June 17, 2008

Second Annual

Comparative & Experimental Medicine Research Symposium

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Program & Schedule

Sponsored by the College of Veterinary Medicine, Graduate School of Medicine, Tennessee Agricultural Experiment Station, and the UTK Office of Research

Welcome

With this symposium, the UT Agricultural Campus is hosting a large group of biomedical and veterinary scientists, including 63 presenters representing 16 different UT departments and programs. The speakers 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 *scholars* in seven different languages, which are spoken by one or more of the presenters.

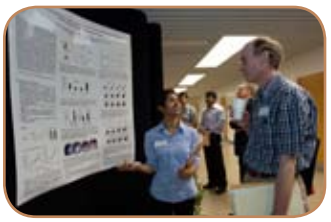
The Comparative and Experimental Medicine Research Symposium provides an excellent venue for students and new investigators to gain experience presenting their work as oral presentations. In addition, the gathering of UT investigators with animal and human health interests provides opportunities for 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 Graduate School of Medicine, the Tennessee Agricultural Experiment Station, and the UTK Office of Research is unprecedented and signifies shared recognition of the need for such a symposium, and there are plans for continuing to expand the symposium in future years.

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







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Event Schedule

8:00-8:45	Poster set-up and continental breakfast	PBB 156
9:00-10:00	Keynote presentation	PBB 156/157
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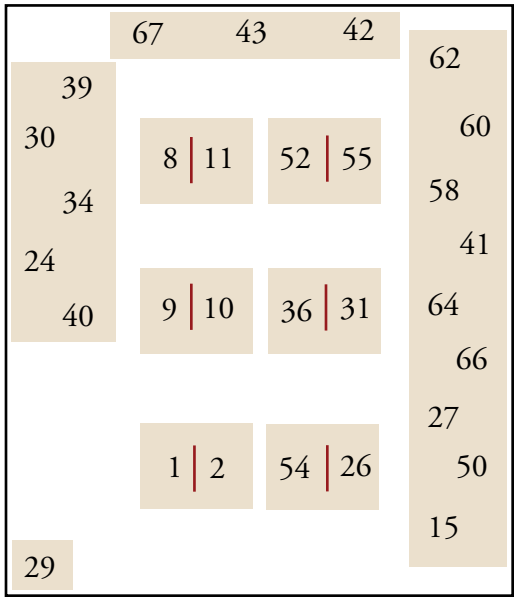
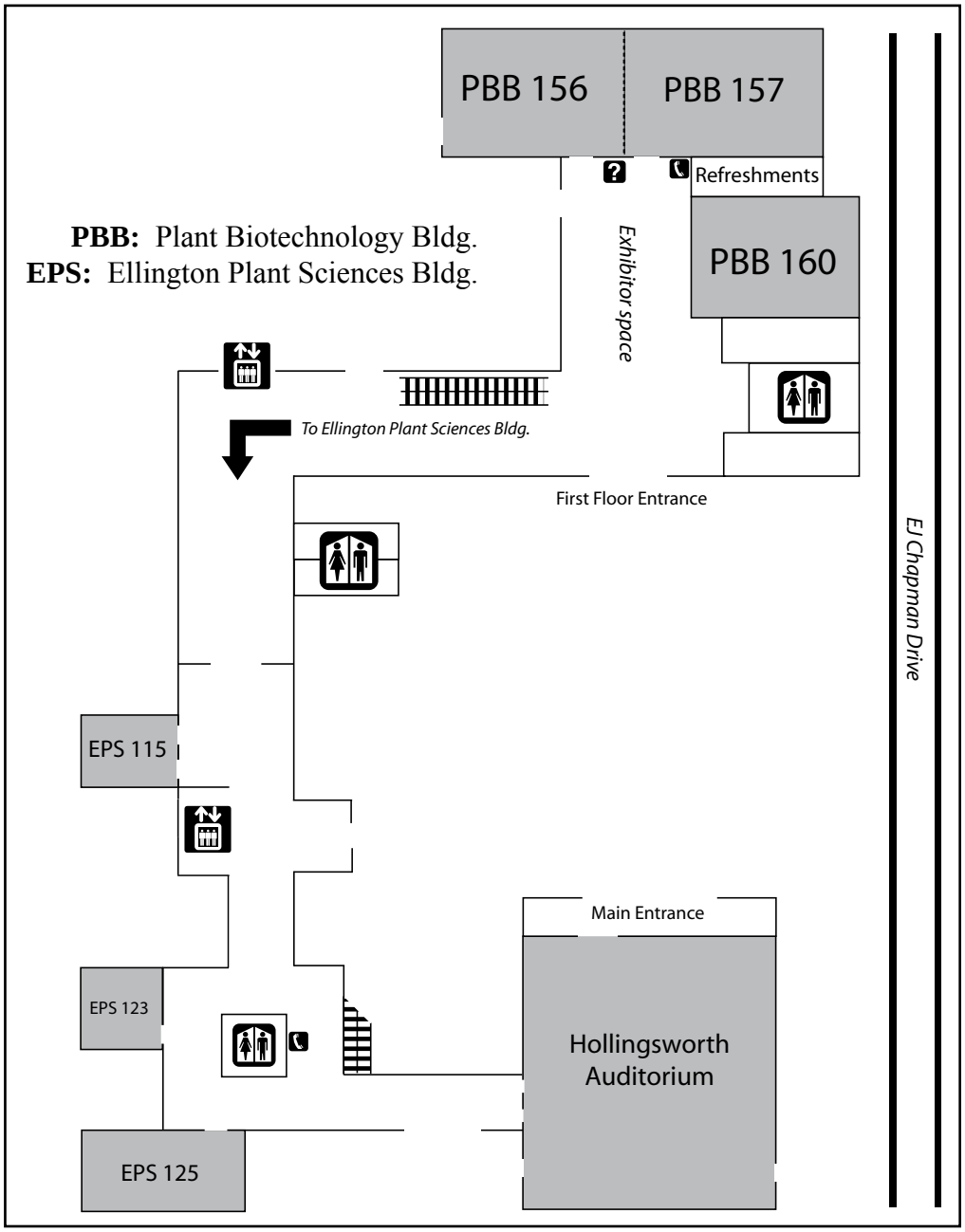
12:00-1:00	Presenter lunch (Emory University Office of Postdoctoral Education presentation)	PBB 160
1:15-2:15	Poster session	PBB 156
2:30-5:15	Session 2	See session matrix
6:30	Awards banquet (ticket required)	Hollingsworth Auditorium

Poster Session–PBB 156



*Numbers correspond with title numbers in session matrix.



Parking

Faculty & staff with a valid parking permit for a main (east) campus lot may park in lot 66–across from Hollingsworth–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 p. 11-34)

	Bacterial Virulence, Diagnostics, & Transmission **Rm. PBB 157**		Viruses, Parasites, & Immunity **Rm. PBB 160**		Cancer Cell Biology **Rm. EPS 125**		Evidence-Based Clinical Sciences **Rm. EPS 115**		Proteins & Technology **Rm. EPS 123**	
10:30 am	1. Comparison of Antimicrobial Susceptibility Patterns of Fecal *Escherichia coli* from Healthy Dogs and Their Owners **(Stenske)**	**Moderator: Maria Prado**	13. A Novel Game-Theoretical Framework for Modeling Host-Pathogen Interactions **(Bewick)**	**Moderator: David Brian**	28. Tolfenamic Acid Induces Apoptosis Through ESE-1/EGR-1/NAG-1 in Colorectal Cancer Cells **(Lee)**	**Moderator: Hwa-Chain Robert Wang**	41. Advancements in Kinematic Data Acquisition: Reduction of Skin Movement Artifact and Further Improvements Towards the Inverse Dynamics Method **(Headrick)**	**Moderator: Karla Matteson**	56. The Role of GXXXG Motif in the Interaction of APP with γ-Secretase and Formation of Aβ **(Mao)**	**Moderator: Robert Donnell**
10:45 am	2. Comparison of Clonal Relatedness of Fecal *Escherichia coli* Isolates from Dogs and Their Owners and Epidemiological Analysis of Within Household Sharing of Bacteria **(Stenske)**		14. Acute Virulence of *Toxoplasma gondii* in Mice is Strongly Associated with Uncontrolled Growth **(Hill)**		29. Design and Synthesis of Sutent, a Novel Tyrosine Kinase Inhibitor for the Treatment of Gastrointestinal Stromal Tumors and Renal Cell Carcinoma **(W Zeng)**		42. Subarachnoid Anesthesia in Caesarian Section: A Study on Objective Measurement with Bispectral Index **(Higley)**		57. Effects of γ-Secretase Cleavage Region Mutations on APP Processing and Aβ Formation **(Tan)**	
11:00 am	3. Identifying Mechanisms Associated with Neutrophil Migration in Cows Genetically Susceptible to Mastitis **(Elliott)**		15. The Impacts of Extreme Population Structure in Big Brown Bats (*Eptesicus fuscus*) on Rabies Virus Adaptation **(Turmelle)**		30. Target Endpoints for Dietary Prevention of Breast Cancer **(Siriwardhana)**		43. Absolute Clot Strength of Multi-Trauma Patients Increases During Transit to the Emergency Department **(Clanton)**		58. Lysophosphatidic Acid in Inflammation and Atherosclerosis **(Hao)**	
11:15 am	4. *Staphylococcus pseudintermedius* Induced Oxidative Burst by Canine Neutrophils **(Black)**		16. Functional Analysis of Polymorphisms within Human Cytomegalovirus Viral Chemokine, vCXCL-1 **(Heo)**		31. The Role of Non-steroidal Anti-inflammatory Drug Activated Gene-1 (NAG-1) in Lung Tumorigenesis **(Cekanova)**		44. Positron Emission Tomography in Normal Hispaniolan Amazon Parrots (*Amazona ventralis*) **(Souza)**		59. Allosteric Interplay in Thyroid Hormone Receptor Transactivation Complex **(Putcha)**	
11:30 am	68. Rapid Detection of *Salmonella typhimurium* from Spiked Lettuce using Real-Time Reverse-Transcriptase-Polymerase Chain Reaction **(Miller)**		17. Generation of Recombinant MCMVs Overexpressing the MHV-68 Chemokine-Binding Protein, M3, Using the BAC System **(Benedict-Hamilton)**				45. Kinematics of Stair Ascent Versus Trotting in Healthy Dogs **(Durant)**			

	Bacterial Virulence, Diagnostics, & Transmission **Rm. PBB 157**		Viruses, Parasites, & Immunity **Rm. PBB 160**		Cancer Cell Biology **Rm. EPS 125**		Endocrine & Metabolic Disorders **Rm. EPS 115**		Proteins & Technology **Rm. EPS 123**	
2:30 pm	5. The Challenge of Johne's Disease Diagnosis: UTK-Flow Cytometry Method and EV-ELISA **(Scott)**	**Moderator: Maria Prado**	18. Prevalence of Malignant Catarrhal Fever White-Tailed Deer Variant in Tennessee Hunter-Harvested Deer **(Cissell)**	**Moderator: Melissa Kennedy**	32. Reactive Oxygen Species-Dependent Anticancer Therapeutics **(Rathore)**	**Moderator: Seung Joon Baek**	46. Effects of Short- and Long-Term Hypoxia on Hemolymph Gas Values in the American Horseshoe Crab (*Limulus polyphemus*) **(Allender)**	**Moderator: Madhu Dhar**	60. Lysophosphatidic Acid Induction of Matricellular Protein CYR61 Expression in Aortic Smooth Muscle Cells and in Neointimal Lesions **(D Wu)**	**Moderator: Robert Donnell**
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3:00 pm	7. Identifying Gene Markers for Respiratory Disease Resistance in Cattle **(Prado)**	**Moderator: Jun Lin**	20. ST6Gal-I Gene Expression is Required for Optimal Viral-Specific CD8 T Cell Expansion *in vivo* **(J Patel)**		34. Histone Deacetylase Inhibitors for Selective Anticancer Therapeutics **(Choudhary)**		48. Effects of a Combined Chromium, Magnesium, and Herbal Dietary Supplement on Insulin Sensitivity in a Genetically Diverse Population of Insulin Resistant Horses **(Chameroy)**		62. Knockdown of Immunoglobulin Light Chain Production by RNA Interference **(Phipps)**	
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3:45 pm	9. Expression of the Recombinant Form of a Novel Surface Protein (SUAM) of *Streptococcus uberis* **(Prado)**	**Moderator: Jun Lin**	Break	**Moderator: David Gangemi**	Break	**Moderator: Thomas Masi**	Break	**Moderator: Nicholas Frank**	64. Development of an Autonomous Mammalian Lux Bioreporter **(Close)**	**Moderator: Terry W. Schultz**
4:00 pm	10. Vaccination with *Streptococcus uberis* Adhesion Molecule Induces Isotypic Antibody Responses in Bovine Serum and Colostrum **(Prado)**		23. Unexpected Role of Selectin Ligands in T cell Trafficking to the Lung Mucosa **(Harp)**		37. Resveratrol Increases ATF3 Expression Mediated by Egr-1 and KLF4 **(Whitlock)**		51. Metabolic and Genomic Changes Induced by Macronutrient Composition vs. Caloric Restriction in C57/BL6 Mice **(Kalupahana)**		65. Chromodomain Helicase DNA Binding Protein 2 and DNA Damage Response Signalling **(Rajagopalan)**	
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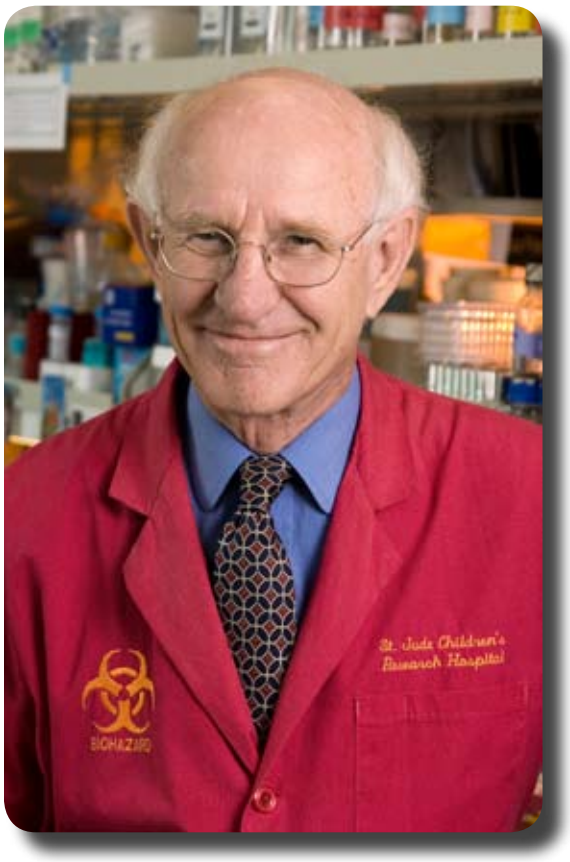
Keynote Presentation



H5N1 Influenza: Has the Risk Been Overblown?

A native of New Zealand, Dr. Robert G. Webster received his BSc and MSc in microbiology from Otago University in New Zealand. In 1962, he earned his PhD from the Australian National University and spent the next two years as a Fulbright Scholar working on influenza in the Department of Epidemiology at the University of Michigan, Ann Arbor.

During most of his career Dr. Webster has been director of the World Health Organization Collaborating Center for Studies on the Ecology of Influenza in Animals and Birds, one of only five such collaborating centers in the world. His interests include the emergence and control of influenza viruses, viral immunology, the structure and function of influenza virus proteins, and the development of new vaccines and antivirals. The major focus of his research is the importance of influenza viruses in wild aquatic birds as a major reservoir of influenza viruses and their role in the evolution of new pandemic strains for humans and lower animals. His curriculum vitae contains over 500 original articles and reviews on influenza viruses, and he has trained many scientists who now contribute to our understanding of the evolution and pathogenesis of influenza.

Robert G. Webster, PhD, FRS

Professor & Rose Marie Thomas Chair
Division of Virology
Department of Infectious Diseases
St. Jude Children's Research Hospital

Honors

Fellow of the Royal Society, London, 1989
Fellow of the Royal Society of New Zealand, 1990 (Honorary)
National Academy of Sciences of the United States of America, 1998
12th Annual Bristol-Myers Squibb Award for Distinguished Achievement in Infectious Diseases, 2002
New Zealand Biotech Distinguished Biotechnologist Award, 2006

On the Backs of Serpents: Prehistoric Cave Art in the Southeast

Jan Simek, PhD

Distinguished Professor of Anthropology & Interim Chancellor
University of Tennessee

An anthropologist with an MA and PhD from the State University of New York at Binghamton, Dr. Jan Simek has been at the University of Tennessee since 1984.

Throughout his career, he has focused on ancient human use of caves for habitation, exploration, and religion. Since 1976, he has worked in France studying the relationships between Neanderthals and Modern *Homo sapiens* between 100,000 and 25,000 years ago. For 18 years, he co-directed extensive excavations in the Grotte XVI, a cave with four meters of stratified occupation deposits spanning 100 millennia.

In 1995, Simek became interested in prehistoric cave use in Tennessee and the South, and has since documented more than 50 prehistoric cave art sites in the region, representing the first cave art tradition ever discovered in North America. He has also located numerous other cave sites used for burial, ritual, and mineral mining by Tennessee's prehistoric inhabitants.

Simek has received some 35 grants, has 85 publications to his credit, and since 1981 has presented more than 100 papers and lectures here and abroad. His research interests include quantitative and spatial analysis, paleolithic archaeology and Old World prehistory.

Honors

Distinguished Professor of Science, 2001
TVA Certificate of Appreciation for Valuable Service to our National Heritage, 1996
Visiting scholar and lecturer at the Universitat Autonoma de Barcelona, Spain, 1991
Elected member of Commission 8 on the Upper Paleolithic of Europe, International Union of Prehistoric and Protohistoric Sciences, 1989

Acknowledgements

We wish to acknowledge the following university programs, without whom this day could not be possible:

College of Veterinary Medicine
Graduate School of Medicine
Tennessee Agricultural Experiment Station
UTK Office of Research

Thanks also go to the symposium organizers and staffers:

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Robert Holland
Stephen Kania
Claudia Kirk
Karla Matteson
Robert Moore
Kim Rutherford
Linda Sangster
Lucy Simpson

We appreciate the contributions of session moderators:

Seung Joon Baek,
Asst. Prof., Pathobiology

David Brian,
Prof., Microbiology & Pathobiology

Madhu Dhar,
Res. Assoc. Prof., Large Animal Clinical Sciences

Robert Donnell,
Asst. Prof., Pathobiology

Nicholas Frank,
Assoc. Prof., Large Animal Clinical Sciences

David Gangemi,
Prof., Biological Sciences, Clemson University

Melissa Kennedy,
Assoc. Prof., Comparative Medicine

Jun Lin,
Asst. Prof., Animal Science

Thomas Masi,
Res. Asst. Prof., Microbiology

Karla Matteson,
Prof., Medical Genetics

Maria Prado,
Asst. Prof., Large Animal Clinical Sciences

Terry W. Schultz,
Prof. Emeritus, Comparative Medicine

Hwa-Chain Robert Wang,
Assoc. Prof., Pathobiology

Thanks also to the symposium judges, the UTCVM chapter of Phi Zeta, 2008 Center of Excellence Summer Student Research Program participants, the Emory University Office of Postdoctoral Education, and our sponsors and exhibitors.

Leon Potgieter, *Interim Dean*
College of Veterinary Medicine

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

James Neutens, *Dean*
Graduate School of Medicine

Thomas Klindt, *Interim Dean*
Tennessee Agricultural Experiment Station

Abstracts

학자 Eruditos Gelehrte

Pandit 学者 Tudósok Scholars

THE UNIVERSITY of TENNESSEE

1. Comparison of Antimicrobial Susceptibility Patterns of Fecal *Escherichia coli* from Healthy Dogs and Their Owners

Katherine A. Stenske (CEM),[1] David A. Bemis,[2] Karla J. Matteson,[3] F. Ann Draughon,[4] Joseph W. Bartges[1]
[1]Department of Small Animal Clinical Sciences; [2]Department of Comparative Medicine; [3]Department of Medical Genetics; [4]Department of Food Science and Technology

Routine contact between human beings and companion animals may allow transmission of pathogenic and antimicrobial resistant bacteria between species. The goal of this study was to determine antimicrobial susceptibilities of *E. coli* isolates from healthy dogs and their owners. Fecal swabs were collected from 61 healthy dog and human owner pairs and a control group of non-dog-owners. Volunteers were excluded if they had received antimicrobial therapy within 2 weeks. Three representative *E. coli* colonies were isolated from each sample. Susceptibilities were determined using a disc diffusion method for 17 antimicrobials included in the National Antimicrobial Resistance Monitoring System. In all groups, lowest percent susceptibility was to cephalothin (48% susceptibility in dogs, 59% in owners, 60% in controls), ampicillin (67% in dogs, 61% in owners, 50% in controls), and amoxicillin-clavulanic acid (80% in dogs, 87% in owners, 80% in controls). Imipenem was the only antimicrobial to which all isolates were susceptible. Susceptibility patterns of isolates from dog owners and controls, and from paired dog owners and dogs, were compared using Chi-square and McNemar Chi-square analyses, respectively, and no significant differences were found between groups or within households. Multiple drug resistance (MDR), defined as resistance to 3 or more antimicrobial agents, was seen in 4/61 (7%) dogs, 10/61 (16%) owners, and 5/30 (17%) controls; differences between groups were not significant (P=0.10). In conclusion, antimicrobial resistance, including MDR, was common among fecal *E. coli* isolates from healthy dogs and human beings. Dog ownership did not increase risk of harboring resistant fecal *E. coli*.

2. Comparison of Clonal Relatedness of Fecal *Escherichia coli* Isolates from Dogs and Their Owners and Epidemiological Analysis of Within Household Sharing of Bacteria

Katherine A. Stenske (CEM),[1] Barbara E. Gillespie,[2] Stephen P. Oliver,[2] David A. Bemis,[3] Karla J. Matteson,[4] F. Ann Draughon,[5] Joseph W. Bartges[1]
[1]Department of Small Animal Clinical Sciences; [2]Department of Animal Science; [3]Department of Comparative Medicine; [4]Department of Medical Genetics; [5]Department of Food Science and Technology

With the increasing human animal bond, cross-species bacterial transmission has become a concern among pet owners, veterinarians, and public health officials. Improved epidemiologic understanding of bacterial sharing may help minimize this risk. The goals of this study were to determine the prevalence of fecal *E. coli* sharing between dogs and their owners and to analyze potential epidemiological risk factors involved in inter-host transfer. Fecal swabs and a survey were collected from 61 healthy dog and human owner pairs and a control group. Three representative *E. coli* colonies were isolated from each fecal sample. Pulse field gel electrophoresis (PFGE) using restriction endonuclease XbaI was performed on DNA from each isolate, and similarity matrices were used to compare profiles within households. Surveys questioned frequency of behaviors including sleeping in the same bed, kissing on the face, washing hands after petting and before feeding, disposal of feces, drinking out of toilets, and time spent awake together. Chi-square and Mann-Whitney analyses were used to compare fingerprint and survey results. A wide array of *E. coli* PFGE profiles was observed in all groups. Fecal *E. coli* isolates from only one dog-owner pair had identical profiles. Isolates from twelve other dog-owner pairs had >90% fingerprint similarity. No behaviors surveyed were found statistically more often in households with >90% fecal *E. coli* similarity than households without similar strains. Although sharing of genetically similar fecal *E. coli* is uncommon between dogs and their owners, proper hygiene should be encouraged to minimize potential transmission.

3. Identifying Mechanisms Associated with Neutrophil Migration in Cows Genetically Susceptible to Mastitis

Alexandra Elliott, Gina M. Pighetti
Department of Animal Science

The largest loss in profit for dairy farmers occurs with mastitis, an inflammation of the mammary gland. Our prior research has identified a genetic marker in the gene (CXCR1) associated with mastitis and decreased neutrophil migration. Because neutrophil migration is critical for eliminating most infections, our ongoing research seeks to identify the specific mechanisms causing impaired migration. One of the first steps in neutrophil migration is actin polymerization, which determines the rate and direction of migration. This study evaluated actin polymerization in neutrophils of cows with different genotypes at position +777 in the CXCR1 gene. Neutrophils from Holstein dairy cows with GG (n=10) and CC (n=10) genotypes were isolated from whole blood and stimulated with zymosan-activated serum. Cells were fixed and stained for F-actin at times 0, 15, 30, 60, 90, 120, and 180 seconds. F-actin fluorescence was measured by flow cytometry. An increase in actin polymerization from 0 through 60 seconds was observed for both genotypes. Four out of five assays resulted in neutrophils of the CC cow having lower fluorescence, meaning less F-actin polymerization than the GG cow. These results help explain why neutrophil migration is decreased, potentially leading to an increase in 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.

4. *Staphylococcus pseudintermedius*-Induced Oxidative Burst by Canine Neutrophils

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

Coagulase-positive hemolytic *Staphylococcus* species, particularly *Staphylococcus pseudintermedius*, are frequently associated with clinically important infections in dogs. Little is known about the role Staphylococcal virulence factors have in the severity of clinical disease or evasion of host immune response. Some community-associated, methicillin resistent *S. aureus* and analogous antibiotic resistent *S. pseudintermedius* strains have a propensity to cause greater tissue damage. This may be due to virulence factors with an ability to alter the effectiveness of neutrophil phagocytosis. The host immune response to *S. pseudintermedius* involves phagocytosis and killing by neutrophils. In this study we examined the role of opsonisation on phagocytosis and on generation of an oxidative burst in canine neutrophils. Flow cytometry was used to measure antibody binding to the surface of *S. pseudintermedius*. The effect of opsonisation on neutrophil response was examined on *S. pseudintermedius* strains with different genotypic fingerprints and antibiotic resistance profiles, and those collected from different body sites.

68. Rapid Detection of *Salmonella typhimurium* from Spiked Lettuce using Real-Time Reverse-Transcriptase-Polymerase Chain Reaction

Nathan Miller
Department of Food Science & Technology

Recent outbreaks of *Salmonella* linked to fresh produce emphasize the need for rapid detection methods to help control outbreaks. Reverse-transcriptase-PCR (RT-PCR) detects the presence of mRNA (shorter half-life than DNA) with greater potential of detecting viable pathogens and eliminates the need for gel electrophoresis. Our previous real-time RT-PCR (rt-RT-PCR) studies with SYBR green I using overnight *Salmonella typhimurium* cultures showed detection limits of up to 10^2 log CFU/ml (Tm at 87.5°C; Ct values from 16-29). Our objective was to apply this rt-RT-PCR method to extract and rapidly detect *Salmonella typhimurium* from spiked lettuce (ranging from 10^8 to 10^2 CFU *Salmonella*). Outer leaves of iceberg lettuce were removed, and 3 X 3 cm^2 inner leaves were thoroughly washed with sterile water, rinsed with 70% ethanol, and kept under ultraviolet light for 15 min in sterile petri dishes, before inoculating with *S. typhimurium*. RNA extraction using 0.05M glycine saline-buffer (0.05% Tween, 3% beef extract) and a Qiagen RNeasy Mini kit was compared to the Trizol method. RNA quality and quantity was determined using the Nanodrop spectrophotometer, and amplification was carried out using the Superscript III one-step RT-PCR kit. Our results showed that the glycine saline-Qiagen method gave higher yields and quality of RNA than the Trizol method. RT-PCR using the Qiagen method detected up to 10^3 CFU *Salmonella* from inoculated lettuce. The results of this study showed improved and rapid detection of *Salmonella* from lettuce within a day. This method has tremendous potential to be applied to outbreak samples and real-world scenarios.

5. The Challenge of Johne's Disease Diagnosis: UTK-Flow Cytometry Method and EV-ELISA

Cathy Scott, Shigetoshi Eda
Center for Wildlife Health; Department of Forestry, Wildlife and Fisheries

Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease (JD), a widespread and economically significant infectious disease of cattle and other ruminants. Effective control of JD in U.S. herds remains elusive due to difficulties in diagnosis. Several years post-infection are required before animals become patent and shed sufficient MAP in their feces to be detected by culture or PCR. Prepatent animals typically do not show signs of disease, so the majority of MAP infections are unnoticed and undiagnosed. Enzyme-linked immunosorbent assays (ELISAs) to identify cattle for follow-up fecal culture testing and/or culling is the currently recommended approach to JD control. The performance of such tests is generally poor due to low sensitivity and antigenic cross-reactivity with other mycobacterial and non-mycobacterial organisms–in particular *Mycobacterium avium* subsp. *avium*. Several recent reports have estimated diagnostic sensitivities of currently-available JD ELISAs to be only 14% to 28%. In 2005, our laboratory developed a new JD serological diagnostic method based on secondarily labeled, whole MAP bacilli that are reacted with serum and measured using a flow cytometric (FCM). Diagnostic sensitivity and specificity of the FCM are estimated to be 95% and 97%, respectively. The FCM is able to detect MAP infections as early as 170 days after experimental inoculation of calves and did not react with calves inoculated with other mycobacteria. Based on our FCM findings, we have subsequently developed an equally-sensitive Ethanol Vortex-ELISA using extracted MAP surface antigens, which is a high-throughput method that does not require expensive instrumentation.

6. Novel Bacterial Diagnostic Methods for Problematic Disease Diagnoses: FUTURE Directions

Shigetoshi Eda, M. Cathy Scott
Center for Wildlife Health; Department of Forestry, Wildlife and Fisheries

The long-term goal of our research is to contribute to control and/or eradication of bacterial diseases in wild, captive, and livestock animals and humans through development, evaluation, and application of simple, highly accurate diagnostic tests for these diseases. Bacterial infections are leading causes of health concerns in human and animals worldwide. For example, human tuberculosis, caused by *M. tuberculosis* and *M. bovis*, occurs in more than 10 million people and worldwide is estimated to be responsible for the death of 2 million people annually. Wild animals infected with *M. bovis* are reservoirs of the bacteria and threaten governmental efforts to eradicate *M. bovis* infections from livestock. Accurate diagnosis of infection is the key to successful control of bacterial diseases. Short turnaround time, safety, and a simple procedure are additional important criteria for a practical diagnostic test. However, current diagnostic tests for mycobacterial infections suffer numerous drawbacks, including long turn-around time and low sensitivity. Our research group has discovered recently that antigens extracted from the surface of *Mycobacterium avium* subsp. *paratuberculosis* can be used for the development of rapid and highly sensitive diagnostic tests for Johne's disease. We are currently examining whether this method is applicable to diagnosis of other infectious diseases. Further, employing a state-of-art technology, microfluidics, we are developing a hand-portable (on-site, bed-side) diagnostic device for bacterial infections in animals and humans. Recent progress and future directions for our research will be presented.

7. Identifying Gene Markers for Respiratory Disease Resistance in Cattle

Maria E. Prado,[1,2] Kathryn E. Oliff,[2] Kelly L. Grimes,[2] Cheryl J. Kojima,[2] Gina M. Pighetti[2]
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Bovine Respiratory Disease (BRD) is the most economically important disease affecting feedlot cattle, costing the North American beef cattle industry $1 billion each year. *Mannheimia haemolytica* is the number one bacterium isolated from cases of BRD, causing an often fatal fibrinous pleuropneumonia. The pathogenesis for *M. haemolytica*-induced pneumonia is not well understood; however, production of leukotoxin (LKT), a virulence factor, is known to be involved. Following successful invasion and colonization of the bovine lung, *M. haemolytica* secretes LKT, and a massive influx of neutrophils takes place. Previous research has shown that the neutrophils are responsible for the extensive lung tissue damage observed in cattle with *M. haemolytica* pneumonia. Host genes that are known to interact with *M. haemolytica* LKT include the β_2-integrin (CD11a/CD18) receptors ITGAL and ITGB2, and indirectly, the neutrophil IL8 receptor, CXCr1. We hypothesized that single nucleotide polymorphisms (SNPs) at the level of these receptors may be used as genetic markers for increased resistance of cattle to BRD. We selected regions that were more likely to include SNPs based on the sequence of these receptors. Subsequently, these regions were amplified by PCR using genomic DNA from a population of cattle that had high incidence of BRD (44%). We found one SNP in the ITGB2 receptor sequence that may be associated with BRD resistance. In conclusion, association of gene markers with resistance to *M. haemolytica* pneumonia might be an alternative used to control the incidence of BRD in cattle.

8. Identification, Isolation, and Characterization of *Streptococcus uberis* Adhesion Molecule (SUAM)

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In many well-managed dairy farms, environmental streptococci, particularly *Streptococcus uberis*, account for a significant proportion of subclinical and clinical mastitis in lactating and nonlactating cows. Virulence factors that promote adherence to and internalization of *S. uberis* into host cells are likely to play a central role in the persistence of the pathogen in the host. Research from our laboratory led to the identification of SUAM and to the delineation of its role in adherence to bovine mammary epithelial cells. Using a bacterial surface protein extraction protocol and affinity chromatography, a 112-kDa protein that had affinity for bovine lactoferrin (a whey protein synthesized by mammary epithelial cells) was discovered. To further characterize SUAM, its N-terminal amino acid sequence was elucidated. A protein query versus translated database TBLASTN search of the National Center for Biotechnology's non-redundant database with the SUAM N-terminal amino acid sequence showed no significant similarity with previous entries. Antibodies directed against SUAM and a 15-amino-acid-long N-terminal sequence (pep-SUAM) inhibited adherence to and internalization of *S. uberis* in bovine mammary epithelial cells. Data presented suggest that we have discovered a novel bacterial protein involved in the pathogenesis of *S. uberis* mastitis.

9. Expression of the Recombinant Form of a Novel Surface Protein (SUAM) of *Streptococcus uberis*

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Mastitis is the most prevalent disease affecting the dairy cattle industry worldwide. In the United States, economic losses due to mastitis have been estimated at $2 billion per year. Several pathogens have been implicated as causative agents of environmental mastitis; however, *Streptococcus uberis* is the most commonly isolated environmental pathogen infecting mammary glands when favorable conditions arise. Information regarding *S. uberis* virulence factors is limited and not well understood. Using in vitro models, we have identified and partially characterized the *S. uberis* adhesion molecule (SUAM). We demonstrated that SUAM was involved in adherence, internalization, and persistence of *S. uberis* in bovine mammary epithelial cells and may be an excellent target for vaccine development. To further test SUAM's potential as a vaccine candidate would require sufficient quantities of protein to conduct in vitro and in vivo studies. The objective of this work was to clone and express a recombinant form of SUAM (rSUAM) and to determine if convalescent sera from cows exposed to *S. uberis* recognized rSUAM. Our results showed that: 1) we successfully amplified, sucloned and expressed SUAM as a fusion protein (rSUAM), and 2) rSUAM was readily recognized by bovine antibodies produced in vivo against *S. uberis* UT888. In conclusion, the recombinant form of SUAM appears to be an alternative for obtaining milligram quantities and could be used in proof-of-concept studies to vaccinate cattle against this novel protein.

10. Vaccination with *Streptococcus uberis* Adhesion Molecule Induces Isotypic Antibody Responses in Bovine Serum and Colostrum

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Despite implementation of mastitis control practices that have significantly reduced the incidence of contagious mastitis, recent studies have shown that as the prevalence of contagious mastitis pathogens decreased, the incidence of environmental mastitis pathogens increased. *Streptococcus uberis* is one of the most commonly isolated environmental pathogens infecting mammary glands when conditions are favorable. During the last decade, our research efforts have concentrated on understanding the mechanisms used by *S. uberis* to invade the mammary gland. Using in vitro models, we have identified and partially characterized the *S. uberis* adhesion molecule (SUAM). We demonstrated that SUAM was involved in adherence, internalization, and persistence of *S. uberis* in bovine mammary epithelial cells and may be an excellent target for vaccine development. The objective of this study was to evaluate the isotypic antibody responses of cattle vaccinated with recombinant SUAM (rSUAM) in serum and colostrum. We used ELISA and Western blotting to measure total IgG, and/or IgG1 and IgG2 responses following vaccination with 3 doses of rSUAM or a placebo. Results from these experiments suggest that: 1) rSUAM effectively induced an immune response in vaccinated cows, 2) anti-rSUAM antibodies were detected in serum and colostrum of vaccinated cows, and 3) rSUAM induced IgG1 and IgG2 isotypes in the serum of vaccinated cows. In conclusion, rSUAM appears to be a good immunogen and should be considered further as a potential vaccine candidate for the prevention and control of *S. uberis* mastitis.

11. Blocking Effect of *Streptococcus uberis* Adhesion Molecule Affinity Purified Antibodies on Adherence to and Internalization of *Streptococcus uberis* into Bovine Mammary Epithelial Cells

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Streptococcus uberis is an important environmental mastitis pathogen and one of the leading causes of intramammary infections in well-managed dairy farms. Research conducted in our laboratory suggests that *Streptococcus uberis* adhesion molecule (SUAM) and an N-terminal fragment of SUAM (pepSUAM) are involved in *S. uberis* pathogenesis. The objective of this study was to evaluate the blocking effect of recombinant SUAM and pepSUAM affinity purified bovine antibodies on adherence to and internalization of *S. uberis* in bovine mammary epithelial cells. Two clinical strains of *S. uberis* (UT888 and UT366) were pre-treated with several dilutions of rSUAM and pepSUAM antibodies and co-cultured with bovine mammary epithelial cells, and adherence to and internalization of *S. uberis* in bovine mammary epithelial cells were measured. Inhibition of adherence and internalization for both stains ranged from 60 to 90% and from 45 to 94%, respectively, when rSUAM antibodies were used. Similarly, inhibition of adherence and internalization for both strains of *S. uberis* caused by affinity purified pepSUAM antibodies ranged from 65 to 90% and from 46 to 86%, respectively. These results suggest that rSUAM and pepSUAM might be involved in the pathogenesis of *S. uberis* mastitis.

12. Characterization of *Streptococcus uberis* Transposon Mutants Deficient in Mammary Epithelial Cell Entry

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Streptococcus uberis is a significant cause of environmental bovine mastitis throughout the world and causes significant economic loss to the dairy industry. Research has shown that current methods of mastitis control are less effective for controlling mastitis caused by environmental mastitis pathogens, particularly *S. uberis*. Ongoing research in our lab focuses on discovery of virulence factors involved in the pathogenesis of *S. uberis* mastitis. Discovery of chromosomally encoded bacterial factors could provide avenues for developing better strategies for the prevention of *S. uberis* mastitis. To accomplish this objective, a mutant library was prepared with a strain of *S. uberis* (UT888) used extensively in our lab. Screening of this library using a bovine mammary epithelial cell line identified five mutants that were deficient in mammary cell adhesion and internalization. We hypothesize that adhesion and internalization are important initial events in the pathogenesis of *S. uberis* intramammary infection. Further characterization of mutants will help elucidate virulence factors of *S. uberis* associated with mammary gland infection. Protein expression and Western blot analysis of mutants using sera from cows challenged with *S. uberis* indicated the expression of unique proteins and a unique immunological profile. Sequencing of three mutants identified putative *S. uberis* genes associated with virulence. In vivo and in vitro characterization of identified mutants could provide a molecular basis for *S. uberis* infection with a possibility to develop control strategies.

13. A Novel Game-Theoretical Framework for Modeling Host-Pathogen Interactions

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The immune system and its response to invading pathogens have been the focus of experimental study for many years. Recently, progress has been made towards developing mathematical models to describe host-pathogen interactions. Unfortunately, while quantitative descriptions of pathogen behavior have been successful, a simple mathematical understanding of the host immune response has proven difficult to obtain. Although several differential equation-based models have been suggested, mathematical tractability limits the number of components that can be considered. We propose a new and unique game-theory framework for understanding host-pathogen interactions. Interpreting pathogen invasion and the immune system response as a conflict allows us to focus mathematical scrutiny on the underlying, diametrically opposed optimization problems faced by both sides. In the current study, we consider resource allocation within the immune system. By altering the numbers and types of immune cells dispatched to fight the pathogen(s), the immune system can optimally defend against different invasion strategies. In response, the pathogen(s) can vary properties like replication rate and state (latent, lytic, etc.). The complex interactions and strategies employed by both sides would be impossible to analyze using the standard differential equation framework. By employing game theory, however, we simplify the model and consider only the crucial forces that determine the nature of the host-pathogen system. Ideally, this will yield a quantitative analysis that can be used to understand the immune system accurately enough to manipulate it through various drug delivery strategies.

14. Acute Virulence of *Toxoplasma gondii* in Mice is Strongly Associated with Uncontrolled Growth

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In North America and Europe, most natural isolates of *T. gondii* have been genotyped into three predominant clonal lineages (Type I, II and III). In mice, Type I strains are acutely virulent; Type II and III strains are considered non-virulent. Limited data indicated that virulent strains grow faster and give high parasite load in vivo, but it is not clear if this phenomenon is common to all virulent strains regardless of their genotypic lineages. Is there a correlation between growth rate and virulence? Here we tested in vivo parasite loads by real time PCR (RT-PCR) to quantitate parasite load of non-virulent and virulent strains in mice. Mice were inoculated by peritoneal injection (IP) with tachyzoites from virulent, non-virulent, and offspring resulting from a genetic cross of virulent and non-virulent strains. Spleens were collected, and RT-PCR was performed to quantitate parasite load. Results showed that the virulent strains grew significantly more than non-virulent strains and offspring crosses. RT-PCR was also used to quantitate parasite load of spleens collected during our competition assay. In the competition assay, mice were inoculated with a 1:5 ratio of Type I to Type III tacyzoites. At various days, spleens were collected, and RT-PCR was used to determine increased growth of parasites in tissue samples. Identity of strains present in tissue samples were revealed using genotyping. Our data from both assays showed that the virulent strain outgrew the non-virulent, indicating a correlation between growth rate and virulence.

15. The Impacts of Extreme Population Structure in Big Brown Bats (*Eptesicus fuscus*) on Rabies Virus Adaptation

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Big brown bats (*Eptesicus fuscus*) are common throughout the United States and rank highest in the numbers of bats submitted for rabies diagnosis. This trend suggests frequent exposure of the public to big brown bats, which is consistent with the fact that they commonly roost in man-made structures such as residential homes. Genetic studies on the population structure of *E. fuscus* have revealed deeply split regional mitochondrial (mtDNA) genetic lineages in North America, with similar phylogenetic structure in *E. fuscus* rabies viruses (RABV). Nuclear DNA data suggest recent male-mediated gene flow between regional host populations, which could lead to exposure with divergent isolates of *E. fuscus* RABV. Theory predicts that immunological cross-protection against RABV infection scales inversely with phylogenetic distance. We experimentally compared the pathogenicity of eastern and western *E. fuscus* RABV in 92 eastern U.S. big brown bats. The data were fit to a sigmoid response model to statistically compare the pathogenicity between isolates across multiple challenge doses. Preliminary modeling of the experimental results indicates increased pathogenicity of western *E. fuscus* RABV to eastern big brown bats. Our results suggest that significant population structure within a host may lead to host-virus coevolution and reduced pathogenicity of local endemic RABV.

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

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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 CXC chemokine family member that is predicted to be an immune modulator and 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. In these clades, the four cysteine residues that provide the structure to vCXCL-1 were conserved while the important receptor-binding region, the N-loop region, was variable. One clade also contained a modified ELR motif, which is important for receptor activation. Based on this sequence information, these proteins might function differently in the host. To address the functional differences of this hypervariable protein, we produced and isolated the vCXCL-1 protein from 9 of 11 clades using the baculovirus expression system. We will assess functional differences using chemotaxis, receptor binding, and calcium flux assays. Currently, we have shown several differences between three of the vCXCL-1 clades in calcium flux potency on neutrophils. This analysis could provide insight into the role of vCXCL-1 in HCMV pathogenesis.

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

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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 of humans. 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 a wide range of host chemokines including CC, CXC, and CX_3C chemokines. 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 *in vivo*.

18. Prevalence of Malignant Catarrhal Fever White-Tailed Deer Variant in Tennessee Hunter-Harvested Deer

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Malignant catarrhal fever (MCF) is a lymphoproliferative and inflammatory syndrome that affects many ruminant species. This syndrome is caused by several gammaherpesviruses within the rhadinovirus subgroup, the most prevalent of which is believed to be ovine herpesvirus-2 (OvHV-2). In 2000, a novel variant associated with the white-tailed deer was described, designated white-tailed deer variant-malignant catarrhal fever virus (MCF-WTD). MCF-WTD is present in Tennessee wild deer populations. Prevalence of the virus within the state of Tennessee, as well as within individual TWRA regions (1-4) will be calculated. Convenience samples of formalin-fixed lymph nodes, fresh lymph nodes and blood samples obtained from deer harvested in the 2006 and 2007 hunting season are being used to test this hypothesis. Real-time polymerase chain reaction methodology was developed to determine the presence of MCF-WTD. The specificity of this assay was tested using OvHV-2 positive sheep samples. MCF-WTD real-time PCR positive samples are being sequenced and compared to previously published MCF-WTD sequences.

19. Novel Role of ST6Gal-1 in the Regulation of CD4 T Cell and B Cell Differentiation Following Viral Infection

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An effective immune response to viral infection is coordinated by various cells of the immune system. Defects in the post-translational modification of proteins on the cell surface could lead to defects in cell-cell interactions or cell signaling, impairing cellular function. Glycosylation is one of the major post-translational modifications. The impact of glycosylation changes of immune cell glycoproteins on viral immunity is not completely understood. Studies have suggested post-translational modifications may serve as novel mechanisms of immune regulation. We are studying the role of ST6Gal-1 on the early signaling events in programming of T and B cells. ST6Gal-1 is a mammalian glycosyltransferase, highly expressed by B and T cells, that catalyzes the addition of α2,6 sialic acids to Galβ1-4GlcNAc. Using LCMV (lymphocytic choriomeningitis virus) infection of ST6Gal-1 knockout mice, we show impaired B cell primary responses, with reduced viral-specific IgM and IgG at day 8 and 15 post-infection compared to wild type mice. FACS analysis also revealed reduced numbers of activated CD4 T cells and B cell subsets in the spleen. In LCMV immune mice at day 49 through 330 p.i., ST6Gal-1 knockout mice continued to display reduced LCMV-specific IgG. The decreased antibody levels in the serum and the lower number of CD4 T cells in the ST6Gal-1 knockout mice following LCMV infection suggest that modification of T and B cell glycoproteins by ST6Gal-1 is required for optimal differentiation of B cells to form long-lived plasma cells and/or memory B-cells. Future studies will examine CD4 T cell intrinsic vs. B cell intrinsic contributions to the observed defects in humoral responses.

20. ST6Gal-I Gene Expression is Required for Optimal Viral-Specific CD8 T Cell Expansion *in vivo*

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ST6Gal-I is a sialyltransferase, predominantly expressed by T and B lymphocytes, that participates in the post-translational modification of N-linked glycoproteins by attaching alpha-2,6 sialic acid to galactose on cell glycoproteins. Glycosylation plays an important role in the function and localization of glycoproteins on the cell membrane, which is critical for cell-to-cell signaling. We found that in response to lymphocytic choriomeningitis virus (LCMV), ST6Gal-I knockout mice have lower numbers of antigen-specific CD8 T cells at day 8, the peak of the T cell response, as compared to the wild-type mice. We performed *in vivo* adoptive transfer experiments comparing TCR transgenic wild-type versus knockout cells transferred into non-transgenic recipient wild-type mice. We found reduced numbers of antigen-specific ST6Gal-I knockout CD8 T cells compared to wild-type T cells, suggesting that defective expansion of knockout CD8 T cells is CD8 T cell intrinsic. Reduced numbers can be due to increased cell death or decreased proliferation. To examine this, we performed *in vivo* CFSE assays, where we purified wild-type or knockout transgenic CD8 T cells, labeled them with CFSE, and transferred them into non-transgenic, wild-type recipient mice. After day 3 post infection with LCMV, we discovered that ST6Gal-I knockout CD8 T cells showed substantially reduced proliferation. These studies suggest that differential expression of surface carbohydrates can significantly impair CD8 T cell activation and proliferation *in vivo*.

21. Post-Transcriptional Subgenomic Messenger RNA Amplification by Coronaviruses

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Coronaviruses replicate by making a 3'-coterminal nested set of subgenomic mRNAs that are also 5'-coterminal by virtue of a common leader sequence encoded only at the 5' end of the genome. We favor the Sawicki model (Sawicki and Sawicki, *Curr Top Microbiol Immunol*, 287) for explaining the origin of sgmRNAs from the viral genome: (-)-strand templates for sgmRNAs are first made by the viral RdRp, which switches templates at intergenic donor signals (UCUAAAC in the bovine coronavirus) to place a template for the leader onto the 3' end of the sgmRNA (-)-strand. The acceptor template for the leader is the 5' end of the genome. Here, we demonstrate that when a bovine coronavirus (+)-strand sgmRNA carrying an in-frame 30-nt reporter is made as a T7 RNA polymerase transcript and transfected into coronavirus-infected cells, a sgmRNA (-)-strand copy of the sgmRNA is made. Thus, the sgmRNA (+) strand can serve as a template for sgmRNA (-) strand synthesis. There is no evidence to date that the sgmRNA undergoes replication as we had once postulated. However, when the reporter-containing (+)-strand sgmRNA contains an internal template switching signal for making a sgmRNA (-) strand of smaller size, a smaller sgmRNA is made. That is, a smaller sgmRNA is found with the predicted leader-body fusion site as observed by RT-PCR. In principle, the mechanism exists for larger coronavirus sgmRNAs to serve as templates for sgmRNAs of smaller size encoded within. This mechanism of sgmRNA amplification may be an important factor in the rapid appearance of coronavirus sgmRNAs and in the establishment of the relatively higher abundance of 3'-proximal sgmRNAs.

22. Coronavirus 2'-O-Methyltransferase Binds a Stem-Loop in the Genomic 3' Untranslated Region

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The coronavirus genome, the largest known of any RNA virus (32 kilobases long), is a single-stranded, positive-strand RNA molecule that replicates in the cytoplasm of the infected cell. Genome replication and synthesis of the 3'-terminal nested set of subgenomic mRNAs take place within viral replication complexes comprised of up to 16 viral proteins. One of the 16 proteins is an *S*-adenosylomethionine-dependent ribose 2'-O-methyltransferase (2'-O-MT), as determined by bio-informatics analyses. By analogy, the coronaviral 2'-O-MT is presumed to methylate 5' cap structures, resulting in enhanced ribosomal binding and translation of the viral genome and subgenomic mRNAs. Also by analogy, the 2'-O-MT is presumed to bind its RNA substrate near the site of cap methylation. To characterize enzymatic and RNA binding properties, we have purified recombinant bovine coronavirus 2'-O-MT from *E. coli* and performed enzyme and binding assays in vitro. While enzyme activities have not yet been confirmed, we have learned, surprisingly, that the 2'-O-MT does not bind 5'-terminal structure genomic or subgenomic RNA but does bind a site within the 3'-terminal 162 nt with high affinity (50 nM) and specificity. This site is part of a phylogenetically-conserved octamer-associated bulged stem-loop (8mer-BSL), a demonstrated cis-acting RNA replication element. RNA footprinting revealed a higher-order RNA structure-dependent interaction between the 2'-O-MT and the 8mer-BSL. We postulate a two-fold function for the 2'-O-MT positioned at this site: (1) It plays a role in assembly of the replication complex for initiating (-)-strand synthesis. (2) It methylates the 5' end cap structure after the replication complex has traveled to the 3' end of the (-)-strand RNA.

23. Unexpected Role of Selectin Ligands in T Cell Trafficking to the Lung Mucosa

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Post translational modification of proteins can play an important role in cell-cell interactions. Fucosyltransferase (FucT) -IV and -VII are two glycosyltransferases expressed by lymphocytes that catalyze the addition of an alpha 1,3 fucose to form the sialyl Lewis X moiety, which is essential for selectin binding and T cell entry to lymph nodes. Previous work has established that deficiencies in alpha1,3 fucosyltransferase expression results in defective T cell migration to peripheral lymph nodes. In our study, we examined T cell trafficking to secondary lymphoid organs and non-lymphoid compartments *in vivo* using FucT-IV and -VII double knockout (dKO) mice. As expected, uninfected FucT dKO mice contained small lymph nodes evident by significantly reduced numbers of T cells in the CLN, MedLN, and ILN, but not the spleen. We showed that T cells found in the lymph nodes of dKO mice exhibit an activated $CD44^{hi}$ phenotype. Surprisingly, we also observed significantly increased T cell numbers in the lung parenchyma of FucT dKO mice. Despite increased T cell numbers in the lung, analysis of lung sections by H&E and confocal microscopy revealed no formation of inducible bronchus-associated lymphoid tissue, as has previously been shown in mice following respiratory infection. These initial studies demonstrate impairment of naïve T cell migration to lymph nodes, but not other secondary lymphoid organs as expected, and reveal an unexpected role of selectin ligands in T cell migration to the lung.

24. Exploiting Regulatory T cells to Control Viral-Induced Immunopathology

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Recent years have witnessed an increased interest in understanding the biology of regulatory T cells (Treg) that has opened up the prospect of using these cells immunotherapeutically. A major focus has been on generating and expanding specific, self-reactive Treg *in vitro* and using them *in vivo* to modulate host responses to self or allo-antigens. Such approaches, however, are cumbersome and extremely expensive. A better way would be to expand the Treg population *in vivo* to the antigen of choice. We demonstrate that FTY720, a fungal metabolite immunosuppressive drug, may also act by causing the conversion of TCR-stimulated non-regulatory $CD4^+$ T cells to $Foxp3^+CD4^+$ regulatory T cells and enhancing their suppressive activity. In a model in which mice were ocularly infected with herpes simplex virus (HSV), daily treatment with FTY720 resulted in significantly diminished ocular lesions. The treated animals showed increased frequencies of $Foxp3^+$ T cells in lymphoid organs and at two inflammatory sites viz. cornea and trigeminal ganglia. The phenomenon of conversion of conventional $CD4^+Foxp3^-$ cells into $Foxp3^+$ Treg by FTY720 was also shown *in vitro* as well as *in vivo* in TCR transgenic x RAG2-/- mice, which are known to possess few, if any, $Foxp3^+$ Treg. Thus, as is well known, FTY720 has a potent anti-inflammatory activity because of its known effect on lymphocyte sequestration. However, its ability to expand and activate $Foxp3^+$ Treg to an antigen of choice could prove particularly useful, since this should eliminate the unwanted side effects that polyclonal Treg populations might exert.

25. Defective Influenza-Specific B Cell Responses in Mice Lacking Expression of ST6Gal-1

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Post-translational modification of proteins can affect the localization of cell surface glycoproteins on the cell membrane, impacting cell signaling and function. ST6Gal-1 is a glycosyltransferase, expressed by T and B cells, that catalyzes the addition of alpha-2,6 sialic acid to galactose, a modification typically found on N-linked glycoproteins. In this study, we show that in contrast to activated T cells, ST6Gal-1 expression remains high on plasma blasts and germinal center B cells following viral infection. To determine the *in vivo* role of the loss of this enzyme during viral infection, we intra-nasally infected ST6Gal-1 null mice with influenza A/HK x31. The loss of ST6Gal-1 expression resulted in similar infectivity in the lung at day 3, but significantly reduced influenza-specific IgM and IgG levels in the serum and antibody-forming cells (AFCs) in the CLN, MedLN and spleen in the acute phase of influenza infection. At memory time-points, overall levels of influenza-specific serum antibody were comparable, and numbers of influenza-specific memory B cells in ST6Gal $1^{-/-}$ mice were similar. These studies suggest that loss of ST6Gal-1 expression significantly impairs the acute viral-specific B cell immune response, but has little effect on memory. We propose that ST6Gal-1 expression by B cells amplifies signals through the B cell receptor, which may favor extra-follicular B cell differentiation of short-lived plasma blasts, resulting in the initial burst of protective antibodies. In the absence of this modification, weaker signaling events may result in recruitment into the germinal center where long-lived plasma cells and memory B cells arise to sustain the humoral response.

26. Mucosal B Cell Response to a Persistent Respiratory Virus

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Antibody (Ab) responses against viruses that infect mucosal surfaces typically include substantial IgA production. This is a well-characterized feature of the Ab response to influenza virus infection of the respiratory tract in mice. Influenza infection results in vigorous IgA responses in draining lymphoid tissues and establishment of a long-maintained population of IgA Ab-secreting cells (ASCs) throughout the respiratory tract. Local influenza-specific IgA production in the respiratory tract contributes to the clearance of infectious virus and provides a barrier to re-infection. Murine gammaherpes virus-68 (MHV-68) is a persistent virus considered a murine model for the study of immune responses to human gammaherpes viruses like Epstein Barr. MHV-68 also replicates well in the respiratory tract of mice following intranasal administration. However, unlike influenza virus, MHV-68 spreads systemically and establishes lifelong latency in B cells, macrophages and dendritic cells. Our analysis of the B cell response to MHV-68 intranasal infection demonstrated a surprising absence of IgA production, in marked contrast to the response to influenza. This absence of IgA was observed in the draining lymph nodes and organized nasal-associated lymphoid tissue. In additional, this deficiency in the virus-specific response extended to body fluids like serum, nasal wash and lung wash. There was reduced influx of virus-specific IgG plasma cells to the lungs and diffused nasal-associated lymphoid tissue of MHV-68 infected mice. This study revealed a deficiency in mucosal MHV-68-specific B cell response that may facilitate virus spread through mucosal secretions following reactivation.

27. Influenza Virus-Specific B Cell Memory Induced by Inactivated Virus Vaccination

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The B cell response to the current influenza vaccine of choice, a preparation of inactivated viruses administered intramuscularly, generates the two cellular components of B cell memory: (i) antibody-secreting plasma cells, and (ii) memory B cells (MBCs). MBCs are quiescent cells that respond to a "recall" antigen with rapid and vigorous antibody production and contribute substantially to vaccine effectiveness, especially when virus-specific antibody levels begin to fall. Our objective was to quantitatively analyze B cell memory generated in mice by intramuscular (IM) vaccination with inactivated influenza virus. Initial studies analyzed the primary response to vaccination. The virus-specific antibody-secreting cell (ASC) response in the draining lymph nodes and the spleen was characterized by an early peak of IgM ASCs, followed by increased numbers of IgG2b and IgG2c ASCs. Responses in the lymph nodes and spleen waned as a stable population of virus-specific ASCs was established in the bone marrow. In contrast to the situation following infection, virus-specific ASCs did not populate the respiratory tract following IM vaccination. Influenza-specific IgG MBC frequencies were determined by limiting dilution analysis 8-12 weeks after vaccination. Virus-specific IgG MBCs dispersed broadly to secondary lymphoid tissues throughout the body. The Peyer's patches and mediastinal lymph node were notable as sites of MBC concentration, even though they did not participate in the primary response to vaccination. Interestingly, MBCs also migrated to lung tissue, as was the case following infection. Our findings indicate differential regulation of plasma cell and MBC trafficking to the lung following IM vaccination.

28. Tolfenamic Acid Induces Apoptosis Through ESE-1/EGR-1/NAG-1 in Colorectal Cancer Cells

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Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the incidence of colorectal cancer. Anti-tumor effects of NSAIDs are due to inhibition of cyclooxgenase (COX) activity; however, there is increasing evidence that COX-2-independent mechanisms can also play an important role. The early growth response-1 (*EGR-1*) gene is a member of the immediate early gene family and has been identified as a tumor suppressor gene. Tolfenamic acid (TA) is an NSAID that exhibits anti-cancer activity in a pancreatic cancer model, and in the present study, we investigated the anti-cancer activity of TA in human colorectal cancer cells. TA treatment inhibited cell growth and induced apoptosis as measured by caspase activity and bioelectric impedance. TA induced EGR-1 expression at the transcription level, and analysis of the *EGR-1* promoter showed that a putative ETS binding site (EBS) in the *EGR-1* promoter, located at -400 and -394 bp, was required for activation by TA. The electrophoretic mobility shift assay and chromatin immunoprecipitation assay confirmed that this sequence specifically bound to the ETS family protein ESE-1 transcription factor. TA also facilitated translocation of endogenous and exogenous ESE-1 to the nucleus in colorectal cancer cells, and gene silencing using *ESE-1* siRNA attenuated TA-induced EGR-1 expression and apoptosis. Overexpression of EGR-1 increased apoptosis and decreased bioelectrical impedance, representing increasing anoikis formation, and silencing of endogenous EGR-1 prevented TA-induced apoptosis. These results demonstrate that activation of ESE-1 via enhanced nuclear translocation mediates TA-induced EGR-1 expression, which plays a critical role in the activation of apoptosis.

29. Design and Synthesis of Sutent, a Novel Tyrosine Kinase Inhibitor for the Treatment of Gastrointestinal Stromal Tumors and Renal Cell Carcinoma

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Receptor tyrosine kinases (RTKs) have a key role in tumor growth and survival. Sutent is a multiple RTK inhibitor approved by the Food and Drug Administration for the treatment of imatinib-refractory gastrointestinal stromal tumors and renal cell carcinoma. The overexpression of RTKs in tumors indicates that RTKs are the suitable target for tumor imaging agent development. Positron emission tomography (PET) is a functional biomedical imaging modality that can probe tumor cell physiology. PET and a positron-labeled biomarker may prove to be a useful tool for monitoring RTK levels in tumor tissues and for evaluating the effectiveness of the antitumor drug. Herein, we report the design of a developed synthetic route to Sutent and its analogues from easily handled chemicals. The key aldehyde is prepared from easily handled ᵗbutyl and ethyl acetoacetate, instead of easily explosive diketene. Then the aldehyde is activated by 1,1'-carbonyldiimidazole (CDI) to form an imine *in situ*. Addition of *N,N*-diethylethylenediamine leads to a carboxamide. Coupling the carboxamide with 5-fluorooxindole gives Sutent, while with 4-nitrooxindole yields a nitro compound, the precursor for [^{18}F]-

Sutent. The syntheses of Sutent and the nitro-precursor were produced in a yield of 81% and 75%, respectively. Moreover, the byproducts are easily removed from the reaction mixture. This advantage, coupled with the simple procedure and relatively low cost of CDI, renders this method attractive. In summary, a new multi-step synthetic route was developed, providing a versatile means for Sutent and its analogues with excellent yields. The PET studies of [^{18}F]Sutent pharmacokinetics is currently underway.

30. Target Endpoints for Dietary Prevention of Breast Cancer

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To investigate dietary prevention of human breast cell precancerous carcinogenesis, we studied biological, biochemical, and transcriptomic target endpoints induced by chronic, accumulated exposure to low doses of tobacco and environmental carcinogens in human breast epithelial cells. Immortalized, non-cancerous human breast epithelial MCF10A cells were repeatedly exposed to the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) combined with the environmental carcinogen benzo[a]pyrene (B[a]P), each at picomolar concentrations, to induce increasing acquisition of cancer-related biological, biochemical, and transcriptomic changes as cancerous targets for prevention of precancerous carcinogenesis. We then used this cellular model to reveal the ability of dietary grape seed proanthocyanidin extract and green tea catechin extract to block acquisition of i) biological target endpoints, ii) a biochemical target endpoint, and iii) transcriptomic target endpoints in the suppression of combined NNK- and B[a]P-induced carcinogenesis of MCF10A cells. Our approach provides a human breast, precancerous carcinogenesis-cellular model to serve as a cost-efficient, *in vitro* system equipped with biological, biochemical, and transcriptomic target endpoints to identify dietary agents that are able to suppress human breast cell carcinogenesis associated with long-term exposures to carcinogens.

31. The Role of Non-Steroidal Anti-Inflammatory Drug Activated Gene-1 (NAG-1) in Lung Tumorigenesis

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Non-steroidal anti-inflammatory drug activated gene-1 (NAG-1) protein inhibits gastrointestinal tumorigenesis in NAG-1 transgenic mice. In the present study, we investigated whether NAG-1 protein would alter urethane-induced pulmonary adenomas (PAs) in NAG-1 transgenic mice (NAG-Tg, FVB background). NAG-Tg mice showed decreased numbers of urethane-induced PAs compared to control littermates (NAG-Tg = 16 ± 4 per mouse versus WT = 20 ± 7 per mouse, $p<0.05$). Urethane-induced PAs in both the wild-type and NAG-1-Tg mice highly expressed both cyclooxygenases 1 and 2, prostaglandin E synthase, prostaglandin E_2 receptor, phospho-Raf-1, phospho-Erk1/2, and phospho-Akt. However, urethane-induced p38 MAP kinase phosphorylation was decreased, and cyclin-dependent kinase inhibitors (CdkIs) p21 and p27 were increased in adenomas isolated from NAG-Tg mice. These results were confirmed with human A549 pulmonary carcinoma cells. Less phosphorylated p38 MAPK and increased CdkIs after various stimulus treatments were observed after ectopic expression of NAG-1 compared to control cells. Furthermore, significantly increased apoptosis was observed in NAG-Tg adenomas compared to WT as measured by caspase 3/7 and TUNEL assays. Our study revealed for the first time that NAG-1 protein inhibits urethane-induced pulmonary adenomas and suggests a possible new target for lung cancer chemoprevention.

32. Reactive Oxygen Species-Dependent Anticancer Therapeutics

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Ectopic expression of oncogenic H-Ras increases intracellular reactive oxygen species (ROS) in human urinary bladder J82 cancer cells. More than 35% of human urinary bladder cancers involve oncogenic H-Ras activation. Expression of oncogenic H-Ras promotes J82 cells to acquire tumorigenic ability and increases susceptibility of J82 (J82-Ras) cells to histone deacetylase inhibitor FK228 for inducing apoptosis. We detected that FK228 treatment results in significantly higher levels of ROS and cell death in J82-Ras cells versus parental J82 cells. It is therefore important to understand the role of ROS in FK228-induced selective cell death of oncogenic H-Ras-expressed cells for therapeutic control of Ras-involved human urinary bladder cancers with FK228. Using biochemical and biological approaches, our data led us to a suggestion that ROS plays an essential role in FK228-induced selective apoptosis of oncogenic H-Ras-expressed cells versus parental cells in a dose-dependent manner. Elevation of intracellular ROS plays a pivotal role in the pro-apoptotic ability of oncogenic H-Ras to facilitate FK228-induced selective apoptosis of human urinary bladder cancer cells.

33. Precancerous Cellular Model for Studying Breast Cancer Associated with Environmental Carcinogenesis

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Department of Pathobiology

Breast cancer is the most common type of cancer and the second leading cause of cancer deaths among women in the United States, Canada, and northern Europe. More than 70% of sporadic breast cancers are attributable to environmental factors, such as exposures to chemical carcinogens, dietary habits, etc. Resulting from these exposures, carcinogenic transformation of breast cells from non-cancerous to precancerous and cancerous stages is a multiyear, multistep, and multipath disease process with progressive genetic and epigenetic alterations. We have developed a precancerous carcinogenesis cellular model to understand chronic carcinogenesis of human breast epithelial cells associated with environmental pollution. Immortalized, non-cancerous human breast epithelial MCF10A cells were repeatedly exposed to low doses of tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the environmental carcinogen benzo[a]pyrene (B[a]P), each at picomolar concentrations, to induce increasing acquisition of cancer-related biological, biochemical, and transcriptomic changes as target endpoints for quantitative and qualitative measurements of the progression of cellular carcinogenesis. Our approach represents *a new paradigm* providing biological, biochemical, and molecular evidence that carcinogenesis of non-cancerous cells progressively produces precancerous cells in an accumulative manner as likely occurs in the development of human breast cancer.

34. Histone Deacetylase Inhibitors for Selective Anticancer Therapeutics

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Department of Pathobiology

More than 35% of human urinary bladder cancers involve oncogenic H-Ras activation. Ectopic expression of oncogenic H-Ras not only promotes human urinary bladder J82 cancer cells to acquire tumorigenic ability, but also increases susceptibility of J82 (J82-Ras) cells to histone deacetylase inhibitor FK228 for inducing apoptosis. Thus, it is very important to understand the novel pro-apoptotic ability of oncogenic H-Ras to increase cell susceptibility to FK228 for inducing selective apoptosis in order to advance current therapeutic strategies for controlling Ras-involved human urinary bladder cancers. By using biological and biochemical approaches, our results led us to a suggestion that intracellular reactive oxygen species (ROS) play an important role in the induction of the extrinsic caspase-8 and the intrinsic caspase-9 pathways, leading to activation of executor caspase-3/-7 for FK228-induced selective apoptosis of oncogenic H-Ras-expressed J82 cells.

35. Effects of 17 Beta Estrodiol on G Protein Inwardly Rectifying Potassium Channels (GIRK) in Breast Cancer*

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Breast cancer is a leading cancer in women, and estrogen receptor (ER) (-) cancers tend to have a poorer prognosis compared to ER (+) breast cancers. Estrogen is required for normal female development and reproduction, but long-term consistent exposure has been implicated in breast cancers. Previous work in our lab indicated that ER (+) and ER (-) breast cancer cells may respond to specific carcinogens via different signaling mechanisms, and that G protein inwardly rectifying potassium channels (GIRK) are associated with cellular signaling pathways implicated in tumorigenesis. In the present study, we investigated the effects of treating ER (+) MCF-7 and ER (-) MDA-MB453 breast cancer cells with estrogen or the anti estrogen compound ICI 182780 on GIRK and estrogen receptor expression by Western Blot analysis. In ER (+) MCF-7 breast cancer cells treated with estrogen, we found increased GIRK 1 expression and decreased ERα expression. However, 100nM ICI 182780 treatment increased GIRK1, GIRK 2, and ERα expression. In ER (-) MDA-MB-453, GIRK and ERα expression are fairly consistent with both treatments. The data indicates a correlation between estrogen signaling and GIRK.

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36. An N-terminal Single Point Mutation in CXCR2 Induces Constitutive Activity and Results in Cellular Transformation of NIH 3T3 Cells*

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Although there are many contributing factors in tumorigenesis, chemokines and their receptors have previously been shown to play a role in tumorigenesis and metastasis. Constitutively active mutant (CAM) receptors continually induce an intracellular signal transduction cascade in the absence of ligands and lead to deregulation of the ligand/receptor signaling mechanism. The best example of a chemokine receptor with constitutive activity linked

to cancers is the Kaposi's sarcoma-associated herpesvirus-encoded GPCR ORF74. ORF74 has homology to human CXCR2 and leads to transformation of NIH3T3 cells and tumors in nude mice. CXCR2 is expressed in various cancers, including lung carcinomas, melanomas, colorectal cancers, and adult myeloblasitc leukemias. In a screen for constitutively active mutants of CXCR2, using the yeast *S. cerevisiae*, we identified a single point mutant in the ninth residue (D9) of the N-terminus. Mutation of Asp 9 to histidine (D9H), as well as other substitutions at this residue, resulted in constitutive activity analyzed by increased-galactosidase activity in yeast. Moreover, expression of these mutants in NIH3T3 cells led to transformation as indicated by foci formation and the anchorage independent growth assay. These data demonstrate mutations of residue D9 result in cellular transformation. Interestingly, a D9N mutation was identified in CXCR2 from a small cell lung cancer cell line, H69. This mutation also induced cellular transformation of NIH3T3 cells. This is the first evidence that a single residue in the N-terminus of a chemokine receptor plays an important role in receptor activation and may contribute to the development of cancers and their metastasis.

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37. Resveratrol Increases ATF3 Expression Mediated by Egr-1 and KLF4

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Resveratrol, a dietary phytoalexin found in red grapes and wine, is known to possess anti-tumorigenic activities. Reports indicate resveratrol inhibits cell proliferation via the promotion of pro-apoptotic mechanisms as well as by inducing a number of anti-cancer genes, such as the NSAID-activated gene, NAG-1. Our lab performed two independent microarrays using HCT-116 human colorectal carcinoma cells treated with 50 and 100 μM resveratrol. Microarray data revealed activating transcription factor 3 (ATF3) as the gene most highly upregulated by the treatment. ATF3 has been postulated to be a tumor suppressor gene. The expression of ATF3 is repressed in human colorectal tumors compared to normal adjacent tissue. Analysis of ATF3 transcriptional regulation by resveratrol revealed a possible resveratrol-responsible element located within the -514/+34 region of the ATF3 promoter. After further characterization, we found that early growth response-1 (Egr-1) and krüppel like factor 4 (KLF4) sites were located in this region. Co-transfection of the ATF3 promoter with Egr-1 or KLF4 expressing vector showed that ATF3 promoter activity is indeed increased by this trans-acting element. Thus, resveratrol induction of ATF3 may be mediated by activation of both Egr-1 and KLF4.

38. Effects of Non-Steroidal Anti-Inflammatory Drugs on Mitochondrial NADP(+)-Dependent Isocitrate Dehydrogenase

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively over the counter to treat headaches and inflammation as well as clinically to prevent cancer among high-risk groups. The inhibition of cyclooxygenase (COX) activity by NSAIDs plays a role in their anti-tumorigenic properties. NSAIDs also have COX-independent activity that is not fully understood. Our lab has previously shown that NSAIDs also induce other anti-tumorigenic genes including NSAID-activated gene (NAG-1) and activating transcription factor 3 (ATF3). In this study, we report that sulindac sulfide (SS), a conventional NSAID, down-regulates mitochondrial NADP(+)-dependent isocitrate dehydrogenase (IDH2) in HCT-116 human colorectal cells in two independent microarrays confirmed by reverse transcriptase polymerase chain reaction. Western blot analysis shows that SS also reduces IDH2 protein levels in HCT-116 cells in a time- and dose-dependent manner. This effect is not unique to SS, but is seen in several other NSAIDs tested for IDH2 inhibition. IDH2 plays a role in intermediary metabolism and energy production and is a key source of mitochondrial NADPH, an important element in protection against reactive oxygen species. One group recently demonstrated that IDH2 may be involved in cell growth, using small interfering RNA of IDH2. Thus, down-regulation of IDH2 by NSAIDs could be a vital factor in the ability of NSAIDs to induce apoptosis. Elucidating the mechanisms involved in this process has the potential to drive development of new derivatives that exploit this action.

39. NAG-1 Binds to Latent TGF-beta Binding Protein and Regulates the TGF-beta Signaling Pathway

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NAG-1 (nonsteroidal anti-inflammatory drug-activated gene-1) is a member of the TGF-β super family, which is involved in many physiological pathways. Recent studies showed that NAG-1 expression is involved in cell growth arrest and apoptosis in several cancer cells. However, the molecular mechanism by which NAG-1 affects biological activity has not been elucidated. NAG-1 may bind to a receptor and mediate its biological activity. To investigate NAG-1's role in anti-tumorigenesis, we have performed the T7 phage display screening, using the human colon cDNA library, to identify NAG-1 binding protein. Briefly, a NAG-1 binding protein encoded by human colon cDNA was displayed on the outside of the phage coat protein. To obtain a large amount of the NAG-1 protein, the FLAG-tagged NAG-1 was overexpressed

in the insect cell system by the baculovirus vector. The purified NAG-1 protein was immobilized on the FLAG-agarose column as a bait to isolate NAG-1 binding protein. After several rounds of screening, we found that latent TGF-β binding protein-1 (LTBP1) binds to NAG-1. The interaction between NAG-1 and LTBP1 was confirmed by immunoprecipitation. Subsequently, we observed that serum TGF-β concentration in NAG-1 KO mice was 20% less than wild-type mice, indicating that NAG-1 may activate the TGF-β signaling pathway. Overall, the NAG-1/LTBP1 interaction studies may provide a molecular mechanism by which NAG-1 affects anti-tumorigenesis in human colorectal cancer.

40. Characterization of a Novel Tumor Promoting Gene NUDT6 and its Suppression by Green Tea Catechins

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Department of Pathobiology

Green tea has received much attention as a suitable dietary agent because of its anti-tumorigenic activity. One of the most commonly consumed beverages in the world, green tea contains catechins, a group of polyphenols. The most active constituents of green tea are the catechins EGCG, EGC, ECG and EC. Many laboratories, including ours, have reported anti-cancer effects of green tea components in cancers of the gastrointestinal tract, lung, skin, prostate, and breast. During the course of our EGCG study in anti-tumorigenesis, we found that EGCG suppressed the gene NUDT6/GFGF, whose function is not yet known. The main aim of this study was to modulate the regulation of NUDT6 by catechins and characterize the biological function of NUDT6. At the post-transcriptional level, EGCG affected the RNA stability of NUDT6, indicating that EGCG stabilizes NUDT6 mRNA. To further define the underlying mechanism of the biological function of NUDT6 in human colorectal cancer cells, we constructed the NUDT6 correct (pool) and reverse (control) orientation clones, tagged with V5. These plasmids were transfected and selected under G418 in HCT-116 cells to generate stable cell lines for control and NUDT6. Using these cell systems, we found that NUDT6 expression increased cell growth, soft agar cloning efficiency, and colony formation, compared to the control cells. There was also decreased 3/7-caspase activity in NUDT6 overexpressing cells, compared to controls. We conclude that NUDT6 is a novel cell proliferator in colorectal cancer and is down-regulated by the green tea catechin EGCG.

41. Advancements in Kinematic Data Acquisition: Reduction of Skin Movement Artifact and Further Improvements Towards the Inverse Dynamics Method

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The ultimate goal of biomechanical analysis is the knowledge of what muscles and joints are doing throughout movement: the timing of their contractions, the power of these contractions (whether they are concentric or eccentric), the subsequent amount of force generated about a joint, and the movement of the joint. The process used to derive these joint forces, or moments, is known as inverse dynamics, so-named because we work back from the known kinematics and ground reaction force (GRF) to derive the forces responsible for motion. The inverse dynamics solution depends on accurate kinetic data and an appropriate kinematic model. The current commonly used kinematic model is often criticized for soft tissue movement artefact resulting in possible inaccurate measurements. The goals of this presentation are to discuss the results of a study looking at a skin cluster model commonly used in human biomechanical research to reduce skin movement artefact and thus more accurately represent skeletal and joint movement. This is an important first step in developing an appropriate kinematic model for use with inverse dynamics. The hypothesis was that this new skin cluster model would not affect the gait when compared to the standard model used. Furthermore, other advancements in the kinematic model will be discussed in order to promote discussion on further inverse dynamics applications and research.

42. Subarachnoid Anesthesia in Caesarian Section: A Study on Objective Measurement with Bispectral Index

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Patients undergoing spinal anesthesia demonstrate a decrease in the level of alertness without sedative drugs. Blockade of the ascending cortical projection pathways reduces excitability, decreases arousal, and reduces dosages of sedative drugs. The Bispectral Index (BIS) monitor detects cortical EEG activity via a strip applied to the forehead. It is routinely employed in the OR by anesthetists as part of the assessment of depth of general anesthesia in surgical subjects. The purpose of this study was to determine if there is lowered BIS in patients undergoing cesarean section with spinal anesthesia. This was an IRB-approved prospective, non-blinded, non-randomized study of pregnant women presenting for scheduled cesarean section. The spinal

anesthetic was delivered in the usual fashion, and BIS data were collected for 30 min. Twenty-eight patients were enrolled, nine were excluded, and two withdrew. A paired *t* test was employed to test the null hypothesis that no difference in BIS would be observed. Mean values +/- the standard deviation were determined at time zero (T0), then every 5 min (T5, T10, T15, etc.) for 30 min. There was no significant decrease in BIS values following subarachnoid injection. Explanations include sedation associated with spinals are countered by stimulus from the OR, emotional stimulus of birth, and/or inadequate BIS sensitivity. Average signal quality and motion artifact adversely affected accuracy. Limitations of the study include small sample size, non-randomized, and no control group.

43. Absolute Clot Strength of Multi-Trauma Patients Increases During Transit to the Emergency Department

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Hemorrhage associated with coagulopathy is a major cause of morbidity and mortality in trauma patients. Coagulopathy can occur as a result of several etiologies, including hypothermia, consumption of clotting factors, and resuscitation. Early identification of coagulopathy may lead to better outcomes. Thromboelastography (TEG) provides a point-of-care method of identifying patients with coagulopathy and can lead to earlier intervention. TEGs were performed on 92 successive trauma patients brought to a level-one trauma center by air ambulance over a 9-month period. Two samples were collected from each patient, one in the field and a second within one hour of arrival in the emergency department (ED). Field and ED samples were compared for reaction (R) time, clot formation (K) time, clotting rate (alpha angle), strength of clot (maximal amplitude [MA]), and fibrinolysis at 60 min (LY60). There was no difference between the two samples in R time, K time, alpha angle, or LY60. However, the ED samples had a significantly higher MA compared to the field samples (P-value-0.0001), and this difference persisted after controlling for patients with head injury. Conclusions: 1) The increase in clot strength represents an increase in platelet aggregation and correlates with other studies that indicate initial hypercoagulability in trauma patients; 2) Initial resuscitation during transport does not appear to contribute to coagulopathy seen in trauma patients; and 3) TEG may be used in the evolving clinical scenario to assess clotting and may be useful to identify coagulopathy at an early stage.

44. Positron Emission Tomography in Normal Hispaniolan Amazon Parrots (*Amazona ventralis*)

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Positron emission tomography (PET) scans are used in human oncology cases for surgical planning, to stage cancers prior to treatment, and to evaluate response to therapy. The most commonly used radiopharmaceutical is ^{18}F-fluorodeoxyglucose (^{18}F-FDG), which is concentrated and trapped within cells that use glucose as their energy substrate. Tissues with higher metabolic rates such as in the brain, liver, heart, neoplastic lesions or areas of inflammation will have increased ^{18}F-FDG uptake compared to surrounding tissues. This increased metabolic activity is detected by the PET scanner as areas of increased radioactivity. No research detailing diagnostic PET imaging of normal birds is available. Ten apparently healthy Hispaniolan Amazon parrots (*Amazona ventralis*) had static, whole body PET scans performed to evaluate the normal distribution of ^{18}F-FDG at steady state (60 min post injection). Relative metabolic activity of various structures was determined. ^{18}F-FDG that is not trapped in cells is cleared from the blood by the kidneys. Significant variability was present in the intestines because avian species reflux fluid and electrolytes from their cloaca into their colon. Because of this variability, dynamic PET scans were performed on the coelomic cavity of six Hispaniolan Amazon parrots to evaluate the distribution of ^{18}F-FDG from time 0 to 60 min post injection. Reflux of radioactive material from the cloaca into the colon occurred in all birds to varying degrees and occurred before steady state was reached. To evaluate the intestinal tract of birds, dynamic scans must be performed so that increased radioactivity due to metabolism can be differentiated from increased radioactivity due to reflux of fluid from the cloaca.

45. Kinematics of Stair Ascent Versus Trotting in Healthy Dogs

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The purpose of this study was to define range of motion of pelvic limb joints in orthopedically normal dogs during stair ascension. Eight healthy adult hound-type dogs were fitted with reflective spheres over landmarks on the right and left pelvic limbs. Dogs were walked up a set of stairs, consisting of four steps, and then trotted across the calibrated test space. The mean maximum, minimum and range of motion were calculated for all joints. Comparisons were obtained using a paired *t* test, with a P-value <0.05 considered significant. A difference in range of motion of all joints in the pelvic limb during stair ascent was detected when compared to range of motion at a level trot. There was

greater extension of the coxofemoral and tibiotarsal joints during ascension, whereas the stifle joint underwent less extension. Maximal flexion of the coxofemoral joint during stair ascent was not greater than at a level trot; however, the maximal flexion of the stifle and tarsal joints was greater in ascension. No significant difference in the range of motion of pelvic limb joints could be detected in the right limb when compared to the left limb. As was hypothesized, all subjects required a greater range of motion of all joints of the pelvic limb in order to ascend stairs. The coxofemoral joint had significantly increased extension and overall range of motion when the subject ascended the stairs. The stifle and tarsal joints underwent a greater extension, flexion and range of motion during stair ascent.

46. Effects of Short- and Long-Term Hypoxia on Hemolymph Gas Values in the American Horseshoe Crab (*Limulus polyphemus*)

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We determined resting hemolymph gas parameters in 22 adult, healthy horseshoe crabs from Ripley's Aquarium of the Smokies to evaluate the effect of short- and long-term hypoxia in the American horseshoe crab (*Limulus polyphemus*). In group 1, baseline vascular pH, PO_2, PCO_2, HCO_3, base excess, TCO_2 and lactate concentrations were determined from hemolymph samples collected from 10 horseshoe crabs submerged in water. Baseline results were compared to values following removal from water for 5 min, and following recovery in water for 10 and for greater than 60 min (range: 61-221 min). In group 2, hemolymph gas parameters were determined in 12 horseshoe crabs following shipment out of water for 24 hours and compared to values obtained from group 1 animals. Following removal from water for 5 min, all crabs developed severe hypoxia with PO_2 levels falling below the detectable limit of the analyzer. No significant changes in PCO_2 concentrations were seen over time in group 1 animals. Group 2 crabs had pronounced respiratory acidosis (pH = 6.7; PCO_2 = 46 mmHg) following transport, and their PO_2 concentrations were significantly below baseline values of Group 1 animals. Baseline hemolymph gas values of the American horseshoe crab are within range for other aquatic vertebrates. Short-term removal of crabs from their aquatic environment causes severe hypoxia and metabolic changes. While crabs have the ability to compensate for a changing hypoxic environment if removed for long periods of time (24 hours), severe hypercapnia and respiratory acidosis will still be present.

47. Influence of Gender and Sexual Alteration Status on Feline Adiponectin

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Adiponectin is a hormone secreted from adipocytes that correlates with insulin sensitivity. Unlike other adipokines, adiponectin levels decrease as fat mass increases. Although strong associations between obesity and gender and/or reproductive status exist in cats, the role of adiponectin has not been evaluated. The purpose of this study was to identify the impact of gender and reproductive status on adiponectin and insulin resistance in the cat. Four groups of lean (BCS 4-6/9), healthy, young adult cats (1-6 years; n=40) were used in the study. The following groups consisted of 10 cats each: intact female, intact male (IM), neutered female (NF), neutered male. Health status was assessed through physical exam, complete blood count, and chemistry panel with electrolytes. Body weight and body condition scores (BCS) were also recorded. Serum samples for adiponectin were collected once from fasted cats and stored at -80ºC. An ELISA kit was used to measure adiponectin. A linear ANOVA model was used for statistical analysis (SAS v.9.1) comparing gender, reproductive status, and body weight with total adiponectin. Body weight differed between all groups except IM and NF ($P<.05$); BCS did not differ. No significant difference existed between adiponectin levels among groups ($P<.05$); however, NF tended to have greater adiponectin levels compared to IM ($P=0.07$), and neutered animals tended to have higher adiponectin levels compared to intact animals ($P=0.06$). While this indicates total adiponectin levels are not influenced by gender or gonadectomy in cats, there are strong trends that may be influenced by sample size.

48. Effects of a Combined Chromium, Magnesium, and Herbal Dietary Supplement on Insulin Sensitivity in a Genetically Diverse Population of Insulin Resistant Horses

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

Horses develop insulin resistance (IR) in association with obesity, regional adiposity, and laminitis, and these problems can be grouped together and referred to as equine metabolic syndrome. Laminitis is a painful disease of the hoof that can result in permanent damage to hoof structures. Insulin resistance and laminitis may be associated because in humans, altered insulin signaling leads to vasoconstriction, which could pre-dispose hoof tissues to nutrient deprivation in horses. Glucose availability may be reduced as a consequence, and there is *in vitro* evidence to suggest that hoof lamellae separate in conditions of low glucose.

The purpose of this study was to evaluate the effectiveness of a dietary supplement comprised of chromium, magnesium, and herbs on insulin sensitivity in a diverse population of insulin resistant horses with prior histories of laminitis. The population consisted of 14 horses shown to suffer from IR by resting hyperinsulinemia (>30 µU/mL) or an abnormal combined intravenous glucose-insulin tolerance test (CGIT) result (>45 min to return to baseline). Six animals served as controls, and eight received the supplement daily in feed. Testing consisted of frequently sampled intravenous glucose tolerance tests (FSIGT) performed at time 0 weeks, 8 weeks, and 16 weeks. Plasma blood glucose was measured by colorimetric assay on an automated analyzer, and serum insulin was measured by radioimmunoassay. Glucose and insulin area under the curve (AUC) values were calculated to evaluate changes in insulin sensitivity and glucose tolerance in response to supplementation. Results were pending at the time of publication.

49. Effects of Pretreatment With Dexamethasone or Levothyroxine Sodium on Endotoxin-Induced Insulin Resistance in Horses

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Endotoxemia has been associated with laminitis, and transient insulin resistance (IR) develops after administration of exogenous lipopolysaccharide (LPS) to horses. We hypothesized that resting insulin sensitivity would affect the magnitude of IR induced by LPS. Horses were pretreated with dexamethasone (20 mg/day PO) to induce IR or levothyroxine sodium (LT4; 48 mg/day PO) to increase insulin sensitivity. Twenty-four adult mares were randomly assigned to control (no pretreatment; n = 8), dexamethasone (n = 8), and LT4 (n = 8) groups. After the 14-day pretreatment period, horses were challenged by intravenous administration of 20 ng/kg body weight *Escherichia coli* O55: B5 LPS. Frequently-sampled intravenous glucose tolerance test procedures were performed at -14 days, -3 hours, and 20 hours relative to LPS administration. Areas under the plasma glucose (AUCg) and serum insulin (AUCi) curves were calculated. Significant treatment × time effects were detected for AUCg (P = 0.018) and AUCi (P < 0.001) for the 14-day pretreatment period. Treatment with dexamethasone for 14 days significantly (P < 0.001) increased pre-LPS mean AUCg and mean AUCi values by 24% and 364%, respectively, suggesting a significant decrease in insulin sensitivity over time. Furthermore, pretreatment with dexamethasone exacerbated IR induced by LPS. Mean AUCg and AUCi values did not change significantly over 14 days in the LT4 group, and this drug prevented LPS-induced IR. Results suggest that horses already suffering from IR are likely to show greater disturbances in insulin sensitivity when endotoxemia develops, and LT4 pretreatment ameliorates these responses to LPS.

50. Retinyl Ester Stimulates Glucokinase Gene Expression in Primary Hepatocytes

Yifei Gao, Guoxun Chen
Department of Nutrition

Vitamin A (retinol and retinyl ester) is well absorbed in the human intestine. Retinyl esters taken up by the liver are hydrolyzed to retinol, which is released as holo-RBP back into the plasma. Glucokinase (GK) is the key enzyme in controlling hepatic glucose usage. In the liver, GK activity is mainly regulated by transcription of its genes in response to hormonal or nutritional stimuli. It has been shown that activation of the retinoid acid receptor can induce GK gene expression in hepatocytes. The object of this project is to find out whether retinyl ester induces GK gene expression. Primary hepatocytes were isolated from rat liver and treated with vehicle control or retinyl acetate in the absence or presence of 1 nM insulin for 6 hours. Total RNA was extracted, and cDNA was synthesized. The expression level of GK gene was analyzed by real-time PCR using the 36B4 gene as an invariable control. Compared to the vehicle control group, retinyl acetate at 20 ug/ml, insulin, and insulin + retinyl acetate induced GK expression by 4.7-, 15.2-, and 49-fold, respectively. These results demonstrate that retinyl ester induces GK gene expression in primary hepatocytes and synergizes with insulin. Future experiments include analyzing the purity of the retinyl acetate samples used in the assay, the catabolism of retinyl acetate in hepatocytes, and the responsive elements in the GK gene promoter.

51. Metabolic and Genomic Changes Induced by Macronutrient Composition vs. Caloric Restriction in C57/BL6 Mice*

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The aim of this study was to investigate differential effects of dietary fat and energy restriction on metabolic and genomic differences in adipose tissue. Eight week-old C57/BL6 mice were either given free access to a low fat (LF) or high fat (HF) diet, or were initially fed HF for 16 weeks followed by 8 weeks of caloric restriction (HFR, 70% of HF ad lib caloric intake). As expected, the HF group gained more body and fat mass than the LF group, and energy restriction resulted in loss of body fat and weight. Although fasting glycemia was comparable between the 3 groups, the HFR group had lower insulinemia, compared to HF and LF groups. Analysis of serum adipokines indicated that plasma leptin levels were lowest in HFR mice; surprisingly, plasma adiponectin levels were also lowest in this group, despite their apparent

enhanced insulin sensitivity. Microarray analysis of gonadal fat indicated upregulation of genes involved in fatty acid synthesis, oxidation and glycolysis in the LF group. Differentially expressed genes in the HF group included several oncogenes, while those in the HFR group included genes related to insulin sensitivity and apoptosis. These studies demonstrate differential effects of low fat diet and caloric restriction on fatness and insulin sensitivity, and on the molecular responses in adipose tissue.

*Supported by TN Agricultural Experiment Station and USDA-CSREES, NRI grant 2005-35200-15224.

52. Polymorphisms in Energy Metabolism Genes as Predictors of Response to Diet

Julia S. Gouffon (CEM), Michael Zemel
Department of Nutrition

Our previous data demonstrate that dietary calcium modulates adipocyte and skeletal muscle energy metabolism and thereby attenuates obesity risk. However, there is clearly heterogeneity in clinical response to calcium interventions. Since polymorphisms in key energy metabolism gene sequences exist, they may responsible for variability in adipose tissue deposition. Accordingly, we have now evaluated this possibility using data and samples from a large-scale population study, the Quebec Family Study, which had already confirmed an anti-obesity effect of dietary calcium. Variations in adiposity response to high calcium (>1000 mg/day) versus low calcium (<600mg/day) intake was evaluated as a function of single nucleotide polymorphisms (SNPs) in key genes involved in lipogenesis, lipolysis, fatty acid oxidation, mitochondrial function and energy partitioning. Data demonstrate a significant difference between calcium responders and non-responders in adiponectin SNP rs822396, -2971A>G (frequencies of 22% and 66% for the minor homozygous allele and heterozygous allele in the responders, $p<0.02$) and in perilipin SNPrs894169, 11482G>A (frequencies of 42% for the minor homozygous allele and 61% in the heterozygous allele in responders) and in perilipin SNP rs2289487, 6209T>C (56% for the minor homozygous allele, 58% for the heterozygous allele and 40% for the major homozygous allele in the responder group). These data demonstrate that polymorphisms in adiponectin, an adipocyte-derived cytokine that modulates skeletal muscle fatty acid oxidation, and in perilipin, which regulates lipase access to adipocyte lipid droplets, predict adiposity responsiveness to dietary calcium interventions. Current work will define the differential functionality of these polymorphisms *in vitro* and their population distribution.

53. The Relationship Between Obesity and Markers of Oxidative Stress in Dogs

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Obesity, a serious epidemic affecting much of our pet population, increases the risk of developing numerous diseases. It has been demonstrated that obesity increases oxidative stress in obese children, cats, and other species. Oxidative stress can result in DNA damage with subsequent alterations in gene expression, cell signaling, mutations, cell death, or cell transformation. These effects of oxidative damage predispose animals and humans to numerous disease processes and cancer. The objective of the study was to determine whether obese dogs are under oxidative stress resulting in DNA damage and decreased endogenous antioxidant protection, measured by serum glutathione levels and the ratio of reduced to oxidized glutathione. In this case-control study, 10 obese dogs were compared to aged-matched healthy control dogs. Dogs were evaluated by history, physical exam, body condition score (BCS), complete blood count, serum biochemical analysis and total T4. Dogs with a BCS of 7 or greater (9-pt. scale) were considered obese. Single-cell gel electrophoresis (COMET assay) was used to measure DNA damage, and high performance liquid chromatography was used to measure serum glutathione. Glutathione levels were significantly different between groups. The results of this pilot study suggest that obesity is associated with oxidative stress in dogs and that justification exists for a larger study population to achieve significance. The greater implication of a larger study will be to show the potential for antioxidant supplementation of weight loss diets.

54. Dietary Calcium and Dairy Modulation of Oxidative Stress and Life Span in Mice

Antje Bruckbauer, Michael B. Zemel
Department of Nutrition

Dietary Ca attenuates adiposity, blood pressure, reactive oxygen species (ROS) production and inflammatory stress, while milk exerts a greater effect than Ca. These effects have the potential to extend lifespan. We have now examined the effects of these dietary treatments on lifespan and related biomarkers in aP2-*agouti* transgenic mice (a model of diet-induced obesity) and littermate controls. Mice were fed low Ca (0.4% Ca), high-Ca (1.2% Ca from $CaCO_3$), or milk (1.2% Ca from non-fat dry milk) obesigenic diets until their death. Randomized subgroups of each diet group were sacrificed at 28, 52 and 78 weeks of age for biomarker analysis. Weight gain in the milk group was up to 46% ($p=0.003$) lower than in controls, and this was associated with a significant decrease in adiposity ($p<0.035$) and higher

muscle mass (p=0.032) with increasing age. Survival analysis to date demonstrate a significant protective effect of the milk diet in wild-type mice; the 75% survival rate for wild-type mice is 546 days for the milk diet group, compared to 483 days (p=0.014) and 424 days (p=0.018) in the control and high calcium groups, respectively. Moreover, the milk diet prevented ROS production in adipose tissue by 50% compared to the control group (p=0.005) and attenuated the rise in ROS production with age. These data demonstrate dairy attenuation of ROS production and extension of lifespan in wild-type mice.

55. The Adipocyte Renin Angiotensin System (RAS) Mediates the Effects of Calcitriol on Oxidative Stress and Cytokine Expression

Christina Caserio, Michael Zemel
Department of Nutrition

Upregulation of the adipocyte RAS is associated with increases in inflammatory cytokine expression and reactive oxygen species (ROS) production. We recently demonstrated calcitriol stimulates adipocyte ROS production and inflammatory stress (IS), while dietary calcium suppression of calcitriol exerted the opposite effect. These effects are mediated, in part, by calcitriol modulation of Ca^{2+} signaling and mitochondrial potential. Since adipocytes contain a functional RAS and angiotensin II (AT) modulates ROS and IS, the purpose of this study was to determine the mechanism whereby calcitriol interacts with adipose RAS to produce oxidative stress. We investigated the role of AT in mediating calcitriol effects in 3T3-L1 adipocytes and assessed expression of specific ROS-producing transcripts. Calcitriol (1 nM) stimulated NOX4 gene expression and ROS production in 3T3-L1 adipocytes by 67% ($p<0.01$), but these effects were reversed by ACE inhibition (enalapril) or antagonism of either AT receptor1 (AT1R) or AT2R. Similarly, AT (0.1 – 1.0 nM) stimulated NOX4 expression ($p<0.05$), an effect reversed by AT1R or AT2R antagonism. Calcitriol and AT both suppressed adiponectin expression ($p<0.04$) and increased IL6 and MCP-1 expression ~ 2-fold ($p<0.03$); these effects were reversed with enalapril or AT2R, but not AT1R, antagonism. These data demonstrate that calcitriol modulation of adipocyte ROS production and IS is affected, in part, by the adipocyte RAS. Strategies designed to inhibit RAS should reduce inflammatory and oxidative burden, thus reducing the risk of cardiovascular disease. Milk contains peptides that inhibit RAS; it is anticipated that milk will reduce cardiovascular risk to a greater extent than other sources of calcium.

56. The Role of the GXXXG Motif in the Interaction of APP with γ-Secretase and Formation of Aβ

Guozhang Mao,[1] Jianxin Tan,[1] Sangram S. Sisodia,[2] Mei-Zhen Cui,[1] Xuemin Xu[1]

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γ-secretase-mediated processing of amyloid precursor protein (APP) is a crucial step in the formation of β-amyloid peptide (Aβ). Little is known about how the substrate APP interacts with the γ-secretase complex. To understand the molecular events involved in γ-secretase-mediated APP processing and Aβ formation, in the present study we determined the role of a well conserved GxxxG motif in the transmembrane domain of APP. Our data clearly demonstrate that substitution of aspartic acid for the first glycine in this GxxxG motif almost completely abolished the formation of Aβ. Furthermore, our data revealed that substitution of aspartic acid for the first glycine in this GxxxG motif disrupts the interaction of APP with the γ-secretase complex. Thus, the present study revealed an essential role for the GxxxG motif in the interaction of APP with the γ-secretase complex and the formation of Aβ.

57. Effects of γ-Secretase Cleavage Region Mutations on APP Processing and Aβ Formation

Jianxin Tan,[1] Guozhang Mao,[1] Mei-Zhen Cui,[1] Bruce T. Lamb,[2] M.-S. Sy,[2] Xuemin Xu[1]
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Beta amyloid peptide (Aβ) is the dominant component of the senile plaque, a hallmark of Alzheimer's disease (AD). The accumulation of Aβ is believed to be the causative event in AD pathogenesis. Thus, understanding the mechanism of the formation of Aβ is a central issue in AD research. In recent studies we identified a new ζ-cleavage site at Aβ46 between the known γ-cleavage site and the ε-cleavage site. More importantly, our findings also suggested a sequential relationship of these cleavages, i.e. ε-cleavage occurs first, followed by ζ-cleavage and γ-cleavage, commencing at the site closest to the membrane boundary and proceeding toward the site within the middle of the transmembrane domain of APP. These findings prompted us to determine how the mutations around these cleavage sites, and specifically, the ε-cleavage site, affect the sequential ε-, ζ- and γ-cleavages and Aβ formation. Our data demonstrated that in living cells, all mutants can be processed by γ-secretase with different efficiency to generate different amounts of intracellular $Aβ_{46}$

and secreted Aβs in various patterns. Among those mutations, substitution of phenylalanine for residues at the ε-, ζ-, and γ-cleavage sites caused dramatic changes in the ratio of long versus short Aβ species. Moreover, mutations around the ε-cleavage site, the initial cleavage site, have strong effects on the efficiency of APP processing and the formation of Aβ. Our data suggest that the sequence around the ε-cleavage site at Aβ49 plays a critical role in γ-secretase-mediated APP processing and Aβ formation.

58. Lysophosphatidic Acid in Inflammation and Atherosclerosis

Feng Hao, Dongwei Wu, Michael McEntee, Xuemin Xu, Mei-Zhen Cui
Department of Pathobiology

Lysophosphatidic acid (LPA) is formed in oxidized low density lipoprotein and secreted by activated platelets. Evidence supports the role of LPA in the development of various diseases, including cardiovascular and inflammatory diseases. LPA induces expression of cytokines, chemokines and growth factors, which in turn promote cell proliferation, migration, and differentiation. Inflammation, vascular cell proliferation, migration and differentiation are critical processes in the development of atherosclerosis. To determine the molecular mechanisms by which LPA contributes to the pathogenesis of atherosclerosis, we discovered that LPA markedly induces early growth response factor-1 (Egr-1) expression in vascular smooth muscle cells (*in vitro*) and in vascular neointimal lesions (*in vivo*). The importance of our finding is due to the critical property of Egr-1, which is a master transcription factor and regulates an array of atherogenic genes in atherosclerotic lesions. Therefore, our data support our hypothesis that LPA, via the key transcription factor Egr-1, regulates the expression of an array of atherogenic genes. Our data reveal that two nuclear factors, serum response factor and CREB, up-regulate Egr-1 expression in response to LPA. We also discovered that downstream of Egr-1, LPA strikingly induces pro-inflammatory cytokine interleukin-6 (IL-6) production and secretion in human primary aortic smooth muscle cells (*in vitro*) and vascular neointimal lesions (*in vivo*). Our data demonstrate that LPA-specific receptor 1 mediates LPA induction of IL-6. Our results further identify that the specific PKC and MAPK are responsible for LPA-induced IL-6 secretion. These results provide new evidence on how LPA promotes inflammation and atherosclerosis and may contribute to the treatment and prevention of atherosclerotic disorders.

59. Allosteric Interplay in Thyroid Hormone Receptor Transactivation Complex

Balananda D. Kumar Putcha, Elias J. Fernandez
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Thyroid hormone receptors (TRs) are transcription factors involved in a variety of physiological and developmental processes. TRs undergo large conformational changes upon ligand (3,3',5 triiodo-L-thyronine, T3) binding, which provides binding surfaces for the interaction of a variety of co-regulators, which ultimately results in transcription of downstream genes. Transactivation mediated by the thyroid receptors is an integrated response to diverse allosteric inputs. We used isothermal titration calorimetry (ITC) and cell based assays to demonstrate the allosteric regulation of TR by its heterodimeric partner, retinoid X receptor (RXR), ligands and the response elements (TREs). RXR and TREs synergistically modulate the recruitment of the coactivator peptide SRC-1 by TR through inter and intra receptor allosterism. Binding properties of architecturally different TREs determine the differential quantitative response *in vivo*. Our data suggest that multi-level regulation is operated to fine-tune the quantitative response by RXR:TR heterodimers. *In vitro* studies using reconstituted nuclear receptor transactivation complex give mechanistic insights into the allosteric interplay, in the absence of structural information for such multi-component systems.

60. Lysophosphatidic Acid Induction of Matricellular Protein CYR61 Expression in Aortic Smooth Muscle Cells and in Neointimal Lesions

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

Lysophosphatidic acid (LPA), a bioactive phospholipid, is produced by activated platelets and formed during the oxidation of low density lipoprotein. Accumulated evidence supports LPA's role in the development of atherosclerosis. In this study, we found that LPA, at very low concentrations, markedly and rapidly induces expression of CYR61 in vascular smooth muscle cells and in vascular neointimal lesions. CYR61 is a matricellular protein and has been shown to up-regulate smooth muscle cell proliferation and migration. Our data reveal a regulatory relationship between LPA and CYR61 in smooth muscle cells and in vascular walls. To determine the intracellular signaling pathway leading to LPA-induced CYR61 expression, we found that the specific pan protein kinase C (PKC) inhibitors, GF109203X or Go6983, dose-dependently blocked CYR61 expression induced by LPA, suggesting the involvement of PKC. Go6976, a specific inhibitor of both PKCalpha and PKCbeta, had no effect on LPA-

induced CYR61 expression, suggesting that PKC subtypes other than PKCalpha and beta mediate LPA-induced CYR61 expression. In contrast, although LPA markedly induces phosphorylation of MAPKs including ERK, p38 and JNK, inhibition of these MAPKs had no effect on LPA induction of CYR61 expression. These data support the hypothesis that LPA induces the expression of CYR61 in smooth muscle cells via a specific PKC pathway.

61. Calpains Affect the Degradation of Amyloid Beta (Aβ) Protein

Xin Lu (CEM), Mei-Zhen Cui, Xuemin Xu
Department of Pathobiology

Abnormal accumulation of β-amyloid peptide (Aβ) is believed to be the primary causative event in the pathogenesis of Alzheimer's disease (AD). Two cellular mechanisms could contribute to the abnormal accumulation of Aβ in the brain: over production and/or failure in clearance of the Aβ peptide. In an effort to identify the cellular system that is involved in Aβ clearance, we conducted experiments to investigate the effects of calpain inhibitors on the production of secreted Aβ and the intracellular accumulated derivatives of APP, using a culture cell model. Our results revealed that at a low concentration, calpain inhibitors caused increased production of both Aβ40 and Aβ42. At a high concentration, calpain inhibitors led to a decline in Aβ production and an increase in intracellular accumulation of C-terminal fragments, including CTF-beta, CTF-alpha, and CTF-epsilon generated by β -, α-, and ε-cleavages, respectively. These results suggest that calpain enzymes, which are a highly conserved superfamily of calcium-dependent, papain-like cysteine proteases, are involved in the metabolism of APP and the formation and accumulation of Aβ. To further identify the enzyme(s) that is(are) responsible for calpain inhibitor-regulated Aβ formation and accumulation, we employed the small interference RNA approach to investigate the effect of knockdown of calpains on the formation and accumulation of Aβ. Our recent results suggest that different isoforms of calpain enzymes may function differently in Aβ production and accumulation. This information may lead to a better understanding of the mechanism underlying the abnormal accumulation of Aβ peptide in the AD brain and provide new insight into the pathogenesis of the disease.

62. Knockdown of Immunoglobulin Light Chain Production by RNA Interference

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Human Immunology and Cancer Program, Graduate School of Medicine

Synthesis and aggregation of monoclonal free immunoglobulin light chain protein (IgLC) contribute to morbidity and mortality in patients with monoclonal plasma cell dyscrasias. Current treatment focuses on reducing the monoclonal plasma cell population, thereby decreasing the concentration of circulating IgLC protein and slowing aggregation and deposition. RNA interference is a relatively new technique by which the translation of mRNAs can be prevented in a sequence-specific manner. For this study, small interfering RNA (siRNA) sequences were designed to target the variable and constant domains of λ IgLC proteins. This study used a mouse myeloma cell line stably transfected with a construct encoding an amyloidogenic human λ6 IgLC as well as commercially available, human myeloma-derived cell lines. Cells were cultured in the presence of experimental or control siRNAs for 24, 48, or 72 hours and assayed for transfection efficiency, cell viability, IgLC mRNA synthesis, and IgLC protein secretion. Following treatment with fluorescent-labeled siRNA, more than 70% of the cell population was transfected, as evidenced using flow cytometry. SiRNA treatment resulted in the reduction of IgLC mRNA and secreted protein levels, compared to control cells. Throughout the 72-hour experiment, cell viability remained high, indicating the measured decrease in IgLC mRNA and protein was not due to cell death associated with transfection. These data demonstrate the efficacy of IgLC-directed siRNA treatment as a means to reduce the production of IgLC protein in clinically relevant myeloma cell lines and provide a basis to investigate its application *in vivo* using a mouse model of plasma cell dyscrasia.

63. Control Facilitated Electroporation for Drug Delivery

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Department of Mechanical, Aerospace and Biomedical Engineering

The procedure of electroporation uses an electric pulse to temporarily disrupt the phospholipid bilayer of a cell, forming pores, which then reassemble after a period of time. Electroporation has the ability to be employed in a number of areas. It can be used as a method of drug delivery, as an alternative to oral medications that may become dissolute in digestion or to injections that are unpleasant for the patient. In gene therapy, electroporation could allow for the incorporation of DNA into the cell in order to treat genetic disorders. However, these diverse purposes make use of different molecules and therefore require varying pore sizes. One difficulty in the advancement

of the electroporation technique is developing a protocol that allows for the creation of a pore size appropriate to the application for a sufficient amount of time while minimizing cell damage and death. Resolving this issue is a vital factor in making electroporation an even more attractive method for getting molecules into the desired cells. This work presents a feedback control algorithm that is able to achieve any desired pore size. Numerical examples demonstrate the control strategy is robust. The control algorithm will improve the operation of electroporation in drug delivery.

64. Development of an Autonomous Mammalian Lux Bioreporter

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The bacterial luciferase, lux, cassette is a six gene enzyme system isolated from the insect pathogen *Photorhabdus luminescens*. The proteins of the Lux system interact to produce visible light in an autonomous fashion. This is an obvious advantage of the Lux system for the creation of bioreporters with real-time monitoring capabilities. Unlike fluorescent proteins and firefly luciferase, Lux does not require exogenous stimulation for detection nor the addition of exogenous substrate. While the structure and function of these genes, as well as their interactions *in vivo*, have been well characterized, the function and expression of the entire cassette in mammalian cells has yet to be demonstrated. We hypothesize that assembly of the complete Lux cassette into a single, regulatable vector using versions of the genes that are codon-optimized for mammalian expression will result in an autonomous bioreporter in human derived cells and potentially whole animal models. Construction of this vector will be accomplished using a variety of established molecular biology techniques including restriction digestion, sub-cloning, and polymerase chain reaction. Following creation, the Lux vector will be optimized to function in the mammalian cell environment through proper targeting and conditional expression in order to allow for efficient reporter activity without the disruption of normal cellular function. Upon functional completion of the vector, this bioreporter system will serve as proof in principal of a self-contained biological mammalian sensor, capable of detecting and reporting any number of compounds within the host cell in real time.

65. Chromodomain Helicase DNA Binding Protein 2 and DNA Damage Response Signalling

Sangeetha Rajagopalan,[1] Prabakaran Nagarajan,[2] Robert Donnell,[3] Sundar Venkatachalam[2]
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Alterations in chromatin structure allow various nuclear factors to access certain regions of DNA to carry out DNA replication, repair, recombination, and transcription. The chromatin modifiers are classified broadly into two classes: histone-modifying enzymes and ATP-dependent chromatin-remodeling factors. Chromodomain helicase DNA binding proteins (CHD) are a group of highly conserved proteins sharing sequence motifs and functional domains and are classified under the group of ATP-dependent chromatin-remodeling factors. CHD2 is one of the poorly characterized CHD proteins. The human gene encoding CHD2 is mapped to chromosome15q26.2, which has been associated with rare genetic disorders that lead to growth retardation, cardiac defects, and early post natal lethality. In an effort to understand the functional role of the chromodomain helicase DNA binding protein 2 (Chd2) in mammals, we have generated a Chd2 mutant mouse model. Our data indicates that the Chd2 protein plays an important role in haematopoiesis, DNA damage response regulation, and tumor suppression. Interestingly, the Chd2 heterozygous mutant animals are highly susceptible to lymphomas, and the homozygous mutants exhibit prenatal lethality. Chd2-deficient cells exhibit growth defects indicative of cellular senescence, accumulate higher levels of the chromatin-associated DNA damage response mediator γH2AX, and exhibit an aberrant DNA damage response after X-ray irradiation. Chd2 deficient cells exhibit increased chromosomal aberrations after treatment with X-ray irradiation, indicating an important role for Chd2 in DNA damage responses. Experiments have been initiated to identify interacting protein partners of Chd2.

66. Agonism and Inverse Agonism in the Constitutive Androstane Receptor

Sarah Wisecarver, Elias Fernandez
Department of Biochemistry and Cellular and Molecular Biology

The constitutive androstane receptor (CAR) belongs to the nuclear receptor (NR) family of ligand-mediated transcription factors. NRs are modular proteins with an N-terminal domain (DNA binding domain) that binds specific sites on DNA known as response elements. The C-terminal domain (ligand binding domain, LBD) functions as a receptor for hormonal ligands and regulates the activity of the NR. Several NRs function as heterodimers with the promiscuous NR, retinoid X receptor; the dimerization interface is mostly restricted to the LBDs of these

proteins. CAR is different from the classical NR in that CAR is active in the apo state, though inverse agonists such as androstenol can repress its transcriptional activity, while synthetic agonists such as TCPOBOP can enhance the activity of CAR. Remarkably, isoforms of CAR from murine and human origin respond differently to the anti-emetic meclizine. Meclizine functions as an mCAR agonist and as an hCAR inverse agonist. We are using X-ray crystallography and fluorescence to characterize and compare the mechanisms of agonism and inverse agonism that occur in response to meclizine in mCAR and hCAR, respectively.

67. Ligand-Specific Structural Changes of the Antibiotic Resistance Enzyme Aminoglycoside Phosphotransferase (3')-IIIa

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In recent years, the medical world has seen an alarming increase in antibiotic resistance among numerous classes of bacteria. While different mechanisms exist to hinder the effectiveness of various antibiotics, one of the most common is enzyme-assisted covalent modification of aminoglycosides. Aminoglycosides are broad spectrum antibiotics that target 16S RNA of the 30S ribosomal subunit found only in bacteria. This inhibits protein translation leading to cell death. The enzyme aminoglycoside-phosphotransferase(3')-IIIa (APH) from *Enterococcus faecalis*, a drug resistant strain that is a common cause of severe hospital infections, is capable of binding to these types of antibiotics and causing a detoxifying phosphorylation event at the 3'- or 5'-OH group via a metal-ATP complex. Nuclear magnetic resonance (NMR) and other biophysical methods have been employed to study the interactions between this enzyme and its antibiotic substrates. Data show that APH is very flexible in solution when in the apo state, and it may be even intrinsically unstructured. The enzyme, however, acquires a well-defined structure upon aminoglycoside binding and can favorably target several different aminoglycosides that vary greatly in size and structure. Temperature dependence and hydrogen-deuterium exchange studies reveal that amino acids distant from the active site are affected by ligand binding, suggesting that a global structural alteration occurs in APH to favorably accommodate various sized aminoglycosides. Studying APH-antibiotic interactions provides critical information about how this enzyme functions and may give insight into other aminoglycoside modifying proteins. This would create a more intelligent foundation in designing drugs to combat the antibiotic resistance problem.

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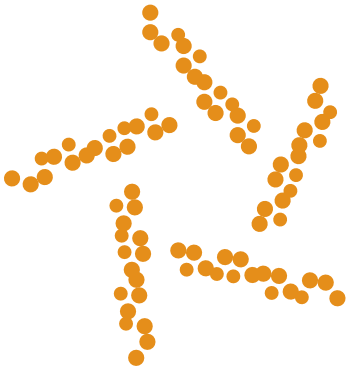


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