Comparative & Experimental Medicine and Public Health Research Symposium

Investigación

연구

Research

Kutatás

研究

Výskun

Ricercare

June 15 & 16, 2009

Program & Schedule

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

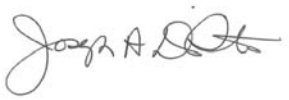
Welcome

Once again, the University of Tennessee (UT) Agricultural Campus is hosting a symposium for UT investigators with animal and human health interests. Interest in this symposium is growing explosively, and the symposium 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. Last year, the symposium was opened to participants throughout the Knoxville campuses, and there was a four-fold increase in presentations with representation from 16 different UT departments and programs. This year, the Center for Public Health has teamed with the CEM to produce a joint *Comparative & Experimental Medicine and Public Health Research Symposium* hosting an even larger group of scientists including 85 presenters representing 19 different UT departments and programs.

The *Comparative & Experimental Medicine and Public Health Research Symposium* provides an excellent venue for students and new investigators to gain experience presenting 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 networks that will enhance health-related research at the Knoxville campuses of the university. The joint sponsorship of the symposium by the College of Veterinary Medicine, the UT Center for Public Health, the Graduate School of Medicine, Tennessee AgResearch, the UT Graduate School, and the UTK Office of Research Administration 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 *research* in seven 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.



Joseph DiPietro, Vice President
University of Tennessee
Institute of Agriculture

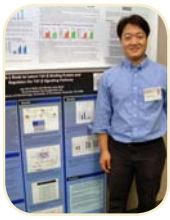
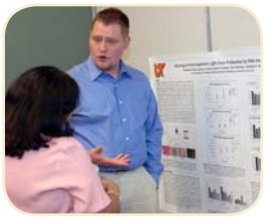
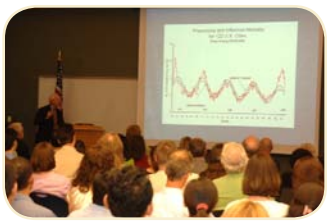






Table of Contents

Schedule at a Glance 4

Location Information 5

Session Matrix 6-9

Featured Speakers 10-12

Abstracts 14-51

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

Graduate School of Medicine

UTK Office of Research

UT Graduate School

Misty Bailey
Joseph Bartges
Tammy Berry
Debra L. Butenko
Paul Campbell Erwin
William Hill
James Lawler
Karla Matteson
Robert N. Moore
Kim Rutherford
Jenny Tinkle
Anik Vasington
Michael Zemel

We appreciate the contributions of session moderators and judges.

Thanks also to the UTCVM chapter of Phi Zeta, 2009 Center of Excellence Summer Student Research Program participants, and our sponsors and exhibitors.

James Thompson, *Dean*
College of Veterinary Medicine

Brad Fenwick, *Vice Chancellor for Research*
UTK Office of Research

James Neutens, *Dean*
Graduate School of Medicine

William F. Brown, *Dean*
Tennessee AgResearch

Carolyn R. Hodges, *Vice Provost*
UT Graduate School

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

Schedule at a Glance

Monday, June 15

	Room	CEM*	PH*	Room
8:30-9:00			Welcome reception with refreshments	PBB* 156/157
9:00-9:45			PH Keynote address: Dr. William Harris, "Omega-3 Fatty Acids and Cardiovascular Disease Risks"	PBB 156/157
10:00†-11:45	VMB* 118	CEM Seminar: Dr. Tairo Oshima, "Functions and Metabolisms of Unusual Polyamines in Extreme Thermophiles" *(†10:30-11:30)*	Workshop: "Research Grants and Manuscripts," Panel with Jay Whelan, David Bassett, Hollie Raynor, Denise Bates, & Ken Phillips	PBB 156/157
12:00-1:15	PBB 160	Workshop luncheon for presenters: Dr. Diane Klotz, "Why Choose the NIH for a Postdoctoral Fellowship?"	Featured address & luncheon: Dr. Roger Cone, "The Central Melanocortin System and Energy Homeostasis"	Hollingsworth Auditorium
1:30-4:00	See session matrix (p. 6)	New investigator presentations	New investigator presentations	See session matrix (p. 6)
4:00-5:00			Panel: "Community-Based Participatory Research," featuring Mr. Bruce Behringer	Hollingsworth Auditorium

THE UNIVERSITY of TENNESSEE
INSTITUTE of AGRICULTURE
COLLEGE of VETERINARY MEDICINE

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

Tuesday, June 16

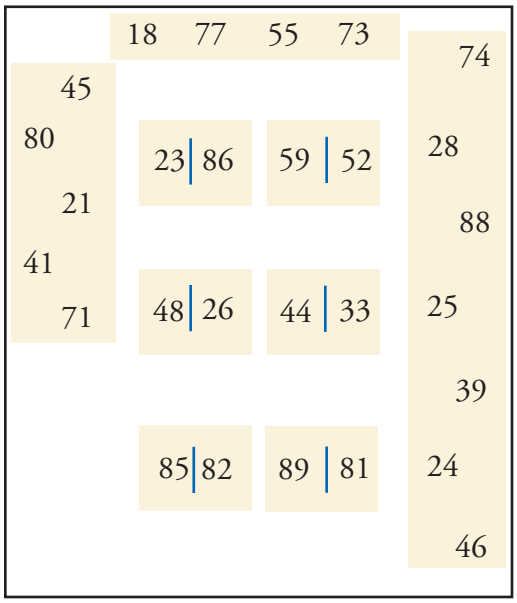
	Room	
9:00-10:00	Hollingsworth Auditorium	CEM keynote address: Dr. Hildegard Schuller, "Nicotine Addiction and Cancer"
10:30-12:00	See session matrix (p. 7)	New investigator presentations
12:00-1:30	PBB 156	Poster session‡ & luncheon
2:00-4:30	See session matrix (pp. 8-9)	New investigator presentations
6:00	Hollingsworth Auditorium	Awards banquet & after-dinner address: Bill Landry, "Herbs, Potions, Magic Cures and Remedies of the Southern Appalachians"

*CEM, Comparative & Experimental Medicine
PH, Public Health
VMB, Veterinary Medicine Building
PBB, Plant Biotechnology Building

‡Poster set-up: Mon, June 15, 1:00-4:00 & Tues, June 16, 8:00-11:00; Take down by Tues, June 16, 5:00

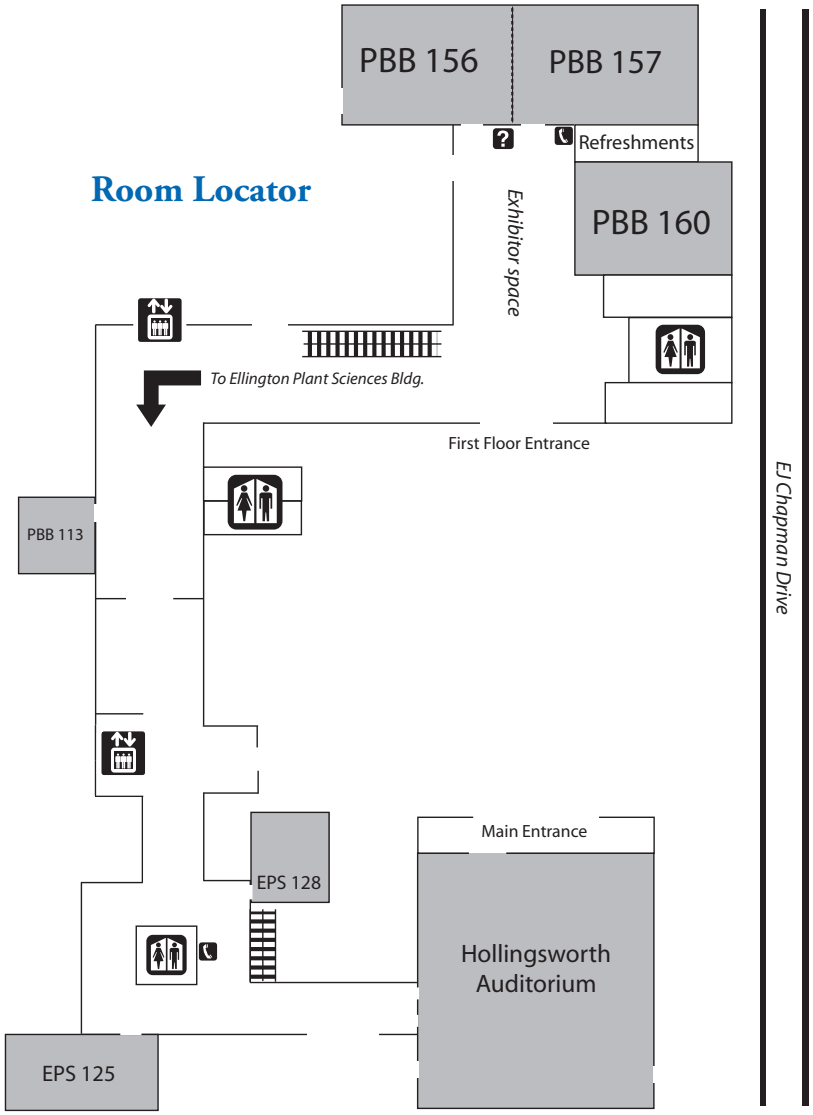
Location Information

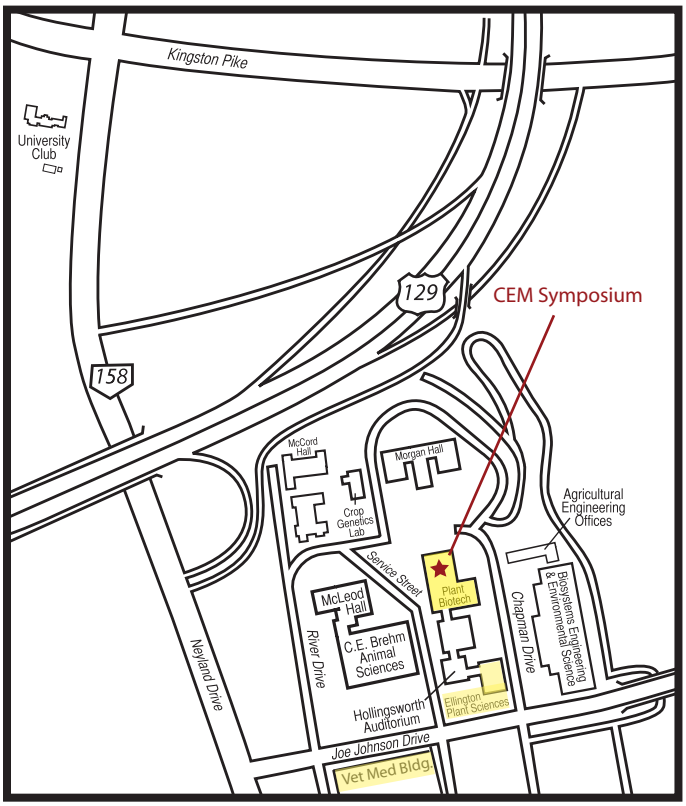
Poster Session–PBB 156



*Numbers correspond with title numbers in session matrix.

Room Locator





University of Tennessee Agricultural Campus

Parking

Faculty & staff with a valid parking permit for a main (east) campus lot may park in lot 66–across from Ellington Plant Sciences–at no additional cost or 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.

Session Matrix (Abstracts on pp. 14-51)

Monday, June 15

	Public Health	Bacterial Virulence & Transmission	Vascular & Deposition Disorders
	Rm. PBB 160	**Rm. PBB 113**	**Rm. EPS 128**
1:30 pm	1. Detrimental Alcohol-related Health Consequences among College Student Binge Drinkers Enrolled in Southern Institutions of Higher Education **(Tallant)**	10. Identification of a Pulsed-Field Gel Electrophoresis Cluster in Clinical Isolates of Methicillin-Resistant *Staphylococcus pseudintermedius* and the Implications of a Regionally Distinct Multi-Drug-Resistant Clone **(Black)**	18. Serum Levels of Matrix Metalloproteinase-2 as a Marker of Intimal Hyperplasia **(Tummers)**
1:45 pm	2. Dietary Supplement Use and Beliefs among College Students Enrolled in an Introductory Nutrition Course **(Webb)**	11. Novel Multilocus Sequence Types among *Staphylococcus pseudintermedius* Strains Isolated from Dogs **(Solyman)**	19. Lysophosphatidylcholine Activates a Novel PKD2-Mediated Signaling Pathway that Controls Human Blood Monocyte Migration **(Hao)**
2:00 pm	3. Comparison of Four Actigraph Accelerometers during Walking and Running **(John)**	12. Effect of Antibiotic Growth Promoters (AGPs) on Intestinal Microbiota in Chickens **(Clark-Hunkapiller)**	20. Dynamic Expression and Cellular Localization of LPA-induced CYR61 in Smooth Muscle Cells and its Novel Role in LPA-induced Cell Migration **(D. Wu)**
2:15 pm	4. Eating Frequency and Healthy Weight Status **(Bachman)**	13. Reverse-Transcriptase Loop-mediated Isothermal Amplification (RT-LAMP) as a Novel Approach for the Detection of *Salmonella Typhimurium* in Pork **(Techathuvanan)**	21. Presenilin Interacts with Nicastrin and APH1, Independent of Subcomplex Formation of Nicastrin and APH1 **(Mao)**
2:30 pm	5. The Effects of a Pedometer Intervention on the Physical Activity Patterns of Phase II Cardiac Rehabilitation Participants **(Shipe)**	14. Chronic *Escherichia coli* Experimental Intramammary Infections in Primiparous Dairy Cows during the Periparturient Period **(Almeida)**	22. Residues at the P2-P1 Positions of e- and ζ-cleavage Sites are Important in Aß Formation **(Tan)**
2:45 pm	**Pathogen Distribution & Prevalence**	15. Pathogenesis of Chronic *Escherichia coli* Mastitis **(Almeida)**	**Break**
3:00 pm	6. Ominous Tadpoles: American Bullfrogs are Suitable Hosts of *Escherichia coli* O157:H7 **(Thompson)**	***16. Presentation Canceled***	23. The Search for Imaging and Therapeutic Agents for Huntington's Disease **(Rowe)**
3:15 pm	7. Blacklegged Ticks in Tennessee: Implications for Lyme Disease **(Harmon)**	***17. Presentation Canceled***	24. Protein Hydration and Association Studied by Small-Angle Neutron Scattering **(Stanley)**
3:30 pm	8. Investigation of Human *Campylobacter* Infection in Georgia Using GIS and Spatial Analysis Techniques **(Weisent)**		25. Structural Study of Polyglutamine Amyloid Fibril Formation by Small-Angle Neutron Scattering **(Perevozchikova)**
3:45 pm	9. Community-Associated Methicillin-Resistant *Staphylococcus aureus* Nasal Carriage in a College Student Athlete Population **(Rackham & Pinn)**		

Tuesday, June 16, *Morning*

	Bovine Mastitis & Theriogenology	Clinical Sciences, Nutrition, & Metabolic Disorders	Innovative Biomedical Technologies	Viral Pathology & Immunity	Oncology & Cancer Cell Biology
	Rm. EPS 125	**Rm. PBB 157**	**Rm. EPS 128**	**Rm. PBB 160**	**Rm. PBB 113**
10:30 am	26. Bulk Tank Milk Quality of Nine Dairy Farms in Tennessee over a 12-Month Period **(Gillespie)**	37. Investigation of Geographic Access to Heart Attack and Stroke Care in the East Tennessee Appalachian Region **(Pedigo)**	52. Development of an Autonomous Mammalian Lux Bioreporter **(Close)**	66. A Stochastic Game Theoretical Approach to Controlled Drug Delivery during HIV Infection **(J Wu)**	79. ODAM as a Novel Biomarker for Human Breast Cancer **(Siddiqui)**
10:45 am	27. Evaluation of Bulk Tank Milk Quality in Tennessee **(Gillespie)**	38. Electroencephalograph in the Default Mode of Brain Function **(Cannon)**	53. Material Design Strategies for Bone and Nerve Regeneration: Controlled Physical Properties and Regulated Cell Responses **(Cai)**	67. Mathematical Model for Immune-Vaccine Interactions **(Yang)**	80. Role of Alternative Splicing in Colorectal Tumorigenesis **(Bahn)**
11:00 am	28. Biofilm Production by *Streptococcus uberis* Isolated from Dairy Cows with Mastitis **(Moore)**	39. Injection Site Pain after Fluoroscopically Guided Epidural Steroid Injection **(Clanton)**	54. Design of a Micro-Electrical-Mechanical-System (MEMS) for Diagnosis and Treatment of Malaria **(Lenaghan)**	68. Yeast Co-expression–A Novel Methodology for Characterization and Engineering of Peptide-MHC II Binding **(Jiang)**	81. Peroxisome Proliferator-Activated Receptor Gamma Ligands Induce Pro-Apoptotic Protein NAG-1 Expression Via KLF4 in a PPAR - Dependent Manner **(Cekanova)**
11:15 am	29. sua Gene Deletion Mutagenesis in *Streptococcus uberis* UT888 Using a Thermosensitive Replicative Plasmid **(XY Chen)**	40. Effect of Therapeutic Hypothermia on Potassium Balance Post-Cardiac Arrest **(Malcom)**	55. Using AC Electrokinetic-enhanced, Microfluidic Magnetoelastic Sensors for Detection of Low Analyte Concentrations in Biological Fluids **(Wilson)**	69. Selectin Ligand Deficiency Confers an Unexpected Increase in Migration of Naïve T Cells to the Lung **(Harp)**	82. Reactive Oxygen Species in the Ability of Pro-apoptotic H-Ras to Enhance Apoptosis Induced by Histone Deacetylase Inhibitors **(Choudhary)**
11:30 am	30. Responses of Different CXCR1 Genotypes after Experimental Challenge with *Streptococcus uberis* **(Elliott)**	41. Arterial Desaturation and Airway Intervention During Deep Propofol Sedation for Outpatient Endoscopy **(DiRuzzo)**	56. Ivy Nanoparticle Characterization and Biomedical Application **(Xia)**	70. ST6GalI Expression is Required for the Optimal Generation of Anti-viral Humoral Responses **(Bheemreddy)**	83. Nonsteroidal Anti-inflammatory Drugs Suppress Structural Protein Nesprin-2 Expression in Colorectal Cancer Cells **(Liggett)**
11:45 am		42. Innate Immune Function in Kittens Fed Raw vs. Commercially Heat-Processed Diets **(Hamper)**	57. In Situ Preconcentrator for Rapid and Ultra Sensitive Nanoparticle Detection **(Yang)**	90.	

Tuesday, June 16, *Afternoon*

	Bovine Mastitis & Theriogenology	Clinical Sciences, Nutrition, & Metabolic Disorders	Viral Replication, Distribution, & Prevalence	Viral Pathology & Immunity	Oncology & Cancer Cell Biology
	EPS 125	**PBB 157**	**EPS 128**	**PBB 160**	**PBB 113**
2:00 pm	31. Presence of ISS1-like Element in Wild Type *Streptococcus uberis* Strains Isolated from Cases of Bovine Mastitis **(Kerro Dego)**	43. Effects of Dietary Calcium and Milk on Oxidative and Inflammatory Stress and Lifespan in Wild-type and aP2-agouti Transgenic Mice **(Bruckbauer)**	58. Ranaviruses in Southern Appalachian Salamanders **(Haislip)**	71. Suppression of HSV-1-induced Corneal Angiogenesis and Vascular Permeability by an src Kinase Inhibitor **(Sharma)**	84. Zyflamend, a Multi-Herb Extract, Enhances Tumor Regression of CWR22 Prostate Cancer Cells in a Xenograft Model when Used in Conjunction with Hormone Ablation Therapy **(Huang)**
2:15 pm	32. Application of a Reproductive Tract Scoring System in Lactating Dairy Cattle **(Young)**	44. Calcitriol Increases Microphage Adherence to Differentiated Adipocytes *in vitro* **(Sun)**	59. Anuran Susceptibilities to the Emerging Amphibian Pathogen *Ranavirus* **(Hoverman)**	72. Modulation of VLA-4 -VCAM1 Interaction Diminishes HSV-1-Induced Corneal Immunopathology **(Suryawanshi)**	85. NUDT6, a Putative Prognostic Marker in Colon Carcinogenesis, is Suppressed by EGCG **(Sukhthankar)**
2:30 pm	33. Estimation of Genetic Parameters for Embryo Transfer Traits **(Di Croce)**	45. Vitamin A Status Affects the Expression of PPARα and ME Gene in Rat Liver **(Li)**	60. Prevalence of BVDV on Alpaca Farms in Eastern and Middle Tennessee **(Buchheit)**	73. Unusual Isotype Skewing of the Influenza-Specific B Cell Response in HLA-DR1 Transgenic Mice **(Huan)**	86. Inhibition of Pathologic Immunoglobulin Light Chain Species by Small Interfering RNA Molecules **(Phipps)**
2:45 pm	34. Pregnancy Retention Rates after Transfer of a Bovine Embryo Following Exposure to a PGF2α Receptor Antagonist during Collection **(Roper)**	46. Vitamin A Status Affects L-PK and S14 Gene Transcription in Rat Liver **(W. Chen)**	***61. Presentation Canceled***	74. Deficient Virus-Specific IgA Production and Mucosal B Cell Memory Following Murine gammaherpesvirus 68 Infection of the Respiratory Tract **(Sundararajan)**	87. Resveratrol Induction of ATF3 is Mediated by EGR-1/KLF4 Interaction **(Whitlock)**

Tuesday, June 16, *Afternoon*

	Bovine Mastitis & Theriogenology	Clinical Sciences, Nutrition, & Metabolic Disorders	Viral Replication, Distribution, & Prevalence	Viral Pathology & Immunity	Oncology & Cancer Cell Biology
	EPS 125	**PBB 157**	**EPS 128**	**PBB 160**	**PBB 113**
3:00 pm	35. Proteomic Analysis of Bovine Oocytes During Maturation Using 2-D DIGE **(Rispoli)**	47. High Molecular Weight Adiponectin Ratio Correlates Better with Body Fat Mass and Indices of Glucose Metabolism than Total Adiponectin in Lean and Obese Cats **(Lusby)**	Break	Break	88. Precancerous Model of Human Breast Epithelial Cells Induced by NNK & Benzo[a]pyrene and the Role of Green Tea Catechins in Breast Cancer Prevention **(Rathore)**
3:15 pm	36. Molecular Features of Gamete RNA **(Payton)**	Break	62. Inactivation of Human Enteric Virus Surrogates by High Intensity Ultrasound **(Su)**	75. Pomegranate Constituents Have Direct Antiviral Activity Against Diverse Influenza Virus Subtypes **(Ganapathy)**	89. Chili Pepper Component Capsaicin in Colorectal Cancer Prevention **(Lee)**
3:30 pm		48. *In vitro* Manipulations of a Putative Phospholipid Translocase Suggest its Role in Glucose Uptake and Adipogenesis **(Hurst)**	63. Molecular Characterization of Feline Papillomavirus with a Close Relationship to Human Papillomaviruses **(Anis)**	76. Generation of Recombinant MCMVs Overexpressing the MHV-68 Chemokine-Binding Protein-M3, Using the Bac System **(Benedict-Hamilton)**	
3:45 pm		49. Effects of Clinical Endotoxemia on Glucose Metabolism in Horses **(Gomez de Witte)**	64. Regulation of Coronavirus Poly(A) Tail Length During Virus Replication **(H-Y Wu)**	77. Functional Analysis of Polymorphisms within Human Cytomegalovirus Viral Chemokine vCXCL-1 **(Heo)**	
4:00 pm		50. Effects of Levothyroxine Sodium on Body Condition, Blood Measures of Metabolic Status, and Glucose Dynamics in Horses with Equine Metabolic Syndrome (EMS) **(Chameroy)**	65. 5'-Proximal Suppressor Mutations for an Incompatible 32-nt Region in the 5' Untranslated Regions of Bovine and Mouse Hepatitis Coronaviruses Identify a New *cis*-Replication Element **(Guan)**	78. Recombinant Murine Cytomegalovirus Overexpression of Host or Viral Chemokine Leads to a Defect in Salivary Gland Dissemination **(Miller-Kittrell)**	
4:15 pm		51. Optimization of the Frequently Sampled Intravenous Glucose Tolerance Test to Reduce Urinary Glucose Spilling in Horses **(Tóth)**		90. The Role of Amino Acid Sequence Variation of the Immuno-dominant Domain of the Attachment Glycoprotein G of Respiratory Syncytial Virus in the Immune Response **(Abd-Eldaim)**	

Featured Speakers

Hildegard Schuller, PhD

Distinguished Professor, Department of Pathobiology, University of Tennessee

"Nicotine Addiction and Cancer" – CEM Keynote Address

Dr. Hildegard Schuller is an established, NIH-funded investigator whose research focuses on the role of neurotransmitter receptors in tobacco-associated cancer. Before joining the University of Tennessee (UT) as a professor in 1985, she held several different positions at the National Cancer Institute. Schuller has a total of 223 peer-reviewed publications (and counting) and since 2001, has brought in over $5 million in research support for UT. These funding dollars, and those before them, have formed a solid foundation for the study of nitrosamines, cancer-causing substances formed from nicotine. The tobacco-specific nitrosamine NNK reacts with nicotinic acetylcholine receptors (nAChRs) in cells, resulting in hyperstimulation of these receptors, a reaction that stimulates intracellular signaling pathways that regulate cancer cells. In her laboratory, Schuller has determined that many of nicotine's biological effects may be caused by the interaction of nitrosamines (like NNK) with nAChRs. Still, a host of factors in the human environment affects the sensitivity of these receptors, making it important to develop tools to identify hyperstimulation in individual patients. Schuller's research provides a foundation for the development of novel, "custom-tailored" cancer prevention and treatment strategies based on that hyperstimulation.

William Harris, PhD

Senior Scientist and Director of the Metabolism and Nutrition Research Center
Sanford School of Medicine, University of South Dakota

"Omega-3 Fatty Acids and Cardiovascular Disease Risks"
Public Health Keynote Address

Dr. Harris is a professor of medicine and co-director of the MD/PhD program at the Sanford School of Medicine. Between 1985 and 1996, Harris was the director of the Lipid Research Laboratory at the Kansas University Medical Center in Kansas City. In 1996, he moved to the University of Missouri-Kansas City as a professor of medicine and held the Daniel J. Lauer/ Missouri Chair in Lipid Metabolism. He also served as the co-director of the Lipid and Diabetes Research Center at the Mid America Heart Center of Saint Luke's Hospital in Kansas City. Over the last 25 years, Harris's research has focused primarily on fish oils (omega-3 fatty acids) and cardiovascular disease, and he has been the principal investigator on three NIH grants focusing on omega-3 fatty acids and human lipid metabolism.

His current project is examining the effects of niacin and omega-3 fatty acids, separately and in combination, on lipid and glucose metabolism in patients with metabolic syndrome. He has also been examining the potential of a blood test he developed to measure omega-3 fatty acid levels as a new risk factor for cardiovascular disease. In collaboration with his new colleagues at the Cardiovascular Research Center, he will be expanding his research program to include studies of the cellular and biochemical mechanisms by which omega-3 fatty acids allow the heart to resist ischemic stress. He has over 120 publications.

Featured Speakers

Bruce Behringer, MPH

Assistant Vice President, Division of Health Science, East Tennessee State University

"Community-Based Participatory Research"

Bruce Behringer, MPH, is Assistant Vice President in the Division of Health Sciences at East Tennessee State University (ETSU) in Johnson City, TN. The division includes the colleges of Medicine, Nursing, Public Health, Clinical and Rehabilitative Health Sciences and Pharmacy. At ETSU, he serves as the Executive Director of the Office of Rural and Community Health and Community Partnerships, which develops community partnerships to promote student learning in rural areas and with minority communities. He has also promoted research and service in subjects of Appalachian health disparities. Prior to moving to ETSU in 1992, Behringer was with the Virginia Primary Care Association and helped many underserved Virginia communities to expand health services. He has also served as administrator of a rural community health center in Eastern North Carolina. He now serves on six state, regional and national boards for veterans' health, rural physician recruitment, health information exchange, public health and cancer control.

Behringer's recent publications have focused on health disparity issues in the Appalachian region. He is responsible for several national grants that have helped to identify and describe dimensions of health disparity issues in several diseases including cancer, substance abuse and diabetes.

Behringer is a graduate of Penn State University and the University of North Carolina's School of Public Health.

Tairo Oshima, PhD

Director, Institute of Environmental Microbiology, Kyowa Kako Company, Tokyo, Japan

"Functions and Metabolisms of Unusual Polyamines in Extreme Thermophiles"

Dr. Tairo Oshima directs the Institute of Environmental Microbiology at the Kyowa Kako Company in Tokyo, Japan, and is a professor emeritus from the Tokyo University of Pharmacy and Life Science and the Tokyo Institute of Technology. From 2002-2007, he was head of the Research Promotion Committee for the Protein 3000 Project, a national research project in Japan aimed at determining over 3,000 protein structures and their important biological and medical functions. Oshima earned his PhD from the University of Tokyo, and during the last 7 years, he has co-authored 45 peer-reviewed publications.

Oshima's research interests include proteins and enzymes from thermophiles, conformational stability of proteins, biochemistry and molecular biology of extremophiles and archaea, and evolutionary molecular engineering of enzymes. His recent work, and the focus of his talk, has been concentrated on polyamines, organic compounds that are essential in both eukaryotic and prokaryotic cells. Inhibition of cellular polyamine synthesis leads to cell growth suspension or severely retards cell growth.

Roger Cone, PhD

Chairman of Molecular Physiology,
Director, Institute for Obesity and Metabolism, Vanderbilt University

"The Central Melanocortin System and Energy Homeostasis"

Dr. Roger Cone joined Vanderbilt in 2008, but previously, he was director of the Center for the Study of Weight Regulation (CSWR) at Oregon Health and Science University, and senior scientist at the Vollum Institute. Cone received his PhD in biology from the Massachussetts Institute of Technology. He began his studies in endocrinology as an assistant professor at New England Medical Center and then spent 18 years at the Vollum Institute, before starting the CSWR research center in 2006. Cone and his colleagues cloned melanocortin receptors, characterized their roles in pigmentation and energy homeostasis, and demonstrated that the agouti protein was an endogenous antagonist of these GPCRs. This led to their discovery of the function of the central melanocortin system, the critical leptin-responsive neural circuit that coordinates much of feeding behavior and energy expenditure in mammals to achieve long-term energy homeostasis. Mutations in the melanocortin-4 receptor are now known to be a major cause of severe obesity in humans, responsible for about 5% of cases. Dr. Cone has received numerous awards for his research, including the Ernst Oppenheimer Award of the U.S. Endocrine Society, the Berthold Memorial Award of the German Endocrine Society, and the Ipsen Prize in Endocrinology.

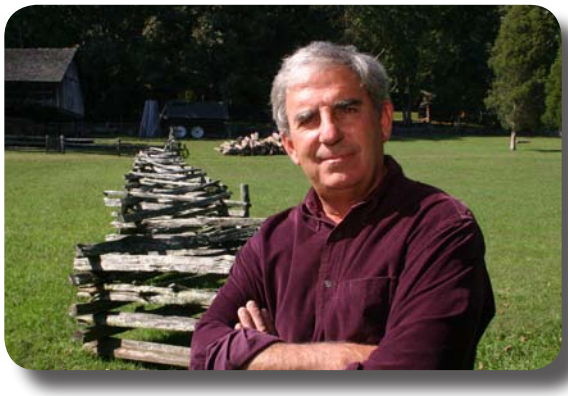
Featured Speakers

Diane Klotz, PhD

Director, Office of Fellows' Career Development
National Institute of Environmental Health Sciences (NIEHS, NIH)

"Why Choose the NIH for a Postdoctoral Fellowship?"
Workshop Luncheon for Presenters

Director of the Office of Fellows' Career Development (OFCD) at NIEHS, Dr. Diane Klotz was an NIEHS postdoc herself. Postdoc training and mentoring is the focus of the OFCD, and Klotz's team has made NIEHS one of the "Best Places to Work for Postdocs" in the United States; for the 4th year in a row, NIEHS has ranked first among NIH institutes in the international survey conducted by *The Scientist*. In particular, NIEHS was noted by respondents to have strengths in facilities and infrastructure, as well as in training and mentoring. In 2007, NIEHS rose to number seven in the survey and has held steady at number 16 for the last 2 years. The OFCD at NIEHS provides predoctoral and postdoctoral fellows at the institute with information and professional skills to help them obtain training positions and excel in their scientific careers. (Photography: Steve McCaw, NIEHS Web site)



Bill Landry, MA

Host and co-producer of the Heartland Series, WBIR Channel 10, Knoxville

"Herbs, Potions, Magic Cures and Remedies of the Southern Appalachians" – Featured After-dinner Address

Bill Landry has been the host, narrator, and co-producer of *The Heartland Series* since it was conceived in 1984. Since its beginning, over 1,900 short features have been produced and aired on Knoxville's WBIR-TV, Channel 10, an NBC affiliate.

The Heartland Series has won numerous national and international awards, including 5 Emmy Awards. In 1999 and 2000, Landry was awarded Emmy Awards for directing particular episodes. It is generally agreed that in East Tennessee, *The Heartland Series* is the most highly regarded local television program on the region's history.

WBIR has announced that *The Heartland Series* will stop producing new shows in September of this year. As many changes occur and plans evolve, Landry, meanwhile, is working on a number of projects that continue to focus on our rich heritage, our spectacular land, and our unique people. Bill is a graduate of *Trinity University, the Dallas Theater Center*, with a Master of fine Arts degree, and a BA in literature from the *University of Tennessee at Chattanooga*. For over 30 years, he has also written, produced, and performed his one-man play, *Einstein, the Man*, which has been presented in 38 states and Canada.

Abstracts

Investigación

Research 연구

Kutatás

Výskun

研究

Ricercare

THE UNIVERSITY of TENNESSEE

1. Detrimental Alcohol-related Health Consequences among College Student Binge Drinkers Enrolled in Southern Institutions of Higher Education

April C. Tallant
College of Education, Health, and Human Sciences (Human Ecology)

The purpose of this research was to examine the relationship between binge drinking and health consequences among college students in the South. College student binge drinking is a public health problem. Research using national samples has shown that the likelihood of alcohol-related problems increases with the frequency of binge drinking, yet region-specific research is lacking. A secondary analysis of the 2006 National College Health Assessment (NCHA) aggregate database was conducted. The Institutional Review Board exempted this research. Analyses were delimited to Southern higher education institutions. The final sample consisted of 5,155 undergraduate students who reported being occasional binge drinkers (consumed five or more drinks 1-2 times during the previous 2 weeks) or frequent binge drinkers (consumed five or more drinks three or more times during the previous 2 weeks). Descriptive statistics showed that of binge drinkers, 57% and 43% were occasional and frequent binge drinkers, respectively. Chi-square analyses showed significant relationships between binge status and gender (χ^2=65.986, *df*=1, *p*=.000) and the following: Self-injury (χ^2=291.423, *df*=1, *p*=.000), injury of another (χ^2=143.254, *df*=1, *p*=.000), fighting (χ^2=176.203, *df*=1, *p*=.000), regretful action (χ^2=181.196, *df*=1, *p*=.000), forgetting (χ^2=367.049, *df*=1, *p*=.000), and unprotected sex (χ^2=165.732, *df*=1, *p*=.000). There was no significant relationship between binge status and being forced into or threatened with force for sex (χ^2=3.008, *df*=1, *p*=.083). Results demonstrated that the likelihood of experiencing detrimental alcohol-related health consequences increases with the frequency of binge drinking among college students enrolled in Southern institutions. Future research should explore methods to moderate detrimental alcohol-related consequences.

2. Dietary Supplement Use and Beliefs among College Students Enrolled in an Introductory Nutrition Course

Amy Webb,[1] Marsha Spence,[1] Betsy Haughton,[1] Trena Paulus[2]
[1]Department of Nutrition; [2]Department of Educational Psychology and Counseling

The study purpose was to assess differences in dietary supplement use and beliefs related to their use based on college major, physical activity frequency, and weight status among college students enrolled in an introductory nutrition class. A secondary database consisting of introductory nutrition students at the University of Tennessee, Knoxville, during spring semester 2008 was used and contained 306 respondents. Data were taken from results of a two-part survey. The first section asked participants to respond about their use of dietary supplements, and the second asked participants to respond to beliefs statements regarding supplements. Dietary supplements were assessed in three categories: vitamins and minerals, herbals, and ergogenic aids. Results found vitamins and minerals were the most commonly used, with 228 (74.5%) respondents reporting they consumed at least one in the last 12 months. While only 23 (7.5%) respondents reported using ergogenic aids, their use varied the most based upon major, weight status, and physical activity. Non-health-related majors (19.6% versus 9.0% of health-related majors, $p<0.01$), overweight and obese individuals (26.7% versus 8.2% of normal and underweight respondents, $p<0.001$), and those who exercised daily (21.7% versus 8.2% who exercised weekly or less, $p<0.001$) were more likely to take them. Major played no role in beliefs scores; however, individuals who exercised daily and overweight or obese individuals had higher mean scores, indicating stronger health beliefs related to dietary supplements. Use of ergogenic aids varied the most based upon study variables. Therefore, future research should focus on determining reasons behind this.

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

3. Comparison of Four Actigraph Accelerometers during Walking and Running

Dinesh John, Brian Tyo, David R. Bassett
Department of Exercise, Sport and Leisure Studies

Actigraph motion sensors are commonly used in physical activity research. Currently, researchers can use the 7164 or one of three different versions of the GT1M to objectively measure physical activity. Our purpose was to determine if differences exist among activity counts from four Actigraph activity monitors at given walking and running speeds. Ten male participants (23.6±2.7yrs) completed treadmill walking and running at ten different speeds (3-min stages) while wearing either the 7164 and the latest GT1M (GT1M-V3) or GT1M version one (GT1M-V1) and GT1M version two (GT1M-V2). Participants walked at 3, 5, and 7 km/hr^{-1} followed by running at 8, 10, 12, 14, 16, 18, and 20 km/hr^{-1}. The accelerometers were worn on an elastic belt around the waist over the left and right hips. Testing was performed on different days using a counterbalanced within-subjects design to account for potential differences attributable to accelerometer placement. At each speed, a one-way repeated measures ANOVA was used to examine differences between accelerometer activity counts in counts·min^{-1}(cpm). Post-hoc pairwise comparisons with Bonferroni corrections were used where appropriate. There were no significant differences between accelerometer activity counts at any given walking or running speed (p<0.05). At all running speeds, activity counts from the 7164 and GT1M-V2 displayed the lowest and highest values, respectively. The mean difference score at peak output between the 7164 and GT1M-V2 was 439±565 cpm. There were no significant differences between outputs from all the accelerometers, indicating that researchers can select any of the four Actigraph accelerometers to do research.

4. Eating Frequency and Healthy Weight Status

Jessica Bachman,[1] Hollie Raynor[2]
[1] College of Education, Health, and Human Sciences (Human Ecology); [2]Department of Nutrition

Previous research demonstrates eating frequency may be negatively related to body mass index (BMI). This study examined eating frequency in overweight individuals (OW: BMI ≥ 27), long-term weight loss maintainers (WLM: BMI< 25) who had lost ≥ 30 lbs and kept it off for ≥ 5 years, and normal weight individuals who had never been overweight (NO: BMI < 25). We hypothesized that eating frequency would be lower in OW than WLM and NO. Three, 24-hr dietary recalls (2 weekdays, 1 weekend day) were collected by phone from 286 adults on randomly selected days using NDS-R dietary software (OW = 97: 57% female, 51.0 ± 9.2 years, BMI = 34.6 ± 4.2 kg/m^2; WLM = 104: 84% female, 49.7 ± 11.8 years, BMI = 22.1 ± 1.7 kg/m^2; NO = 85: 94% female, 46.3 ± 11.9 years, BMI = 21.1 ± 1.4 kg/m^2). One-way ANCOVAs, adjusting for group differences in age, gender, and physical activity, showed daily energy intake (kcals) was higher in OW than WLM (2022 ± 689 vs. 1712 ± 493; p <0.05), with no differences between NO (1781 ± 408) and the other groups. There were no differences in meals/day consumed in the three groups (2.68 ± 0.40). OW ate fewer snacks/day than NO (1.55 ± 0.86 vs. 2.18 ± 1.07; p < 0.00), with no differences between WLM (1.88 ± 1.08) and the other groups. Greater eating frequency may be important for a healthy weight status.

5. The Effects of a Pedometer Intervention on the Physical Activity Patterns of Phase II Cardiac Rehabilitation Participants

Michael F. Shipe, David Bassett, Jr.
Department of Exercise, Sport and Leisure Studies

The purpose was to assess whether the provision of a pedometer and exercise diary could significantly increase the activity levels of phase II cardiac rehabilitation program (CRP) patients on the days they did not attend the program. Seventy patients participated in the study. During their first visit to a phase II CRP, control patients were given a blinded pedometer (n = 34), while experimental subjects received a pedometer that they could view (n = 36). The baseline activity patterns of the experimental patients were determined during their first week of phase II CRP enrollment. Next, they were encouraged to increase their step counts gradually on the days they did not attend the phase II CRP until they were accumulating 2,000 steps/day above their baseline levels and to sustain this increase throughout their enrollment. At baseline, men took more overall steps than women, and all patients took more steps on days they attended the phase II CRP versus days they did not. The experimental

patients took significantly more steps and increased their step counts at a faster rate than the control patients. Phase II CRP patients who use a pedometer and exercise diary significantly increase their overall and aerobic step counts on the days they do not, as well as on the days they do, attend a phase II CRP program versus patients who receive usual care. Thus, pedometers can be used by allied health professionals who work in a phase II CRP to more effectively monitor and increase the activity patterns of their patients.

6. Ominous Tadpoles: American Bullfrogs are Suitable Hosts of *Escherichia coli* O157:H7

William L. Thompson,[1] Matthew J. Gray,[1] Sreekumari Rajeev,[2] Debra L. Miller,[1,3] Alan G. Mathew[4]
[1]Center for Wildlife Health, Department of Forestry, Wildlife and Fisheries; [2]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia; [3]Department of Pathology, College of Veterinary Medicine, University of Georgia; [4]Department of Animal Science

Escherichia coli O157:H7 is a zoonotic pathogen that can be transmitted to humans through contaminated beef and vegetables. Amphibian larvae may function as a spill-over reservoir for *E. coli* O157:H7 in aquatic environments used by livestock. We tested whether American bullfrog (*Lithobates catesbeianus*) tadpoles could become naturally infected with *E. coli* O157:H7 via exposure to this pathogen in outdoor aquatic mesocosms. Cattle feces inoculated with *E. coli* O157:H7 (10^6 CFU g^{-1}) were added daily at environmentally relevant levels to mesocosms that included bullfrog tadpoles, and tadpoles were euthanized and tested for infection. After 7, 14, and 28 days of exposure, 23%, 35%, and 51% of tadpoles, respectively, tested positive for *E. coli* O157:H7. Maximum likelihood estimates revealed a 12% linear increase in the predicted odds of infection with each consecutive week of continuous pathogen exposure. Further, we determined that survivability of *E. coli* O157:H7 in mesocosm water was minimal beyond 3 days, suggesting that tadpoles can become infected quickly after exposure. We also found that 25% of the tadpoles that metamorphosed prior to the end of the experiment tested positive for *E. coli* O157:H7, providing preliminary evidence that infected metamorphs may transport the pathogen overland during dispersal. Together, our results suggest that American bullfrog tadpoles, and perhaps other amphibian larvae, could serve as a spill-over reservoir for *E. coli* O157:H7. Given that ruminants are the primary reservoir for this foodborne pathogen, we recommend that livestock are fenced when possible from water sources where amphibian larvae are present.

7. Blacklegged Ticks in Tennessee: Implications for Lyme Disease

Jessica Harmon,[1] Michelle E. Rosen,[2] Graham J. Hickling,[2] Jean I. Tsao,[3] Cathy Scott[2]
[1] Department of Entomology & Plant Pathology; [2]Department of Forestry, Wildlife, and Fisheries; [3]Center for Wildlife Health, Department of Fisheries and Wildlife, Michigan State University

Lyme disease (LD) is caused by the spirochete *Borrelia burgdorferi*, which in the Eastern United States is transmitted by the bite of the blacklegged tick, *Ixodes scapularis*. Whether LD is endemic in Tennessee and adjacent states has been a topic of much recent debate, in part because current tick distribution maps suggest *I. scapularis* is rarely found in this region. From 2006– 2008, we assessed the distribution, abundance, and pathogen status of this tick species in Tennessee by examining ticks collected from hunter-harvested deer at check stations across the state. We detected adult *I. scapularis* in 32 Tennessee counties that had no prior occurrence record of this tick. At a study site in Marshall County, monthly vegetation "drag" sampling indicated that questing adult *I. scapularis* were readily detectable at a rate that peaked at 10.3/1000m^2 in November 2007. Nymphal *I. scapularis* were also readily draggable in the same habitats at a rate that peaked at 4.9/1000m^2 in March 2008. Here we show for the first time in the Southeastern United States, that nymphal *I. scapularis* ticks in Tennessee exhibit the same host-seeking behavior that results in an elevated risk of human LD as in the Northeast. Laboratory assays and continued field studies are underway to determine the extent to which the LD pathogen may be present in Tennessee and to test the tick phenology and host-preference hypotheses for absence of endemic LD in the state. The results of these investigations will be presented.

8. Investigation of Human *Campylobacter* Infection in Georgia Using GIS and Spatial Analysis Techniques

Jennifer Weisent (CEM), Barton Rohrbach, Agricola Odoi
Department of Comparative Medicine

Campylobacteriosis is the leading cause of bacterial gastroenteritis in the United States and many other developed countries. The objective of this study is to use Geographic Information Systems (GIS) to investigate the distribution of *Campylobacter* infection in the U.S. state of Georgia at the county level. Secondly, the study aims to test whether there are geographical differences in the risk of *Campylobacter* infection and visualize these risks as they correspond to county farm animal densities and human demographic factors. Population data were obtained from the U.S. Census Bureau, and distribution of *Campylobacter* cases was supplied by FOODNET, an active surveillance system of The Center for Disease Control and Prevention in Atlanta, GA. These associations were identified using spatial clustering and regression analysis techniques. Study results are intended to provide information that will enable public health personnel to focus disease prevention and control strategies on subpopulations based on geographic and socioeconomic characteristics and improve the cost effectiveness of disease control.

9. Community-Associated Methicillin-Resistant *Staphylococcus aureus* Nasal Carriage in a College Student Athlete Population

Daniel M. Rackham,[1] **Tanika M. Pinn,**[2] Shaunta' M. Ray,[1] Andrea S. Franks,[1] Kenneth M. Bielak[2]
[1]Department of Pharmacy; [2]Department of Family Medicine, Graduate School of Medicine

Participation in athletics has been identified as a risk factor for infection with community-associated, methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Reports of outbreaks of CA-MRSA skin and soft tissue infection among athletic teams are well documented in the medical literature. The Centers for Disease Control and Prevention (CDC) has identified contact with those colonized or infected as a main mode of transmission for CA-MRSA. The most recent national survey conducted by the CDC demonstrated a 1.5% prevalence of nasal colonization with MRSA in the United States population. This study is designed to evaluate the prevalence of CA-MRSA nasal carriage, risk factors for nasal carriage, and antibiotic susceptibility patterns in college student athletes. IRB approval was obtained. From February to April 2009, 278 college student athletes were evaluated for CA-MRSA nasal carriage using swabs of both anterior nares, followed by cultures and susceptibilities. Subjects completed a brief questionnaire to assess potential risk factors for nasal carriage including sharing personal items, use of whirlpools, previous treatment with antibiotics, and exposure to others with skin infections. Investigators analyzed the prevalence of CA-MRSA nasal carriage, risk factors for nasal carriage, and antibiotic susceptibility patterns in the studied population. Five of 278 (1.8%) college student athletes sampled tested positive for nasal carriage of CA-MRSA. Risk factor analysis is underway. It does not appear college student athletes have higher rates of CA-MRSA nasal carriage than that of the general population.

10. Identification of a Pulsed-Field Gel Electrophoresis Cluster in Clinical Isolates of Methicillin-Resistant *Staphylococcus pseudintermedius* and the Implications of a Regionally Distinct Multi-Drug-Resistant Clone

Chad C. Black (CEM),[1] Samar M. Solyman,[1] David A. Bemis,[1] A.M. Woron,[2] Stephen A. Kania[1]

[1]Department of Comparative Medicine; [2]Tennessee Department of Health, Nashville, TN

Methicillin resistance encoded by the *mecA* gene is increasingly observed in *Staphylococcus pseudintermedius*. Little is known about the population genetics of veterinary staphylococci bearing methicillin resistance. The aim of this study was to determine the relatedness of resistant isolates and to compare them with methicillin-susceptible organisms. *SmaI* macrorestriction fragment profiling was performed on 60 isolates obtained from canine pyoderma samples collected by the UT Veterinary Teaching Hospital bacteriology service between 2006 and 2008. Forty of the 60 samples were *mecA* positive and classified as methicillin-resistant using the disc diffusion method. Analysis of pulsed-field data revealed a single major cluster of 37 resistant isolates with 22 subtypes. Methicillin-resistant isolates were predominantly ST 68 and fell within the largest PFGE

cluster, whereas methicillin-susceptible strains were more genetically diverse. This suggests that most methicillin resistance within the geographic area serviced by the Veterinary Teaching Hospital originated from a single source that has persisted and expanded for several years.

11. Novel Multilocus Sequence Types among *Staphylococcus pseudintermedius* Strains Isolated from Dogs

Samar M. Solyman (CEM), David A. Bemis, Chad C. Black, Stephen A. Kania
Department of Comparative Medicine

Methicillin resistance is increasingly noted among *Staphylococcus pseudintermedius*, a newly described species that is commonly isolated from dogs and occasionally from other animals, including human beings. Multilocus sequence analysis was performed on 60 isolates of *S. pseudintermedius* from dogs with pyoderma. Isolates were representative of different groups with respect to oxacillin susceptibility patterns and year isolated (2006, 2007, and 2008). Among 20 different sequence types (ST) detected, 16 novel types were described. A majority of the methicillin resistant strains tested belonged to a single ST (ST 68), which suggests that clonal expansion of the successful clone occurred. Phylogenetic analysis for each of five housekeeping genes revealed five alleles found among the phosphate acetyltransferase gene (*pta*), including one novel *pta* sequence; Chaperonin or heat shock protein 60 gene (*cpn60*) was most diverse with nine alleles, including a novel *cpn60* sequence for the same strain with the novel *pta* sequence; two alleles were found among the elongation factor gene (*tuf*); four previously known alleles of the accessory gene regulatory (*agrD*) were found among the isolates with type IV predominance, and small ribosomal subunit (*16 S rRNA*) sequences contained a single allele. Oxacillin-resistant *S. pseudintermedius* isolates from the Southeastern United States were predominantly of ST 68, while oxacillin susceptible strains were more genetically diverse.

12. Effect of Antibiotic Growth Promoters (AGPs) on Intestinal Microbiota in Chickens

Andree Clark Hunkapiller,[1] Kelly R. Robbins,[1] Alice Layton,[2] Arnold Saxton,[1] Jun Lin[1]
[1]Department of Animal Science; [2]Department of Microbiology

Intestinal microbiota play a variety of roles in host growth performance with respect to food digestion, nutrient utilization, and induction of host innate immunity. Antibiotic growth promoters (AGPs) have been used as feed additives to improve average daily weight gain and feed efficiency in food animals for more than five decades. Although it is widely accepted that the growth promoting effect of AGPs is mediated through intestinal microflora, little is known about microbial diversity and dynamics in the animal intestine in response to AGP treatment. In this project, chickens were treated under similar industrial conditions and fed either AGP supplemented or control (non-medicated) feed. Luminal contents were collected from the ileum at the day when the most significant growth promoting effect was observed and were used to identify bacterial diversity changes in response to AGP treatment using a 16S rDNA-based molecular approach. Briefly, total DNA was extracted from luminal fecal samples (four chickens per group) and amplified using broad-range eubacterial primers (530f/1392r). PCR products were cloned into pCR4-TOPO vector for construction of 16S rDNA libraries (96 clones for each chicken sample). Plasmid DNA from each transformant was purified, and the 16S rDNA nucleotide sequence was sequenced using M13F/M13R primers. Bi-directional sequences were aligned to the small subunit rRNA Ribosomal Database Project (RDP-II), and transformants were identified. Phylogenetic analysis showed that AGP treatment influenced the diversity of intestinal microbiota in chickens.

13. Reverse-Transcriptase Loop-mediated Isothermal Amplification (RT-LAMP) as a Novel Approach for the Detection of *Salmonella* Typhimurium in Pork

Chayapa Techathuvanan, F. Ann Draughon, Doris H. D'Souza
Department of Food Science and Technology

Reverse-transcriptase, loop-mediated isothermal amplification (RT-LAMP) is a novel molecular method

with advantages over PCR, with amplification at one temperature within 90 min in a simple water bath. Our objective was to compare the detection sensitivity of *Salmonella* Typhimurium from pork products by RT-LAMP to traditional and molecular PCR assays. Pork chops and sausages (25g) were inoculated with *S.* Typhimurium at high (10^8-10^6 CFU) and low (10^3–10^1 CFU) inocula levels. Serially diluted samples were plated on XLT4 agar either immediately before or after 10 h enrichment in 225-ml tetrathionate broth at 37°C. RNA was extracted using the TRIzol method from 5 ml samples. RT-LAMP assay using six *Salmonella*-specific *inv*A gene primers, *Bst* DNA polymerase and reverse-transcriptase was conducted at 62°C/90 min in a water bath. Detection was by agarose gel electrophoresis and visual turbidity. Real-time RT-PCR analysis was performed using a SYBR Green I kit with *inv*A gene primers and an internal amplification control. Reaction conditions were 50°C/30 min and 45 cycles of PCR at 95°C/30 s, 58°C/30 s, 72°C/30 s in a BioRad iCycler, followed by melt temperature analysis. Improved *Salmonella* detection of 10^2 CFU/25g for both pork samples by RT-LAMP assay was obtained after 10 h enrichment, compared to 10^2 CFU/25g pork and 10^4CFU/25g sausage by RT-PCR. Even without enrichment, *Salmonella* could be detected at 10^6 CFU/25g for pork and sausage by RT-LAMP and at 10^6 and 10^7 CFU/25g by RT-PCR, respectively. The cultural assay with positive results in all tested samples takes several days. This RT-LAMP assay shows promise for improved and rapid *Salmonella* detection from pork products within 1 day.

14. Chronic *Escherichia coli* Experimental Intramammary Infections in Primiparous Dairy Cows during the Periparturient Period

Raul A. Almeida,[1] Susan I. Headrick,[1] Mark J. Lewis,[1] Barbara E. Gillespie,[1] Lisa M. Bauer,[2] David L. Johnson,[2] Kenneth C. Lamar,[2] Stephen P. Oliver[1]
[1]Department of Animal Science; [2]Tennessee AgResearch

Experimental infection models have been an important tool for studying pathogenesis and for developing and evaluating strategies for disease prevention and control. The goal of the present study was to develop a chronic *Escherichia coli* mastitis experimental infection model that mimics naturally-occurring, chronic *E. coli* mastitis in dairy cows. One uninfected mammary quarter of seven Holstein heifers was inoculated with 50 colony forming units (cfu) of a strain of *E. coli* isolated from a cow with naturally-occurring, chronic *E. coli* mastitis near calving. Six of 7 heifers became infected following challenge. All heifers that became infected following challenge developed mild cases of clinical mastitis. Rectal temperatures were elevated in all infected heifers approximately 9 to 18 hr after challenge, remained elevated for up to 24 to 36 hr after challenge, and then decreased to normal. *Escherichia coli* was isolated in milk from all infected mammary quarters frequently during the first 7 to 14 days after challenge, and three animals continued to shed *E. coli* intermittently during the 2 month sampling period. One animal shed *E. coli* in milk intermittently for 172 days after challenge and developed clinical mastitis three times, which was caused by the challenge strain as evaluated by pulsed-field gel electrophoresis typing. Results of this study showed that experimental infection of heifer mammary glands during the periparturient period with a strain of *E. coli* isolated originally from a cow with chronic intramammary infection caused mild clinical mastitis that mimics naturally-occurring, chronic *E. coli* mastitis in dairy cows.

15. Pathogenesis of Chronic *Escherichia coli* Mastitis

Raul A. Almeida, Douglas A. Luther, Stephen P. Oliver
Department of Animal Science

Escherichia coli intramammary infections (IMI) are generally thought to be of short duration, resulting either in rapid bacterial clearance or death of the cow. More recent reports have described *E. coli* IMI where the course of infection was chronic and recurrent rather than acute and transient. Work conducted in our lab showed that strains of *E. coli* isolated from cows with chronic mastitis internalize into bovine mammary epithelial cells better than strains of *E. coli* isolated from cows with acute mastitis. Further investigations showed that chronic strains persisted intracellularly longer than acute strains, which led us to investigate intracellular trafficking pathways of acute and chronic strains of *E. coli*. Treatment of bovine mammary epithelial cells with inhibitors of receptor-mediated (clathrin) endocytosis and lipid raft/caveolae-mediated endocytosis (CME) showed that chronic strains of *E. coli* internalized and moved intracellularly, exploiting CME and thus evading deleterious intracellular bactericidal mechanisms such as endocytic acidification

and lysosome fusion. These results could explain why chronic strains of *E. coli* persist intracellularly longer than strains of *E. coli* from acute mastitis. Recent proteomic work conducted by our group showed that acute and chronic strains of *E. coli* have different membrane surface protein make-ups, suggesting that acute and chronic strains of *E. coli* are two different clonal types that cause very different pathogenic processes. Collectively, results from our investigations showed that strains of *E. coli* associated with chronic mastitis have specific virulence attributes that exploit defined host cell processes, which allow avoidance of bactericidal mechanisms and bacterial intracellular persistence.

16. Presentation Canceled

17. Presentation Canceled

18. Serum Levels of Matrix Metalloproteinase-2 as a Marker of Intimal Hyperplasia

A. Mike Tummers, Deidra J.H. Mountain, J. William Mix, Stacy S. Kirkpatrick, David C. Cassada, Scott L. Stevens, Michael B. Freeman, Mitchell H. Goldman, Oscar H. Grandas
Department of Surgery

A primary component in the development of intimal hyperplasia (IH) in response to vascular injury is basement membrane remodeling. Matrix metalloproteinases (MMPs) play a major role in this process by degradation of basement membrane proteins, mainly collagen type IV. Vascular injury initiates an inflammatory cascade with the release of tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and C-reactive protein (CRP). We hypothesize serum levels of these elements may serve as biomarkers of the development of IH. At baseline, 2, 7, 10, and 14 days post-balloon angioplasty of the carotid artery, rat tissue samples were stained with Masson Trichrome elastin to examine IH. Intima:media ratios (I:M) increased significantly over time post-injury. Serum samples were collected at the time of tissue sampling, and levels of MMP-2, MMP-9, collagen type IV, TNFα, IL-1β, and CRP were assayed using sandwich ELISA immunoassay. MMP-2 serum levels at 7, 10, and 14 days post-injury were significantly elevated compared to baseline. Other elements were not significantly elevated. Early and persistent elevation in the serum levels of MMP-2 may be a useful biomarker of basement membrane remodeling and the presence of IH.

19. Lysophosphatidylcholine Activates a Novel PKD2-Mediated Signaling Pathway that Controls Human Blood Monocyte Migration

Feng Hao, Mingqi Tan, **Dongwei Wu, Xuemin Xu, Mei-Zhen** Cui
Department of Pathobiology

Monocyte activation is important in the development of atherosclerosis and other inflammatory diseases. We report here that lysophosphatidylcholine (lysoPC), which is present in arterial lesions and is a prominent component of oxidized low density lipoprotein, induces rapid and marked protein kinase D (PKD) activation in human peripheral blood monocytes. Immunoblot data confirm that of the three members of the PKD family (PKD1, PKD2 and PKD3), PKD2 is the predominant isoform in monocytes. Using the small interfering RNA knock-down method, we found that lysoPC-induced endogenous PKD2 activation is required for the activation of both ERK and p38 MAPK. Interestingly, the lysoPC-induced PKD2-p38 pathway, but not the PKD2-ERK pathway, controls monocyte migration. Thus, the present study reveals that PKD is a novel and functional intracellular regulator in the lysoPC signaling pathway and monocyte activation. Our data also reveal a novel functional role for the endogenous PKD2 in cell migration in response to an extracellular stimulus. This study suggests a new role of PKD2 in the development of atherosclerosis and possibly other inflammatory diseases.

20. Dynamic Expression and Cellular Localization of LPA-induced CYR61 in Smooth Muscle Cells and its Novel Role in LPA-induced Cell Migration

Dongwei Wu (CEM), Feng Hao, Xuemin Xu, Mei-Zhen Cui
Department of Pathobiology

Lysophosphatidic acid (LPA), a bioactive phospholipid produced by activated platelets and formed during the oxidation of low density lipoprotein (LDL), has been found to accumulate in high concentrations in atherosclerotic lesions and to induce vascular smooth muscle cell (SMC) proliferation and migration and neointimal formation in carotid arteries. Therefore, LPA is being increasingly recognized as a risk factor for atherosclerosis. Our results demonstrate that LPA markedly and rapidly induces matricellular protein CYR61 expression in mouse smooth muscle cells. CYR61 has been shown to induce smooth muscle cell proliferation and migration, which are crucial events in the development of atherosclerosis. Our immunofluorescence results demonstrate a dynamic movement of CYR61; LPA-induced CYR61 protein first accumulates in the endoplasmic reticulum and then localizes to the extracellular matrix. Interestingly, our data reveal for the first time that LPA-induced SMC migration is inhibited by both a CYR61-specifc antibody and integrin-specific antibodies. Thus, this CYR61-bridged LPA/GPCR pathway and the CYR61/integrin pathway define a novel convergence mechanism that may underlie vascular wall remodeling in atherosclerosis.

21. Presenilin Interacts with Nicastrin and APH1, Independent of Subcomplex Formation of Nicastrin and APH1

Guozhang Mao,[1] Jianxin Tan,[1] Mei-zhen Cui,[1] Tong Li,[2] Bart De Strooper,[3] Huaxi Xu,[4] Xuemin Xu[1]
[1]Department of Pathobiology; [2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD; [3]Center for Human Genetics, Department of Molecular Genetics, Katholieke Universiteit Leuven, Leuven, Belgium; [4]Center for Neuroscience and Aging, Burnham Institute for Medical Research, La Jolla, CA

Accumulating evidence supports the hypothesis that the abnormal accumulation of β-amyloid peptide (Aβ) in the brain is a causative event in the development of Alzheimer's disease. Aβ is proteolyticaly produced from a large amyloid precursor protein (APP). In the amyloidogenic pathway, APP is first cleaved by β-secretase to produce a soluble ectodomain, sAPPβ, and a membrane-associated C-terminal fragment, CTFβ. CTFβ is subsequently cleaved within the membrane domain by γ-secretase to produce the full-length Aβ. Thus, γ-secretase-mediated processing of APP is a crucial step in the formation of Aβ. Despite extensive investigation of the mechanisms by which Aβ is produced, many unanswered questions still exist regarding the assembly of the γ-secretase complex per se and the interaction of γ-secretase with its substrate. In the present study, using knockout cell lines in which some components of the γ-secretase complex have been eliminated, we determined some aspects of how the multisubunit γ-secretase complex is assembled. Our data clearly show that presenilin, the key component of the γ-secretase complex, can co-immunoprecipitate with nicastrin, another major component of the γ-secretase complex, in the absence of Aph1α, the other major component of the complex. Vice versa, presenilin can also co-immunoprecipitate with Aph1α in the absence of nicastrin. The information obtained from this study could provide new insight into the molecular mechanisms of γ-secretase-mediated APP processing.

This work was supported by National Institutes of Health grant R01AG26640 to XX and supported by the Alzheimer's Association grant 11RG-05-14584 to XX.

22. Residues at the P2-P1 Positions of e- and ζ-cleavage Sites are Important in Aß Formation

Jianxin Tan,[1] Guozhang Mao,[1] Mei-Zhen Cui,[1] Bruce Lamb,[3] Man-Sun Sy,[2] Xuemin Xu[1]

[1]Department of Pathobiology; [2]Department of Neuroscience, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH; [3]Department of Pathology, Case Western Reserve University, Cleveland, OH

Several Alzheimer's disease (AD)-linked mutations in amyloid precursor protein (APP) cause abnormal production of ß-amyloid (Aß) peptide, which is believed to be the causative material in the development of AD. It is interesting to note that most of the AD-linked APP mutations are localized at the major ß-secretase- and γ-secretase-mediated cleavage sites. To determine the

mechanism by which the mutations alter γ-secretase processing resulting in abnormal Aß production, we have generated a series of mutations around the ε-cleavage site at Aß49 and the ζ-cleavage site at Aß46. By characterizing these mutations, our data demonstrated that residues at the P2-P1 positions of the ε-cleavage site and ζ-cleavage site have strong effects on γ-secretase-mediated processing of APP. Specifically, the structures of the substituting residues at these key positions strongly determine cleavage efficiency, and the sizes of the side chain of the substituting residues determine the cleavage preference of γ-secretase-mediated APP processing at the final step, which determines the ratio of different secreted Aß species. Thus, these findings may provide a biochemical explanation for why the disease-linked mutations are always found at the major processing sites of APP.

23. The Search for Imaging and Therapeutic Agents for Huntington's Disease

Erica L. Rowe,[1,4] H. Penney Koeppen,[1] Alan Stuckey,[2] George W. Kabalka,[3] Stephen J. Kennel,[2] Jonathan S. Wall,[2] Valerie Berthelier[1]

[1]Conformational Diseases and Therapeutics Research; [2]Preclinical and Diagnostic Molecular Imaging; [3]Department of Chemistry/Radiology, Graduate School of Medicine; [4]Neutron Scattering Science Division, Oak Ridge National Laboratory

Huntington's disease (HD) is caused by the presence of an abnormal stretch of polyglutamine (polyGln) repeat in the huntingtin (htt) protein. A pathological hallmark of HD is the neuronal formation and deposition of insoluble cytoplasmic and/or nuclear aggregates containing the mutant htt with its long polyGln stretch. Various morphologies of aggregates/fibrils have been described. The debate continues, however, whether these various polyGln aggregated states contribute to disease pathogenesis or protect against neuronal death, suggesting that the aggregation pathway is complex and may involve many discrete or intertwined pathways. In a high-throughput screening assay, we identified curcumin as an effective inhibitor of polyGln fibril formation. Curcumin is not only known for its therapeutics effects–anti-inflammatory and anti-oxidant properties–but also for its ability to prevent Aβ and α-synuclein fibril formation, Aβ and α-synuclein being amyloid proteins involved in Alzheimer's and Parkinson's, respectively. Importantly, curcumin can also disrupt already-formed fibrils. We investigated the effects of curcumin toward mutant htt fibrils *in vitro*, using polyGln peptides, and *in vivo*, using an htt-inducible cellular model. *In vitro*, curcumin impeded polyGln aggregation and disrupted formed fibrils. *In vivo*, curcumin significantly reduced the number of recruitment-competent aggregation foci (≥50%) without altering the number of recruitment-inert aggregates. Based on these results, curcumin appeared to be a promising candidate to image fibrils in HD mouse, and therefore studies were carried out. While our results of the imaging data were not encouraging, curcumin remains a candidate as an HD therapeutic agent and therefore needs to be explored further.

Funding provided by the Physicians' Medical Education and Research Foundation.

24. Protein Hydration and Association Studied by Small-Angle Neutron Scattering

Christopher Stanley,[1] Hugh O'Neill,[2] Erica Rowe,[1,3] Valerie Berthelier[3]

[1]Neutron Scattering Science Division, Oak Ridge National Laboratory; [2]Center for Structural and Molecular Biology, Oak Ridge National Laboratory; [3]Graduate School of Medicine

Nature uses a variety of small solutes, called osmolytes, to protect proteins against thermal and chemical denaturation. Osmolytes often act indirectly through an exclusion from water, hydrating biomolecular surfaces, cavities, and pores. This equivalently results in the preferential hydration of these regions, where osmolyte competition for this water significantly affects the stability and binding reactions of these biomolecules. We are using small-angle neutron scattering (SANS) combined with osmotic stress to study the preferential hydration of the monomer and dimer forms of hexokinase (HK) in the presence of several osmolytes that vary in size and chemistry. HK is the first enzyme in the glycolytic pathway and catalyzes the phosphoryl transfer from ATP to glucose. The HK monomer-dimer equilibrium plays a regulatory role, but its importance to function is not entirely clear. With SANS, three regions of scattering contrast are created upon osmolyte addition: protein, protein-associated water, and bulk water/osmolyte solution. SANS coupled with osmotic stress allows us to address critical questions about protein preferential hydration and the importance of water in governing protein-protein interactions.

25. Structural Study of Polyglutamine Amyloid Fibril Formation by Small-Angle Neutron Scattering

Tatiana Perevozchikova,[1,2] Chris Stanley,[3] Helen P. Mc Koeppen-Williams,[1] Valerie Berthelier[1]
[1]Graduate School of Medicine; [2]Genome Science Technology Program; [3]Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, TN

The presence of an abnormally expanded polyglutamine (polyGln) repeat in the huntingtin (htt) protein sequence is a hallmark of Huntington's disease (HD), where the mutant htt undergoes conformational changes leading to formation of amyloid-like fibrils. Intermediate structures preceding the mature fibrils are also formed during the aggregation process, but as of today, neither their structure(s) nor their role(s) in neuronal toxicity has been established. Obtaining this information is critical in searching and designing therapeutics for HD. We used time-resolved, small-angle neutron scattering (SANS) to follow polyGln aggregation in solution and investigated the effects of protein context, polyGln length, and hydration. Osmotic stress is also imposed using natural osmolytes to mimic the crowded cellular environment and probe changes in the protein hydration profile. From Guinier analysis of the SANS data, we obtained the radius of gyration and apparent mass of the early intermediates and the cross-sectional radius and mass per length of the growing fibrils. Our SANS results on polyGln Q_{42} fibrils indicate a more open and relaxed structure, resembling the Perutz β-helix model, while polyGln Q_{22} tends to form denser fibrils. This knowledge on the structural differences will provide insight into the relationship between polyGln length and toxicity in Huntington's disease. Overall, the current research will provide a better understanding of the process of the mutant htt protein aggregation and yield details on the structural differences of the oligomeric intermediates.

Funding provided by the National Institutes of Health-NINDS (1R21NS056325-01A1)

26. Bulk Tank Milk Quality of Nine Dairy Farms in Tennessee over a 12-Month Period

Barbara E. Gillespie,[1] Sukolrat Boonyayatra,[2] Mark J. Lewis,[1] Arnold M. Saxton,[1] Stephen P. Oliver[1]
[1]Department of Animal Science; [2]Department of Animal Science, Washington State University, Pullman, WA

Parameters of bulk tank milk (BTM) quality include somatic cell count (SCC), standard plate count (SPC), preliminary incubation count (PIC) and laboratory pasteurization count (LPC). The purpose of this study was to evaluate BTM quality of nine dairy farms and to determine the relationship between these milk quality parameters. Farms were selected based on their PIC history. Three farms with a PIC of <10,000 colony forming units (cfu)/ml, three farms with a PIC >10,000 cfu/ml, and three farms with consistently high PIC were chosen. BTM samples were collected weekly from June 2006–May 2007. Samples were analyzed for SCC, SPC, PIC, LPC, *Staphylococcus* count, *Streptococcus* count, and coliform count. Identification of bacteria from PIC petrifilms with counts >10,000 cfu/ml of milk was performed using standard microbiological procedures. BTM samples were also evaluated to determine the effect of sample storage; samples were collected and set up for analysis either on the day of collection (0), 1 day after collection, or 2 days after collection. Results of this study indicated that PIC, SPC and LPC are all interrelated and are reflective of *Streptococcus* counts and *Staphylococcus* counts. Coliform counts and SCC were relatively independent of all other variables. BTM analysis should be conducted within 1 day of sample collection to get an accurate assessment of BTM quality. Samples analyzed 2 days after collection had significantly lower *Staphylococcus* and coliform counts and significantly higher PIC and LPC.

27. Evaluation of Bulk Tank Milk Quality in Tennessee

Barbara E. Gillespie,[1] Mark J. Lewis,[1] Megan L. Maxwell,[1] E. Miller,[2] Arnold M. Saxton,[1] Stephen P. Oliver[1]
[1]Department of Animal Science; [2]Consolidated Lab Services, Knoxville, TN

Several methods are used to assess milk quality. Some methods, such as the somatic cell count (SCC) and standard plate count (SPC), are mandated by the Pasteurized Milk Ordinance, which specifies safety standards of Grade A milk. Other methods, such as preliminary incubation count (PIC) and laboratory pasteurization count (LPC), while not mandated, are useful to monitor milk quality and to help diagnose on-farm problems/deficiencies associated with high counts and poor quality milk. The purpose of this study was to evaluate bulk-tank milk (BTM) quality on ~30% of dairy farms in Tennessee, representing size and management styles typical for Tennessee dairy operations, as well as BTM quality of herds with a high, average, or low SCC. BTM samples were obtained from Consolidated Lab Services (CLS), Knoxville, TN, from each herd every 3 months for 1 year; SCC, SPC and PIC were analyzed by CLS. Our laboratory analyzed the same BTM sample for LPC, *Staphylococcus* count, *Streptococcus* count, coliform count, and for the presence of *Mycoplasma*. BTM samples (n=742) from 175 different dairy farms were analyzed. Prevalence of *Mycoplasma* and *Streptococcus agalactiae* from these herds was <1%. The presence of *Staphylococcus aureus* and/or a high streptococcal count in BTM was highly correlated with a high SCC. No seasonal effect was seen with SCC; however, farms designated with low, average, and high SCC prior to the start of the study remained consistent over the duration of the study. SPC was highly correlated with *Streptococcus* count and SCC, and PIC and SPC were strongly related.

28. Biofilm Production by *Streptococcus uberis* Isolated from Dairy Cows with Mastitis

Glenis E. Moore,[1] Maria E. Prado,[1,2] Aurie P. Bouchard,[1] John R. Dunlap,[3] Mark J. Lewis,[1] Stephen P. Oliver[1]
[1]Department of Animal Science; [2]Department of Large Animal Clinical Sciences; [3]Division of Biology

Streptococcus uberis is an important etiologic agent of mastitis in dairy cows. This important mastitis pathogen is not controlled effectively by current methods. Our research has concentrated on understanding mechanisms utilized by *S. uberis* to colonize and invade the mammary gland. One such mechanism might be formation of biofilms. The objective of the present study was to determine whether *S. uberis* could form biofilms in vitro. Strains of *S. uberis* (n=27) were screened by qualitative and quantitative assays (microtiter plate [MP], air-liquid interface [ALI] and Congo Red agar [CRA]), and for presence of genes associated with biofilm formation such as competence genes (*comEA*, *comEC*, *comX*) and the quorum sensing gene (*luxS)* by PCR. A known biofilm producer, *Staphylococcus epidermidis*, was used as a positive control. The ability of *S. uberis* to produce biofilms varied according to the method used. All strains produced biofilms when tested by the CRA method. Most strains were positive when ALI (22 of 27) and MP (24 of 27) assays were used. Strains were further classified as negative (3, 11%), weak (4, 15%) and strong (20, 74%) biofilm formers based on the MT assay. *comEA*, *comEC*, *comX* and *luxS* genes were amplified by PCR in 96% (26/27) of strains evaluated. Results suggest that *S. uberis* is capable of forming biofilms in vitro on abiotic surfaces. Genes associated with biofilm formation appear to be highly conserved in the strains evaluated. Additional research is needed to link in vitro findings with events that take place in the bovine mammary gland.

29. sua Gene Deletion Mutagenesis in *Streptococcus uberis* UT888 Using a Thermosensitive Replicative Plasmid

Xue Yan Chen, Raul A. Almeida, Douglas A. Luther, Oudessa Kerro Dego, Stephen P. Oliver.
Department of Animal Science

Streptococcus uberis is an important environmental pathogen that causes mastitis in dairy cows and is associated with significant economic losses worldwide. Research from our lab showed that *S. uberis* adhesion molecule (SUAM) is involved in adherence to and internalization of *S. uberis* into bovine mammary epithelial. To elucidate the role of SUAM in *S. uberis* pathogenesis, *sua* deletion in *S. uberis* UT888 was achieved using a thermosensitive plasmid. The *sua* deletion cassette was prepared by overlap extension PCR and cloned into pGhost9, which was electroporated into UT888. Transformant was grown at 37°C to facilitate integration. The integrant was then incubated with erythromycin (Em) at 28°C to facilitate excision. Following excision, the replicating plasmid (now with wild type *sua* gene) was eliminated by growing at 37°C without Em. Em-sensitive colonies were analyzed for *sua* deletion by PCR, Southern blot and sequencing, and the strain with *sua* deletion was designated Δ*sua S. uberis* UT888. Compared to the parent strain, *Δsua S. uberis* UT888 did not produce SUAM based on SDS-PAGE gel and Western blot. Lack of SUAM almost completely eliminated the ability of the gene deletion mutant to internalize into mammary epithelial cells. These results confirm the central role of SUAM in internalization of *S. uberis* into host cells. The gene deletion *S. uberis* mutant will be a valuable tool in experimental infection studies in dairy cows to confirm the role of SUAM in pathogenesis and to define the value of SUAM as an immunogen to control *S. uberis* mastitis in dairy cows.

This research was supported by USDA/NRI/CSREES grant 2007-35204-18300.

30. Responses of Different CXCR1 Genotypes after Experimental Challenge with *Streptococcus uberis*

Ali A. Elliott, Stephen P. Oliver, Gina M. Pighetti
Department of Animal Science

Mastitis, an inflammation of the mammary gland, accounts for the largest loss in profit for dairy farmers. Our prior research has identified a polymorphism in the CXCR1 gene associated with mastitis, and research is ongoing to identify what makes some cows genetically more vulnerable to infection. This study evaluated cows with different genotypes at position +777 in the CXCR1 gene experimentally challenged with *Streptococcus uberis*. Holstein dairy cows with GG (n=12), GC (n=21), and CC (n=15) were challenged intramammarily with *S. uberis* strain UT888, and samples were collected twice daily for 3 days and once daily for 3 days thereafter. After the challenge, 83% of cows with the GG genotype, 80% of cows with the CC genotype and only 43% of cows with the GC genotype had ≥ 1 colony-forming unit of *S. uberis* for at least two sampling periods (P=0.02). Of those cows that became infected, 27% of the GG genotype, 25% of the GC genotype and 58% of the CC genotype required antibiotics (P=0.25). Average somatic cell counts over all collection times were 1266, 824, and 1976 ($x10^3$ cells/ml) from GG, GC, and CC genotypes, respectively (P=0.55). The significant differences in infection susceptibility observed between genotypes are likely due to variations in the early immune response allowing quick elimination of *S. uberis* and preventing infection. Finding the reasons behind what makes some cows more genetically vulnerable to infection will provide an understanding that will help develop targeted strategies to prevent and treat mastitis infections.

31. Presence of ISS1-like Element in Wild Type *Streptococcus uberis* Strains Isolated from Cases of Bovine Mastitis

Oudessa Kerro Dego, Raul A. Almeida, Douglas A. Luther, X. Chen, Stephen P. Oliver
Department of Animal Science

Streptococcus uberis is a major cause of environmental mastitis worldwide. Current programs aimed at controlling *S. uberis* mastitis are only partially effective. In spite of significant economic losses caused by *S. uberis* in many well-managed dairy herds, virulence factors and mechanisms associated with the pathogenesis of *S. uberis* mastitis are not well known. The ability of *S. uberis* to attach to and internalize into mammary epithelial cells and subsequent intracellular survival enables it to avoid host immune responses. One of our research approaches is to create a random chromosomal mutant library of *S. uberis* strains from cases of bovine mastitis using thermosensitive pGh9:ISS1 plasmid to find genetic factors that enable this pathogen to survive in the face of a massive infiltration of inflammatory cells during mastitis. During Southern blot analysis of the mutant library, we found endogenous transposable elements similar to ISS1 of *Lactococcus lactis*. ISS1 is a transposable bacterial insertion sequence (IS) isolated originally from *L. lactis*. Insertion sequences are small (0.8 – 2.5 kb), phenotypically cryptic sequences of DNA with a simple genetic organization and capable of inserting at multiple sites in a target molecule. They are flanked by inverted repeats, generally encode their own transposition functions, and are mobile elements that can carry virulence genes. We found that seven of eight wild type strains of *S. uberis* carried the ISS1-like element. The presence of the ISS1-like element in some wild type strains of *S. uberis* might allow these strains to become more virulent than others.

This research was supported by USDA/NRI/CSREES grant 2007-35204-18300.

32. Application of a Reproductive Tract Scoring System in Lactating Dairy Cattle

Charles D. Young,[1,2] Fernando A. Di Croce,[1] David A. Roper,[1] Kenneth C. Lamar,[2] Lisa M. Bauer,[2] David L. Johnson,[2] Hugh D. Moorehead,[2] Nancy R. Rohrbach,[1] J. Lannett Edwards,[1] F. Neal Schrick[1]
[1]Department of Animal Science, UTIA Reproductive Research Program; [2]Tennessee AgResearch

Reproductive tract scoring (RTS) systems have been effective in evaluating fertility of heifers. A similar RTS system has not been reported in lactating dairy cows (LDC) pertaining to fertility. Objectives of this preliminary study include development of an RTS system for LDC and determining if RTS has an effect on artificial insemination (AI) success with semen type (conventional [CS] vs. sexed [SS]). Prior to estrus, cows were assigned an RTS (1, 2, or 3) by at least two experienced palpators. Reproductive tracts for RTS 1 were small and compact; RTS 2 were intermediate and slightly larger and deeper then RTS 1; and RTS 3 were very large and deep, considered outside the pelvic cavity. Following RTS, cows (n=179) were randomly assigned to conventional or sexed semen. Ultrasonography was performed at 28–45 days post AI for determination of conception rate (CR). Preliminary results are presented as numerical differences since no statistical significance was found with current experimental numbers. The highest CR was observed in RTS 1, followed by RTS 2 and RTS 3 (37.5%, 24.9%, and 23.0%, respectively). Regardless of RTS, conception rates of CS (33.4%) were higher than SS (24.0%). Similar trends were noted in the interaction of RTS and semen type, with CS having higher CR vs. SS in RTS 1 (46.2 vs. 29.6%), RTS 2 (30.6 vs. 20.0%), and RTS 3 (25.0 vs. 23.1%). These preliminary results indicate that uterine tract size may affect fertility of LDC, and SS may be more effective in smaller tracts.

33. Estimation of Genetic Parameters for Embryo Transfer Traits

Fernando Di Croce, Arnold M. Saxton, Nancy R. Rohrbach, J. Lannett Edwards
F. Neal Schrick
Department of Animal Science, UTIA Reproductive Research Program

Multiple ovulation/embryo transfer (MOET) technology rapidly increases genetic progress, reduces risk of disease transmission, and expands number of progeny from genetically superior parents. However, critical limitations of MOET include variability in superovulatory response of donor animals and pregnancy success from transferred embryos. Although significant genetic variation for fertility is generally accepted, development of breeding values for fertility traits has mostly been ignored. Strategies that elevate fertility in cattle by means of genetic/genomic selection will become increasingly important. The objective of the present research was to estimate genetic parameters associated with MOET. Data were analyzed on 10,425 transferred embryos (2,900 collections) from 611 donor animals using semen from 215 bulls. Phenotypic traits examined included pregnancy status of the recipient following transfer (ET-PREG), number of transferable embryos per collection (ET-TRANS), and number of unfertilized ova at collection (ET-UFO). Genetic parameters were estimated for a single-trait animal model using restricted maximum likelihood (REML) procedures in Wombat. The EPD for ET-PREG ranged from −6.1 to 4.4% (SE=2.2 to 4.2) for semen sires and −5.3 to 3.8% (SE=3.2 to 4.2) for donor animals. Heritabilities were 0.03, 0.00, and 0.05 for ET-PREG, ET-TRANS, and ET-UFO respectively. EPD values for ET-UFO ranged from −0.6 to 0.8 (SE=0.3 to 0.6) for semen sires and −0.4 to 1.1 (SE=0.5 to 0.6) for donor cows. Genetic gain estimate, assuming 50% selection for ET-PREG and estimated by (i=0.8)*(SD=4 9.962)*(h2=2.9), was = 1.16 %. Genetic improvement in fertility by selection of embryo transfer traits is possible, but progress would be slow.

34. Pregnancy Retention Rates after Transfer of a Bovine Embryo Following Exposure to a $PGF_{2\alpha}$ Receptor Antagonist during Collection

David A. Roper, Fernando Di Croce, Arnold M. Saxton, Nancy R. Rohrbach, J. Lannett Edwards, F. Neal Schrick
Department of Animal Science, UTIA Reproductive Research Program

Pregnancy rates of bovine embryo transfer recipients have previously been improved with inclusion of a prostaglandin $F_{2\alpha}$ receptor antagonist in collection media. The current objectives were to determine pregnancy retention rates (PRR) of recipients and calf normality/viability when receiving fresh or frozen (ethylene glycol) embryos exposed during collection to commercial medium plus 1 mL DMSO (VEH), medium plus 100 nM of AL 8810 (AL100), or medium plus 1000 nM of AL 8810 (AL1000). From 1,734 transfers across 17 replicates, 910 confirmed pregnancies (VEH, n=294; AL100, n=267; AL1000, n=349) with calf data were used for analysis. Pregnancy retention rates were defined as the percentage of recipients calving that were diagnosed pregnant (30-90 days of gestation) after transfer. Pregnancy retention rates did not differ between AL100 and AL1000 (95% ± 0.10 and 91% ± 0.02, respectively; $P = 0.72$); therefore, these groups were combined. Addition of AL 8810 to collection media of embryos increased PRR compared to VEH (92% ± 0.02 vs. 83% ± 0.03, respectively; $P = 0.01$). Furthermore, a trend was noted for improved average daily gain between fresh and frozen embryos (1.13 kg ± 0.03 vs. 1.02 kg ± 0.05, respectively, $P = 0.07$). Analysis of other production traits (gestation length, sex ratio, birth weight, weaning weight, death loss) revealed no differences due to media treatment, transfer type (fresh vs. frozen), or interactions. Therefore, inclusion of a prostaglandin $F_{2\alpha}$ receptor antagonist to collection media of embryos increased initial pregnancy rates and subsequent pregnancy retention rates without influencing calf normality/viability.

35. Proteomic Analysis of Bovine Oocytes during Maturation Using 2-D DIGE

Louisa A. Rispoli,[1] Christina R. Rozanas,[2] T.A. Stoming,[2] Brooke W. Middlebrooks,[1] Nancy R. Rohrbach,[1] F. Neal Schrick,[1] J. Lannett Edwards[1]
[1] UTIA Reproductive Research Program, [2]Department of Animal Science; GE Healthcare Bio-Sciences, Piscataway, NJ

Reduced protein synthesis was previously noted in heat-stressed oocytes, which was correlated with reduced developmental competence, an indicator of poor oocyte health. Objective was to evaluate relative abundance of individual proteins during oocyte maturation (i.e. germinal vesicle [GV-] stage versus matured) and to assess the impact after heat stress (HS). Oocytes were collected on 3 days and randomly distributed into three treatment groups: GV-stage, 24 hr control (38.5ºC), or 24 hr HS (41.0ºC). A subset was matured, fertilized, and evaluated for developmental competence. Protein content of each sample was assessed prior to shipment to GE Healthcare for DIGE (Fluorescence Difference Gel Electrophoresis) analysis. Statistical significance of the average ratio (i.e. degree of difference between the abundance of a protein in two different groups) was determined by the paired Student *t* test. Data were filtered to include only proteins with an average ratio of at least 1.3. Heat stress reduced developmental competence (blastocyst rate 25.2 ± 1.6% for control, 16.4 ± 3.8% for HS). More than 2,400 proteins were visualized, with 551 matched across all treatment groups. The relative abundance of most proteins was similar in the GV stage versus matured oocytes, but oocyte maturation was coincident with a reduction in 21 proteins and an increase in eight proteins compared to the GV stage ($P<0.05$). While most proteins were of similar abundance in control and HS oocytes, HS reduced six proteins and increased nine proteins ($P<0.05$). This study demonstrates the usefulness of 2-D DIGE for oocyte proteomic analysis and the need for further analysis using mass spectrometry.

36. Molecular Features of Gamete RNA

Rebecca R. Payton, Louisa A. Rispoli, Brooke W. Middlebrooks, Nancy R. Rohrbach, F. Neal Schrick, J. Lannett Edwards
Department of Animal Science, UTIA Reproductive Research Program

Oocyte health, which is critical for female fertility, relies upon maternal stores of RNA accumulated during oocyte growth. Despite numerous studies reporting examination of gamete RNA integrity before further RNA characterization, few were found to provide data regarding general features of total RNA (i.e., rRNA ratio or the size distribution of RNA). Results herein provide additional insight regarding molecular features of gamete RNA and how they compare to cumulus cell total RNA. In particular, RIN values and ratios for rRNA, 18S/fast region and 18S/inter region were significantly lower in total RNA from oocytes versus cumulus cells after isolation with a commercially-available kit (PicoPure). Lower values for the 18S/fast region ratio suggested that oocyte total RNA had an increased abundance of smaller RNA sizes relative to 18S rRNA than RNA from surrounding cumulus. While actual values varied, extensive efforts demonstrated that oocyte RNA features were repeatable whether maturation occurred in vitro or in vivo, and were similar between the nuclear stages examined (i.e., GV stage vs. MII). Features of oocyte RNA were conserved across six mammalian species examined (bovine, ovine, porcine, canine, feline and murine) yet differed from surrounding cumulus. Profiles of sperm RNA ranged in size from approximately 25 to 4,000 nucleotides, had no discernible ribosomal RNA peaks, and were conserved across species (bovine, porcine, ovine, and human). Because both the oocyte and the spermatozoon are highly specialized cells representing different molecular entities required for proper embryo development, features obtained likely represent real differences in gamete versus cumulus RNA.

37. Investigation of Geographic Access to Heart Attack and Stroke Care in the East Tennessee Appalachian Region

Ashley Pedigo (CEM),[1] Melany Noltenius,[2] Bruce Ralston,[2] Agricola Odoi[1]
[1]Department of Comparative Medicine; [2]Department of Geography

Stroke and heart attack are serious conditions that have serious effects in the United States, with prevalence of 2.6% and 3.7% and estimated costs of $65.5 and $156.4 billion in 2008, respectively. Although geographic and socioeconomic factors are known to be important determinants of access to emergency care for these conditions, little is known regarding access of different neighborhoods in the Tennessee Appalachian region, which has one of the highest heart attack and stroke rates in the nation. Therefore, the goal of this study was to identify neighborhoods lacking timely access to appropriate heart attack and stroke care in the Tennessee Appalachian region. Since both conditions require time-sensitive treatments, timely access to appropriate care is important in improving health outcomes. Neighborhoods lacking timely access to appropriate care centers were identified using the network analyst service area solver in ArcGIS 9.3. Travel times to the nearest care centers were computed while taking into consideration travel distance, speed limits, degree of road connectivity, and turn impedances. Buffers of 30, 60, and 90 min from each care center were determined to classify neighborhood access. Preliminary results show that 15% and 9.8% of the study area population do not have access to emergency cardiac and stroke care, respectively, within a reasonable time. The populations with poor access were located in the very rural areas. These findings will provide invaluable information for population health planning initiatives that seek to address health inequities associated with access to emergency cardiac and stroke care.

38. Electroencephalograph in the Default Mode of Brain Function

Rex L. Cannon, Debora Baldwin
Biological Psychology/Neuroscience Laboratory, Department of Psychology

Recent research exploring cortical functional connectivity defines a default network (DNt) of brain function. The DNt is a set of dynamically coupled brain regions that are shown more highly active at rest than during cognitively demanding tasks. This study aimed to examine EEG frequency-specific activity within and between 12 regions of interest in both resting-state and active tasks. This study was conducted with 70 non-clinical participants, 40 of them female with a mean age of 20. We contrasted eyes-closed baseline (ECB) with eyes-opened baseline (EOB) and EOB with active tasks (AT) for each frequency domain. We estimated EEG source localization with standardized low resolution electromagnetic tomography (sLORETA) in 12 of the 13 regions of interest (ROI) used by other default mode studies. The ECB resting condition showed higher activity in delta and theta frequencies for all ROI. Contrarily, alpha 1, alpha 2, and beta showed the effect in some but not all of the ROIs. Likewise, the active tasks showed differential effects for increased activity as compared to EOB for each ROI in each frequency domain. The inter-rater reliability for the subjective reports showed Cohen's Kappa of .83. The data are in agreement with other neuroimaging techniques (fMRI/PET) investigating the default mode of brain function. The functional core regions show increased delta and theta during eyes-closed resting; however, this pattern does not extend all ROIs in the higher frequencies. The subjective reports offer evidence that this difference represents functional connectivity relating to endogenous/exogenous attentional states as opposed to the description of "resting-state."

39. Injection Site Pain after Fluoroscopically-Guided Epidural Steroid Injection

Colin Clanton, Matthew Vance
Department of Anesthesiology

Back pain has been reported as the most common complication of epidural injection procedures. Studies have reported the incidence of back pain or tenderness as high as 30% after epidural anesthesia for obstetric and non-obstetric procedures. Factors such as catheter placement and use of fluoroscopic guidance may influence complication rates. No studies have specifically evaluated injection site tenderness after fluoroscopically-guided lumbar epidural steroid injection (LESI). Our goal was to determine the incidence of injection site tenderness after LESI. We also measured the correlation between tenderness and patient satisfaction. One day post LESI,

113 patients were contacted by phone and asked to rate their tenderness at the epidural injection site (1- no discomfort, 2- minor discomfort, 3- major discomfort) and their satisfaction with the procedure (1- dissatisfied, 2- somewhat satisfied, 3- very satisfied). LESIs were performed via interlaminar approach with fluoroscopic guidance. Thirty-five of 113 patients (31%) reported discomfort at the injection site 1 day after LESI. Eighty-two percent (93 of 113) were very satisfied with the procedure. Eleven (14%) of the 78 patients who reported no discomfort at the injection site 1 day after LESI were less than very satisfied with the procedure. Eight out of the 33 patients (24%) with mild discomfort at the injection site were less than very satisfied with the procedure. Only two patients of 113 reported major discomfort at the injection site and one of these patients was less than very satisfied with the procedure. Pain at the injection site is common after LESI. Our reported incidence of 31% is not significantly different from reports for other epidural injections performed without use of fluoroscopic guidance. Experiencing discomfort at the injection site following epidural steroid injection may have an impact on patient satisfaction. Any technique or treatment that decreases the incidence/severity of injection site tenderness may improve patient satisfaction.

40. Effect of Therapeutic Hypothermia on Potassium Balance Post-Cardiac Arrest

Daniel R. Malcom, Mary Ellen Cox
Department of Pharmacy

Therapeutic hypothermia is becoming an important component in the care of many post-cardiac arrest patients with poor neurologic function. Disorders of potassium balance have been reported in the literature, but hypothermia's effect on potassium levels has not been extensively studied in a controlled setting. The purpose of this study was to determine if there is a significant difference in the average serum potassium of hypothermic vs. normothermic patients in the ICU setting. This was a single-center, nonblinded, retrospective chart review. Serum potassium levels from all hypothermia patients during the year following inception of the hypothermia protocol were evaluated and compared with normothermic post-cardiac arrest patients who fit protocol criteria in the previous year. Patients were assessed on age, gender, and cardiac rhythm upon presentation to medical care. Potassium data were reviewed and compared at 4 hr intervals over a 28–32 hr period, depending on lab availability. Eight patients were in the hypothermia group, and ten patients were in the normothermia group. There were no statistically significant difference in gender, age, or presenting cardiac rhythm between the two groups ($p>0.05$). No significant difference was found in average potassium between the two groups ($p>0.05$), and both groups stayed within normal range (3.5-5.0 mEq/L) for serum potassium over a 32–hr period. In our study population, hypothermic patients' potassium levels were similar to normothermic patients. Overall potassium balance in these patient populations should be viewed as comparable. Patient-specific parameters should be taken into account for treatment.

41. Arterial Desaturation and Airway Intervention during Deep Propofol Sedation for Outpatient Endoscopy

Jonathan DiRuzzo, Daniel Bustamante, Carolyn Snider, Roger Carroll, Patrick Dakin
Department of Anesthesiology

The purpose of this study was to determine the incidence of significant arterial desaturation ($SaO2 < 90\%$) during outpatient endoscopy using propofol for deep sedation or general anesthesia and to determine the frequency with which airway interventions occurred during these procedures. After IRB approval and informed consent, 200 patients (ASA Class 1-3, age 18 or greater) scheduled for outpatient colonoscopy, esophagogastroduodenoscopy (EGD) or both were enrolled. Propofol was administered as necessary to facilitate the successful completion of the procedure. ECG, non-invasive blood pressure and continuous pulse oximetry were monitored using a GE Dash 4000. Each patient received oxygen by nasal cannula, and nasal capnography was monitored. If SaO2 was less than 90% at any time during the procedure, this occurrence was noted. Airway maneuvers used during the procedure were also recorded. Arterial desaturation (<90%) occurred in 17.5% of all subjects. Airway maneuvers were used in 28.5% of patients. Sixty-three airway maneuvers were used in 57 patients (57 jaw thrust/chin lift, 3 nasal airways, 1 oral airway, 1 LMA, 1 bag-mask ventilation). Arterial desaturation significantly correlated with obesity, BMI≥30, ($P=0.0006$, Chi-square), with a history of sleep apnea ($P<0.0001$), and with increasing ASA level ($P=0.016$). Obesity ($P<0.0001$), sleep apnea ($P<0.0001$), and increasing ASA classification ($P=0.0003$) significantly correlated with the use of airway maneuvers. This observational study demonstrated

that arterial desaturation is a frequent occurrence during deep propofol sedation or general anesthesia for outpatient endoscopic procedures. Airway maneuvers were frequently used during the performance of these procedures. The most commonly used airway maneuver was a simple chin lift or jaw thrust, but a smaller number of patients (3%) required a greater degree of airway assistance.

42. Innate Immune Function in Kittens Fed Raw vs. Commercially Heat-Processed Diets

Beth Hamper (CEM),[1] Melissa Kennedy,[2] Stephen Kania,[2] David Bemis,[2] Joseph Bartges,[1] Claudia Kirk[1]
[1]Department of Small Animal Clinical Sciences; [2]Department of Comparative Medicine

Claims of increased immune function are attributed to feeding raw food diets. The goal of this study is to determine if two commercially produced raw food diets enhance immune function compared to a heat-processed diet in felids. In a pilot study, 11 kittens were allotted into three feeding groups. Diet A was a commercially available heat-processed diet. Diet B was a combination of raw chicken and commercial supplement. Diet C was a balanced commercial raw diet. Innate immune function was analyzed at weeks 0, 5, and 10 using flow cytometry to quantify neutrophil oxidative burst to added probol myristate acetate (PMA) stimulant and *Escherichia coli* and to measure neutrophil phagocytosis. There were no significant differences in innate immune function between the three diets at week 0, 5 and week 10. There were immune function differences between the diets when examined individually over time. Diet B showed significantly improved phagocytosis intensity from week 0–5. A possible trend toward significant phagocystosis intensity from week 5–10 and significant phagocystosis percentage from week 0–10 was also seen in Diet B. At this point, very little differences are seen in innate immune function between the raw and cooked diets when compared at specific time intervals. The raw diets do show a marked improvement in several immune parameters when examined over time.

43. Effects of Dietary Calcium and Milk on Oxidative and Inflammatory Stress and Lifespan in Wild-type and aP2-agouti Transgenic Mice

Antje Bruckbauer, Michael B. Zemel
Department of Nutrition

Dietary calcium reduces oxidative and inflammatory stress, while milk exerts a greater effect than supplemental calcium. To examine the effects on lifespan and related biomarkers, aP2-*agouti* transgenic (model of diet-induced obesity) and wild-type mice were fed low calcium (0.4% Ca), high-calcium (1.2% Ca from $CaCO_3$), or milk (1.2% Ca from non-fat dry milk) obesigenic diets until their death. Randomized subgroups were sacrificed at 28, 52, and 78 weeks of age for biomarker analysis. The milk diet attenuated weight and adiposity gain and prevented muscle loss ($p<0.04$) and the age-related rise in reactive oxygen production (ROS) in adipose tissue ($p=0.01$). This was associated with an increase in Superoxide dismutase (SOD) 3 (in wild-type, $p=0.04$) and glutathione peroxidase gene expression ($p<0.013$) in soleus muscle, and liver SOD activity ($p<0.04$). In male mice, the milk diet decreased Interleukin (IL) 6 ($p<0.05$) and TNFα ($p<0.03$) gene expression in adipose tissue. The survival analysis showed an overall shorter lifespan in transgenic vs. wild-type mice. Although no diet effect was found on lifespan, the milk diet resulted in a significant increase in the 75% survival rate in wild-type mice ($p<0.02$). These data demonstrate that the milk diet attenuates adiposity, protects against muscle loss, and reduces oxidative and inflammatory stress. Although these effects seem not to influence the maximum lifespan, they may suppress early mortality.

44. Calcitriol Increases Microphage Adherence to Differentiated Adipocytes *in vitro*

Xiaocun Sun, Michael B. Zemel
Department of Nutrition

Adipocyte reactive oxygen species (ROS) production to be modulated by mitochondrial uncoupling status and Ca^{2+} signaling, and consequently, calcitriol regulates ROS production in adipocytes by inhibiting adipocyte uncoupling protein 2 (UCP2) expression and increasing

cytoslic Ca^{2+} signaling. Furthermore, we have also observed that calcitriol stimulated the expression and production of multiple adipokines which, together with ROS, play key roles in regulating macrophage function. Consistent with these concepts, our *in vivo* studies show that suppression of calcitriol by increasing dietary calcium attenuated diet-induced oxidative and inflammatory stress in white adipose tissue. Notably, infiltration and differentiation of adipose tissue-resident macrophages, which also contribute to obesity-related oxidative and inflammatory stress, are under the local control of cytokines produced by adipocytes. Accordingly, to further test the physiological consequences related to the effect of calcitriol in regulating oxidative stress and cytokine production, we investigated macrophage adherence to differentiated adipocytes using an *in vitro* perfusion system. Fluorescently-labeled macrophages were continuously perfused across adherent differentiated adipocytes for varying periods of time, and the non-adherent cells were washed off prior to imaging and quantification of macrophage adherence. Calcitriol significantly increased macrophage adherence to differentiated adipocytes by 87% ($p<0.01$), providing further evidence for calcitriol regulation of adipocyte-macrophage cross-talk and modulation of adipose tissue metabolic stress.

45. Vitamin A Status Affects the Expression of PPARα and ME Gene in Rat Liver

Rui Li, Guoxun Chen
Department of Nutrition

It has been observed that hyperlipidemia is associated with subjects taking an excessive amount of retinoic acid (RA) as a therapeutic agent for acne. Peroxisome proliferator-activated receptor alpha (PPARα) and malic enzyme are proteins involved in fatty acid metabolism. In this research, we aimed to examine the effects of vitamin A (VA) status on the transcription levels of PPARα and ME gene in liver. Male Zucker lean rats (eight in each group) were fed a vitamin A-sufficient (VAS) diet or vitamin A-deficient (VAD) diet for 11 weeks after their weaning at 3 weeks of age. Total liver RNA was isolated from individual rats and subjected to real-time PCR (RT-PCR) analysis. The levels of indicated transcripts are expressed as the minus difference of the cycle threshold ($-\Delta Ct$ = Ct of 36B4 – Ct of *gene X*). The larger the $-\Delta Ct$ number, the higher the expression level of the indicated transcripts. Hepatic transcriptional levels of *Ppara* in rats fed the VAD diet were significantly lower than that in rats fed a VAS diet (-1.56 VS 0.18). The mRNA level of the *Me* gene was -4.50 and -2.74 in the VAD and VAS groups, respectively. There were significant differences between the two groups ($P<0.05$). The results demonstrate for the first time that VA plays a key role in regulation of the expression of genes involved in fatty acid metabolism, such as *Ppara* and *Me*.

46. Vitamin A Status Affects L-PK and S14 Gene Transcription in Rat Liver

Wei Chen, Guoxun Chen
Department of Nutrition

Vitamin A (VA) and its metabolites are critical to the normal function of many physiological events, such as vision sensation, immune response, and glucose metabolism. In the liver, vitamin A has been shown to affect glucose metabolism in our lab. It may be achieved through regulating the transcription of a variety of retinoid responsive genes. In this study, the expressions of Liver Pyruvate Kinase (L-PK) and Thyroid Hormone-Responsive SPOT 14 (S14) were examined. Zucker lean rats were fed a vitamin A-sufficient (VAS) diet or a vitamin A-deficient (VAD) diet before liver total RNA was extracted and analyzed individually by quantitative real-time PCR (RT-PCR) with 36B4 as control. The levels of indicated transcripts are expressed as the minus difference of the cycle threshold ($-\Delta Ct$ = Ct of 36B4 – Ct of *gene X*). The larger the $-\Delta Ct$ number, the higher the expression level of the indicated transcripts. The results showed that both L-PK and S14 mRNA levels were significantly higher ($P<0.001$) in the liver tissues from rats fed a VAS diet than from rats fed a VAD diet. The data implied that vitamin A status was able to regulate the transcription of both L-PK and S14 genes in rat liver, which might be of great importance in glucose metabolism.

47. High Molecular Weight Adiponectin Ratio Correlates Better with Body Fat Mass and Indices of Glucose Metabolism than Total Adiponectin in Lean and Obese Cats

Angela Lusby (CEM),[1] Claudia A. Kirk,[1] Stephen Kania,[2] Susan Lauten,[2] Raymond Boston,[3] Joseph Bartges[1]
[1] Department of Small Animal Clinical Sciences; [2]Department of Comparative Medicine; [3]University of Pennsylvania School of Veterinary Medicine

Adiponectin is a hormone secreted from adipose tissue that paradoxically decreases as fat mass increases. Adiponectin improves insulin sensitivity in people by increasing glucose uptake and fatty acid oxidation in skeletal muscle. Serum adiponectin forms multimeric complexes that range in size from 30 to >500 kDa. The high molecular weight (HMW) multimers (>200kDa) of adiponectin appear to be more active and are better associated with insulin sensitivity in people than total adiponectin. In this study, twenty adult (1-11 years) laboratory cats were classified as overweight (BCS 6-9/9, n=10) or lean (BCS 3-5/9, n=10). Overweight cats had food restricted to lose 1-2% BW_{kg}/week. Lean cats were fed ad lib. Body fat mass, total adiponectin, HMW adiponectin, and insulin sensitivity using the FSIVGTT method were measured at the start and end of the study. The ratio of HMW (HMWR) adiponectin was measured using a combination of size exclusion chromatography and ELISA. HMWR is defined as HMW/(HMW+LMW). Total adiponectin did not correlate with body fat mass or markers of insulin sensitivity, but trended toward lower concentrations with higher fat mass ($p=0.09$). The HMWR of adiponectin negatively correlated with body fat mass ($R=-0.4$, $p<0.05$) and positively correlated with two markers of improved glucose metabolism, glucose: insulin ratio ($R=0.4$, $p<0.05$), and glucose effectiveness (S_G) ($R=0.4$, $p<0.05$). Results from this study suggest the HMWR of adiponectin is more closely associated with improved glucose metabolism and body fat mass than total adiponectin.

48. *In vitro* Manipulations of a Putative Phospholipid Translocase Suggest its Role in Glucose Uptake and Adipogenesis

Sarah E. Hurst (CEM),[1] Amanda Peretich,[1] Steven C. Minkin,[2] John Biggerstaff,[2] Madhu Dhar[1]
[1]Department of Large Animal Clinical Sciences; [2]Electron Microscope Facility, Division of Biology

Atp10c is a putative phospholipid translocase that encodes for a type IV P-type ATPase. The *Atp10c* gene, located on mouse chromosome 7, appears to be a strong candidate for diet-induced obesity and type II diabetes mellitus. *In vitro* studies using a 3T3 L1 murine adipocyte cell line revealed that when *Atp10c* expression was silenced or knocked down using siRNA, glucose uptake and fat accumulation increased, while insulin resistance decreased. Furthermore, the silencing effect of *Atp10c* caused an increase in *Glut 1* and *CEBP-α* gene expression and a decrease in *Glut 4* and *PPAR-γ* gene expression. To further investigate the role of *Atp10c* in insulin resistance and glucose uptake, immunolocalization of *Atp10*c by fluorescence microscopy was employed. Data from these experiments suggest that *Atp10c* not only co-localizes with caveolin-1 and GLUT4 at the plasma membrane, but also can be visualized as a punctate pattern in and around the nucleus. This finding suggests that *Atp10c* additionally may localize to the trans-Golgi network or endosome, strengthening its biological role in protein trafficking in the exocytic and endocytic pathways.

49. Effects of Clinical Endotoxemia on Glucose Metabolism in Horses

Fiamma Gomez de Witte, Nicholas Frank
Department of Large Animal Clinical Sciences

Endotoxemia is an established risk factor for laminitis in hospitalized horses and may play an important role in the development of laminitis. In experimental models of endotoxemia, blood glucose concentrations rise as insulin resistance develops after intravenous infusion of lipopolysaccharide, and this alteration develops at the same time as the systemic inflammatory response. However, these relationships have not been examined in horses with clinical endotoxemia, so this study was undertaken to address that question. We hypothesize that the magnitude of glucose metabolism alterations is

significantly greater in hospitalized horses with clinical endotoxemia, when compared with horses that are hospitalized because of other medical conditions that are not accompanied by endotoxemia. Horses with clinical endotoxemia are being compared with hospitalized horses with medical colic, surgical colic, or lameness. Glucose metabolism is being assessed over 72-hr time periods by measuring blood glucose and insulin concentrations every 6 hr and interstitial glucose concentrations every 5 min, using a continuous glucose monitoring system (CGMS). This system is also being validated by assessing repeatability of measurements across 6-hr time periods for 6 days in healthy mares. Measures of systemic inflammation include physical examination findings and complete blood count analysis results. Preliminary results from four horses with clinical endotoxemia and four healthy mares included in the validation study indicate that CGMS can be successfully used to assess glucose metabolism in horses, but repeatability depends largely upon maintenance of interstitial probes. Horses with clinical endotoxemia are exhibiting hyperglycemia and marked increases in interstitial glucose concentrations.

50. Effects of Levothyroxine Sodium on Body Condition, Blood Measures of Metabolic Status, and Glucose Dynamics in Horses with Equine Metabolic Syndrome (EMS)

Kelly Chameroy (CEM), Nicholas Frank, Sarah B. Elliott, Lisa Tadros
Department of Large Animal Clinical Sciences

Obesity, regional adiposity ("cresty neck"), insulin resistance (IR), and laminitis are clinical manifestations of equine metabolic syndrome (EMS) in horses. Insulin resistance is associated with obesity, and affected horses have an increased risk of developing laminitis. Previous work has shown that levothyroxine sodium induces weight loss and improves insulin sensitivity in healthy horses, so the purpose of this study was to assess the efficacy of this treatment in horses with EMS. It was hypothesized that levothyroxine sodium would significantly alter physical characteristics, glucose dynamics, and markers of metabolic status in horses with EMS. Ten horses with chronic EMS were evaluated over a 9-month period with body weights and morphometric measurements obtained at the same time points as frequently-sampled intravenous glucose tolerance tests (0, 3, 6, and 9 months). Seven horses received levothyroxine sodium orally at a dosage of 48 mg/day (6 months) and then 72 mg/day (3 months), while three other horses remained untreated. Analysis of variance revealed significant time × treatment effects for body weight ($P = 0.049$) and mean neck circumference ($P = 0.027$), but there was no significant change in BCS ($P = 0.350$). Area under the curve for glucose values measured at 0, 3, and 6 months were not affected by treatment (time × treatment; $P = 0.834$). Levothyroxine induces weight loss and reduces neck circumference in horses with EMS, but area under the glucose curve values remain unaffected. Results pertaining to insulin metabolism are pending.

51. Optimization of the Frequently Sampled Intravenous Glucose Tolerance Test to Reduce Urinary Glucose Spilling in Horses

Ferenc Tóth,[1] Nicholas Frank,[1] Sarah B. Elliott,[1] Kelly Perdue,[2] Raymond J. Geor,[3] Raymond C. Boston[4]
[1]Department of Large Animal Clinical Sciences; [2]College of Veterinary Medicine; [3]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI; [4]University of Pennsylvania, Kennett Square, PA

The frequently-sampled intravenous glucose tolerance test (FSIGTT) is used to evaluate glucose and insulin dynamics in horses, but it is unknown whether urinary glucose spilling affects results. We hypothesized that urinary glucose spilling occurs in horses undergoing a FSIGTT, and this problem can be minimized by adjusting dextrose and insulin dosages. Six adult mares were included in this study comprising four phases. In the first phase, six FSIGTT procedures were performed in each horse to evaluate six different dextrose dosages. Six different insulin dosages were evaluated during the second phase after administration of 300 mg/kg dextrose. Area under the glucose (AUCg) and insulin (AUCi) curves were calculated, and minimal model analyses were performed. Urinary glucose spilling was measured in the third and fourth phases during the combined glucose insulin test and established FSIGTT. A new FSIGTT was developed and evaluated. Positive linear effects of dextrose dosage on AUCg, AUCi and acute insulin response to glucose were detected, with AUCg reaching a plateau at doses ≥ 200 mg/kg. Insulin

dosage had an inverse linear effect on AUCg. Urinary glucose spilling occurred during all three tests and was the highest for the established FSIGTT and the lowest for new FSIGTT. The type of FSIGTT performed did not affect minimal model results. A new FSIGTT involving the administration of 100 mg/kg dextrose followed by 20 mU/kg insulin 20 min later is recommended for use in horses because this test provides adequate data for minimal model analysis while minimizing urinary glucose spilling.

52. Development of an Autonomous Mammalian Lux Bioreporter

Dan Close,[1,2] Stacey Patterson,[2] Gary S. Sayler[2]
[1]Genome Science and Technology Program; [2]Center for Environmental Biotechnology, University of Tennessee, Knoxville

Current mammalian cell biosensors are limited in their usefulness because of the inability to monitor changes in real time. In addition, these sensors often require either the destruction of the cell or the addition of a potentially influential exogenous substrate. To overcome these problems, the bacterial luciferase-based *lux* cassette is being developed into an autonomous mammalian reporter system. The *lux* cassette is a series of five genes (*luxCDABE*) that work in concert to autonomously produce light in the visible spectrum. Expression of the two genes forming the bacterial luciferase heterodimer (*luxA* and *luxB*) has previously been demonstrated through addition of an exogenous aldehyde substrate. Here we show, for the first time, that the remaining three genes (*luxC*, *luxD*, and *luxE*) are capable of performing their endogenous function in mammalian cells. Bioluminescence levels were, on average, 5,375% (± 1,323) greater in HEK293 cells expressing the entire *lux* cassette than in those transfected with only the *luxA* and *luxB* genes. It has been demonstrated that in the mammalian system, the limiting reagent for bioluminescent production is the availability of reducing power. Addition of exogenous NAD(P)H:FMN oxidoreductase protein leads to an average bioluminescent production increase of 11,881% (± 3,422). These results indicate that expression of the entire bacterial luciferase cassette in its codon-optimized form is capable of bioluminescent production in mammalian cells. Through localization of these genes to a single, regulatable vector, it will be possible to develop an autonomous *lux* reporter system capable of performing real-time detection in mammalian cells.

53. Material Design Strategies for Bone and Nerve Regeneration: Controlled Physical Properties and Regulated Cell Responses

Lei Cai, Shanfeng Wang
Department of Materials Science and Engineering

In an effort to develop suitable biomaterials with controllable physical properties for different tissue-engineering applications such as bone and nerve regeneration, we present a facile synthetic method to achieve self-crosslinkable poly(ε-caprolactone) diacrylates (PCLDAs) and triacrylates (PCLTAs). This novel method uses potassium carbonate (K2CO3) as the proton scavenger rather than triethylamine in the literature to avoid side reactions and make purification significantly easier. Furthermore, we employ a material design strategy of combining a crystallite-based physical network and a crosslink-based chemical network together to modulate material properties and cell responses. PCLDAs with different molecular weights are blended with another crosslinkable biomaterial, poly(propylene fumarate) (PPF), to regulate the physical properties and photocrosslinking characteristics. Since different PCLDAs have different crystallinites and melting points while PPF is amorphous with a higher density of crosslinkable segments, the mechanical properties of photo-crosslinked blends can be modulated efficiently yet distinctively by varying both crosslinking density and crystallinity with the PPF composition in the blends. Thermal properties such as glass transition temperature (Tg), melting temperature (Tm), and the heat of fusion (ΔHm) have been measured and correlated with their mechanical and rheological properties. Surface characteristics such as surface morphology, hydrophilicity, and the capability of adsorbing serum protein from cell culture medium have also been examined for the crosslinked polymer disks. MC3T3 cells and the Schwann precursor cell line (SPL201) have been applied to evaluate the *in vitro* biocompatibility of this series of polymeric networks and the roles of surface chemistry, crystallinity, and stiffness in regulating cell responses.

54. Design of a Micro-Electrical-Mechanical-System (MEMS) for Diagnosis and Treatment of Malaria

Scott Christopher Lenaghan, Ruoting Yang, Lijin Xia, Mingjun Zhang
Department of Mechanical, Aerospace and Biomedical Engineering

Malaria is one of the deadliest diseases in the world, with 350-500 million cases occurring every year, and children are among those affected the greatest. The purpose of this study was to design a micro-electrical-mechanical-system (MEMS) device for rapid diagnosis of infection with malaria and immediate treatment upon infection. A novel immuno-sensor will be developed that incorporates antibodies specific for parasite lactate dehydrogenase (pLDH) from the four species of human malaria, *Plasmodium falciparum*, *vivax*, *ovale*, and *malariae*. Briefly, blood will be taken up through capillary action using a series of micro-needles. The blood will be lysed in a lysis buffer, and pLDH will be released into solution. The antibody 6C9 conjugated to gold-nanoparticles will first bind to the pLDH and then become immobilized by binding to a secondary antibody, 17E4 or 19G7, attached to a substrate. A charge will be constantly passed along this substrate and the change in conductivity obtained by the binding of the gold-nanoparticles via the antibody linkers will correlate to the concentration of pLDH in solution and the level of infection. When the immuno-sensor detects infection with malaria, it sends a signal to the wearer via an auditory beep and triggers a micro-fluid pump to dispense treatment. This micro-fluid pump dispenses chloroquine into the blood stream using a second set of micro-needles. This rapid diagnostic and treatment device will play a significant role in reducing the number of malaria-related deaths worldwide.

55. Using AC Electrokinetic-enhanced, Microfluidic Magnetoelastic Sensors for Detection of Low Analyte Concentrations in Biological Fluids

James Wilson, Jayne Wu, Kia Yang, Quon Yuan
Department of Electrical Engineering and Computer Science

The motivation for this research is exemplified by the need to quickly and accurately analyze biological samples with low analyte concentrations. Our research is focused on the development of a wireless, passive, and remotely interrogative sensor with a low unit cost that can be used on a disposable basis. The approach described here employs a microfluidic device containing a magnetoelastic (ME) sensor. Magnetoelastic thin-film sensors (the magnetic analog of an acoustic bell) "ring" in response to an externally applied magnetic field impulse. The "ringing" produces an inductive flux that is at its maximum when the sensor oscillates at the mechanical resonant frequency (RF). This unique capability (wireless remote interrogation) allows ME sensors to be monitored from inside sealed, opaque containers (such as food or medicine packages, or people) without the need for wire or mechanical connections. ME sensors can be sized from micrometer to millimeter dimensional scales, and have a material cost of approximately $0.001, allowing for their use on a disposable basis. Further, an AC electrokinetic (ACEK) signal is applied so that the sensor's detection sensitivity can be improved. The ACEK signal accomplishes this by transporting and mixing the bulk fluid and by trapping analytes onto the sensor. The application of an ACEK signal greatly increases the ME sensor's ability to detect low analyte concentrations without mechanical implementation.

56. Ivy Nanoparticle Characterization and Biomedical Application

Lijin Xia, Scott Christopher Lenaghan, Mingjun Zhang
Department of Mechanical, Aerospace and Biomedical Engineering

Recent discovery of nanoparticles secreted from ivy drew a lot of scientific interest and opened the possibility of their biomedical and engineering applications. However, before they can be used in these fields, basic characteristics of these ivy nanoparticles need to be investigated. The objective of this study is to characterize the nanoparticles secreted from ivy. Specifically, we use different imaging techniques, including atomic force microscopy and laser scanning confocal microscope, to track the source of the production of these nanoparticles in ivy and to define their secretion pathway from inside to the outer surface of specific ivy cells. These nanoparticles are then isolated depending on their specific size and are examined for their physical and chemical properties including their chemical composition and adhesion ability. The understanding of their adhesion

ability allows us to exploit their actual biomedical use in bioglue, bioadhesive, and suture. The understanding of the mechanisms that control nanoparticle secretion will provide insightful information for understanding the basic characteristics of these nanoparticles and lead to our intentional control of their production for future applications. The information gained from chemical analysis will then allow us to specifically modify these nanoparticles for our purpose. Overall, the information gained from this study will guide us in later studies, which finally will allow us to monitor the production and modification of these nanoparticles for the purpose of their actual applications in biomedical and engineering fields in the future.

57. In Situ Preconcentrator for Rapid and Ultra Sensitive Nanoparticle Detection

Kai Yang, Jie Wu
Department of Electrical Engineering and Computer Science

The design and fabrication of a rapid detection and diagnostic micro-system is highly in demand in many fields, especially for biomedical and environmental applications. The conventional way is to wait for targets to diffuse toward sensors. This slow diffusion process has imposed a bottleneck, and current real-time detection sensitivity is several orders of magnitude away from practical applications. We present a sensor design that can achieve rapid and ultra sensitive nanoparticle detection based on a new AC electrothermal effect (ACET) that can operate even in a highly conductive solution for which current methods such as dielectrophoresis, electrophresis, or electroosmosis will not function effectively. The preconcentrator consists of a micropump and a pair of stirring electrodes. The micropump is used to realize a flow-through system, enabling the system to handle more fluid volume and to eventually trap more targets. Our results show that the collection of analytes goes down with channel diameter in a diffusion-limited region, indicating the need for stirring. The stirring electrodes generate swirls that bring the fluid toward the gap of the electrodes where the detection sensor is located. Our simulation shows that the binding rate at the reaction surface is greatly enhanced (by 10 times in 5 sec) since swirls generate a large velocity perpendicular to a reaction surface. ACET force directs particles (~100μm/sec or higher) toward sensors to break the diffusion barrier for rapid detection. This preconcentrator works well with nanoparticles to cells in micrometers. It is especially suitable for biological solutions with high conductivities because ACET force scales with fluid conductivity.

58. Ranaviruses in Southern Appalachian Salamanders

Nathan A. Haislip,[1] Matthew J. Gray,[1] Debra L. Miller,[1,2] Jason T. Hoverman,[1] Richard G. Secrist[3]
[1]Center for Wildlife Health, Department of Forestry, Wildlife and Fisheries; [2]Department of Pathology, College of Veterinary Medicine, University of Georgia; [3]Division of Natural Sciences, Maryville College

Ranaviruses are a group of pathogens that have been linked to amphibian die-offs in North America and elsewhere. The first known die-off from ranaviruses in the Southern Appalachian Mountains occurred in the late 1990s in anurans and newts. However, to date, no studies have investigated the occurrence of ranaviruses in plethodontid salamanders, which is the most diverse group of salamanders in the region. Therefore, we collected samples (i.e., skin swabs and tail clips) from 174 salamanders of 13 species during April 2007 and 2008 from three sites that differed in elevation. In 2008, samples were collected from two additional sites. Samples were tested for *Ranavirus* DNA using traditional and quantitative PCR. In 2007, 81% of individuals of 10 species tested positive for *Ranavirus* infection, and the likelihood of infection increased with decreasing elevation. In 2008, 14% of six species tested positive, and no relationship with elevation was evident, suggesting possible annual and spatial variation in *Ranavirus* prevalence or species susceptibility. In 2007, a sequenced 500-bp region of the virus major capsid protein for four infected plethodontid species showed distinct protein sequence differences from known *Ranavirus* species, suggesting the possibility that an undescribed *Ranavirus* may be present in the Southern Appalachians. Given that ranaviruses can cause catastrophic die-offs in amphibian populations, and they are classified as a notifiable disease by the World Organization for Animal Health, surveillance of this pathogen should be expanded in the Southern Appalachians and perhaps elsewhere.

59. Anuran Susceptibilities to the Emerging Amphibian Pathogen *Ranavirus*

Jason T. Hoverman,[1] Debra L. Miller,[1,2] Matthew J. Gray[1]

[1]Center for Wildlife Health, Department of Forestry, Wildlife, and Fisheries; [2]Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA

The pathogen *Ranavirus* has been implicated as a major cause of reported amphibian die-offs in the United States. One of the hypothesized factors in the recent emergence of ranaviruses in amphibian populations is novel strain introduction (i.e., pathogen pollution). While pathogen pollution has been identified as a significant concern, studies are needed that compare the relative susceptibility of amphibian species to novel versus endemic strains. The goals of our study were to 1) determine the susceptibility of three anuran species to ranaviruses and 2) assess the degree of susceptibility to a novel *Ranavirus* isolate. Using controlled laboratory experiments, we exposed tadpoles of pickerel frogs (*Rana* [*Lithobates*] *palustris*), Cope's gray tree frogs (*Hyla chrysoscelis*), and eastern narrow-mouthed toads (*Gastrophryne carolinensis*) to a known laboratory strain of *Frog virus* 3 (FV3) and a novel *Ranavirus* isolate from a bullfrog ranaculture facility in Georgia. We found that the species varied in their susceptibility to the virus isolates. Pickerel frogs exposed to the novel isolate experienced 80% mortality, but there was no increase in mortality following exposure to FV3. Gray tree frogs experienced 65% and 35% mortality following exposure to the novel isolate and FV3, respectively. Eastern narrow-mouth toads were relatively resistant to infection by both virus isolates. In addition to species-specific susceptibilities to *Ranavirus*, this research demonstrates that novel *Ranavirus* isolates can be highly pathogenic to naïve populations. Thus, strategies that reduce pathogen pollution may reduce the likelihood of *Ranavirus* emergence.

60. Prevalence of Bovine Viral Diarrhea Virus on Alpaca Farms in Eastern and Middle Tennessee

Teresa M. Buchheit,[1] Sarel R. Van Amstel,[1] Kathy V. Thomas,[2] Mohamed Abd-Eldaim[2]

[1]Department of Large Animal Clinical Sciences; [2]Department of Comparative Medicine

The purpose of this study was to survey the seroprevalence of Bovine Viral Diarrhea Virus (BVDV) and possible presence of persistently infected (PI) animals in alpaca herds. Blood was collected from 244 alpacas in five herds with a negative BVDV vaccination history. All animals in each herd were tested for the presence of antibodies to BVDV by use of immunofluorescence assay and for the presence of viral RNA by conventional polymerase chain reaction assay using reverse transcriptase RT-PCR. Overall, antibodies to BVDV were detected in 1.6% (4/244) of the samples, which is similar to most other BVDV prevalence studies. Two herds had at least one BVDV-positive animal with a titer ≥ 1:80, with three of the four seropositive alpacas from the same herd (Farm 1). All 244 samples were found virus-negative by PCR. No animals younger than 2 years of age were found to be seropositive, and none of the BVDV-positive animals were pregnant at the time of testing. Only one of the herds tested had cattle on the farm; however, none of the alpacas in this herd tested BVDV-positive. No suspected PI animals were identified. The presence of three seropositive animals on Farm 1 may be due to unrestricted comingling of animals. In conclusion, although no animals with viremia were identified in the area, BVDV should still be considered as a cause of recurring illness, ill-thrift, low birth-weight, or premature crias and abortion.

61. Presentation Canceled

62. Inactivation of Human Enteric Virus Surrogates by High Intensity Ultrasound

Xiaowei Su, Svetlana Zivanovic, Doris H. D'Souza
Department of Food Science and Technology

Human noroviruses are a growing international concern for food safety and public health, being one of the leading causes of nonbacterial gastroenteritis worldwide. They remain persistent in the environment for long periods and are quite resistant to most current inactivation methods. Novel approaches for the efficacious inactivation of human enteric viruses in liquid foods (such as fruit juices, purees) are of great importance to control disease spread. Ideally, these approaches should be cost-effective and retain nutritive value and sensory attributes of the product for consumer acceptability. High intensity ultrasound (HIUS) is a featured alternate process for microbial inactivation of liquids in continuous flow systems coupled with aseptic filling. HIUS effects have not been studied on enteric viruses. Our objective was to determine the effect of HIUS on the infectivity of three human enteric viral surrogates. Murine norovirus (MNV-1), feline calicivirus (FCV), and MS2 bacteriophage (10^6 PFU/ml each) were subjected to HIUS by sonicating at 20 kHz in ice-water for a duration of 2, 5, 10, 15, 20 and 30 min followed by plaque assays. HIUS was found to be effective in inactivating FCV and MS2 from ~1 log PFU/ml after 10 min to ~3 log PFU/ml after 30 min. However, MNV-1 was not sensitive to ultrasound treatment for up to 30 min. For MNV-1, hurdle approaches involving HIUS and approved food grade chemicals that break down viral capsids may need to be explored. HIUS shows promise for significant reduction of human noroviruses in liquid foods as part of a hurdle approach.

63. Molecular Characterization of Feline Papillomavirus with a Close Relationship to Human Papillomaviruses

Eman Anis,[1] Sarah O'Neil,[2] Kim Newkirk,[3] Rupal Brahmbhatt,[1] Mohamed Abd-Eldaim,[1] Linda A. Frank,[2] Stephen A. Kania[1]
[1]Department of Comparative Medicine; [2]Department of Small Animal Clinical Sciences; [3]Department of Pathobiology

Papillomaviruses (PV) are highly species-specific with different viral types that infect a broad spectrum of mammals and birds. Recently human papillomavirus (HPV) was detected in feline lesions. In this retrospective study, 175 paraffin embedded samples from 115 cats showing tumor lesions were examined. Two pairs of polymerase chain reaction (PCR) primer sets were used for screening for papillomavirus DNA from these cats. PV DNA was amplified and sequenced from 21 samples. Six of these products had 100% homology with feline papillomavirus (FPV), 71% homology with canine papillomavirus (CPV), and 68% with HPV. Two of the 21 samples had 71% and 68% homology to CPV and HPV, respectively. The other PV sequences showed 71%–100% homology with HPV. More primer pairs were designed to sequence about 2000bp from three randomly chosen samples. Phylogenetic analysis of two samples (2000bp genome) showed 99% homology to HPV type 38b, subtype FA125, complete genome. The other sample showed 84% homology with HPV type 80 E6, E7, E1, E2, E4, L2, and L1 genes. Thus, this PV sequence is considered to be a new PV type, since their sequence identity is less than 90% in the L1 gene. In conclusion, this study strongly suggests an interspecies transmission of papillomavirus between humans and cats.

64. Regulation of Coronavirus Poly(A) Tail Length During Virus Replication

Hung-Yi Wu, David A. Brian
Department of Pathobiology

The ~30 kilobase coronavirus genome is a single-stranded, (+)-strand RNA molecule with a 5' cap, 5' and 3' untranslated regions of ~200 and ~300 nucleotides, respectively, and a 3' poly(A) tail. The genome functions first as a messenger RNA for synthesis of replicase enzymes, and second as a template for genome replication. The poly(A) tail in mRNAs has been generally shown to protect RNAs from 3' terminal degradation and to enhance translation via interactions between poly(A)-binding protein and 5' terminal structures. By ligating head-to-tail genomic RNA (-) strands from infected cells and sequencing across the ligated junction, we have learned that the poly(U) on genomic (-) strands remains a stable 24–44 nucleotides throughout a 144 h infection. By contrast, when the same technique was applied to determine poly(A) tail length on the (+)-strand genome, a surprising variation in poly(A) length was found throughout the same period. That is, poly(A) tail length was 46–50 nt at 0–2 hpi, 50–68 nt at 2–12 hpi, and slowly decreased to ~24 nt by 144 hpi.

This means that maximal poly(A) length corresponds to time of peak (-)-strand RNA synthesis. In vitro translation in rabbit reticulocyte lysate, however, showed maximal poly(A) length corresponding to minimal translation rate. Since translation (requiring 5'→3' ribosomal movement) and (-)-strand synthesis (requiring 3'→5' RdRp movement) cannot simultaneously occur on the same (+)-strand molecule, we hypothesize poly(A) tail length may contribute to the switch between genome translation and replication in coronaviruses. How poly(A) tail length is regulated remains to be determined.

65. 5'-Proximal Suppressor Mutations for an Incompatible 32-nt Region in the 5' Untranslated Regions of Bovine and Mouse Hepatitis Coronaviruses Identify a New *cis*-Replication Element

Bo-Jhih Guan,[1] David A. Brian[1,2]
[1]Department of Microbiology; [2]Department of Pathobiology

Higher-order RNA secondary structures in the 5'- and 3'-untranslated regions of the coronavirus genome have been shown to be *cis*-replication elements. In group 2 coronaviruses, the ~300-nt 3' UTR of BCoV and SARS-CoV can functionally replace the 3' UTR in the MHV genome. It has also been shown that MHV functions as a helper virus for replication of BCoV DI RNA in cell culture, leading us to hypothesize interchangeability among the 5' *cis*-acting elements as well. In the BCoV 210-nt 5' UTR, four higher-order, *cis*-acting structures have been identified (stem-loops I [comprised of smaller stem-loops 1 and 2], II, III, and IV), and these have predicted counterparts in the MHV 209-nt 5' UTR. Here, with the MHV reverse genetics, we show that replacement of the entire 5' UTR of MHV with that of BCoV is lethal. The region of incompatibility maps to a hypervariable 32-nt stretch between stem-loops III and IV, which has little predicted higher-order structure. The 32-nt segment from BCoV in the MHV background yielded viable progeny with small plaques and slow growth rates after two blind passages. Although the entire genome of phenotypic revertants has yet to be examined, no potential suppressor mutations were found in the 3' UTR but were repeatedly found at specific sites in stem-loop 2 in the 5' UTR and within a 5'-proximal region of ORF 1. Reconstruction experiments suggest these are the sole suppressor mutations. These results reveal a previously unidentified *cis*-acting element in the 5' UTR required for coronaviruses replication.

66. A Stochastic Game Theoretical Approach to Controlled Drug Delivery during HIV Infection

Jing Wu, Ruoting Yang, Mingjun Zhang
Department of Mechanical, Aerospace, and Biomedical Engineering

Human immunodeficiency virus (HIV) infection is fatal because it directly damages humans' immune system by infecting CD4+ T cells. Treatment for HIV infection is a complex process that involves interactions among the virus, the immune system, and antiretroviral drugs. However, HIV infection is difficult to treat because its high mutation rate makes it possible for HIV to escape from drug attacks. We formed a stochastic game between host-drug coalition and virus. The purpose of this game was to seek an optimal drug dosage strategy that can decrease the side effects of medication burden placed on patients while maintaining control over HIV replication in the immune system. Game theory is an excellent mathematical tool to describe the interaction between HIV and the drug-host coalition. Because the virus mutates at a rather high level, it is impossible to completely eliminate the virus by drug delivery. Thus, Nash Equilibrium is sought so that the T cell and virus can coexist, and we use a stochastic model to describe the host-drug dynamics due to the randomness of virus mutation and T cell death. The simulation results show that a Nash equilibrium exists where the drug can keep HIV from explosively mutating into dangerous drug resistant ones while the population of CD4+ T cells remains at a proper level. This brings a new drug therapy during HIV infection to prolong the life of patients with HIV co-existence.

67. Mathematical Model for Immune-vaccine Interactions

Ruoting Yang, Scott C. Lenaghan, Lijin Xia, Mingjun Zhang
Department of Mechanical, Aerospace and Biomedical Engineering

The current design of adjuvant-based vaccine strategies is heavily dependant on trial-and-error-type experimental studies, which are not directed to a specific strategy. Often times a "shotgun" approach is employed to test potential adjuvants with vaccines of interest. This approach has significantly limited the optimization of vaccine strategies by consuming a significant amount of time and effort that often leads to dead ends before reaching an ideal strategy. Similarly, most mathematical models that attempt to optimize vaccine-based strategies fall short due to a highly theoretical approach that may not be closely tied with experimental data. In this work, we create a predictive model that can allow researchers to directly input a vaccine of interest and predict the responses of the immune system through the addition of different adjuvants. This will allow researchers to predict the input of different interleukins as adjuvants, as well as other known adjuvants, and predict their subsequent effect on the immune system. The proposed model is structured around the major types of T cells (Th1, Th2, Th17, and Treg) and responses of the secreted cytokines. This research is centered on development of such a complex nonlinear dynamic model and employment of a genetic algorithm for model parameter identification. This research will advance the understanding of immune-vaccine interactions via a systems biology approach; moreover, it will benefit clinical research by developing more efficient vaccines and reduce the amount of time spent by researchers pursuing potentially faulty leads.

68. Yeast Co-expression–A Novel Methodology for Characterization and Engineering of Peptide-MHC II Binding

Wei Jiang[1], Eric Boder[2]

[1]Department of Chemical and Biomolecular Engineering, University of Pennsylvania; [2]Department of Chemical and Biomolecular Engineering

MHC II-restricted T cell responses are related to a great number of diseases, including autoimmunity, graft rejection, and atypical immune response. In-depth characterization of peptide binding by MHC II is critical to understanding issues in vaccine design, autoimmune disease, infectious disease progression, and transplantation rejection. The lack of a high throughput quantitative methodology for characterizing the peptide binding specificity and promiscuity of MHC alleles remains a bottleneck. A yeast co-expression system, which can surface display peptides from influenza hemagglutinin (FLU) bound by soluble HLA-DR1 secreted by the same cell, has been developed for studying the peptide binding properties of MHC II. Initial studies focused on characterizing the peptide side chain preferences at the dominant anchor position (P1) of FLU/HLA-DR1 complexes. A set of yeast surface tethered FLU peptide analogues containing all natural amino acids (except for Cys) at the P1 position has been tested for their ability to bind and anchor soluble HLA-DR1 to the cell surface. Flow cytometry analysis enabled discrimination of P1 residues supporting binding to MHC and further allowed evaluation of relative binding affinities, which follow the order Phe > Tyr ≥ Trp > Met ≥ Leu ≥ Ile > Val, with all other amino acids tested, yielding no detectable binding, in agreement with binding preferences determined using phage display and/or other methods. We are extending this system to develop and validate an approach for retargeting defined MHC toward novel peptides for possible applications in vaccine technology.

69. Selectin Ligand Deficiency Confers an Unexpected Increase in Migration of Naïve T Cells to the Lung

John R. Harp,[1] Kim M. Newkirk,[2] Thandi M. Onami[1]
[1]Department of Microbiology; [2]Department of Pathobiology

Mechanisms that determine the migration of naïve T cells from the vasculature into non-lymphoid tissues remain unclear. Fucosyltransferase –IV and –VII are two glycosyltransferases that catalyze the addition of alpha 1,3 fucose to form the sialyl Lewis X moiety, essential for selectin binding and extravasation into tissues. In our study using fucosyltransferase –IV and –VII double knockout mice (FucTdKO), we have observed an increase in naïve T cell infiltrates in lung parenchyma compared to WT mice. Examination of lung sections from these uninfected FucTdKO mice revealed no evidence of iBALT. Adoptive transfer of bonafide naïve or memory CD8 T cells into FucTdKO mice illustrated a dramatic increase of transferred cells into non-lymphoid compartments, including lung and liver. This result suggests a general phenomenon where naïve as well as memory CD8 T cells have an increased capacity to enter non-lymphoid compartments when lymph node access is reduced. Thus, contrary to the current paradigm, which suggests only effector and memory T cells enter non-lymphoid compartments, our data support a paradigm where naïve T cells normally migrate into non-lymphoid tissues such as the lung. When access to the lymph node is blocked, these tissues become sites of T cell accumulation. Consequently, this could represent increased risk of clinical disease due to increased T cell influx into these tissues.

70. ST6GalI Expression is Required for the Optimal Generation of Anti-viral Humoral Responses

Rajini Bheemreddy, Junwei Zeng, Thandi M. Onami
Department of Microbiology

Post-translational modification of proteins present on the cell surface plays an important role in cell-cell interaction and thus coordination between cells. Glycosylation is one of the major post-translational modifications that proteins undergo. We have examined the role of ST6GalI, a mammalian glycosyltransferase enzyme in T and B cell programming. ST6GalI is highly expressed by B and T cells and catalyzes the addition of α2,6 sialic acids to Galβ1-4GlcNAc. Our studies of viral infection of ST6GalI knockout mice have shown impaired primary B cell responses. The antibody levels and the number of antibody producing cells (ASC) after LCMV infection are significantly reduced in ST6GalI knockout mice when compared to wild type mice. This reduction in antibody levels was also seen in immune mice. We observed reduced viral-specific CD4 T cells and reduced germinal center B cells. Immunohistochemistry revealed defects in CD4 trafficking to the T cell zone of B cell follicles in the spleen. These observations suggest that the modification of T and B cell glycoproteins by ST6GalI affect their interaction and thus influence the generation of antiviral humoral responses. Future studies will determine the role of ST6GalI expression in the differentiation of T follicular helper cells, which play a crucial role in providing help to germinal center B cells.

71. Suppression of HSV-1-induced Corneal Angiogenesis and Vascular Permeability by an src Kinase Inhibitor

Shalini Sharma (CEM), Sachin Mulik, Sharvan Sehrawat, Barry Rouse
Department of Pathobiology

Neovascularization of the otherwise avascular cornea is a critical event in HSV-1 induced immunopathology, a process that principally involves the cytokine VEGF (vascular endothelial growth factor). Signaling through VEGF activates multiple cascades, leading to proliferation, migration, and survival of endothelial cells. One of these cascades involves activation of the src family of tyrosine kinases, resulting in tyrosine phosphorylation of adhesion junction components, important to endothelial cell adhesion. Using an src kinase antagonist, we showed the relevance of src inhibition to VEGF-mediated corneal angiogenesis and alterations in vascular permeability following HSV-1 infection. Topical application of src kinase inhibitor TG100801 (an inactive prodrug that generates the active form, TG100572, by de-esterification) significantly reduced the leucocytic infiltration and angiogenesis in mouse cornea following HSV-1 infection. Moreover, src blockade diminished FAK (focal adhesion kinase) phosphorylation at tyrosine residue 861 (biomarker of src activity) in the corneal lysates. However, the drug had no effect on the replication

of HSV-1 in cell culture (vero cells).Taken together, our findings suggest that src kinase inhibition in combination with other approaches may be evaluated for therapeutic effects against herpatic stromal keratitis.

72. Modulation of VLA-4 -VCAM1 Interaction Diminishes HSV-1-Induced Corneal Immunopathology

Amol Suryawanshi (CEM)
Department of Microbiology

HSV-1 infection of the cornea results in an immunoinflammatory condition of the eye termed herpetic stromal keratitis (HSK), which is considered to be orchestrated by infiltrating CD4 T cells into the cornea. Thus, blockade of infiltration of CD4 T cells into the cornea represents a potentially useful therapeutic strategy for controlling HSK. Here, we show that about 80% of CD4 T cells infiltrating into the cornea after HSV-1 infection express the integrin VLA-4. Furthermore, infiltration of CD4 T cells into the cornea was reduced dramatically by using a monoclonal antibody against VLA-4, and antibody-treated, HSV-infected animals showed diminished SK severity compared to control, untreated animals at day 15 post-infection. To further confirm the role of VLA-4 integrin, we used mice bearing a mutation in the α_4 subunit of VLA-4 (alpha4 Y991A), which restricts the binding of paxillin, inhibiting the downstream signaling essential for leukocyte migration. These animals, when infected ocularly with HSV-1, showed diminished HSK severity compared to WT animals, similar to alpha-4 blockade. Thus, these findings explain the indispensable role of VLA-4 in migration of CD4 T cells in the cornea, which can be exploited for therapeutic intervention of HSK, a major cause of infectious blindness in the Western world.

73. Unusual Isotype Skewing of the Influenza-Specific B Cell Response in HLA-DR1 Transgenic Mice

Lifang Huan,[1] Hye Mee Joo,[1] Richard Webby,[2] Andrea Sant,[3] Mark Sangster[1]
[1]Department of Microbiology; [2]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN; [3]David H. Smith Center for Vaccine Biology and Immunology, Aab Institute, and Department of Microbiology and Immunology, University of Rochester, Rochester, NY

HLA-DR1 transgenic mice provide a model for evaluating CD4 T cell responses that may develop in humans following influenza infection or vaccination. Recent studies identified a tremendously broad HLA-DR1-restricted CD4 T cell response in HLA-DR1 transgenic mice infected intranasally with influenza A/New Caledonia/99 (H1N1). Our study was undertaken to establish how B cell response correlates with CD4 T cell activation in this system. Influenza-specific B cell responses following infection were compared in HLA-DR1 transgenic mice and in the genetically matched *H-2*^{b} strain C57BL/10 (B10). The response in B10 mice featured strong IgG2b and IgG2c production and was typical of previously described B cell responses to a variety of mouse-adapted influenza strains. In contrast, the response in transgenic mice followed delayed kinetics and was strongly skewed to IgG1 production, consistent with Th2 polarization of the CD4 T cell response. Viral clearance from the lung was more rapid in B10 mice, correlating with the earlier B cell response. The different antibody isotype profiles in B10 and transgenic mice were evident in antibody-secreting cell frequencies and in circulating antibody levels. Early in the response, serum Ab levels measured by ELISA were higher in B10 mice compared with transgenic mice. Surprisingly, however, transgenic mice at this time had higher viral neutralizing Ab levels in serum, suggesting a relatively greater contribution of the germinal center pathway to Ab production. A mechanistic understanding of the unusual B cell response in the transgenic mice may have implications for the optimal control of influenza infection.

74. Deficient Virus-Specific IgA Production and Mucosal B Cell Memory Following Murine gammaherpesvirus 68 Infection of the Respiratory Tract

Aarthi Sundararajan, Hye Mee Joo, Mark Y. Sangster
Department of Microbiology

B cell response to viruses that replicate in the respiratory tract typically includes a substantial IgA component. B cell memory consists of Ab-secreting cells (ASCs) and memory B cells (B_{Mem}), localized in the respiratory tract. Murine gammaherpesvirus 68 (MHV-68) is a naturally-occurring virus of rodents that replicates well in the respiratory tract after intranasal (i.n.) administration. Many features of MHV-68 infection in mice parallel Epstein-Barr virus infection in humans. The respiratory mucosa is a critical environment for MHV-68 entry, replication, latency establishment, reactivation, and transmission, but little is known about B cell-mediated mucosal immunity to the virus. We conducted a comprehensive analysis of the MHV-68-specific B cell response to i.n. infection using the ELISPOT assay and ELISA to measure ASC numbers and Ab levels, respectively. B_{Mem} were quantified using an ELISPOT-based LDA. MHV-68 infection generated a strong virus-specific IgG response in draining lymph nodes, but surprisingly, no virus-specific IgA ASCs. Serum and respiratory secretions did not comprise virus-specific IgA. Very few virus-specific ASCs or B_{Mem} (IgG) migrated to the respiratory tract at any time, indicating substantial deficiency in local B cell memory. Interestingly, co-infection of mice with MHV-68 and influenza virus resulted in an increased influx of MHV-68-specific ASCs into the lung, pointing to an influence of influenza infection on local factors that influence ASC trafficking. Our study identifies defects in MHV-68-specific B cell response and the establishment of local B cell memory, which may reflect a high level of viral adaptation to the respiratory tract to favor survival and transmission.

75. Pomegranate Constituents Have Direct Antiviral Activity against Diverse Influenza Virus Subtypes

Radha Ganapathy, Aarthi Sundararajan, Mark Sangster
Department of Microbiology

The pomegranate fruit has a long history of traditional use as a folk remedy. Recent research has associated constituents of the fruit with a variety of medical benefits, many of which are attributed to the potent antioxidative activity of pomegranate components. Pomegranates contain high levels of polyphenolic compounds, which are largely responsible for the fruit's antioxidant properties. A number of studies have demonstrated that polyphenolic complexes derived from other plants have antiviral effects, suggesting that antiviral activity may also reside in the polyphenol (PP) fraction of pomegranates. The current study was undertaken to evaluate the direct anti-influenza activity of pomegranate constituents present in commercially available juice and PP-rich extracts. A standard dose of infectious virus was incubated with the pomegranate products. Viral infectivity was measured by titration in susceptible MDCK cells, and hemagglutinating activity was determined using chicken red blood cells. Both pomegranate juice and a concentrated liquid extract had anti-influenza activity that was distinct from the effect of the acidic pH of the test materials. A PP extract of pomegranates at concentrations >100 μg/ml had a rapid antiviral effect on H3N2 and H1N1 influenza viruses. PP concentrations that decreased viral infectivity also eliminated hemagglutinating activity, raising the possibility of PP interactions with the viral hemagglutinin. Our findings demonstrate rapid anti-influenza activity in pomegranate PPs that is equally potent against diverse viral subtypes. A possible mechanism is direct coating of viral particles by reactive PPs.

76. Generation of Recombinant MCMVs Overexpressing the MHV-68 Chemokine-Binding Protein-M3, Using the Bac System

Heather Benedict-Hamilton, Tom Masi, Tim E. Sparer
Department of Microbiology

Human cytomegalovirus (HCMV) causes congenital infections as well as disseminated disease in immunocompromised hosts. CMVs are species specific,

making it necessary to use murine CMV (MCMV) infection of mice as a model for HCMV infection. Dissemination of CMVs is immune cell associated in both hosts. Our hypothesis is that host chemokines contribute to the attraction of immune cells and subsequent viral dissemination. In order to test our hypothesis, recombinant MCMVs containing the M3 binding protein of murine gammaherpesvirus-68 (MHV-68) were generated. M3 is a potent chemokine scavenger that binds to and inhibits a wide range of host chemokines, including CC, CXC, and CX_3C. By using MCMV expressing the M3 protein, we will determine whether host chemokines are important for dissemination in vivo. Two recombinant M3 MCMV viruses, one inserted into the ie2 region and the other inserted into the mck2 locus, and the corresponding control viruses were generated using homologous recombination between a shuttle vector and the K181 BAC (pARK25). We have generated and confirmed these BAC recombinants. Once proper in vitro growth and M3 expression is confirmed, we will begin to assess the role of host chemokines on MCMV dissemination. Understanding the role that chemokines play in CMV dissemination will provide an additional target for the development of novel anti-CMV drugs to prevent CMV-induced morbidity.

77. Functional Analysis of Polymorphisms within Human Cytomegalovirus Viral Chemokine vCXCL-1

Jinho Heo, Casey Dauw, Tom Masi, Tim E. Sparer
Department of Microbiology

The human cytomegalovirus (HCMV) viral chemokine gene *UL146* shows a high degree of hypervariability in clinical isolates. The *UL146*-produced viral chemokine vCXCL-1 is a member of the CXC chemokine family and is predicted to be an immune modulator that may contribute to the pathogenesis of HCMV infections. In the analysis of clinical isolates from congenitally-infected infants, we found 11 distinct genotypic clades of vCXCL-1. The group designation did not strictly correlate to the clinical outcomes, but in clades 8, 10, and 12, the majority of congenitally-infected infants were asymptomatic. In these clades, the four cysteine residues that provide the structure to vCXCL-1 were conserved while the N-loop region, important for receptor-binding, was variable. One clade also contained a modified ELR motif, which is important for receptor binding and activation. Based on this sequence information, we hypothesize that these proteins differentially activate neutrophils, which may have a role in HCMV pathogenesis. To address these functional differences, we produced representative vCXCL-1 proteins from each of the 11 clades using the baculovirus expression system. We have examined functional differences between the vCXCL-1s using chemotaxis, apoptosis, and calcium flux assays. We have found differences in the potency of seven of the vCXCL-1s for neutrophil activation. This analysis could provide insights into the role of vCXCL-1 in HCMV pathogenesis.

78. Recombinant Murine Cytomegalovirus Overexpression of Host or Viral Chemokine Leads to a Defect in Salivary Gland Dissemination

Mindy Miller-Kittrell, Tom Masi, Tim E. Sparer
Department of Microbiology

Cytomegaloviruses (CMVs) have evolved multiple different proteins for manipulating the immune response for increasing the viruses' survival and dissemination. One of these proteins, vCXCL-1, is a chemokine homolog that attracts and activates neutrophils. In order to study the role of this viral chemokine on viral dissemination, we generated recombinant murine cytomegaloviruses (MCMVs) overexpressing either vCXCL-1 from chimpanzee CMV or its murine chemokine homolog, mCXCL1. These recombinants were inoculated into mice in order to study their role on viral dissemination *in vivo*. We found these chemokines did not affect MCMV replication or clearance at the site of infection or primary dissemination to the spleen, lymph node, or lung. In contrast, we did not identify either of the chemokine-expressing MCMVs in the salivary gland. In order to test whether chemokine activation induced the adaptive immune response leading to clearance from the salivary gland, we inoculated SCID mice with our recombinants. Even without the adaptive arm of the immune response, the chemokine-expressing recombinants were not recovered from the salivary glands. To determine whether neutrophils were responsible for the salivary gland defect, mice were depleted of neutrophils following MCMV infection. Surprisingly, even without neutrophils, MCMVs were not recovered from the salivary gland. From this data we conclude that there

is an additional cell type that is responsive to the CXC chemokines that either masks viral dissemination or leads to clearance solely from the salivary gland.

79. ODAM as a Novel Biomarker for Human Breast Cancer

Sabina Siddiqui,[1] C. Todd Bruker,[2] Daniel P. Kestler,[3] James S. Foster,[3] Keith D. Gray,[1] Alan Solomon,[3] John L. Bell[1]
[1]Department of Surgery; [2]Department of Pathology; [3]Human Immunology and Cancer Program, UT Medical Center

Odontogenic ameloblast-associated protein (ODAM) is a novel protein produced in ameloblasts during odontogenesis. ODAM expression was identified in breast cancer, but its significance remains to be defined. The purpose of this study was to determine if ODAM expression can serve as a prognostic marker and provide information regarding treatment effects in human breast cancer. Breast cancer patients were identified in our tumor registry for 1993-2003. The archived fixed breast tissue of 243 of these patients (Stage 0=53, Stage I=51, Stage II=53, Stage III=47, Stage IV=39) was stained using a monoclonal antibody for ODAM. The presence or absence of immunostaining was correlated with disease stage, histologic grade, response to chemotherapeutic regimen, and survival using chi-square and logistic regression analyses. A statistically significant increase in nuclear staining was observed for ODAM with increased group stage ($p<.001$). Staining did not correlate with histologic grade or chemotherapy ($p=.558$, $p=.093$); however, statistically significant ($p<.001$) improved survival was demonstrated between group stages in the presence of ODAM protein. There was statistical significance within individual group stages 0, I, and II, ($p<.001$, $p=.003$, $p=.003$), but we were unable to show significance in III or IV ($p=.724$, $p=.059$) due to power. These results show that ODAM may help predict survival in breast cancer as improved outcomes were observed based on stage when ODAM protein was detected in the nuclei of the tumor cells. Research is ongoing to determine ODAM's role in carcinogenesis and its potential clinical utility.

80. Role of Alternative Splicing in Colorectal Tumorigenesis

Jae Hoon Bahn (CEM), Seong-Ho Lee, Seung Joon Baek
Department of Pathobiology

Alternative splicing is an important post-transcriptional regulation mechanism to allow generation of multiple gene products from a single transcript. More than 70% of human genes undergo alternative splicing, and many abnormal splicing processes are associated with tumorigenesis. Krüppel-like factor 4 (KLF4) is a member of the C2H2-type zinc finger protein family, acts as a transcription regulator, and is involved in cell differentiation, proliferation, and apoptosis. In this study, we found a novel alternative spliced isoform of KLF4 by exon skipping in several human cancer cell lines. RT-PCR analysis revealed that KLF4 generates two major alternative spliced forms, $KLF4_L$ and $KLF4_S$. Both $KLF4_L$ and $KLF4_S$ proteins are dominantly expressed in the nucleus, compared to cytoplasm. Cyclin D1 and PAI-1 promoter assay revealed that overexpression of $KLF4_S$ inhibits $KLF4_L$ transcription activity. Interestingly, sulindac sulfide (SS) treatment restores $KLF4_L$ expression in HCT-116 colorectal cancer cells. To further investigate molecular mechanisms of alternative splicing, we designed a KLF4 minigene construct containing three exons and two introns of KLF4 genomic DNA and transfected minigene to identify a cis-acting element to control KLF4 alternative splicing. We found that mutation of 3' splice site in the second intron decreases splicing events. These data are supported by less expression of RNA binding motif protein 5 (RBM5) that regulates exon skipping in normal tissues, compared to tumor. Overall our data suggest that the regulation of alternative splicing in KLF4 is mediated by RBM5 and cis-acting elements located in 3' splicing site and provide a novel mechanism by which alternative splicing affects tumorigenesis.

81. Peroxisome Proliferator-Activated Receptor Gamma Ligands Induce Pro-Apoptotic Protein NAG-1 Expression via KLF4 in a PPARγ-Dependent Manner

Maria Cekanova,[1] Seong-Ho Lee,[2] Michael F. McEntee,[2] Seung Joon Baek[2]
[1]Department of Small Animal Clinical Sciences; [2]Department of Pathobiology

Peroxisome proliferator-activated receptor gamma (PPARγ) plays a central role in differentiation, metabolism ,and tumorigenesis. We identified Krüppel-like factor 4 (KLF4) as a novel PPARγ target gene, which subsequently induces the expression of non-steroidal anti-inflammatory-activated gene-1 (NAG-1) at the transcription level. We used siRNAs of PPARγ and KLF4 and PPARγ antagonist to confirm that NAG-1 expression was affected by these two upstream proteins. The promoter study of NAG-1 reveals that there are functional KLF4 binding sites in the -807 and -558 bp region of the NAG-1 promoter. Transient transfection of KLF4 transactivates the NAG-1 promoter in HCT-116 cells, and specific deletion clones (-807/-803 and -558/-554) showed the reduced promoters' activities in the presence of KLF4 expression, indicating that these sites play a pivotal role in KLF4-induced NAG-1 expression. Gel shift assay further confirmed that KLF4 protein binds to these KLF4-binding sites. In the MCC-555-treated $Apc^{Min/+}$ mice, samples of small intestine and colon MCC-555 induced the expression of Klf4 in both mRNA and protein levels in polyps as assessed by RT-PCR and immunohistochemistry. In conclusion, we have identified KLF4 as a novel regulator of NAG-1 expression at the transcription level, and KLF4 is regulated by PPARγ ligands in human colorectal cancer.

82. Reactive Oxygen Species in the Ability of Pro-apoptotic H-Ras to Enhance Apoptosis Induced by Histone Deacetylase Inhibitors

Shambhunath Choudhary (CEM), Hwa-Chain Robert Wang
Department of Comparative Medicine

Oncogenic induction of the *ras* genes is widely involved in human cancers. Expression of oncogenic H-Ras increases susceptibility of human and mouse cells to histone deacetylase inhibitors (HDACIs), such as FK228 and trichostatin A, for inducing caspase activation and selective apoptosis. HDACIs make up a new class of structurally diverse anticancer agents and have been shown to exhibit antimetastatic and antiangiogenic activities toward malignantly transformed cells. To understand the mechanisms behind the proapoptotic ability of oncogenic H-Ras to enhance cell susceptibility to HDACIs, we detected that expression of oncogenic H-Ras potentiated intracellular reactive oxygen species (ROS) in human urinary bladder cancer J82, human colorectal cancer HT29, and mouse embryo fibroblast 10T1/2 cells to enhance HDACI-induced ROS, thereby contributing to the induction of selective apoptosis and caspase activation. Expression of oncogenic H-Ras also increased cell susceptibility to hydrogen peroxide (H_2O_2) for inducing apoptosis and caspase activation. By studying IC_{50} values of FK228 and H_2O_2 for oncogenic H-Ras-expressing and parental cells and by quantifying cell death and caspase activation induced by FK228 and H_2O_2, we demonstrated evidence to indicate, for the first time, that intracellular ROS was cooperatively up-regulated by oncogenic H-Ras and FK228 treatment to induce apoptosis and caspase activation in a dose-dependent manner. Considering intracellular ROS as involved in the novel proapoptotic ability of oncogenic H-Ras as a potential target is important in developing therapeutic strategies to control oncogenic H-Ras-involved human cancers.

83. Nonsteroidal Anti-inflammatory Drugs Suppress Structural Protein Nesprin-2 Expression in Colorectal Cancer Cells

Jason L. Liggett (CEM),[1] Chang K. Choi,[1,2,*] Robert L. Donnell,[1] Anthony E. English,[2] Jong-Sik Kim,[3] Kenneth D. Kihm,[2] Angelika Anna Noegel,[4] Seung Joon Baek[1]

[1]Department of Pathobiology; [2]Department of Mechanical, Aerospace and Biomedical Engineering; [3]Department of Biology, Andong National University, Andong, Korea; [4]Institute for Biochemistry, University of Cologne, Cologne, Germany; [*]Current address: Microscale Electrical and Optical Sensing (micro-EOS) Laboratory, Michigan Technological University, Houghton, MI

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to have anti-tumorigenic effects in colorectal cancer. A structure protein nesprin-2 was further characterized as an NSAID-suppressed gene, based on microarray data. Human colorectal cancer cells were treated with the NSAID sulindac sulfide (SS) and examined by differential interference contrast microscopy (DICM), interference reflection contrast microscopy (IRCM), epi-fluorescence microscopy, and by micro-impedance measurements. Two microarray analyses were performed using HCT-116 cells treated with SS. Genes common to both microarrays were confirmed through reverse-transcriptase polymerase chain reaction (RT-PCR). Tissue array was performed. Silencing of mRNA was performed with shRNA for micro impedance measurement. Western blot showed downregulation of truncated nesprin-2 isoform after SS treatment. HCT-116 cells showed dramatic morphological changes under microscopic examination caused by SS treatment. Microarray analysis of SS treated cells revealed nesprin-2 is a down-regulated gene. Immunohistochemistry showed higher levels of nesprin-2 in tumors of many tissues. Knockdown of nesprin-2 mRNA caused a proportional reduction of cellular adhesion. Nesprin-2 is down-regulated by NSAIDs, is highly expressed in many cancers, and may be a potential novel oncogene and a molecular target of NSAIDs in colorectal cancer.

84. Zyflamend, a Multi-Herb Extract, Enhances Tumor Regression of CWR22 Prostate Cancer Cells in a Xenograft Model when Used in Conjunction with Hormone Ablation Therapy

E-Chu Huang,[1] Michael F McEntee,[2] Julia S Gouffon,[1,3] Jay Whelan[1]

[1]Department of Nutrition; [2]Department of Pathobiology; [3]Affymetrix Core Facility

Zyflamend is an extract of multiple herbs with anti-cancer properties reported previously, but the mechanisms are not known. We hypothesize that Zyflamend synergizes the effect of hormone ablation therapy (HAT) on tumor regression. In this study, the inhibitory effect of Zyflamend was examined using a CWR22 human prostate cancer cell xenograft model, allowing us to evaluate the impact of HAT with Zyflamend. CWR22 human prostate tumor cells were implanted in 60 castrated, athymic male nude mice (containing an implanted testosterone pellet), and 30 animals were randomly placed on a diet containing Zyflamend after tumor growing. We demonstrate that HAT resulted in significant regression of prostate tumors within 4 days, in part, by reducing the expression of pro-tumorigenic androgen receptors and pAKT. Zyflamend, when supplemented at human equivalent doses, significantly enhanced tumor regression *($p<0.0001$)*. In addition, Zyflamend protected against body weight loss as a result of HAT. The overall cellular mechanisms of cancer cells responding to HAT with and without adjuvant therapy using Zyflamend have yet to be fully investigated. When regressing tumors were subjected to Affymetrix gene array analysis, Zyflamend shifted global gene expression by significantly modifying ~1,000 genes. These results indicate that adjuvant therapy with a combination of medicinal herbs can enhance the beneficial effects of standard therapy for the treatment of prostate cancer by coordinately modifying, in part, prostate tumors at a global genomic level.

This research is funded by a grant from New Chapter, Inc. Brattleboro, VT, and in part from Tennessee AgResearch.

85. NUDT6, a Putative Prognostic Marker in Colon Carcinogenesis, is Suppressed by EGCG

Mugdha Sukhthankar (CEM), Maria Cekanova, Seung Joon Baek
Department of Pathobiology

Epigallocatechin (-) gallate (EGCG), a major constituent of green tea, is reported to exert anti-tumorigenic activities through multiple mechanisms, most of which are not well characterized. Here we show that one mechanism of anti-tumor activities involves the novel cell proliferator gene *NUDT6*, nudix (nucleoside diphosphate linked moiety X)-type motif 6. Microarray analysis shows EGCG decreases *NUDT6* mRNA. EGCG treatment affects NUDT6 at the post-transcriptional level as indicated by decreased RNA stability. Luciferase assays using cloned 3'UTR of human *NUDT6* mRNA showed reduced promoter activity after EGCG treatment mediated by ERK and p38 MAPK pathways. Furthermore, stable cell lines overexpressing NUDT6 showed increased cell proliferation, cell growth in soft agar, and decreased caspase activity. Because NUDT6 is localized in the mitochondria, we investigated NUDT6's effect on other mitochondrial genes and found pro-apoptotic genes Bax and p53 were decreased, whereas Bcl2 was increased in NUDT6 expressing cells. *In vivo* studies showed that NUDT6 desensitized tumor cells to EGCG treatment as measured by tumor volume, caspase activity, and immunohistochemistry. Measurement of caspase activity using NUDT6 siRNA further supported NUDT6-mediated desensitization to EGCG. Additionally, analysis of multidrug resistance (MDR) genes, such as ABC genes, showed that NUDT6-expressing cells had increased expression of these genes. Together, these findings suggest NUDT6 acts as an anti-apoptotic protein and may serve as a prognostic marker for colorectal cancer.

86. Inhibition of Pathologic Immunoglobulin Light Chain Species by Small Interfering RNA Molecules

Jonathan Phipps (CEM), Daniel Kestler, James S. Foster, Alan Solomon, Jonathan S. Wall
Graduate School of Medicine

The aggregation and deposition of immunoglobulin light chain (LC) protein are a major contributing factor in the morbidity and mortality of patients with plasma cell dyscrasia. Current treatments rely on ablative chemotherapies to reduce clonal plasma cell number and, thus, toxic LC products. Recently, a novel system of post-transcriptional gene regulation, RNA interference (RNAi), has been proposed as a therapeutic modality in a number of disease states. To this end, we have investigated the use of small interfering RNA (siRNA) molecules as a method for inhibiting LC synthesis in immortalized plasma cell lines. Experimentally, the λ2 LC-secreting human myeloma cell line RPMI 8226 and an IgĢκ3-producing cell line, designated Bur, were treated with siRNA targeting the V or C region of each LC product. Analysis of culture supernatant from both cell lines by ELISA revealed that treatment with siRNA significantly reduced LC protein production at 24, 48, and 72 hr compared to control cells treated with an irrelevant siRNA. Furthermore, flow cytometric analysis of RPMI 8226 cells stained with λ LC-reactive antibodies demonstrated a marked reduction in the intracellular LC pool. Analysis of RPMI 8226 cells by Real-Time PCR revealed an accompanying decrease in LC mRNA following exposure to the targeted siRNA as compared to controls. The reduction in LC concentration was accomplished without inducing overt cytotoxicity. These results are encouraging and support testing these reagents in a murine model of plasma cell dyscrasia, and also indicate that siRNA treatment may prove beneficial in patients with plasma cell dyscrasias.

87. Resveratrol Induction of ATF3 is Mediated by EGR-1/KLF4 Interaction

Nichelle C. Whitlock (CEM), Jae Hoon Bahn, Seung J. Baek
Department of Pathobiology

Resveratrol, a phytochemical readily available in the diet, is reported to possess anti-tumorigenic properties

in several cancers, including colorectal. However, the mechanism by which resveratrol exerts its anti-tumor activities is not completely understood. Microarray analysis using HCT-116 colorectal cancer cells identified activating transcription factor 3 (ATF3) as most highly induced after resveratrol treatment, and this was confirmed by reverse transcription-PCR and Western analysis. ATF3 promoter analysis revealed a major resveratrol response-element located between the -514 and -132 bp region of the promoter. Sequence analysis identified four putative transcription factor binding sites: p53, specificity protein 1 (Sp1), early growth receptor 1 (Egr-1), and Krüppel-like factor 4 (KLF4). Co-transfection with these factors showed increased ATF3 promoter activity after resveratrol treatment, with the greatest induction observed in cells over-expressing Egr-1 and KLF4. Internal deletions of the Egr-1 and KLF4 binding sites confirmed the importance of these *cis*-acting elements in resveratrol-induced ATF3 expression. Western analysis and cycloheximide experiments further suggest that EGR-1 and/or KLF4 may be important in resveratrol induction of ATF3. Competition assays showed Egr-1 and KLF4 act synergistically in ATF3 promoter transactivation, which is enhanced by resveratrol, suggesting Egr-1 and KLF4 interaction. Furthermore, we found that Egr-1 and KLF4 bind to each other, which may facilitate ATF3 transcription. This is amplified by resveratrol. To our knowledge, this is the first report indicating that two zinc-finger transcription factors bind and enhance transcriptional activity. Taken together, these results provide a new molecular mechanism by which resveratrol affects anti-tumorigenesis in human colorectal cancer cells.

88. Precancerous Model of Human Breast Epithelial Cells Induced by NNK & Benzo[a]pyrene and the Role of Green Tea Catechins in Breast Cancer Prevention

Kusum Rathore,[1] Hwa-Chain Robert Wang[1,2]
[1]Genomic Science Technology Program; [2]Department of Comparative Medicine

To investigate dietary prevention of human breast cell precancerous carcinogenesis, we want to study the biological and biochemical target endpoints induced by chronic, accumulated exposure to low doses of environmental carcinogens such as [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] and [Benzo[a]pyrene] in human breast epithelial cells. We introduced a precancerous carcinogenesis-cellular model using combined NNK and B[a]P to induce chronic carcinogenesis of MCF10A cells. Immortalized, non-cancerous, human breast epithelial MCF10A cells were repeatedly exposed to combined carcinogens NNK and B[a]P, each at picomolar concentrations, to induce increasing acquisition of cancer-related biological and biochemical changes. We then used this cellular model to reveal the ability of green tea catechins to block acquisition of target endpoints in the suppression of combined NNK- and B[a]P-induced carcinogenesis of MCF10A cells. Dietary polyphenolic compounds (DPPCs) possess anticancer, antioxidant, anti-proliferative, apoptotic, and other activities that inhibit bio-activating enzymes and induce detoxifying enzymes. We used green tea catechin extract GTC (epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate) to suppress cellular carcinogenesis. Catechins were first isolated from the plant extract catechu, from which they derive their name. Epigallocatechin and gallocatechin contain an additional phenolic hydroxyl group when compared to epicatechin and catechin. Catechin gallates are gallic acid esters of the catechins, such as EGCG (epigallocatechin gallate).We used (a) reduced dependence on growth factors (b) anchorage dependence, and (c) acinar formation to study precancerous progression and its suppression with GTCs. This model reveals identifiable precancerous sub-stages and associated biochemical profiles in the progression of cellular carcinogenesis and serves as a target system to identify dietary constituents for precancerous prevention.

89. Chili Pepper Component Capsaicin in Colorectal Cancer Prevention

Seong-Ho Lee, Chutwadee Krisanapun, Seung Joon Baek
Department of Pathobiology

Capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide), a natural product of the *Capsicum* species of red peppers, is known to induce apoptosis and suppress cell growth in various cancer models. The chemopreventive mechanism of capsaicin is cell-specific, and its efficacy is dependent on cell or tissue context. Non-steroidal anti-inflammatory drug (NSAID)-activated gene-1 (NAG-1) is a cytokine associated with pro-apoptotic and anti-tumorigenic properties in colorectal cancer. Our data demonstrate

that capsaicin leads to suppression of cell growth and up-regulation of NAG-1 in human colorectal cancer cells. Capsaicin treatment resulted in stimulation of *NAG-1* promoter activity. Overexpression of C/EBPβ caused a dramatic increase of basal and capsaicin-induced luciferase activity of the NAG-1 promoter. The ChIP assay confirmed binding of C/EBPβ to the *NAG-1* promoter. We identified potential binding sites for C/EBPβ and ATF3, which are necessary for capsaicin-induced NAG-1 transactivation. Capsaicin treatment resulted in an increase of phosphorylated serine/threonine residues on C/EBPβ, and immunoprecipitation study showed that capsaicin enhanced association of C/EBPβ and ATF3, which are suppressed by inhibition of PKCδ and GSK3β pathways. Knockdown of C/EBPβ ameliorates NAG-1 expression and apoptosis induced by capsaicin treatment. These data indicate that C/EBPβ phosphorylation through PKCδ and GSK3β may mediate capsaicin-induced expression of NAG-1 and apoptosis through cooperation with ATF3 in human colorectal cancer cells.

90. The Role of Amino Acid Sequence Variation of the Immuno-dominant Domain of the Attachment Glycoprotein G of Respiratory Syncytial Virus in the Immune Response

Mohamed Abd-Eldaim, Stephen A. Kania, Leon Potgieter
Department of Comparative Medicine

Bovine respiratory syncytial virus (RSV) is a major cause of respiratory diseases in ruminants. The G protein of RSV serves as the viral attachment protein, and its amino acid sequences have been used to classify the ruminant RSV into bovine and ovine RSV. RSV immunity is incomplete, and re-infections occur. The existence of variants may be responsible for this phenomenon. Vaccine trials using the 174-187 amino acid region of G protein, which represents the immuno-dominant domain, have protected against viral infection in both mice and calves. Based on the amino acid sequences of this domain, we designed four synthetic peptides from the 174-187 amino acid region of G protein representing four possible subgroups of ruminant RSV isolates (including the 391-2 strain and related isolates, Maryland strain BRSV, a group of European BRSV isolates, and ORSV). Each peptide was conjugated to KLH, emulsified in incomplete Freund's adjuvant, and inoculated into a group of 10 mice. The antibodies raised against each peptide were examined using ELISA and flow cytometry. ELISA was done using non-conjugated peptide as antigen to evaluate the reactivity of the antibodies raised against linear antigen. Flow cytometry, using MDBK infected with RSV subgroups, was done to evaluate the reactivity of the antibodies raised against the native form of the G protein. Based on ELISA, antibodies reacted with homologous and heterologous peptides, but flow cytometry analysis indicated the antibodies raised against peptides representing two of the bovine subgroups reacted only with homologous virus. The antibodies against the peptide corresponding to the Maryland strain reacted slightly with homologous and heterologous viruses. The antibodies to ORSV peptide did not react with homologous or heterologous RSV. The results obtained revealed that the native form of G glycoprotein might be antigenically distinct. Thus, incomplete immunity and recurrent infection of RSV may be attributed to differences of antibodies against the immuno-dominant domain of the attachment glycoprotein G.

Sponsor & Exhibitor Directory

Aurogene
Chad Thatcher
(865)671-2166
cthatcher@tds.net

Charles River Laboratories
Lorie Boyd
(919) 341-7283
Lorie.Boyd@crl.com

Fisher Safety
Crystal Stepp
(615) 484-3236
crystal.stepp@thermofisher.com

Millipore
Shannon Eaker
(865) 567-9014
Shannon_Eaker@millipore.com

Myrtle's Rabbitry
Rebecca Dodson
(800) 424-9511
rebecca@myrtles.com

RefWorks–Community of Science
Hunter Wagner
(210) 231-0238
Hunter.Wagner@refworks.cos.com

UT Federal Credit Union
Teri Branam
(865) 971-1971 (Ext. 115)
tbranam@utfcu.org

UT Press
Tom Post
(865) 974-5466
tpost@utk.edu


RefWorks COS


UT
Federal Credit Union


Fisher Safety


THE UNIVERSITY of TENNESSEE PRESS


AUROGENE™


charles river
accelerating drug development. exactly.


MILLIPORE


MYRTLE'S
RABBITRY
INCORPORATED