses of various genes in the TGF-beta signaling pathway, we found that Myoglianin (Myo), a ligand TGF-beta, and its type I receptor Baboon play an essential role in vCrz PCD, whereas other components, including type II receptors, Punt, and Wit, are only marginally required for this process. Although Smad2 is likely a downstream effector of the Babo for vCrz PCD, our genetic data suggest that Myo does not signal exclusively through Babo, indicating a complicated signaling pathway of Myo. Interestingly, Sara, a known adaptor protein for Smad2 phosphorylation and cytoplasm retention factor, is also required in vCrz PCD and functions as a concentration-dependent modulator of TGF-beta signaling.

74. EcR and Usp-mediated Programmed Cell Death of Corazonergic Neurons in *Drosophila melanogaster*

Ritika Sehgal, Gyunghee Lee, Jae Park

Biochemistry and Cellular Molecular Biology

The steroid hormone ecdysone is known to signal the onset of metamorphic processes, including the stage-specific programmed cell death of larval tissues. Ecdysone is the critical developmental cue orchestrating the metamorphic reformation of CNS, resulting in the formation of adult-specific neural circuitry. Ecdysone signaling is transduced by a heterodimeric receptor complex between EcR and Ultraspiracle (Usp), which on activation, results in the coordinated transcriptional regulation of a host of transcription factors regulating genes essential for PCD. Usp plays a dual role in ecdysone response, as its function is necessary for both activation and repression of ecdysone primary response genes. The functions of Usp are thus heterogeneous in different tissues that respond to ecdysone during metamorphosis. We have developed a possible dominant-negative mutant Usp (usp3), and expressed it in flies using the GAL4/UAS system to illustrate the role of Usp in ecdysone-mediated PCD of vCrz neurons. Targeted expression of Usp3 in corazonin neurons resulted in a complete blockage of the PCD pathway. Another interacting partner of Usp, Drosophila Hormone Receptor 38, however, showed no involvement in PCD of vCrz neurons. Lastly, we have also designed an ecdysone sensor to monitor the developmental timing of EcR activation in these neurons.

75. Analysis of Gene Structure and Expression of the Neuropeptide Corazonin and Corazonin Receptor in the House Fly, *Musca domestica*

Kai Sha, William G. Conner, Seung-Hoon Ohio, Jae H. Park

Biochemistry and Cellular and Molecular Biology

The corazonin gene (Crz) encodes for the neuropeptide corazonin, with its mature form of 11 conserved amino acids. The mature peptide sequence is well conserved among species. However, its described functions vary greatly or remain unknown. This study broadens our understanding of Crz by studying its expression patterns as well as its receptor (CrzR), in the house fly, *Musca domestica*. The *M. domestica* Crz (MdCrz) and CrzR (MdCrzR) gene sequence and structure were determined by RACE and inverse PCR. MdCrz was found to possess two introns, one in the 5' UTR and the other in the open reading frame. Comparable gene structures were also found in mosquitoes and *Drosophila virilis*, but not in

D. melanogaster, which lacks the 5'UTR intron. MdCrz expression was analyzed by in situ hybridization (ISH) and immunohistochemistry (IHC) in the central nervous system (CNS) in both the larval and adult stages. These expression levels were found to be comparable to the previously studied *D. melanogaster* and *D. virilis* in the larval stage. However, in the adult stage, *M. domestica* was shown to have a slightly reduced CNS expression and lacked expression in a pair of dorso-medial Crz neurons. MdCrzR was found to share a very high sequence similarity with DmCrzR in the transmembrane regions. Tissue-specific transcript expression of CrzR showed differences between them. In *D. melanogaster*, high CrzR expression was found in the CNS and epidermis, while in *M. domestica*, CrzR was found mostly in the gut and Malpighian tubule.

76. Mechanisms Underlying Cell-specific Expression of Pigment Dispersing Factor in *Drosophila melanogaster*

Presentation Canceled

Sudershana Nair, Jae Hoon Bahn, Jae Park

Biochemistry, Cell and Molecular Biology

All living organisms display daily rhythms, controlled by an endogenous clock that runs with a period of approximately 24 hr that can persist without environmental time cues. External zeitgebers (e.g. food, light, temperature) can synchronize daily rhythms, providing the organism with an internal representation of the external time. A circadian oscillator keeps circadian time and activates rhythmic outputs at the appropriate time of the day. Disturbance of the biological rhythms can cause serious health problems, including insomnia, seasonal affective disorder, delayed or advanced sleep phase syndrome, and other temporary problems caused by a jet lag and work shifts. Pigment dispersing factor (Pdf) is an important neuropeptide that regulates the circadian pace-making system and is a modulator of the clock output pathways in various insects. We have previously shown that *Drosophila melanogaster* lacking the Pdf gene shows altered circadian locomotor activity rhythms, suggesting that Pdf is an essential component of the circadian clock output pathway. Pdf is also important for intercellular communication for the synchronization of different groups of clock neurons. Hence, appropriate levels of Pdf within the key pacemaker neurons are very important for normal clock functions in *D. melanogaster*. Pdf expression is regulated and maintained in the core clock neurons, but how exactly it acts on downstream circuits to mediate rhythmic behavior is unknown. Our preliminary data from heterologous promoter analysis indicate a homeobox transcription factor, SCARECROW (SCRO), to be involved in negative regulation that limits the Pdf transcription to the ventro-lateral clock neurons.

77. For Driving and Not Drinking: Brewer's Yeast as a Biofuel Production Tool

Dan Close, Gary Sayler

Joint Institute for Biological Sciences, Center for Environmental Biotechnology

While humankind has long taken advantage of *Saccharomyces cerevisiae* as a means for brewing alcohol, it has since been developed into an important tool for the elucidation of myriad biological processes as well. Building upon *S. cerevisiae*'s role as a research tool, we are developing unique strains capable of directly producing alkane compounds for use as liquid fuels. Alkane production in the host proceeds as a two-fold process, beginning with the production of a long chain aldehyde, and then followed by conversion to an alkane upon interaction with an associated aldehyde decarbonylase enzyme. Our system incorporates the bacterial luminescence aldehyde processing genes (luxCDE) to produce the aldehyde precursor, and will incorporate the Nostoc punctiforme aldehyde decarbonylase gene to convert this substrate to an alkane. As a hydrophobic alkane, the final product will be compatible with all existing refining, transportation, and storage infrastructure. Synthesis of the aldehyde precursor has been verified though production of bioluminescence from the *S. cerevisiae* host following the addition of the luxA and luxB genes, and has been shown to be stable for relatively prolonged time periods and across multiple generations. Our focus is now on the integration of the aldehyde precursor with the aldehyde decarbonylase enzyme to elicit and confirm the production of the final alkane product. Successful completion of this project will lead to the development of a novel alternative fuel source that can be cultured and maintained inexpensively while providing a fuel capable of immediate integration into existing infrastructure.

78. A Pilot Assessment of a Novel Technique to Evaluate Intrapulmonary Concentrations of Drugs in Pigs

Nicolas Villarino, Tomas Martin Jimenez

Comparative Medicine (Villarino, Martin Jimenez), Comparative and Experimental Medicine (Villarino)

In the lung, determination of drug concentration is usually accomplished by homogenizing lung tissue and bronchial mucosa. These techniques often provide conflicting information and/or have many shortcomings from the experimental design standpoint. The objective of this study was to evaluate the use of bronchial microsampling (BMS) probes (BC-402C; Olympus; Japan) for sampling

an antimicrobial in bronchial epithelial lining fluid (BELF) in pigs. Four healthy female pigs (20–30 kg) were used. Procedures were approved by IACUC. Animals (3 out of 4) were dosed with the antimicrobial. Blood samples were taken at 24 hr after the intramuscular administration of the drug. Afterwards, animals were euthanized. BELF was gathered using BMS probes from the bronchus at the right and left middle lobes of each experimental animal (six bronchial samples/animal). Then, lung homogenate samples were harvested. Samples were analyzed by liquid chromatography-mass spectrometry. The concentrations (mean ± SD) of the drug in plasma and lung homogenate at 24 hr after dosing were 41.2 ± 15.4 ηg/mL and 3900 ± 431 ηg/g, respectively. Drug concentrations were notably high in the BELF. The concentrations of the drug in BELF were 2710 ± 885 and 2520 ± 881 ηg/mL for the left and right bronchus, respectively. The use of BMS probes represents a fast and simple technique for sampling BELF for quantitation of drugs in pigs. In addition, BMS probes avoid many of the shortcomings and increase the interpretative value of the data when compared with other sampling techniques.

79. Sensitivity and Specificity of Tissue Impedance Determination for Correct Veress Needle Placement in Cadaverous Cats

Sara Hyink, Jacqueline Whittemore, Amanda Mitchell, Ann Reed

Small Animal Clinical Sciences (Whittemore, Mitchell, Hyink), Office of Information Technology (Reed)

Delayed detection of intestinal perforation during Veress needle insertion is associated with high mortality. The purpose of this study was to evaluate the accuracy of tissue impedance measurement interpretation for Veress needle location. Two laparoscopists, blinded to impedance measurements, placed reusable Veress needles in 24 cadaverous cats. Placement order was randomized. A third individual evaluated impedance measurements (SensorMed, Knoxville, TN) to determine placement location. Needle locations were marked using India Ink; tissues were dissected to determine ink locations. Impedance measurement interpretation identified 36/38 correct and 2/10 incorrect placements. All eight undetected incorrect placements were located within the retroperitoneal fat pad. Sensitivity, specificity, accuracy, and precision for correct Veress needle placement were 94.7%, 20%, 79.2%, and 81%, respectively, for both operators combined. Correlation was absent (Kappa -0.15, $P = 0.34$) for placements by operator 1 and substantial (Kappa 0.78, $P < 0.01$) for operator 2. There was no association between correct or incorrect placement and operator on Chi-squared analysis. Failure of impedance measurements to identify placement in the retroperitoneal fat pad resulted in poor accuracy and discordant Kappa statistics. Small cat size limited the number of appropriate placement sites, perhaps resulting in excessively dorsal placement. Comparison of needle placement with and without tissue impedance feedback will be necessary to determine whether impedance measurements increase detection of inappropriate Veress needle placements or decrease installment phase complication rates.

80. Polymer-mediated Therapeutic Delivery for Neural Interface Applications

Yu Cao, Wei He

Materials Science and Engineering (Cao, He), Mechanical, Aerospace, and Biomedical Engineering (He)

The technique of connecting neurons and machines through neural electrodes has many applications including therapeutic intervention for patients with neurological diseases and scientific understanding of brain functions on the cellular lever. The electrode has been implanted in humans for a clinical trial to help paralyzed patients to control machines using their thoughts. However, one major issue still exists and halts progress, and that is chronic tissue response to the neural electrode will suspend normal function. The objective of this study is to provide a comprehensive solution to direct cellular response to preserve the functioning of the neural electrode in an in vitro paradigm. To achieve this goal, a range of polymeric therapeutics including anti-inflammatories and antioxidants will be designed and synthesized. The backbones of polymers are polyvinylactam or polyacrylate. They will be fully characterized by nuclear magnetic resonance (1H-NMR and 13C-NMR), Fourier infrared spectroscopy (FTIR), ultraviolet spectroscopy (UV), gel permeation chromatography (GPC), and thermogravimetric analysis (TGA). The therapeutic effects of these prodrugs will be tested by conducting in vitro studies using neural cells. At the same time, prodrugs will be introduced on model neural electrode substrate (Si wafers) through the layer-by-layer (LBL) technique. The LBL experimental parameters and results will be obtained. Finally, cellular studies will be conducted on the Si wafers with LBL layers containing prodrugs. These in vitro studies can serve as foundations for in vivo experiments and eventual clinical applications. In addition, the results could also be applied to other biomedical sensor applications such as glucose sensors.

81. Parallel FEM Simulation of Electromechanics in the Heart

Henian Xia, Kwai Wong, Xiaopeng Zhao

Mechanical, Aerospace, and Biomedical Engineering(Xia, Zhao), National Institute for Computational Sciences (Wong)

We developed a coupled electromechanical model that integrates properties of cardiac electrophysiology, electromechanics, and mechanoelectrical feedback. The model is implemented on the supercomputer Kraken. Numerical simulations are carried out on cardiac tissue to investigate the interaction of electrical and mechanical functions in the heart and their influences to cardiac arrhythmias. Preliminary simulations have shown significant influence of the mechanical deformation on normal electrical propagations, as well as on arrhythmias.

82. Detection of Traumatic Brain Injury from Scalp EEG Using Event-related Tsallis Entropy Functionals

Joseph McBride, Xiaopeng Zhao, Trent Nichols, Tania Abdul-Ahad, Mary Wilson, Victori Vagnini, Nancy Munro, David Berry, Yang Jiang

Mechanical, Aerospace, and Biomedical Engineering (McBride, Zhao, Abdul-Ahad, Wilson), Oak Ridge National Laboratory (Nichols, Munro), VA Connecticut Healthcare System West Haven Campus (Vagnini), Psychology, University of Kentucky (Berry); Behavioral Science, University of Kentucky College of Medicine (Jiang)

Traumatic brain injury (TBI) is the leading cause of death and disability of children and adolescents in the United States. An estimated 1.4 million Americans suffer from TBI each year. Current methods of detecting TBI, such as computerized tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) scanning are time-consuming and expensive. This work explores the viability of a potentially more cost-effective means of screening for TBI using electroencephalogram (EEG). First, Tsallis entropy of EEG recordings taken during a working-memory task is computed for each lead, and the lead average is computed for each of six regional sites (event-related functionals). Then, support vector machine (SVM) analyses are employed to classify 15 TBI and 15 normal individuals. The analyses demonstrate a strong correlation between the event-related functionals (ERFs) and the presence of TBI, attaining a prediction accuracy of 90%.

83. Kinetic Assessment of Myocardial 14(R,S)-[18F]Fluoro-6-Thia-Heptadecanoic Acid and 18F-Fluorodeoxyglucose Uptake with Positron Emission Tomography/Computed Tomography (PET/CT) in Domestic Cats

Amy K. LeBlanc, Murthy R. Akula, Emily B. Martin, Rebecca E. Gompf, Gina D. Galyon, Tamberlyn D. Moyers, Alan C. Stuckey, Misty J. Long, Stephen J. Kennel, George W. Kabalka, Myrwood C. Besozzi, Jonathan S. Wall

Graduate School of Medicine Molecular Imaging and Translational Research Program (LeBlanc, Akula, Martin, Stuckey, Long, Kennel, Kabalka, Wall), Small Animal Clinical Sciences, (LeBlanc, Gompf, Galyon, Moyers), University of Tennessee Medical Center (Besozzi)

Fatty acid oxidation (FAO) is an important myocardial biochemical pathway with therapeutic and diagnostic applications. The domestic cat provides a robust and relevant model to study myocardial fatty acid metabolism using cardiac PET/CT imaging. Six clinically normal, purpose-bred cats of similar age and body condition were imaged with [18F] fluorodeoxyglucose ([18F]FDG) and fluoro-6-thia-heptadecanoic acid ([18F]FTHA) by using dynamic, cardiac-gated, fused PET/CT for kinetic assessment of myocardial glucose and fatty acid uptake and metabolism, respectively. PET data were acquired over a 1-hr period with the heart in the center of the scanner field of view. The equilibrium biodistribution of both tracers was documented 1 hr post-injection in a whole body PET/CT image. Both radiotracers remained in the plasma fraction; however, [18F]FTHA was cleared more rapidly than [18F]FDG (t1/2 ~ 2 and ~ 20 min, respectively). The tracers were readily visualized within the feline myocardium in dynamic PET images, and analysis of the blood pool clearance from the kinetic image data agreed with blood sampling data. Myocardial uptake of each tracer was best described by a double exponential analysis and was rapid but variable among animals (range 1 – 30 Bq/cc/min), although blood glucose levels were similar in all cats during image acquisition. This study demonstrates the utility of kinetic imaging using [18F]FDG and [18F]FTHA to study feline myocardial metabolism in the fasted state using PET/CT imaging, and provides validation of [18F]FTHA in a relevant in vivo model of myocardial metabolism to support future clinical trials in animals with myocardial disease.

84. Normal Biodistribution of 3'-Deoxy-3'-[18F] Fluorothymidine (18FLT) in Adult Cats

Josh Rowe, Amy LeBlanc, Jonathan Wall, Emily Martin, Stephen Kennel, Murthy Akula, Gina Galyon, Misty Long, Alan Stuckey

Molecular Imaging and Translational Research Program (Rowe, LeBlanc, Wall, Martin, Kennel, Akula, Long, Stuckey), Comparative and Experimental Medicine (Rowe), Small Animal Clinical Sciences (Galyon)

Positron emission tomography (PET) with 3'-deoxy-3'-[18F]fluorothymidine (18FLT), a proliferation tracer, is a useful tool for characterization of both neoplastic disease and bone marrow function. PET/CT is increasingly available in veterinary medicine; as a result, normal biodistribution of 18FLT in veterinary species is needed for lesion interpretation in the clinical setting. The purpose of this study is to describe the normal biodistribution of 18FLT in adult domestic cats. Imaging of six healthy adult male cats was performed using a Biograph mCT scanner (Siemens Molecular Imaging, Knoxville, TN) which combines a 64-slice CT scanner with a whole-body, high-resolution PET scanner. The scanner is used for research as well as clinical patients (quality control and normalization obtained daily via Ge-68 phantom). Cats were sedated and injected intravenously with 108.595 ± 2.090 (mean ± SD) MBq of 18FLT (greater than 99% radiochemical purity by HPLC). General anesthesia was induced and cats placed in sternal recumbency on the scanner's bed. Static images using multiple bed positions (corrected for radionuclide decay) were acquired 60 min post-injection. 32 separate regions of interest (ROIs) were manually drawn by three observers over major parenchymal organs and selected areas of bone marrow. Standardized uptake values (SUVs) were calculated. Notable areas of bone marrow uptake included proximal humeri, caudal ilia, distal femora, sternum, and vertebral bodies. Kidneys, liver, gall bladder, intestinal tract, and urinary bladder had relatively intense uptake consistent with excretion. No appreciable brain or lung uptake was observed. This study demonstrates normal biodistribution of 18FLT in the normal cat.

85. Generation of Three-dimensional Tissue Using Stem Cell Seeding of Polymeric Scaffolds

Christopher P. Stephens, Ryan Hammonds, Pelagie Favi, Liesl Breickner, Nancy Neilsen, Madhu S. Dhar

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This study develops an osteoconductive polymer/ceramic nanocomposite that could be used for bone tissue engineering, treatment of traumatic injury, and remodeling of non-unions. The purpose of this study is to develop an array of microporous scaffolds that are optimized for culture and differentiation of stem cells harvested from large animals (i.e. porcine, equine, and ovine). The scaffolds developed in this study will all be based on bacterial cellulose and have micron-scale (50–200 mm) interconnected pores. The cellular scaffolds used in this study will be native bacterial cellulose (BC) or BC mineralized with biomimetic hydroxyapatite. These scaffolds' morphology will be characterized using scanning electron microscopy and confocal microscopy. Previously isolated and molecularly characterized equine adult stem cells harvested from bone marrow will be transiently transfected with green fluorescent protein and seeded onto these scaffolds with the biochemical signals required for efficient differentiation into bone and cartilage. Differentiation will be monitored and confirmed using alizarin red (bone) and alcian blue (cartilage) staining. This exploratory study will lay the foundation for the development of large animal disease models.

86. Development and Commercialization of a Conformal Ultrawideband Multilayer Lens Applicator (CUMLA) for Therapeutic Hyperthermia

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Small Animal Clinical Sciences (Phillips, Smrkovski), Electrical Engineering Computer Science (Fathy, Liu, Koo), Comparative and Experimental Medicine (Lembcke)

Consumer spending on veterinary healthcare has doubled since 2003 and is expected to be in excess of 15B in 2010. To meet this growing demand, newer and more effective treatment alternatives are needed for the veterinary cancer patient. Hyperthermia has often been referred to as the "fourth arm" of modern cancer therapy and is typically delivered through microwave antenna systems. Traditional systems suffer because they lack directivity (focus energy field onto tumor), have poor penetration due to skin depth phenomenon (limited to superficial tumors), have poor tissue matching abilities (reducing the type of lesions that can be treated), are difficult to use (require invasive thermoprobes and advanced training), and finally are expensive (>$500,000). These problems have resulted in the relative unavailability of traditional hyperthermia to supplement the care of human and veterinary cancer patients. For the past 2 years, UTCVM researchers have documented the safety and activity of a novel antenna system and treatment protocols for therapeutic hyperthermia in veterinary

cancer patients; the results have been impressive. This novel antenna system provides directivity, improved depth of penetration, excellent tissue matching properties and ease of use. These easy-to-use hyperthermia units, along with specific treatment protocols, provide an effective treatment alternative for veterinary cancer patients, which has resulted in objective response rates of over 80% in patients with locally advanced disease. These are the same patients that historically had no other effective treatment options available. We will present our developmental process and some of the exciting clinical results we have seen.

87. Toxicity Screening of Damnacanthal Nanoparticle Using Bioluminescent Yeast-Reporter [CANCELED]

Nutsawan Chaisutatip, Pleumchitt Rojanapanthu, Alice C. Layton, Steven A. Ripp, Gary S. Sayler, Karla J. Matteson, Darunee Lawson

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Damnacanthal, an anthraquinone compound that exhibits anti-tumorigenic activity, was successfully extracted from the root of *Morinda citriforia* L. (Noni) by a simple method (maceration and column chromatography). Chitosan nanosphere was synthesized as a damnacanthal carrier in drug delivery. This study was designed to incorporate damnacanthal, a hydrophobic compound in N-phthaloylchitosan-grafted poly (ethylene glycol) methyl ether (PLC-g-mPEG) to form damnacanthal nanoparticle. This study is a part of drug discovery and development to reduce severe systemic toxicity and enhance antitumor effects in tumor tissue. Moreover, it is commonly known that the microenvironment of tumors has lower pH than healthy tissue, yielding polymeric nanoparticle formula sustained release. The Bioluminescent Yeast-reporter System is a high throughput screening system to assess a compound's effects on hormonal systems and examine its toxicity. Therefore, this study used the capability of the Bioluminescent Yeast-reporter System to screen toxicity of damnacanthal nanoparticles and to study drug delivery efficiency in various pH-simulating microenvironments of tumors. The initial screening results showed that the damnacanthal released from the nanoparticles was not toxic.

88. Development of a Novel High Throughput Screening Method for Estrogenic Compounds

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The bacterial bioluminescence (lux) cassette is a unique reporter system due to its autonomous nature. Recent expression of the full lux cassette in mammalian cells has expanded its potential applications. A mammalian lux bioreporter has the ability to indicate human bioavailability in a way that cannot be represented using prokaryotic-based bioreporters. Here we report the development of a mammalian lux bioreporter for detection of estrogenic compounds. These chemicals were chosen because they can disrupt the endocrine system and because chemicals released to the environment with potential estrogenicity raise a public health concern and require screening. A vector containing three tandem repeats of the estrogen response element and the luxAB genes was constructed and transfected into T-47D breast cancer cells. E2-induced LuxAB activity in transiently transfected T-47D cells increased in a dose-response relationship with increasing exposure time. Induction with 10 nM E2 was observed after 2 hr exposure with a 1.73 (±0.08)-fold increase in bioluminescence relative to control. Peak luminescent expression occurred after 24 hr exposure, leading to a 10.2 (±0.85)-fold increase in bioluminescence. In stable clones, E2-stimulated luciferase activity was present after 48 hr exposure with ~18-fold increase. The stable clones were also able to respond to E2 in a dose-dependent manner. Exposure to 0.01 nM, 1 nM, and 10 nM E2 for 24 hr resulted in 2.0 (±0.13), 4.7 (±0.07), and 10.1 (±0.87) -fold increases in bioluminescence, respectively. Future work to co-express the remaining lux genes will enable development of a fully autonomous estrogen bioreporter for high throughput screening.

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