7th Annual Combined DVM/PhD Degree Colloquium

Forging our Own Paths

August 3rd, 2023; San Juan, PR

NATIONAL ASSOCIATION OF VETERINARY SCIENTISTS

7th Annual Combined DVM/PhD Degree Colloquium

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August 3rd, 2023, San Juan, PR

Featured Speakers

Keynote: Dr. Dara Kraitchman
Prof. of Radiology & Radiological Science
Johns Hopkins

Keynote: Dr. María E. Negrón
Epidemiology Team Lead
Bacterial Special Pathogens Branch, CDC

Workshop: Sonia Gutierrez
Senior Communications Officer
The Denver Foundation

DVM/PhD Alumni Panelists:

- **Dr. Andi Lear**
  - ***Assistant Professor of Large Animal Clinical Sciences, UTCVM***
- **Dr. Frances Chen**
  - ***Assistant Professor of Molecular Medicine, UMass Chan Medical School***
- **Dr. Justin S. Lee**
  - ***Senior Laboratory Advisor, CDC Global Health Center***
- **Dr. Kristen A. Davenport**
  - ***Clinical Research Scientist, IDEXX Laboratories, Inc.***

Schedule

August 2nd, 2023

6:00-8:00pm	Welcome Reception: Sheraton Puerto Rico Hotel & Casino San Felipe Room; 200 *Convention Boulevard / San Juan, 00907, PR*

August 3rd, 2023

Conference Venue: Puerto Rico Convention Center
100 Convention Boulevard / San Juan, 00907,PR

7:30-8:30am	Registration and Breakfast (Provided)
8:30-8:45am	Welcome Address
9:00-10:00am	Keynote Addresses
10:00-10:50am	Poster Session 1 *Refreshments 10:15-10:45am*
11:00-12:00pm	Student Oral Presentations Session 1
12:00-1:00pm	Lunch (Provided) & Director Meeting
1:00-2:00pm	Science Communication Workshop: Personal Branding
2:00-3:00pm	Student Oral Presentations Session 2
3:00-3:50pm	Poster Session 2 *Refreshments 3:15-3:45pm*
4:00-5:30pm	DVM/PhD Alumni Career Panel
5:30-5:45pm	Closing Remarks
6:00-8:00pm	Dinner (Provided)
8:00pm	Student Social Hour

Program Directory

Keynote Biographies

Dr. Dara Kraitchman, VMD, PhD, MS

Cardiovascular Interventional Section Head, Prof. of Radiology & Radiological Science, Johns Hopkins

Dr. Dara Kraitchman is a Professor of Radiology and Molecular and Comparative Pathobiology at Johns Hopkins University School of Medicine. She received her VMD in 1992 and her PhD in Bioengineering in 1996 from the University of Pennsylvania. She founded the Center for Image-Guided Animal Therapy (CIGAT) at Johns Hopkins University in 2012, which is the first veterinary hospital within an academic medical center without a school of veterinary medicine, that performs advanced diagnostic imaging and minimally invasive procedures in pets on a referral basis. By using spontaneous disease in pets, advanced therapies are being developed as part of a One Health approach at Johns Hopkins Medicine. In addition to being the Director of CIGAT, Dr. Kraitchman is the Cardiovascular Interventional Section Head in the Division of MR Research. Dr. Kraitchman's lab specializes in cardiovascular and oncological applications of image-guided procedures with a special interest in developing methods to label therapeutics including stem cells for image-guided delivery, targeting, and tracking. She has over 100 peer reviewed publications in translational research in imaging, cardiology, and oncology. She is the first veterinarian recognized as a Fellow of the American College of Cardiology (FACC) and International Society for Magnetic Resonance in Medicine (ISMRM) as well as a Distinguished Investigator in Academy of Radiology Research. She is currently principal investigator of three NIH R01 grants that utilize pets with spontaneous disease to develop new imaging techniques and therapies for both people and pets. She is also actively involved in promoting women in STEM and is a member of the Institutional Animal Care and Use Committee.

María E. Negrón, DVM, PhD, MS

Epidemiology Team Lead, Bacterial Special Pathogens Branch, CDC

Dr. María E. Negrón received a DVM degree from Ross University School of Veterinary Medicine, an MS in Comparative Epidemiology from Purdue University, and a PhD in Veterinary Epidemiology from the University of Calgary in Canada. She began her career at the Centers for Disease Control and Prevention as a Steven M. Teutsch Prevention Effectiveness (PE) fellow at the National Center for Immunization and Respiratory Diseases (NCIRD) in the Division of Viral Diseases. During her time as a PE fellow, she acquired additional training in health economics and decision analysis, and some of her projects included quantifying the economic burden of norovirus and assessing the economic impact of norovirus vaccination. Currently, Dr. Negrón serves as the Team Lead for the Epidemiology Team at the Bacterial Special Pathogens Branch. Some of the diseases covered by BSBP include anthrax, brucellosis, leptospirosis, melioidosis, Hansens's disease, and many other zoonotic and non-zoonotic diseases. Dr. Negrón was part of the Ebola Response in Sierra Leone in West Africa in 2015, Hurricane Maria Response in Puerto Rico in 2017, COVID-19 response in 2020-2021, Mpox response in 2022-2023 and Marburg Response in Equatorial Guinea in 2023.

Workshop Coordinator Biography

Sonia Gutierrez, BA

Senior Communications Officer, The Denver Foundation

Sonia Gutierrez is an award-winning, Emmy-nominated storyteller. She worked as a news reporter for 12 years focusing on investigative community stories. Her latest work looked at the sale of mobile home parks and how those impact the families who live there. Sonia has reported for Rocky Mountain PBS, 9NEWS, KHOU in Texas, WLTX in South Carolina, Telemundo, and Univision. She covered several national events including the Charleston church shooting, Hurricane Matthew and Hurricane Harvey. During her time in South Carolina, Sonia became the first reporter to translate emergency response information, live. That earned her a Certificate of Appreciation from the state's Commission for Minority Affairs. Sonia was most recently awarded for her work on the health and safety of farmworkers in Colorado. She not only advocates for the people in her stories but for BIPOC journalists in local newsrooms, just like her. She was awarded the Larry Tajiri Media Award from the ACLU of Colorado for speaking up about her experience as a Latina reporter. Sonia is now a Senior Communications Officer for The Denver Foundation where she helps elevate stories about impact from around the state. Sonia has expertise in multicultural marketing, crafting a brand voice, storytelling and journalism. Born in Parral, Chihuahua Sonia immigrated to this country when she was two years old. She graduated from Metropolitan State University as a DREAMer and started her career with DACA. Sonia is now married with two beautiful children.

Alumni Panelist Biographies

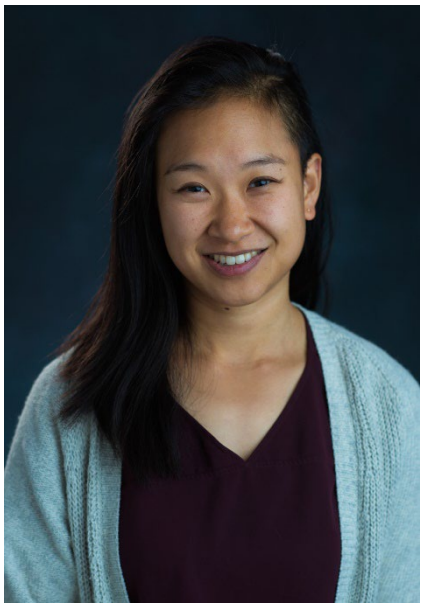
Andi Lear, DVM, MS, PhD, Diplomate ACVIM (Large Animal)

Assistant Professor of Large Animal Clinical Sciences, UTCVM

Dr. Andi Lear holds a DVM from Auburn University College of Veterinary Medicine. While performing a large animal internal medicine residency, with an emphasis in livestock medicine and surgery, at Colorado State University College of Veterinary Medicine, she received a MS. Dr. Lear joined the Farm Animal Field Service team at the University Of Tennessee and concurrently attained her PhD in immunology and infectious disease. She is currently an Assistant Professor in the Department of Large Animal Clinical Sciences at the University Of Tennessee College Of Veterinary Medicine. Her clinical interests include livestock medicine and surgery, small ruminant and camelid herd management, herd disease investigation, and neonatology. Her research interests focus on both basic understanding of infectious disease during pregnancy and clinically relevant diagnostics/interventions.

Frances Chen, DVM, PhD

Assistant Professor of Molecular Medicine, UMass Chan Medical School



Frances is currently an Assistant Professor in the Program of Molecular Medicine at UMass Chan Medical School and non-tenure track faculty member in Dr. Elinor Karlsson's vertebrate genomics research group at the Broad Institute of MIT & Harvard, where she facilities research project management and oversees external collaborations between the lab, industry, and nonprofit partners. Frances trained under an NIH F30 fellowship and received her combined DVM/PhD from Cornell University in comparative biomedical sciences with a genetics focus in 2020. Prior to joining the Karlsson lab, Frances was recruited to the founding team of Loyal, a biotechnology startup developing FDA-approved medicines to extend lifespan and healthspan in dogs. As Head of Veterinary Translational Medicine, she designed and oversaw veterinary clinical studies to evaluate outcome measures for canine healthspan and contributed to regulatory filings and discussion with the FDA Center for Veterinary Medicine. Within her current role, Frances leverages community science initiatives with pet owners and volunteers for working dog organizations to power comparative genomics research in companion animals. Having raised 15 guide/service dog puppies, Frances has specific interests in working dog research not only to lend insight into molecular pathways for canine and human traits relevant to behavior and disease, but also to directly improve canine health and performance in service of the human-animal bond.

Justin S. Lee, DVM, PhD

Senior Laboratory Advisor, CDC Global Health Center

Justin earned a Bachelor's degree in biochemistry from Tulane University in New Orleans and later attended Colorado State University where he earned a PhD in microbiology and a Doctor of Veterinary Medicine degree. He completed a post-doctoral fellowship with a dual appointment at the University of California Davis and Colorado State University. During his graduate and post-graduate training, he studied the ecology and evolution of viruses and their hosts in multiple systems including wildlife, domestic livestock, and vector-borne systems. After his post-doc, he managed the Genomic Sequencing Facility at Colorado State University for several years before joining the Centers for Disease Control and Prevention as the Team Lead of the Genomic Sequencing Lab. During the COVID-19 pandemic, Justin led the CDC's laboratory efforts to monitor viral variants by receiving and sequencing samples from across the United States for the National SARS-CoV-2 Strain Surveillance program. In November 2022, Justin joined the CDC Global Health Center as a Senior Laboratory Advisor where he supports the building of genomics capacity at national public health labs in low- and middle-income countries around the world.

Kristen A. Davenport, DVM, PhD

Clinical Research Scientist, IDEXX Laboratories, Inc.

Dr. Davenport earned her BS in Biochemistry from Tufts University, then completed the combined DVM/PhD program at Colorado State University. Her PhD research, under the mentorship of Dr. Ed Hoover, explored the zoonotic potential of chronic wasting disease compared to other prion diseases. Her graduate work was funded by an NIH NRSA F30. Having grown up on a dairy farm, she was proud to receive the Livestock Clinical Medicine award upon graduation from veterinary school. She moved to the University of Utah for a postdoctoral position studying viral restriction factors and host-pathogen evolution in cell culture and mice, where she received an NIH K01 award. After three years, she accepted a position at IDEXX Laboratories, Inc., as a Clinical Research Scientist. She works in Research and Development to design and execute clinical studies that determine the clinical utility of new diagnostic tests for companion animals.

Student Oral Presentations

Session 1 (11:00am-12:00pm)

Juselyn Tupik

Virginia-Maryland CVM

Juselyn (Jus) Tupik is a 5th year DVM/PhD student at the Virginia-Maryland College of Veterinary Medicine. She is currently finishing her dissertation research on the innate immune response to bacterial diseases, specifically Brucellosis and Lyme Disease. She is planning to start her first year of the DVM program this Fall 2023, with a focus on research and public health. Jus was the former NAVS Coordinator in 2022 and remains passionate about ensuring dual degree careers and support.

Ashlan Jolley

North Carolina State University

Ashlan is a fifth-year combined DVM-PhD student at NC State's College of Veterinary Medicine, with a focus in Epidemiology and Global Health. She earned her double B.S. in Biochemistry and Biological Sciences from NC State University in 2019, along with a minor in Microbiology. She is particularly interested in zoonotic diseases, specifically the mechanisms by which such infections occur at the human-animal interface. Her most recent work focused on antimicrobial use in companion animals, and the impacts that the COVID-19 pandemic had on prescribing practices. She is currently expanding this research for her dissertation work, incorporating data from human outpatient prescribing in order to identify and compare spatiotemporal trends in antimicrobial use across species. She aims to one day work for a national or international health organization such as the CDC or WHO, where she can study transmission dynamics of infectious diseases through the One Health approach. Ashlan enjoys reading and traveling in her free time, along with outdoor activities such as soccer and horseback riding.

Sabina Hlavaty

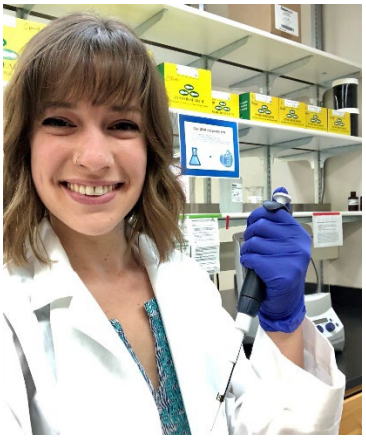
University of Pennsylvania

Sabina Hlavaty is a rising sixth year VMD-PhD student at the University of Pennsylvania, conducting her thesis research on acetate metabolism in the Schug laboratory at The Wistar Institute. She graduated from Princeton University with her bachelor's degree and worked at the National Cancer Insititute as a post-baccalaureate cancer research training award fellow prior to attending veterinary school. Her research interests include cancer metabolism and immunology. In her spare time, she enjoys exploring Philadelphia with her husband.

Session 2 (2:00pm-3:00pm)

Taylor Weary

University of Wisconsin



Taylor Weary is a DVM/PhD candidate at the University of Wisconsin School of Veterinary Medicine. Her research interests include endangered species conservation and emerging viruses, as well as their intersection in the context of reverse zoonotic pathogen transmission. The inaugural recipient of the One Health Commission/Georgia Aquarium Bossart Memorial Scholarship, she utilizes a One Health framework to improve pediatric and wild great ape health outcomes.

Aryana Razmara

University of California, Davis

Aryana Razmara is a DVM/Ph.D. dual degree student at UC Davis and is currently working on her Ph.D. in Immunology in the Robert Canter Laboratory. She honed her interest in comparative oncology while completing a B.S. in Biological Sciences at UC Davis and an M.S. in Comparative Medicine at Stanford University. Her current work used dogs with naturally occurring cancer as models to improve NK cell-targeted immunotherapy across species.

Grayson Walker

North Carolina State University

Grayson was born and raised in the Appalachian foothills of western North Carolina prior to attending NC State in 2013. He completed Bachelor's and Master's degrees in Poultry Science, and his Master's thesis project investigated on-farm strategies for *Salmonella* control in poultry production. He was then accepted into the Combined DVM/PhD program at the NC State College of Veterinary Medicine to pursue his infectious disease research and veterinary interests. His dissertation investigated interactions between pathogenic *E. coli* and *Enterococcus faecalis* in extraintestinal diseases of poultry and dogs. In 2020, he was awarded the National Bio and Agro-defense Facility (NBAF) Scientist Training Program (NSTP) fellowship through USDA APHIS. Grayson's veterinary research interests pertain to microbiology, molecular diagnostics, bacterial and viral diseases of food animals, and zoonotic diseases. After graduation, Grayson is excited to begin his career as a veterinary medical officer at the newly-built National Bio and Agro-defense Facility in Manhattan, Kansas. He is currently completing his clinical training for vet school, where he hopes to spend as much time learning about veterinary diagnostics, regulatory medicine, and pathology as he can. In his free time, Grayson enjoys cooking, using friendly microbes to make mead and kombucha, and doing anything outside (fishing, biking, golfing, backpacking, and SCUBA diving).

Mounting a mitochondrial defense: Metabolic immunoprotection by immune receptor NLRX1 during Lyme disease

Juselyn D. Tupik, Mecaila E. McClune, Julia A. Gregory, Hailey W. Camp, Jules M. Dressler, Margaret A. Nagai-Singer, Brandon L. Jutras, Irving C. Allen

Dept of Biomedical & Veterinary Sciences (Tupik, Gregory, Camp, Nagai-Singer, Allen) and Dept of Biochemistry (McClune, Dressler, Jutras), Virginia Tech, Blacksburg, VA, Dept of Basic Science Education (Allen), Virginia Tech Carilion School of Medicine, Roanoke, VA

Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is an emerging infectious disease of global concern. Roughly 60% of untreated patients will develop inflammation of the joints termed Lyme arthritis. This persistent phenotype results from sustained innate immune signaling. Currently, there are limited treatments for antibiotic-refractory Lyme arthritis, warranting our investigation into how the immune system can mitigate this inflammation. Here, we studied how the anti-inflammatory innate immune receptor NLRX1 regulates host-pathogen interactions in response to *B. burgdorferi*. Expressed almost ubiquitously in mammalian cells, NLRX1 associates with the electron transport chain (ETC) of the mitochondria, playing unique roles in regulating cell metabolism and function. As such, we hypothesized that NLRX1 could have distinct antimicrobial defenses against Lyme arthritis. Following infection in novel *Nlrx1*$^{-/-}$ mice, we found that NLRX1 significantly decreased arthritis severity in wildtype mice when compared with knockouts, modulating bacterial load *in vivo*. Through RNA-seq gene expression analysis, we determined that this protective phenotype may result from NLRX1 regulation of immunometabolism and wound healing during infection. Using murine macrophages, we found that NLRX1 shifted mitochondrial function towards enhanced energy production. Further, this metabolic shift promoted Reactive Oxygen Species (ROS) production and cell death, possibly as a mechanism of host defense against pathogen burden. From these results, we emphasize the importance of NLRX1 regulation of immunometabolism during *B. burgdorferi* infection and encourage its further exploration for new treatments for Lyme arthritis.

Research Support: NIH/NIAID R21AI159800 and the Cohen Foundation
Student Support: VMCVM DVM/PhD Student Support

Impacts of the COVID-19 pandemic on antimicrobial use in companion animals in an academic veterinary hospital

Ashlan Jolley, William Love, Erin Frey, Cristina Lanzas

Department of Population Health and Pathobiology (Jolley, Love, Lanzas) and Department of Clinical Sciences (Frey), College of Veterinary Medicine, NC State University, Raleigh, NC

Antimicrobial resistance in bacterial pathogens reduces the effectiveness of such drugs, making judicious antimicrobial use (AMU) important for its control. The COVID-19 pandemic modified operations in both human and veterinary healthcare, potentially impacting AMU. This research quantified changes in antimicrobial prescribing for companion animals in an academic veterinary hospital during the pandemic. A retrospective study was performed using prescribing data for dogs and cats from the NC State College of Veterinary Medicine pharmacy, which serves the specialty referral hospital and primary care services. 34 antimicrobials prescribed before and during the pandemic were categorized using the FDA's drug classifications for human medical importance. The probability of more important antimicrobials being administered in patients during the pandemic vs. before was modeled. Rates of AMU per week and per patient visit were also estimated. During the pandemic, cumulative antimicrobials prescribed per week were significantly decreased in most services for dogs. Weekly rates for Highly Important antimicrobials were also significantly lower in dogs. For Important and Critically Important antimicrobials, rates per week were significantly decreased in various services overall. Rates of antimicrobial administration per patient visit were significantly increased for Highly Important drugs. Patients in the internal medicine, dermatology, and surgery services received significantly more important antimicrobials during the pandemic than before, while cardiology patients received significantly less. These results suggest that the pandemic significantly impacted prescribing practices of antimicrobials for companion animals in this study.

Research Support: FDA U01FD007057
Student Support: NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070

Impact of acetyl-CoA synthetase expression and acetate availability on melanoma growth

Sabina I. Hlavaty, Kelsey Salcido, Katherine Pniewski, Dominic Duah, Fabrizio Bertolazzi, Dzmitry Mukha, Zachary Schug

Cell and Molecular Biology Program, University of Pennsylvania School of Medicine, Philadelphia, PA (Hlavaty, Salcido), University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA (Hlavaty), and The Wistar Institute, Philadelphia, PA

The median survival of melanoma patients diagnosed with brain metastases is 13 months, highlighting a need for better therapies. Metastatic development involves metabolic changes, such as shifting to using alternative nutrient sources to support vital metabolic pathways. One such nutrient is acetate, which is metabolized into acetyl-CoA by two enzymes: acetyl-CoA synthetase 1 and 2 (ACSS1 and ACSS2) of the mitochondrion and cytosol, respectively. This acetyl-CoA can enter the tricarboxylic acid (TCA) cycle, as well as be used to synthesize lipids required for growth. While targeting acetate metabolism can block primary melanoma growth, the role of acetate in its metastatic spread is unknown. The brain is unique from most organs in the body due to its net production of acetate. This led us to ask whether expression of ACSS1 and ACSS2 enables melanoma cells to exploit acetate as an alternative nutrient in a high acetate environment such as the brain, boosting metastatic growth. I cultured human melanoma cells which do or do not express these enzymes under low (equivalent to murine serum level) or high (equivalent to murine brain level) acetate and performed liquid chromatography / mass spectrometry on extracted cellular metabolites. I found that melanoma cells expressing ACSS1 and exposed to high acetate conditions primarily use acetate as an acetyl-CoA source for the TCA cycle. Furthermore, melanoma cells expressing ACSS1 had improved survival under hypoxic (1% oxygen) and nutrient stressed conditions when supplemented with high acetate. Our study therefore highlights a key role for ACSS1-derived acetyl-CoA in survival under cellular stress in vitro, and studies are ongoing to interrogate their role in vivo.

Research Support: NIH NCI DP2 CA249950-01
Student Support: NIH T32 Training Grant T32CA009171

Reducing respiratory disease transmission from humans to chimpanzees in Uganda

Taylor Weary, Tressa Pappas, Patrick Tusiime, Shamilah Tuhaise, Elizabeth Ross, James Gern, and Tony Goldberg

Department of Pathobiological Sciences, University of Wisconsin School of Veterinary Medicine (Weary, Goldberg), Kibale EcoHealth Project (Weary, Goldberg), Department of Pediatrics, University of Wisconsin School of Medicine and Public Health (Pappas, Gern), and The Kasiisi Project (Tusiime, Tuhaise, Ross)

Respiratory disease is a major cause of morbidity and mortality among people in the developing world and also threatens great apes across Sub-Saharan Africa. Our studies of wild chimpanzees in Kibale National Park, Uganda, have identified the causative agents of respiratory disease outbreaks as “common cold” pediatric human pathogens, but reverse zoonotic transmission pathways have remained unclear. Between May 2019 and July 2022, we collected 2,000 paired respiratory symptoms surveys and nasopharyngeal swabs from 264 people (local children and forest workers) and fecal samples from 55 chimpanzees as part of the One Health "Healthy Children, Healthy Chimps" prospective cohort study. We characterized respiratory pathogens using a multiplex PCR panel and metagenomic sequencing and examined the transmission risk of various pathogens, seasons, social factors, and the individual characteristics of humans and chimpanzees. Children exhibited high incidence rates and symptom severities, whereas adults were largely asymptomatic. COVID-19 lockdown in 2020-2021 significantly decreased respiratory disease incidence. Human symptoms peaked in February. In chimpanzees, the most common month for respiratory disease outbreaks was March. Rhinovirus, which caused a 2013 outbreak that killed 10% of chimpanzees in a Kibale community, was the most prevalent human pathogen throughout the study. Rhinovirus was also most prevalent during February and was the pathogen most likely to be carried asymptomatically by people. Our data suggest that respiratory pathogens circulate in children living near Kibale, and that adults in the same communities become asymptomatically infected and may carry the pathogens into the forest and infect chimpanzees.

Research Support: NIH R01 AG049395, Morris Animal Foundation D21ZO-044, Disney Conservation Fund, Arcus Foundation, One Health Commission, UW-Madison Global Health Institute
Student Support: UW School of Veterinary Medicine Dean's Office

Missing a “missing self” mechanism: Modeling and detection of Ly49 expression in canine natural killer cells

Aryana M. Razmara*, Alicia A. Gingrich*, Phillip W. Gingrich, Robert B. Rebhun, William J. Murphy, Michael S. Kent, C. Titus Brown, Justin Siegel, and Robert J. Canter

Department of Surgery (Razmara AM, Gingrich AA, Canter RJ), Department of Biochemistry and Molecular Medicine (Gingrich PW, Seigel J), Department of Dermatology (Murphy WJ), Department of Surgical and Radiological Sciences (Rebhun RB, Kent MS), University of California, Davis, School of Veterinary Medicine, Davis CA.

Natural killer (NK) cells of the innate immune system are a key focus within the field of immuno-oncology based on their ability to recognize and eliminate malignant cells without prior sensitization. Inhibitory receptors KIR (human) and Ly49 (mouse) bind with MHC-I to inhibit NK cell function and the absence of MHC-I on cell surface allows for attack by NK cells, this has been termed the “missing self” hypothesis. The mechanism for identification of “self” by canine NK cells is currently unknown, and there is evidence KIR is absent in the canine genome while Ly49 contains a mutation on a highly conserved cysteine residue. Our focus was to assess the potential impact of the known Ly49 mutation on protein function. We used computational-based homology modeling to analyze 3-dimensional protein structure of canine Ly49 and protein-protein docking explored the binding mode of canine Ly49 with MHC-I. Bulk and single-cell RNA sequencing analysis was performed to detect gene expression of Ly49 in resting and activated NK cells. Tertiary protein structure demonstrated significant homology and molecular docking of canine Ly49 with MHC-I was favorable, as the Ly49 to MHC-1 docking converged to a single low energy conformation. Gene expression of Ly49 was almost exclusively present in cells within the NK cluster and increased in the activated state when compared to the resting state. Overall, the cysteine to tyrosine mutation in question does not significantly alter the conformation of canine Ly49 or binding to MHC-1, and the Ly49 gene is expressed by canine NK cells with increased expression dependent on activation. Taken together, these data suggest Ly49 is a functional protein to recognize MHC-I in canine natural killer cells.

Research Support: R03CA252793, R03CA270854, U01CA224166, U01CA224166-02S1
Student Support: R03CA252793, R03CA270854, U01CA224166, U01CA224166-02S1

Chicken or the egg: an embryo infection model to decipher virulence mechanisms of avian pathogenic *E. coli*

Grayson K. Walker, Chalise Brown, M. Mitsu Suyemoto, Sid Thakur, Heather Harbottle, Jeffrey M. Gilbert, Marilyn M. Martinez, Steven Foley, and Luke B. Borst

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC (GKW, CB, MMS, ST, and LBB); FDA, Center for Veterinary Medicine, Office of New Animal Drug Evaluation, Rockville, MD (HH, JMG, MM); FDA, National Center for Toxicological Research, Jefferson, AR (SF)

Avian pathogenic *Escherichia coli* (APEC), the agent of colibacillosis, is the leading cause of mortality in poultry. Treatment of APEC infections are confounded by widespread antimicrobial resistance, genetic diversity, and numerous overlapping APEC virulence mechanisms. To develop effective interventions, it is crucial to understand the genetic basis of APEC virulence. In this study, a pangenome-wide association study was used to correlate APEC genotypes with virulence phenotypes to identify APEC genes associated with embryo lethality. The whole-genome sequences of 172 APEC strains isolated from septic chickens and turkeys were annotated to establish a genotype matrix. Binary virulence phenotypes (virulent vs avirulent) were assigned to each strain using an established embryo lethality assay with a survival threshold of 50%. Embryos were challenged with APEC strains at 12 days of incubation and cumulative viability over 5 days was plotted as Kaplan-Meier survival curves. In a pilot study of 27 strains, the genome annotations of the 3 most virulent strains were compared to the 3 least virulent strains using an 80% similarity threshold. Twelve predicted proteins were found to be present in the 3 most virulent strains and absent in the 3 least virulent strains including the aerobactin siderophore biosynthesis proteins and a putative surface-exposed virulence protein homologous to the BigB adhesin of *Brucella abortus*. The BigB adhesin is a known virulence factor in *Brucella abortus* but this protein has not been described in APEC. This genetic screen provides insight into APEC virulence, which is important for the development and evaluation of anti-virulence interventions for colibacillosis.

Research Support: USDA APHIS National Bio and Agro-defense Facility Scientist Training Program
Student Support: None

Student Poster Presentations
August 3rd 2023

Poster Session 1

10:00-10:50 am

Poster Session 1	Poster Number	Title of Presentation
Anderson	1-1	Discovery of a novel variant associated with a late-onset peripheral neuropathy in Labrador retrievers
Astmann	1-2	Pangenomic analysis of secondary bile acid-producing clostridia
Bakhle	1-3	Sensitizing mesenchymal mammary tumors to immunotherapy by targeting cell-intrinsic immune-modulatory factors
Barber	1-4	Bovine Cathelicidins Expression During Bovine Respiratory Syncytial Virus Infection
Brill	1-5	Development of Canine CAR-T Cells Targeting the Disialoganglioside GD2
Burk	1-6	Staphylococcal superantigens suppress pro-inflammatory responses in human aortic endothelial cells
Caldwell	1-7	Neonate enteric glia enhance intestinal epithelial restitution in vitro after exposure to mature lumen content
Cave	1-8	Multi-Year Health Assessment Of Blue-Footed Boobies (Sula Nebouxii Excisa) in the Galápagos Islands
Cherry	1-9	Lactobacillus acidophilus delivered adjuvants drive effector responses in intestinal immune cells
Cockey	1-10	Engineering chimeric antigen receptor (CAR) lymphocytes to target feline infectious peritonitis virus
Cole	1-11	Are blood serum-derived extracellular vesicles a viable Chronic Wasting Disease peripheralization mechanism?
Cook	1-12	Determining Therapeutic Targets for Peripheral T Cell Lymphoma, Not Otherwise Specified in Canines
Curry	1-13	Transcriptomics of Feline Small-Cell Intestinal Epitheliotropic T-cell Lymphoma
Davis	1-14	Validation of automated MRI segmentation protocols of neuroanatomical regions of the rhesus macaque brain
Dombroski	1-15	Polygenic risk scoring of healthy US adults in a cross-sectional metabolic phenotyping study
Erwin-Craig	1-16	iDISCO highlights postnatal changes in enteric glial network development in a comparative pig model
Farias	1-17	Examination of leukocyte coping capacity as a measure of stress in cownose stingrays, Rhinoptera bonasus
Foos	1-18	REDACTED
Gagliardi	1-19	A novel equine in vitro model of osteoarthritis utilizing fibronectin fragment stimulation

Discovery of a novel variant associated with a late-onset peripheral neuropathy in Labrador retrievers

Anderson RS, Momen M, Binversie EE, Hao Z, Gruel J, Baker LA, Rylander H, Cameron S, Eminaga S, Barnes-Heller HL, Patterson M, Kohler N, Ale S, Wilson S, Stilin A, Kearney H, Svaren J, Muir P, Sample SJ

Department of Surgical Sciences (Anderson, Momen, Binversie, Hao, Gruel, Baker, Rylander, Cameron, Eminaga, Barnes-Heller, Patterson, Kohler, Ale, Wilson, Stilin, Kearney, Muir, Sample) and Department of Comparative Biosciences (Svaren), University of Wisconsin School of Veterinary Medicine, Madison, WI

Late-onset peripheral neuropathy (LPN), often referred to as laryngeal paralysis, is a life-limiting, late onset, heritable canine neuropathy common in Labrador retrievers. LPN is clinically characterized by paralysis of the intrinsic muscles of the larynx and pelvic limb paresis. In Labrador retrievers, LPN appears to be inherited in an autosomal dominant fashion based on pedigree analysis, but the genetic basis has not been identified. The objective of this study is to identify the genetic mutation(s) associated with LPN in Labrador retrievers. We hypothesized that LPN in Labrador retrievers was the result of a highly prevalent genomic structural variant. We found an associated locus using a genome-wide association study with 193 Labrador retrievers ($P=1.28x10-7$). Statistical fine mapping narrowed potential causal variants to a single gene that is important for neuronal migration, differentiation, and axonal guidance. Long read whole genome sequencing of 12 Labrador retrievers identified a structural variant in this candidate gene. Using PCR of 128 Labrador retrievers and confirmative Sanger sequencing, we determined that the structural variant significantly associates with greater than 80% of affected Labrador retrievers and yields an odds ratio of 10.23 ($P<0.0001$). Overall, these results significantly advance our understanding of LPN in Labrador retrievers and provide new insights into the genetic basis of neurodegenerative diseases.

Research Support: NIH (K01OD019743-01A1, T32OD010423, R03Od026601-01), The American College of Veterinary Surgeons, The Wisconsin Alumni Research Foundation, and UW-Madison Companion Animal Grants
Student Support: University of Wisconsin School of Veterinary Medicine DVM/PhD Program

Pangenomic analysis of secondary bile acid-producing clostridia

Theresa J Astmann and Daniel P Beiting

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

In the mammalian gut, the transformation of host-derived primary bile acids is a complex metabolic process mediated by the gut microbiome. Interestingly, only a very small subset of gut microbes harbor the complete bai operon encoding the 7α-dehydroxylation pathway required for the generation of secondary bile acids (SBAs). SBAs have been shown to modulate gut microbial community structure and function, as well as to impact host immunity and the outcome of infection by a diverse range of pathogens. In our previous work, *Clostridium hiranonis* was identified as an SBA producer significantly associated with diet-induced remission in a canine model of inflammatory bowel disease. More precise analysis of the molecular mechanisms underlying these observations, however, is currently limited by the extremely low abundance of these organisms and a dearth of genome sequences for SBA producers. To address this knowledge gap, we mined publicly available metagenomic data from over 500,000 samples collected from a range of host species and the environment. Our data show that *C. hiranonis* is uniquely adapted to the canine gut, while other SBA producers are primarily found in the human gut. Abundance of SBA producers across this massive set of public data was then used to prioritize samples to construct metagenome-assembled genomes (MAGs) for both *C. hiranonis* and *C. scindens*. By permitting in silico acquisition of a library of rare clostridial genomes, this study lays the groundwork for pangenome comparison of closely related secondary bile acid producers to derive novel phylogenetic insight on host specificity and metabolic adaptations in these species.

Research Support: None
Student Support: NIH T32 Training Grant 5T32GM007170-48

Sensitizing mesenchymal mammary tumors to immunotherapy by targeting cell-intrinsic immune-modulatory factors

Kimaya Bakhle, Catherine Wheeler, and Anushka Dongre

Department of Biomedical Sciences, Cornell University, Ithaca, NY

Immune checkpoint blockade (ICB) therapy produces durable responses in some patients, but not in others. The underlying reasons for these differential responses remain elusive. We have recently demonstrated that the epithelial-to-mesenchymal transition (EMT) confers tumors with resistance to immunotherapies. Epithelial tumors recruit CD8+ T-cells to the tumor microenvironment and are sensitive to anti-CTLA4 ICB, whereas mesenchymal tumors express multiple cell-intrinsic immunosuppressive paracrine factors relative to their epithelial counterparts. Of these, abrogation of CSF1 or SPP1 from mesenchymal cancer cells generates partial responses to ICB therapy. Strikingly, abrogation of CD73 from mesenchymal tumors completely sensitizes them to immunotherapy. However, the mechanisms that dictate partial versus complete responses of mesenchymal tumor to ICB are unclear. Moreover, whether abrogation of CD73 can also sensitize mesenchymal tumors to other forms of ICB remains unknown. We performed immunofluorescent analysis of tumor sections and observed that knockout of CD73 promoted the greatest infiltration of both CD4+ and CD8+ T-cells in response to anti-CTLA4 ICB. In comparison, tumors knocked out for CSF1 or SPP1 showed only intermediate infiltration. Moreover, treatment of mesenchymal tumor-bearing mice with anti-CD73 generated synergistic responses with anti-CTLA4 ICB, but not with anti-PD1, or combinations of anti-CTLA4 and anti-PD1. In conclusion, our results demonstrate the protection against ICB therapy conferred by the EMT program can be overcome by targeting of these paracrine factors, specifically CD73. These findings could promote translational therapies that sensitize highly refractory mesenchymal tumors.

Research Support: NCI K22 CA255420-01 (Transition Career Development Award)
Student Support: None

Bovine Cathelicidins Expression During Bovine Respiratory Syncytial Virus Infection

Cassandra Barber, Santiago Cornejo, Merrilee Thoresen, and Amelia Woolums

Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University

Bovine respiratory disease (BRD) is an economically devastating disease of cattle worldwide with few efficacious and inexpensive ways to prevent or treat it. BRD is a multi-agent, multifactorial disease because pathogens, the animal's immune readiness, and environmental factors influence the disease's virulence. While vaccinations and antimicrobials can be efficacious, they are not uniformly effective, and the use of antimicrobials is getting pushback from the public and research community for increasing antimicrobial resistance. Thus, a novel prevention or treatment for BRD is needed. Candidates to meet this need are host defense peptides called cathelicidins. Cathelicidins are small cationic peptides with antimicrobial and immunomodulating activities that have been shown to be critical in airway epithelial host defense. However, before cathelicidins can be manipulated to prevent disease, it is necessary to characterize their expression during infection. The objective of this research is to characterize and quantify the endogenous expression of mRNA for bovine cathelicidins 2 and 5 in respiratory tissues of BRSV-infected calves using reverse transcriptase-quantitative real-time polymerase chain reaction (qRT-PCR). Localization will be determined in previously collected tissues from calves experimentally challenged with BRSV: nasal epithelium, trachea, bronchus, and grossly normal and abnormal lung. Positive control will be isolated bovine neutrophils. Primers will be identified using the BLAST Primer design tool. The qRT-PCR will be carried out using SYBR green and compared to a housekeeping gene for reference. The overall hypothesis is bovine cathelicidin expression will be elevated in areas of inflammation.

Research Support: MSU CVM USDA Section 1433 Formula Fund
Student Support: MSU CVM State Graduate Stipend

Development of Canine CAR-T Cells Targeting the Disialoganglioside GD2

Samuel Brill, Michael Yarnell, Lillie Leach, Isabel Brandtjen, Terry Fry, and Douglas Thamm

Cell and Molecular Biology, College of Veterinary Medicine and Biological Sciences, Colorado State University, Fort Collins, CO; Department of Pediatric Hematology/Oncology/BMT, University of Colorado Anschutz Medical Campus, Aurora

Chimeric antigen receptor (CAR)-T cell therapy is a form of immunotherapy which has demonstrated remarkable efficacy in blood cancers, boasting up to 90% remission rates in some forms of leukemia/lymphoma. Despite success in blood cancers, CAR-T cell efficacy in solid tumors is lacking. There are many forces driving the immune-tumor microenvironment that lead to CAR-T cell dysfunction in the context of solid tumors. While mouse models have been integral to CAR-T cell development to date, modeling the complex tumor-immune dynamics scales poorly in mice. Dogs provide a natural animal model to evaluate cell-based immunotherapy owing to their similar immune systems, natural history of cancer, and clinical care. We have developed a system for generating canine CAR-T cells as a first step to develop a canine model for CAR-T cell therapy. We demonstrate that the tumor associated antigen, disialoganglioside GD2, is expressed on canine osteosarcoma and melanoma cell lines. We have optimized culture conditions for T cell proliferation and activation, and demonstrated efficient CAR transduction using a gamma-retroviral vector. Finally, we demonstrate that primary canine T cells transduced with a GD2-targeting CAR can specifically kill GD2+ tumor, while sparing GD2- cells. Together, these data establish a platform for designing and evaluating canine derived CAR-T cells, demonstrate that GD2 is a targetable antigen on canine osteosarcoma and melanoma, and that canine CAR-T cells can specifically target GD2+ tumor cells.

Research Support: NIH T32 GM132057, NIH T32 GM136628, Colorado Clinical and Translational Sciences Institute
Student Support: NIH F31 CA265165

Staphylococcal superantigens suppress pro-inflammatory responses in human aortic endothelial cells

Ariela Burk and Wilmara Salgado-Pabón

Department of Pathobiological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI

Staphylococcus aureus is the leading cause of infective endocarditis, affecting roughly 40,000 individuals each year in the United States in addition to being the second leading cause of bacteremia. *S. aureus* infective endocarditis (IE) is a life-threatening cardiac infection that causes rapid tissue destruction of the cardiac endothelium. The superantigens (SAgs) toxic shock syndrome toxin 1 (TSST-1), staphylococcal enterotoxin C (SEC), and the toxins encoded by the enterotoxin gene cluster (egc) play an essential role in the etiology of S. aureus IE. Previous studies show that TSST-1 directly activates immortalized human aortic endothelial cells (iHAECs) by upregulating the vascular and intercellular adhesion molecules ICAM-1 and VCAM-1, while preventing induction of VCAM-1 to its full potential. Additionally, treatment of cells with purified TSST-1 results in decreased production of pro-inflammatory cytokines that cannot be rescued by pre-treatment with highly inflammatory molecules such as TNF-α and IL-1β. These data suggest that SAgs have the potential to dysregulate and suppress the innate immune response within the microenvironment of the cardiac endothelium, contributing to the establishment of S. aureus IE. What remains unclear is the role of SAgs in suppressing endothelial cell activation caused by the 40+ known exotoxins *S. aureus* produces, and if this suppression contributes to IE pathogenesis. This study utilizes cell free supernatants to evaluate the effect of SAgs on iHAECs within the context of the complete atmosphere of secreted virulence factors S. aureus produces. Suppression of pro-inflammatory responses to supernatant treatment was measured by IL-8 ELISA, and cytotoxicity was evaluated via MTS Assay.

Research Support: NIH R01 AI13469, Dean’s Office Dual Degree Fellow Funds
Student Support: None

Neonate enteric glia enhance intestinal epithelial restitution in vitro after exposure to mature lumen content

Madison Caldwell, Laurianne Van Landeghem, Jack Odle, Courtney Deck, Anthony Blikslager, Amanda Ziegler

Department of Clinical Sciences (Caldwell, Deck, Blikslager, Ziegler) and Department of Molecular Biomedical Sciences (Van Landeghem), College of Veterinary Medicine, NC State University, Raleigh, NC; Department of Animal Science (Odle), College of Agriculture and Life Sciences, NC State University, Raleigh, NC

Neonates exhibit poorer outcomes from intestinal ischemia for unknown reasons. We discovered an epithelial restitution defect in ischemia-injured neonatal intestine that is rescued by application of homogenized juvenile mucosa in a pig model. Enteric glial cells (EGC) have been shown to promote epithelial restitution following injury via paracrine signaling and are abundant in the intestinal mucosa of juvenile but not neonatal pigs. In mice, postnatal maturation and maintenance of the EGC network is purportedly driven by colonization of gut microbiota. Therefore, we believe that changes to pig intestinal microbiota during weaning may play a key role in EGC maturation required for proper epithelial restitution. We hypothesized that treatment with mature weaned (>6 weeks old), but not neonatal (<2 weeks old), luminal content would improve restitution of neonatal epithelial monolayers co-cultured with neonatal EGC. We compared the effect of sterile-filtered neonatal or mature luminal content addition on scratch wound restitution in neonatal porcine IPEC-J2 monolayers in monoculture or co-culture with primary porcine neonatal submucosal EGC using a transwell system (n=9). A two-way ANOVA revealed a significant interaction between luminal content treatment and EGC presence (P=0.0304). Specifically, mature luminal content treatment enhanced IPEC-J2 wound closure in co-culture with EGC but not in IPEC-J2 monoculture (P=0.0020). These data suggest that EGC provide secretory signals to the intestinal epithelium in response to luminal content that promote repair. Future work aims to determine what is driving these differences in restitution by analyzing secretomes from EGC treated with neonatal versus mature luminal content.

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Student Support: NIH 5 T35 OD 11070-12

Multi-Year Health Assessment Of Blue-Footed Boobies (*Sula Nebouxii Excisa*) in the Galápagos Islands

Ashley E. Cave, Jacqueline R. Dillard, Catalina Ulloa, Juan-Pablo Muñoz-Pérez, Alice Skehel, Diane Deresienski, Ronald K. Passingham, Jason Castaneda, Gregory A. Lewbart, Carlos A. Valle.

NC State University, Raleigh, NC, USA (Dillard, Passingham, Lewbart, Deresienski),
Universidad San Francisco de Quito (USFQ) and UNC at Chapel Hill (Ulloa, Muñoz-Pérez, Skehel, Valle), Universidad San Francisco de Quito USFQ, Quito, Ecuador (Ulloa, Valle),
University of the Sunshine Coast, Sippy Downs, Australia (Muñoz-Pérez)

The Galápagos blue-footed booby (*Sula nebouxii excisa*) is a sulid subspecies native to the Galápagos archipelago. Here we present physical examination, hematology, and blood chemistry results from 60 Galápagos blue-footed boobies that were captured by hand from their nests on North Seymour Island in June 2017 and July 2022. A portable blood analyzer (iSTAT) was used to obtain values in the field for hematocrit, hemoglobin, sodium, potassium, chloride, ionized calcium, total CO2, glucose, blood urea nitrogen, creatinine and anion gap for each bird. Blood lactate, total solids, PCV and blood smears were evaluated manually on site. A white blood cell differential was performed in 2017. The breeding status of each bird and the number of chicks in the nests were also recorded. Total CO2, blood urea nitrogen, ionized calcium, potassium, anion gap, hematocrit, and hemoglobin were all higher in 2022 than 2017. There were also more nests with chicks in them in 2022 than in 2017. Lactate, ionized calcium, hematocrit, and hemoglobin were all higher in females than in males, while blood urea nitrogen was higher in males than in females. These results provide a multi-year reference to the baseline health parameters in a free-living population of Galápagos blue-footed boobies that can be used to compare and monitor the health status of this species.

Research Support: Authorized by the Galápagos National Park Service and with support of the Heska Corporation, the Galápagos Academic Institute for the Arts and Sciences (GAIAS)-USFQ and the Galapagos Science Center
Student Support: NC State University Office of the Associate Dean for Research and Graduate Studies

Lactobacillus acidophilus delivered adjuvants drive effector responses in intestinal immune cells

Christian Cherry, Callie Lang, Ben Swartzwelter, Allison Vilander, and Gregg Dean

Department of Microbiology, Immunology, & Pathology, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, CO

Novel vaccine platforms should induce effective adaptive immunity, while also being easily modified, transported, and inexpensive to produce. Most pathogens infecting animals and humans enter through mucosal tissues where local and systemic adaptive immunity is initiated. Our group has developed the probiotic Lactobacillus acidophilus (LA) as an orally delivered mucosal vaccine platform. LA survives gastric acid and bile, accesses immune inductive sites, and activates critical pattern recognition receptors. We have engineered LA to express pathogen antigens relevant to humans and animals and shown induction of antigen-specific immune responses. To increase immune responses to vaccine delivered antigens, we have engineered LA to express the TLR ligands *Salmonella typhimurium* flagellin protein FliC and the type I pilus protein FimH expressed on invasive *E. coli* and *Salmonella spp*. Here, we investigate how these adjuvants influence resident immune cells within the small intestine and associated lymphoid tissue. Mice were orally vaccinated with wildtype LA and LA expressing FliC or FimH. We extracted resident immune cells from the lamina propria and epithelium of the small intestine, Peyer's patches, and mesenteric lymph nodes. These cells were then analyzed by spectral flow cytometry to quantify shifts in frequency of macrophages, specifically M1 and M2 macrophages, dendritic cells, and T cell susbsets induced by our LA vaccine platform. These results provide insight into how adjuvant selection drives local immune responses in the gut mucosa. Furthermore, it can help guide adjuvant selection for probiotic vaccines with the end goal of tailoring mucosal immune responses to various emerging mucosal pathogens.

Research Support: Young Investigator Award Center for Companion Animal Studies [or] NIAID R01 AI141604
Student Support: CSU DVM/PhD Program

Engineering chimeric antigen receptor (CAR) lymphocytes to target feline infectious peritonitis virus

James R. Cockey, Natalia Lopez-Barbosa, Christian Urbina, Gary R. Whittaker, Matthew P. DeLisa, and Cynthia A. Leifer

Department of Microbiology & Immunology, College of Veterinary Medicine (Cockey, Urbina, Whittaker, and Leifer), Robert Frederick Smith School of Chemical and Biomolecular Engineering (Lopez-Barbosa and DeLisa), Cornell University, Ithaca, NY.

The fatal disease feline infectious peritonitis (FIP) currently has no FDA approved treatments. The goal of this study is to design a novel immunotherapy targeting cells infected with FIP virus (FIPV) and thus expressing surface spike protein that can be detected by chimeric antigen receptor (CAR)-engineered immune cells. CAR immunotherapy has been successful in treating some human cancers but has not yet been developed for acute viral infections like FIPV, nor used at all in cats to date. CARs are comprised of two main components: a single chain antibody fragment (ScFv) and signaling domain(s) from immune costimulatory receptor(s). Here we have designed an ScFv specific for FIPV spike protein from the anti-spike clone 18A7.4. We then used this ScFv to create multiple anti-spike CAR constructs with different intracellular signaling domains to determine the optimal construct to direct effector immune cells to seek and destroy FIPV spike-expressing target cells. Because of the lack of feline-specific reagents, the conditions necessary for primary feline T cell growth in culture were not well studied. Here we have optimized the enrichment strategy and growth conditions of primary feline T cells for ex vivo expansion. We also demonstrate successful nucleofection of exogenous plasmid DNA into primary feline T cells. Completion of this study will provide proof-of-principle data using an FIPV model to support the development of FIPV CAR-cell therapy for this devastating disease in cats, and will also determine the feasibility of developing a CAR-based immunotherapy for the potential treatment of acute viral infections in humans or other diseases in cats for which there are few treatment options such as lymphoma.

Research Support: Cornell Feline Health Center
Student Support: Liz Hanson Graduate Scholarship

Are blood serum-derived extracellular vesicles a viable Chronic Wasting Disease peripheralization mechanism?

Jesse Cole, Erin McNulty, Audrey Sandoval, Amy Nalls, Joseph Westrich, and Candace Mathiason

Dept. of Microbiology, Immunology, and Pathology, Colorado State University

Chronic Wasting Disease (CWD) is a rapidly spreading, fatal neurodegenerative or prion disease of cervid species (deer, elk, moose and reindeer). CWD is the most efficiently transmitted of all the prion diseases and is currently detected in captive and free-ranging cervid populations in 30 U.S. States, 4 Canadian Provinces, Europe, and Asia. The effective transfer of CWD among cervids has been largely attributed to horizontal transmission by direct animal-to-animal contact via exchange of bodily secretions (saliva, blood, urine and feces), and by indirect contact with the infectious agent shed in these products to the environment. Prions have been detected in blood, as well as within the pregnancy microenvironment and fetal tissues harvested from CWD-infected cervids. To further investigate CWD peripheralization mechanisms and how prions traffic across the placental barrier, we are assessing the role blood serum-derived extracellular vesicles may play in these processes. Here, EVs were isolated from blood serum collected from experimentally CWD-infected white-tailed deer. Nanoparticle tracking analysis (NTA) was performed to quantify the size distribution and concentration of EV isolates. We are further assessing EV isolates for the presence of prions by western blot (PrPSc) and real-time quaking induced conversion (RT-QuIC) (amyloid seeding activity). These studies will provide the basis for continued studies determining CWD peripheralization in the host, and permit further investigation of EVs as a potential biomarker for CWD diagnostic testing.

Research Support: NIH–NIAD 2R01AI112956-06
Student Support: NIH MSTP T32 Fellowship

Determining Therapeutic Targets for Peripheral T Cell Lymphoma, Not Otherwise Specified in Canines

Sara Cook, Eileen Owens, Janna Yoshimoto, Anne Avery

Department of Microbiology, Immunology, and Pathology (Cook, Owens, Yoshimoto, Avery), College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO

Human peripheral T Cell Lymphoma not otherwise specified (PTCL-NOS) is a proposed subset of T cell lymphoma. PTCL-NOS is a rare neoplasm in people but is relatively common in dogs. We hypothesize that the dog can be a useful pre-clinical model to study human PTCL. The goals of this study were to, 1) identify molecular pathways that are shared with human PTCL that could be targeted by drug therapy, and 2) Develop an in vitro system for evaluating the effect of these drugs on primary PTCL cultures. Using RNA sequencing data from 33 dogs, we found that the PI3K-AKT pathway is upregulated in canine PTCL, similar to findings in the human counterpart. We then developed two methods for determining the ability of drugs to inhibit this pathway and cause cell death. First, we demonstrated that constitutively phosphorylated AKT could be detected in primary PTCL cells using intracellular flow cytometry with an antibody specific for pAKT. This assay will then be used to demonstrate inhibition of AKT phosphorylation following treatment with a PI3K inhibitor. Second, we optimized a Presto Blue cell viability assay, determining the number of cells and days of culture best suited to evaluate cell death in the presence of PI3K inhibitors. These two assays will be used to determine which PI3K inhibitors, and at what concentrations, are most effective in vitro for inducing cell death.

Research Support: none
Student Support: College of Veterinary Medicine and Biomedical Sciences Dean's Office, Colorado State University

Transcriptomics of Feline Small-Cell Intestinal Epitheliotropic T-cell Lymphoma

Shayla Curry, Sydney Bork, Emily Rout, Janna Yoshimoto, Anne Avery

CSU Microbiology, Immunology, and Pathology Department; CSU Clinical Hematopathology Lab; CSU College of Veterinary Medicine and Biomedical Sciences"

Feline small-cell intestinal epitheliotropic T-cell lymphoma (SCL) is a common malignancy in cats. Despite its prevalence, diagnosing and treating this disease remains challenging as its pathogenesis is unclear. SCL is believed to arise from chronic intestinal inflammation but the nature of the T cells causing this disease has not been described. Determining the normal counterpart (cell of origin) of the neoplastic cells will provide insights into the pathogenesis of this disease. In order to investigate the cell of origin, RNA seq was performed on 5 duodenal and 1 ileal sample with a definitive histologic diagnosis of SCL and a positive clonality result, and 5 duodenal samples with no histologic suspicion for SCL and no evidence of clonality (IBD cases). The top five most upregulated KEGG pathways between the cases of SCL and the cases of IBD were: natural killer cell-mediated cytotoxicity, T-cell receptor signaling pathway, primary immunodeficiency, Th1 and Th2 differentiation, and PD-1 checkpoint pathway in cancer. All of these involve T-cell differentiation, with the activity of NK and NKT cytotoxicity being the most upregulated. This observation, if it is recapitulated with a larger dataset, suggests that the neoplastic cells may have NKT-like functions. NKT cells respond to unique microbial antigens; therefore this discovery may help identify the inciting causes of feline SCL.

Research Support: N/A
Student Support: CSU College of Vet Medicine and Biological Sciences

Validation of automated MRI segmentation protocols of neuroanatomical regions of the rhesus macaque brain

Brittany Davis, Joey Charbonneau, Erika Raven, Jeffery Bennet, Eliza Bliss-Moreau

School of Veterinary Medicine, UC Davis, Davis, CA (BD); Neuroscience Graduate Group, UC Davis, Davis, CA (JC); CA National Primate Research Center, UC Davis, Davis, CA (JC, ER, JB, EBM); Department of Radiology, New York University School of Medicine, New York, NY (ER); Department of Psychology, UC Davis, Davis, CA (JB, EBM)

Magnetic resonance imaging (MRI) is an important tool allowing neuroscientists to measure neuroanatomy in vivo. Defining anatomical structures on images has historically been achieved through hand segmentation (HS), a time and labor-intensive process requiring an expert rater to hand-draw structures of interest. While HS is considered the gold standard, automated segmentation (AS) methods are being increasingly utilized because they are more efficient. However, the accuracy of AS methods remains unproven. The aim of this project is to compare the performance of HS and AS methods in delineating cortical and subcortical structures in the rhesus macaque brain. We compare volumetric and morphometric data from these two methods when used to parcellate the amygdala and the insula on T1-weighted images from 36 rhesus macaques (22 males, 14 females; age 1.1-20.3 years). Our results show that HS and AS methods produce different volumetric results when applied to the amygdala: AS methods produce larger relative volumes than those obtained via HS. AS amygdala volume is not predictive of HS amygdala volume. HS and AS methods appear to perform more similarly when applied to the insula: relative volumes between methods overlap more and AS volume is predictive of HS volume in the insula. Finally, we compare the volumetric relationship between the amygdala and insula within HS and AS methods. We find different volume associations between the two structures depending upon the segmentation method used. It is important to assess the accuracy of these tools, as data here indicate that the volumetric relationship between neuroanatomical structures varies by segmentation method.

Research Support: National Institute of Mental Health, National Institute of Child Health and Development
Student Support: Students Training in Advanced Research (STAR) Program through UC Davis SVM Endowment Fund

Polygenic risk scoring of healthy US adults in a cross-sectional metabolic phenotyping study

Catherine Dombroski, Kristen L James, Brian J Bennett

UC Davis School of Veterinary Medicine, Davis, CA (Dombroski), Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, Aurora, CO (James), USDA ARS Western Human Nutrition Research Center, Davis, CA (James, Bennett), Department of Nutrition, UC Davis, Davis, CA (Bennett)

Metabolic marker abnormalities following a meal challenge are important predictors of risk of chronic disease such as diabetes or cardiovascular disease. To comprehensively understand the environmental and physiological factors determining metabolic health, the USDA Western Human Nutrition Research Center conducted a cross-sectional metabolic phenotyping study on 393 healthy human adults in the Davis, CA region. A wide range of data regarding individual's typical diet, hormonal profile, gut microbiome and metabolic markers were collected prior to and at multiple timepoints following a high-fat challenge meal. This study design allows for tracking of the dynamic response to a meal challenge which contrasts the static measurements typical of most metabolic phenotyping studies. Additionally, genotyping data was collected for 238 of these participants, allowing for us to investigate how genetic risk can influence the dynamic metabolic phenotypes observed. Genetic risk can be assessed using polygenic risk scores (PRS), which are calculated values representing the cumulative risk of all single nucleotide polymorphisms (SNPs) predisposing to a particular phenotype weighted by their effect size. Using PRS scores generated for our cohort, we will look at associations between genetic risk and post-prandial lipid metabolism over time. Our findings will help elucidate how cumulative genetic risk influences post-prandial metabolism which can help inform our interpretation of future metabolic phenotyping studies and potentially identify targets for nutritional interventions.

Research Grant: USDA-ARS 2032–51530–025-00D; 2032-51530-026-00D
Student Support: NIH Grant T32GM136559, UC Davis SVM Endowment Funds

iDISCO highlights postnatal changes in enteric glial network development in a comparative pig model

Sara J Erwin-Craig, Sophia P Jodka, Jack Odle, Laurianne Van Landeghem, Anthony T Blikslager, Amanda L Ziegler

Department of Clinical Sciences (Erwin-Craig, Jodka, Blikslager, Ziegler), Department of Animal Science (Odle), and Department of Molecular Biomedical Sciences(Van Landeghem), North Carolina State University, Raleigh, NC

The glial network of the enteric nervous system is instrumental in intestinal repair, but is immature at birth. In mouse models, enteric glia are restricted to the submucosal and myenteric plexuses, and are driven to populate the lamina propria by changes in microbial populations at weaning. Our lab uses a comparative pig model, but early postnatal development of the enteric nervous glial network has not yet been described and measured in the pig. We hypothesized the density and distribution of glial cell subtypes would change within the early postnatal period in our comparative pig model. The immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) technique was used to triple-stain full-thickness jejunum of 1-, 7-, 14-, and 21-day-old pigs against glial markers S100B, Sox10, and glial fibrillary acidic protein (GFAP). Samples were imaged with a light-sheet microscope and glial volumes were calculated in Imaris software. In the lamina propria, density by volume of GFAP+ glia decreases (P=0.1133) while S100B+ glia density increases (P=0.5326). The number of Sox10+ nuclei increases from 1 to 21 days of age (P=0.0147). We believe this indicates GFAP is expressed in more mature glial cells and that S100B, a known inflammatory mediator, is participating in immune responses to colonizing bacteria while Sox10 marks the nuclei of progenitor glia. Understanding this early postnatal development will allow its modulation to accelerate maturation of repair mechanisms.

Research Support: USDA NIFA 1007263 and 07985, NIH K01 OD 028207, NIH R01 HD095876, UNC CGIBD P30 DK034987 and T32 5T32DK007737
Student Support: NIH T35 Training Grant

Examination of leukocyte coping capacity as a measure of stress in cownose stingrays, *Rhinoptera bonasus*

Patrick Farias, Sean Perry, Alexa Delaune, and Justin Stilwell

Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS (Farias, Perry, Delaune, Stilwell); Mississippi Aquarium, Gulfport, MS (Perry, Delaune)

Leukocyte coping capacity (LCC) has been used to quantify real-time physiologic stress in a variety of terrestrial mammalian and avian species in relation to capture and handling, transport, contaminant exposure, surgery, and pain management. However, this method of quantifying stress has never been explored for its feasibility in aquatic vertebrates, particularly elasmobranch (cartilaginous) fishes. The objective of this study is to explore the viability and potentially validate the use of LCC assays in elasmobranch species using the cownose ray (*Rhinoptera bonasus*) as a model species. For this work, an aquarium-housed population of cownose rays will be utilized to draw blood using approved protocols. Blood samples will be collected using a heparinized assay solution. Elasmobranch neutrophils will be stimulated to produce an in vitro respiratory burst through stimulation with phorbol-myristate acetate (PMA) dissolved in dimethyl sulfoxide and diluted with phosphate buffered saline. A Junior LB 9509 portable luminometer and lucigenin, a chemiluminescent superoxide probe, will be used to measure the oxidative free radical production in vitro in PMA-stimulated and unstimulated samples. We hypothesize that elasmobranch neutrophils will be capable of creating a significantly greater respiratory, oxidative burst in response to PMA-stimulation compared to unstimulated controls. This work will potentially validate the use of LCC as an indirect measure of immune system capability and physiologic stress in fish and establish the baseline data needed for future studies examining and mitigating stress impacts related to the husbandry and veterinary care in this group of ecologically important fishes.

Research Support: Office of Research & Graduate Studies, College of Veterinary Medicine, Mississippi State University
Student Support: Office of Research & Graduate Studies, College of Veterinary Medicine, Mississippi State University

A novel equine in vitro model of osteoarthritis utilizing fibronectin fragment stimulation

Rachel Gagliardi[1,2], Drew W. Koch[1,2], Richard Loeser[3,4], Lauren V. Schnabel[1,2,4]

[1]Department of Clinical Sciences, College of Veterinary Medicine, NC State University, Raleigh, NC
[2]Comparative Medicine Institute, NC State University, Raleigh, NC
[3]Division of Rheumatology, Allergy and Immunology, UNC, Chapel Hill, NC
[4]Thurston Arthritis Research Center, UNC, Chapel Hill, NC

Osteoarthritis (OA) is a debilitating disease that impacts millions of individuals. A significant hindrance to therapeutic discovery is the limitations of current in vitro models available for the study of OA. Current models utilize lipopolysaccharide (LPS) or interleukin-1-beta (IL-1β) to stimulate synoviocytes and chondrocytes, the two primary cell types in joints, to induce an OA-like phenotype. Naturally occurring OA is documented to be a disease of chronic low-level inflammation that leads to dysregulation of normal cellular function within the joint. However, current models that utilize LPS and IL-1β invoke an inconsistent, intense, and transient inflammatory response. We hypothesized that utilizing fibronectin fragments (FN-f) in vitro to stimulate synoviocytes and chondrocytes both in monolayer and co-culture may result in a model that more closely mimics naturally occurring OA. Fibronectin is a component of the extracellular matrix and becomes fragmented during OA, which is believed to contribute to OA pathogenesis. In order to induce OA in our model, cells were stimulated with FN-f for 18h and RNA was isolated at the conclusion of stimulation and 24h post-stim. Gene expression of a select set of genes that are widely documented to be altered in an OA disease state was analyzed using a Nanostring nCounter and protein expression was analyzed via ELISA. Several key genes that are altered in OA, such as IL-6, CXCL6, CCL2, MMP3 and MMP13, were also significantly impacted by our FN-f stimulation model. Further analysis to fully characterize the OA phenotype resulting from FN-f stimulation is currently underway. Future studies will aim to compare our FN-f model to traditional LPS and IL-1β models.

Research Support: 2022 NCSU CVM Intramural Research Grant (LVS and RG), R37 AR049003 (RL)
Student Support: NCSU GAANN Biotech Fellowship

Poster Session 2

3:00-3:50 pm

Poster Session 2	Poster Number	Title of Presentation
Gupta	2-1	Artemin/GFRα3 signaling mediates chronic osteoarthritis associated pain
Hallum	2-2	Determining the functional and temporal role of SOX9 protein in two avian species during beak development
Herring	2-3	Impact of the fecal microbiome on subclinical Salmonella shedding in horses
Hommer	2-4	Cellular targeting and functions for the methionine sulfoxide reductases
Jakes	2-5	Dose-dependent effects of an innate immune stimulant on calf respiratory health and immune gene expression
Koch-Laskowski	2-6	MicroRNAs - small molecules with big potential in shaping gut endocrine biology
Lang	2-7	Investigation of canine antigen-specific CD4+ T cells with novel immunological tools
Lutz	2-8	Confirmation and enhancement of a PH-HFpEF murine model
May	2-9	Characterizing the glioblastoma “ablatosome” treated with high-frequency irreversible electroporation
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Artemin/GFRα3 signaling mediates chronic osteoarthritis associated pain

Ankita Gupta, Uma Nair, Connor Thonen-Fleck, Santosh K. Mishra, and B. Duncan X. Lascelles

Translational Research in Pain Program (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Clinical Sciences (Gupta, Nair, Thonen-Fleck, Lascelles), Department of Molecular Biomedical Sciences (Mishra), Comparative Pain Research and Education Centre (Mishra, Lascelles), North Carolina State University, Raleigh, NC

Osteoarthritis (OA) is a leading cause of disability, with ~100 million US adults suffering from chronic joint pain, widespread sensitization, and decreased mobility. Clinically efficacious and safe analgesics for OA pain are limited due to a lack of understanding of neural mechanisms of chronic OA pain. We have linked synovial fluid concentrations of a neurotrophic factor, artemin, to naturally occurring joint pain in dogs. Further, GDNF family receptor alpha 3 (GFRα3, artemin's receptor) expression was increased in dog OA sensory neurons. Despite our compelling data, no studies have elucidated the role of artemin/GFRα3 in OA pain. This study explores the functional role of artemin/GFRα3 signaling in OA pain. We used two mouse models of stifle OA pain, the chemical monoiodoacetate (MIA) and surgical destabilization of the medial meniscus (DMM) in wildtype and transgenic Gfrα3 mutant mice (nonfunctional GFRα3) to evaluate sensitivity to mechanical and thermal stimuli and limb use at chronic pain timepoints (week 6 for MIA; week 16 for DMM). We assessed MIA and DMM-induced hypersensitivity and limb use at 2-24 hrs. post-anti-artemin monoclonal antibody or isotype control administration. Wildtype MIA and DMM mice developed hypersensitivity to mechanical and thermal stimuli and had decreased limb use. Gfrα3 mutant mice did not develop OA-induced changes in hypersensitivity and limb use. Artemin sequestration reversed MIA and DMM-induced hypersensitivity and lameness for up to 24 hrs. at late OA pain timepoints. This is the first evidence demonstrating the functional role of artemin/GFRα3 signaling in models of OA pain. Our ongoing work elucidates putative targets for developing novel and safe analgesics for OA pain.

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Determining the functional and temporal role of SOX9 protein in two avian species during beak development

Juliann Hallum, Crystal Rogers, and Heddy Menendez

Department of Biology (Davis, CA), Department of Anatomy, Physiology, and Cell Biology (Davis, CA), Department of Biochemistry, Molecular, Cellular, and Developmental Biology (Davis, CA), School of Veterinary Medicine, University of California Davis, Davis, CA

The neural crest stem cell population is a vertebrate-specific cellular lineage that differentiates into multiple cell and tissue types during embryogenesis. These cells form in the developing central nervous system, and undergo an epithelial to mesenchymal transition (EMT) to migrate to distant sites where they will form the craniofacial bone and cartilage [4]. Studies show that this complex process is conserved as avian craniofacial development retains similar properties to mammalian development. There are gaps in knowledge about the specific molecular mechanisms and proteins that guide the formation of neural crest derivatives across species [6]. One derivative of neural crest cells is craniofacial bone and cartilage, including the jaw in mammals and the beak structures in birds [13]. NC cells can be identified by the expression of SRY-Box Transcription Factor-9 (SOX9) expression, which drives specification, EMT, and differentiation at different developmental stages [14]. A recent human genetics study demonstrated that differences in levels or timing of SOX9 expression create craniofacial differences [15]. The Rogers' Lab identified that the timing of Sox9 gene and protein expression onset differs between chicken and quail embryos during neural crest development. Therefore, the objective of this project is to: 1) define the role of SOX9 in beak formation in two closely-related organisms, Gallus gallus (chicken) and Coturnix japonica (quail) to determine if the specific mechanisms regulating beak morphogenesis in these species is conserved, and 2) identify molecular changes in neural crest- derived tissues after loss of SOX9.

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Student Support: Boehringer Ingelheim

Impact of the fecal microbiome on subclinical *Salmonella* shedding in horses

Emily C. Herring, Tara N. Gaire, Jessica Brown, Erin S. Groover, Noelle R. Noyes, and Brandy A. Burgess

Dept of Population Health, College of Veterinary Medicine, Univ of Georgia, Athens, GA (Herring, Burgess); Dept of Veterinary Population Medicine, College of Veterinary Medicine, Univ of Minnesota, St. Paul, MN (Gaire, Noyes); Dept of Clinical Sciences, College of Veterinary Medicine, Auburn Univ, Auburn, AL (Brown, Groover)

Subclinical shedding of *Salmonella* in horses is more common than clinical disease and more likely to go undetected, posing a persistent biosecurity risk to equine facilities. Known risk factors for *Salmonella* positivity suggest that disruptions of the gastrointestinal microflora play a key role in initiating fecal *Salmonella* shedding; elucidating these microbial drivers is critical to developing targeted interventions to prevent transmission of *Salmonella* from subclinical horses. This study aimed to characterize the fecal taxonomic profile of horses with subclinical salmonellosis and correlate changes in *Salmonella* shedding status over time with shifts in the fecal microbiome. Six adult horses from a resident herd at a veterinary teaching hospital with fecal culture-confirmed subclinical salmonellosis were included. Samples from a prospective longitudinal study were selected for retrospective analysis. For each horse, serial *Salmonella* fecal cultures were performed weekly for 8 weeks. DNA was isolated from banked fecal samples (n = 48) and subjected to PCR amplification and 16S rRNA amplicon sequencing. No single change in fecal microbial diversity or community composition consistently predicted subclinical *Salmonella* positivity, and fecal microbial community structure varied considerably between individual horses. However, fecal microbial communities were most similar among horses with the same *Salmonella* shedding pattern over time. Therefore, the equine gastrointestinal microbiome may be more useful in predicting long-term *Salmonella* shedding patterns than the immediate likelihood of fecal shedding.

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Cellular targeting and functions for the methionine sulfoxide reductases

Alexandra Hommer and Carolyn Sevier

Department of Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, New York

Reactive oxygen species (ROS) are highly reactive molecules formed upon O2 reduction. ROS-producing enzymes are found throughout the cell. ROS buildup can result in protein oxidation, altering protein function. Post-translational modification by ROS is most appreciated for its association with protein damage and a loss of protein function. However, protein oxidation by ROS can also mediate a change (or gain) of protein function to help cells cope with increased ROS levels. Protein methionines are particularly susceptible to oxidation by ROS, converting methionine to methionine sulfoxide (MetO). Two different enzymes, both referred to as methionine sulfoxide reductases (MSRs) act to reverse MetO. MsrA and MsrB selectively reduce S-MetO or R-MetO isomers, respectively. The enzymology of the MSRs is well understood. Yet, our understanding of the organellar distribution of the MSRs, and the targets of MSR activity, remains limited. The simple eukaryote *S. cerevisiae* with only two MSR-encoding genes (MXR1 and MXR2) provides an ideal background for MSR analysis. We have established that the Mxr1 (MsrA) and Mxr2 (MsrB) proteins are both dual-localized to the cytoplasm and the mitochondria. We are currently focused on establishing whether localization is limited to these locations, or if the MSRs are present in other cellular locations like the endoplasmic reticulum (ER) or nucleus, using a combination of cellular fractionation and microscopy approaches. Once the cellular distribution of the MSRs is fully-established, our goal is to determine MSR targets and how the loss of MSR activity within a specific organelle impacts cell and/or organism function.

Research Support: NIH R01 GM105958
Student Support: None

Dose-dependent effects of an innate immune stimulant on calf respiratory health and immune gene expression

Grace Jakes and Sarah Raabis

Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, CO.

Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in pre-weaned dairy calves. While antibiotics are a primary treatment strategy often used as metaphylaxis, there is a need to reduce antibiotic use in food animals to preserve antibiotic efficacy. Manipulation of the innate immune response via administration of Toll-like receptor (TLR) agonists has been explored as an alternative treatment strategy for cattle at risk for BRD. In a previously implemented field model of BRD, administration of intranasal liposome-TLR-9 agonist complexes (LTC) to weaned Holstein steers was shown to improve clinical illness scores and reduce mortality. However, the application of LTC in healthy calves has not been evaluated. The objective of this study is to determine the optimal dose of intranasal LTC administration in pre-weaned Holstein calves to produce a pro-inflammatory immune response with minimal clinical effects. Twenty healthy pre-weaned dairy calves will be randomly enrolled into 4 intranasal dose groups (n=5): CON (2 ml diluent), Group A (2ml diluent and 0.1 ml LTC), Group B (2ml diluent and 0.05 ml LTC) and Group C (2ml diluent and 0.01 ml LTC). Nasopharyngeal swabs will be collected pre-treatment, 24h, 72h and 7 days post-treatment. Clinical health scores will be collected simultaneously. RNA extracted from swabs will be evaluated for differential expression of IFN-a, IFN-b, INF-g, MCP-1, IL-8 and TNF-a using RT-qPCR. Cumulative scores indicative of respiratory health will be compared among treatment groups. These data will be used to inform future LTC clinical trials in calves to expand understanding of the effects of intranasal immune stimulation on the incidence of BRD in pre-weaned dairy calves.

Research Support: CSU College of Veterinary Medicine College Research Council Grant
Student Support: CSU Department of Microbiology, Immunology, and Pathology

MicroRNAs - small molecules with big potential in shaping gut endocrine biology

Kieran Koch-Laskowski, Heather McCauley, James Wells, and Praveen Sethupathy

Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Koch-Laskowski, Sethupathy); Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH (McCauley, Wells)

Gut enteroendocrine cells (EECs) are key regulators of metabolic health. Scattered among the intestinal epithelium, EECs sense and respond to luminal contents by secreting a variety of hormones that control appetite, digestion, and whole-body energy balance. Such signaling systems have gained attention as pharmacological targets for type 2 diabetes and obesity. However, despite these advancements the mechanisms underlying EEC differentiation and function are not fully understood, limiting the development of more effective treatment strategies for metabolic disease. To address this knowledge gap, we pursued the underexplored role of microRNAs (miRNAs) in EEC biology and sought to define miRNA expression patterns specific to the EEC lineage. As small, non-coding RNA molecules, miRNAs modulate gene expression at the post-transcriptional level and play essential roles in the development and function of tissues throughout the body. Here, we leveraged an inducible, tissue-specific mouse model (Neurog3fl/fl; VillinCreERT2) to deplete EECs in adult animals. Through small RNA-sequencing analysis of intestinal samples isolated from control and EEC-depleted mice, we identified unique miRNA expression profiles of the EEC lineage, spanning differentiating cells located within intestinal crypts to mature cells of the villi. Our preliminary results highlight several miRNAs conserved throughout the EEC differentiation trajectory as well as some miRNAs specific to certain EEC subtypes. Overall, these findings point to potential molecular targets through which miRNAs regulate EECs, which may inform future therapeutics to direct EEC differentiation, function, and ultimately, whole-body metabolism.

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Student Support: N/A

Investigation of canine antigen-specific CD4+ T cells with novel immunological tools

Haeree Park Lang, Thamotharampillai Dileepan, Marc K Jenkins, and Steven G Friedenberg

Center For Immunology (Lang, Dileepan, Jenkins, Friedenberg) and Department of Veterinary Clinical Sciences (Lang, Friedenberg), College of Veterinary Medicine, University of Minnesota

Dogs are diagnosed with diseases like cancer, autoimmune disorders, and allergies at an alarmingly high rate. Yet, very little is known about the underlying immunological mechanisms that drive these common immune-mediated diseases in our canine companions. All of these diseases require T cells, particularly CD4+ T cells, to initiate the adaptive immune response. Therefore, there is significant value in studying the antigens and corresponding antigen-specific CD4+ T cells associated with disease pathogenesis. To better understand how dog CD4+ T cells function in disease, canine immunologists must first understand how these immune cells function in health. As an initial step, we developed canine-specific immunological tools to identify and characterize canine CD4+ T cells in the laboratory in a known system: a vaccine model. Our objective is to use these tools to identify recently activated antigen-specific CD4+ T cells in vitro when stimulated with vaccine antigens derived from the Rabies virus and Canine Parvovirus, two commonly administered core vaccines. Our central hypothesis is that the high frequency of antigen-specific CD4+ T cells present in the blood or spleen from recently vaccinated dogs will be detectable with our canine-specific immunological tools that capture events downstream of CD4+ T cell activation. With this critical validation in a vaccine model, our highly specific tools can be applied to precision medicine: such as discovering neoantigens, autoimmune triggers, or characterizing allergen-specific CD4+ T cells. Ultimately, this work is critical in deepening our understanding of antigen-specific CD4+ T cell immunity and improving novel immunotherapies tailored to the canine patient.

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Student Support: Hanlon/Schmidt O'Brien Residency Fund Award and Graduate Student/Resident Research Grant

Confirmation and enhancement of a PH-HFpEF murine model

Halle Lutz, Dashuai Zhu, Zhenhua Li, Kristen Popowski, Phuong-Uyen Dinh, and Ke Cheng

Department of Molecular Biomedical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC (Lutz, Zhu, Li, Popowski, Dinh, Cheng), and Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, NC, USA (Cheng)

Pulmonary hypertension-associated heart failure with preserved ejection fraction (PH-HFpEF) is estimated to affect two million Americans. It presents as left ventricular diastolic dysfunction that leads to increased pulmonary artery pressure. Even though PH-HFpEF is the most common type of pulmonary hypertension and is associated with high mortality, there are still no effective therapies. One challenge faced by PH-HFpEF is the lack of animal models that accurately reproduce all aspects of the disease since PH-HFpEF arises from several comorbidities. While HFpEF mouse models have been established, stemming from age, hypertension, or metabolic syndrome, only recently was an all-encompassing PH-HFpEF mouse model published. However, this model uses AKR/J mice, which develop thymic lymphoma as early as six months old, with 100% of the mice developing thymic lymphoma by twelve months old. Therefore, confounding variables are introduced and preventative measures for PH-HFpEF can be studied with this model but evaluating potential therapies for the disease becomes difficult and costly. We tracked PH-HFpEF disease progression by measuring body weight and with echocardiography for the proposed twenty weeks after beginning the AKR/J mice on a 60% lipids/kcal high-fat diet (HFD). We found that AKR/J mice develop PH-HFpEF as early as twelve weeks after beginning HFD, maintaining left ventricular ejection fraction but with significantly increased body weight, left ventricular hypertrophy, and tricuspid regurgitation velocity. Shortening the PH-HFpEF murine model to twelve weeks instead of twenty weeks removes the confounding variable of thymic lymphoma and enables researchers to assess efficacy in therapeutic studies accurately.

Research Support: None
Student Support: NIH T35 Interdisciplinary Biomedical Research Program [or] NCSU Provost’s Doctoral Fellowship

Characterizing the glioblastoma "ablatosome" treated with high-frequency irreversible electroporation

James May and John Rossmeisl

Department of Biomedical and Veterinary Sciences, Virginia Maryland College of Veterinary Medicine, Blacksburg, VA

Glioblastoma (GBM) is a highly invasive and aggressive brain tumor in humans and dogs with a low post-treatment survival rate. An effective treatment for GBM will require overcoming the difficulties of treating such a neuroinvasive mass without harming the surrounding brain; replacing an immunosuppressive, tumor-promoting microenvironment with an anti-tumor, pro-inflammatory microenvironment; and creating temporary blood-brain barrier (BBB) disruption to allow for delivery of cancer-targeting drugs to the brain. High-frequency irreversible electroporation (H-FIRE) is a non-thermal tumor ablation method in which an electric field is applied to cells in ultra-short, bipolar electric pulses to disrupt the BBB. Cell survival can be controlled by toggling the strength of the electric field (ie, "reversible" versus "irreversible" electroporation). Characterization of gene expression via pathway analysis in the ablation microenvironment, or "ablatosome," will allow us to better understand the efficacy of H-FIRE for treatment of GBM. Spatial profiling technology was used to compare geographic regions of canine tumor biopsies pre- and post-HFIRE treatment. Transcriptomic data from each region of interest was processed using the Reactome Pathway Analysis with Down-weighting of Overlapping Genes gene analysis method to identify significant changes in gene pathway regulation. We identified 2563 pathways that were significantly different between pre- and post-treatment samples, reflecting a number of expected cellular functions. Current and future investigations include proteomically analyzing the ablatosome via mass spectroscopy to compare with genomic findings and investigation of the implications of specified signaling abnormalities via cross-species comparisons.

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Student Support: Institute for Critical Technology and Applied Science (ICTAS)

Fatty acids alter endocannabinoid and lipid inflammatory mediator profile during lipolysis in cow adipocytes

Madison Myers, Miguel Chirivi, Yossi Tam, Maya Zachut, G. Andres Contreras

Large Animal Clinical Sci Dept, College of Veterinary Medicine, Michigan State U, East Lansing, Michigan (Myers, Chirivi, Contreras); Institute for Drug Research, School of Pharmacy, Hebrew U of Jerusalem, Jerusalem, Israel (Tam); Dept of Ruminant Science, Institute of Animal Sci, Volcani Center, Rishon LeZion, Israel (Zachut)

The periparturient period in dairy cows is characterized by intense lipolysis and inflammation adipose tissue (AT). When protracted, these conditions predispose cows to disease, decreased milk yield, and culling. Bioactive molecules derived from dietary fatty acids (FAs) include oxidized FA metabolites (oxylipids) and endocannabinoids (eCBs) which modulate lipolysis and inflammation in monogastric species' AT. FA supplementation's role on oxylipid and eCB inflammatory/lipolytic profiles in bovine AT is not yet defined. We hypothesized altering FA availability would alter the eCB and oxylipid profiles in the adipocytes of dairy cows following lipolysis. We supplemented cultured adipocytes from four Holstein cows with FAs found abundantly in cow diets-palmitic (C16:0) and oleic (C18:1) acids-for 14 days. Intense lipolysis was mimicked using isoproterenol (ISO). eCB and inflammatory lipid mediators in adipocytes and media were quantified using liquid chromatography-mass spectrometry. Adipocytes supplemented with FA exhibited enhanced production and retention of several eCBs and like compounds including anandamide, 1-arachidonoyl lysophosphatidic acid, and N-oleoyl taurine upon ISO exposure. Interestingly, these conditions favored synthesis and release of pro-inflammatory molecules (thromboxanes 1/2; prostaglandins B2/E2; leukotrienes D4/E4/F4). These findings suggest that FA availability alters the eCB and oxylipid profile of bovine adipocytes, which may modulate AT lipolysis and inflammation. Considering the widespread availability and use of FA supplements, understanding their effects on inflammation and lipolysis is critical to the improvement of current management strategies and development of future interventions.

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Student Support: USDA-NIFA 2021-67037-34657

The cultured swine: the cellular response of primary porcine oviduct epithelial cells to *Chlamydia*

Lydia Poisson, Jessica Proctor, and Tobias Kaeser

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Chlamydia trachomatis is the most common sexually transmitted bacterial infection with sequelae including salpingitis, ectopic pregnancy, and infertility. Its prevalence, severe disease outcomes, and mixed antibiotic treatment results call for a vaccine; however, despite numerous efforts, none have made it to market. To improve the translatability of pre-clinical results, our overall goal is to develop a vaccine in a biologically-relevant large animal model – swine. A lack of translatability of mouse models could be due to the different mechanisms by which the most important immune molecule, the cytokine interferon-γ (IFN-γ), acts in mouse models versus humans: murine IFN-γ acts through iNOS immune pathways, and human IFN-γ through IDO: a pathway which modulates immune function through tryptophan degradation. To further solidify the similarity between the porcine and human immune response to *C. trachomatis*, the specific goal of this project is to determine if porcine IFN-γ also suppresses chlamydial growth in swine through the activation of IDO. To this end, primary porcine oviduct epithelial cells (pOEC) were isolated and infected with C. suis in the presence of increasing amounts of IFN-γ. Our data show that IFN-γ strongly reduced chlamydial propagation in pOECs. Next, we will perform these infection studies in the absence or presence of an IDO inhibitor. Our hypothesis is that this inhibitor can largely prevent the effect of IFN-γ. If true, this would demonstrate that porcine IFN-γ, as in its human counterpart, acts through the activation of IDO. In turn, this similarity between human and porcine IFN-γ would further solidify the use of swine as a biomedical animal model for *Chlamydia* research and vaccine development.

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Student Support: NCSU Comparative Biomedical Sciences Program

Generation of Macrophages from the Jamaican Fruit Bat and Study of Bat Influenza in Antigen Presenting Cells

Clara Reasoner, Bradly Burke, Susanne Kessler, Wenjun Ma and Tony Schountz

Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO USA (Reasoner, Burke, and Schountz); University of Freiburg, 79104 Freiburg, Germany (Kessler), and Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri (Ma)

New World fruit bats serve as reservoirs for rabies virus, H17N10 and H18N11 bat influenza A viruses (IAVs), and coronaviruses. Because bat influenza A viruses were first detected in New World fruit bats, these species likely serve as the natural reservoir hosts. Unlike other IAVs that use sialic acid residues for cellular binding, the bat IAVs use MHC class II beta chains for cellular entry but do not cause meaningful disease in bats. Because of the high sequence conservation of MHC molecules, the use of MHC class II molecules as an entry mediator leads to the possibility of broad vertebrate tropism. Therefore, the characterization and study of H18N11 infection in professional antigen presenting cells (APC) from New World fruit bats will lead to a greater understanding of the mechanisms by which these species control viral infections and how H18N11 infects APCs. APCs were generated with Jamaican fruit bat (*Artibeus jamaicensis*) bone marrow-derived hematopoietic stem cells (HSC) cultured in Egyptian rousette fruit bat macrophage colony stimulating factor (M-CSF). Cells were characterized using flow cytometry and challenged with H18N11. Cell surface expression profiles of H18N11 infected and naïve macrophages were compared. Functionality was studied using pH-sensitive fluorescent yeast to perform phagocytosis assays. MHC class II upregulation was noted in the infected cells relative to uninfected cells, suggesting a possible mechanism of viral pathogenicity. This is the first evidence of cellular tropism of this virus in bats. These cells are important for the activation and augmentation of the adaptive immune response to viruses. The cultured APCs can be used to define function, viral susceptibility and permissibility.

Research Support: National Institute of Allergy and Infectious diseases (R01AI134768)
Student Support: NIH T32 Training Grant T32GM136628-01

Comparative antifungal efficacy across stages of fungal development in an ex vivo model of fungal keratitis

Darby Roberts, Jacklyn Salmon, Marc Cubeta, Brian Gilger

Department of Clinical Sciences, College of Veterinary Medicine (Roberts, Salmon, Gilger) and Department of Entomology and Plant Pathology, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC

Fungal keratitis (FK) is an invasive infection of the cornea primarily associated with *Aspergillus* and *Fusarium* fungal species. FK is treated empirically with a limited selection of topical antifungals with varying levels of success. Though patients typically present with intracorneal infections characterized by sexually mature mycelium, both in vitro as well as ex vivo models of FK are designed to predict antifungal efficacy response in freshly inoculated infections characterized by sexual immaturity. The purpose of this study was to characterize differences in fungal response and treatment efficacy when topical antifungal treatment is initiated at various progressive stages of fungal maturity in an ex vivo model of fungal keratitis. In brief, porcine corneas were inoculated with 20 µL of a 4x10^6 conidia/mL suspension of either Aspergillus flavus or *Fusarium keratoplasticum* via intrastromal injection. Corneas were submerged in culture medium and incubated while the conidia were allowed to mature for a period of either 0, 24, or 48 hours prior to receiving antifungal treatment consisting of either voriconazole, a second-generation triazole and a gold-standard treatment option for FK, or luliconazole, a novel imidazole. Our data demonstrates a suppressed fungistatic effect of either azole when initiated at 48 hours compared to 0 or 24 hours. With these results, we advise that a revised experimental model which evaluates drug efficacy when applied to a sexually mature fungal organism will more closely predict fungal response in the clinical setting where treatment is often not initiated until days after the initial insult.

Research Support: None
Student Support: The Gilger Lab, NCSU CVM

Avian vaccination via recombinant *Lactobacillus*-bound birdseed to curb the spread of West Nile virus

Michelle J. Savran, Benjamin Swartzwelter, Preston Schweiner, Allison Vilander, Gregg Dean, Brian D. Foy

Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

West Nile virus (WNV) is the leading cause of domestically acquired mosquito-borne disease in the United States. Despite significant investment, no effective human WNV vaccines have been developed, so current mitigation efforts remain limited to environmentally toxic insecticidal sprays. While humans and other animals can develop disease, they are dead-end hosts because they do not develop high enough viremia to infect other mosquitoes. Instead, propagation of WNV is primarily maintained between mosquitoes and birds. Thus, we hypothesize that immunizing WNV-susceptible birds will reduce WNV transmission to mosquitoes, protecting both people and animals from infectious bites and disease. To this end, we are genetically modifying a strain of the probiotic *Lactobacillus acidophilus* (LA) to express WNV antigenic proteins premembrane (prM) and envelope (E). The bacteria can be administered orally and deliver intact viral protein to mucosal immune inductive sites. Immunogenicity is enhanced by the addition of a dendritic cell targeting peptide (DCpep). Protein expression by the LA-based vaccine (rLA-WNV) will be assessed by Western blot and flow cytometry. Immunogenicity will be measured by vaccinating chickens and assessing development of anti-WNV antibodies and measuring viremias following WNV challenge. rLA-WNV will then be bound to birdseed for wild bird consumption. We selected this strategy because 1. it is only practical to immunize wild birds orally with food baits in WNV endemic areas, and 2. LA can be lyophilized, preserving its viability and stability such that we can bind it to bird seed. The strategy, if successful, will result in an innovative and cost-effective strategy for control of vector-borne disease.

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Student Support: CVMBS T32 Dual-Degree Medical Scientist Training Program for Veterinarians Training Award

Comparison of the reliability of two owner surveys aimed to assess canine cognitive dysfunction syndrome

Katherine E Simon, Katharine Russell, and Natasha J Olby

Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine Raleigh, NC

Geriatric dogs suffer from Canine Cognitive Dysfunction Syndrome (CCDS), a disorder characterized by disorientation, anxiety and changes in social interaction, elimination behaviors and sleep-wake cycles. CCDS is challenging to diagnose and requires a multifaceted approach including physical examination, blood work and brain imaging. However, owner questionnaires that capture the neurobehavioral changes occurring in the home are key to diagnosis. While scales for dementia have been validated, we observed inconsistencies in owner responses when administered in longitudinal studies. Here, we evaluated the repeatability of two CCDS surveys, the Canine Dementia Scale (CADES) and the Canine Cognitive Dysfunction Rating Scale (CCDR) in a population of 60 senior dogs over a six-month period. We hypothesized that score repeatability will vary between the two surveys. At three separate time points, owners completed both surveys and then repeated each survey utilizing a format which showed their answers from the previous time point (Owner Survey with Prior Answers, OSPA). We compared the owner's initial and OSPA responses using a paired t test. The CADES initial and OSPA responses were extremely variable with a mean difference of 3.56 and standard error of 0.60. The CCDR responses differed less with a mean difference of 0.65 and standard error of 0.26. However, both CADES and CCDR initial survey responses were significantly different from their OSPA responses ($p<0.0001$ and $p=0.0116$ respectively). We conclude that owners provide more consistent answers using CCDR in longitudinal studies when compared to CADES. Future studies are needed to determine which survey captures the effect of therapies most reliably.

Research Support: Animal Biosciences
Student Support: NC State University Provost’s Doctoral Fellowship

Linking and elucidating ontogeny of *Acanthostomum* (Digenea) in *Alligator mississippiensis* using DNA barcoding

James Carey Valentine and Thomas Graham Rosser

College of Veterinary Medicine, Mississippi State University, Starkville, MS T

The genus *Acanthostomum Looss* 1899 (Digenea, Cryptogonimidae) consists of over 20 described trematode species that parasitize fish, alligators and other crocodilians, causing damage to the gut tissue of their hosts. *Acanthostomum* has a complex 2 to 3-host life cycle, including snails, reptiles and fish. Despite a rich history consisting of novel host and geographic records, there are still many questions remaining about their taxonomy and systematics, pathogenesis in each host and their life histories. Morphological characteristics have been used to identify *Acanthostomum* spp., but two areas that require further investigation include (1) elucidating the various life stages and (2) conducting phylogenetic analyses using molecular data to infer evolutionary relationships among different species. Several *Acanthostomum* spp. have been reported from crocodilians globally, but molecular data assessing their diversity and intraspecific variation have not been fully explored. In this study, ribosomal and mitochondrial data were sequenced from adult *Acanthostomum* spp. collected from American alligators *Alligator mississippiensis* and larval metacercariae from the muscle of spotted gar *Lepisosteus oculatus* from the southeastern United States. At least 3 hologenophores were prepared by cutting a section from each worm, extracting its DNA, and then permanently staining and mounting them. Additionally, a whole worm was processed for DNA analysis. The DNA was used to assess the utility of DNA barcoding for linking individuals to specific species and to infer phylogenies using both ribosomal and mitochondrial DNA in conjunction with novel morphological characteristics.

Research Support: None

Student Support: Office of Research and Graduate Studies

Elucidating sex differences in the response to anterior cruciate ligament injury following mechanical rupture

Emily M. Van Zeeland, Brandon Kassel, Travis Montoya, Kelly S. Santangelo, Jeremiah T. Easley, and Katie J. Sikes

College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, Colorado

Following anterior cruciate ligament (ACL) injury, up to 87% of individuals develop post-traumatic osteoarthritis (PTOA) in their knee joint. Although it is well established that females are more likely to experience an ACL injury compared to males, it is poorly understood if sex differences contribute to the development of PTOA. The aim of this project was to examine the variable injury responses of males and females following ACL rupture using a refined mechanical rupture model. Unilateral ACL rupture was achieved via mechanical compression in male and female mice. Longitudinal mobility analyses were conducted prior to ACL rupture and weekly out to eight weeks post-injury. No significant differences were observed between male and female mice at baseline for all parameters. Relative to uninjured baseline values, male mice exhibited decreased voluntary distance traveled compared to female mice at five weeks following ACL injury ($p=0.0086$). Compared to female mice, males showed increased hindlimb stance width on a flat treadmill relative to baseline values ($p=0.0271$). These changes suggest male mice are minimizing voluntary movement after ACL rupture. Further, increased hindlimb stance width seen in male mice compared to females suggests male mice are modifying their gait due to a pain response and/or compensating for decreased stability. These observations could indicate differences in injury responses, pain perception, and/or PTOA development between males and females. Understanding the early injury differences between males and females may elucidate novel therapeutic mechanisms and surgical reconstruction techniques for an overall goal of improving ligamentous healing following rupture to mitigate PTOA progression.

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Myoblast extracellular vesicle production, function, and miRNA cargo are altered by mechanical stimulation

Katherine Williams, Michael Mullen, Thomas LaRocca, Chelsea Bahney, Karyn Hamilton, Nicole Ehrhart

Department of Clinical Sciences (Williams, Ehrhart) and Department of Health and Exercise Science (LaRocca, Hamilton), College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado; Center for Regenerative and Personalized Medicine, Steadman Philippon Research Institute, Vail, Colorado (Mullen, Bahney).

The regenerative capacity of skeletal muscle declines with advanced age, resulting in a decreased ability to recover from injury which contributes to frailty, hospitalizations, and mortality. Accordingly, there is a critical need to develop therapeutics promoting aged muscle repair. Recent findings from our lab demonstrate that in vitro mechanical loading increases C2C12 myoblast extracellular vesicle (EV) production and that these EVs accelerate myoblast proliferation and differentiation– key requisites for myogenesis and regeneration. MicroRNA (miRNA) sequencing revealed that 35 miRNAs are significantly downregulated in mechanically strained EVs compared to static EVs. The gene ontology (GO) terms associated with the mRNA targets of these downregulated miRNAs involve developmental, neural, cell signaling, transcriptional regulation, metabolic, and inflammatory processes. In particular, mechanical loading alters EV-contained miRNAs that target key muscle regeneration signaling pathways, including the MAPK cascade, IGF signaling, and BMP signaling. Current work aims to identify differential miRNAs and their mRNA targets within EVs derived from mechanically loaded primary myotubes, a biologically relevant model for skeletal muscle strain. Future work will determine if delivery of these EVs improves aged muscle regeneration in a mouse model of in vivo muscle injury. Ultimately, this research will guide strategies for the development of EV therapeutics targeting the age-associated decline in skeletal muscle regenerative capacity.

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Synovial fluid proteomics reveals periostin as a biomarker for ACL injury in humans and dogs

Sydney Womack, Camila Carballo, Scott Rodeo, and Heidi Reesink

Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY (Womack, Reesink); Hospital for Special Surgery, New York, NY (Carballo, Rodeo)

Osteoarthritis (OA) following acute trauma to the knee joint is common among multiple species, including dogs and humans, who both experience OA following tear of the anterior cruciate ligament (ACL) (analogous cranial cruciate ligament (CCL) in dogs). Symptoms of post-traumatic OA (PTOA) such as pain and stiffness are conserved across these species, supporting dogs as spontaneous animal models for human PTOA following ACL injury. Proteomics has been used previously to reveal possible biomarkers of human knee OA but this technique has not been used extensively in veterinary species and never through a multispecies lens, comparing two species. Synovial fluid from stifle joints of 8 dogs with spontaneous ACL injury, 8 dogs with no history of joint disease, and 8 humans with unilateral ACL injury (with contralateral knee serving as a control) was collected, and liquid chromatography/tandem mass spectrometry was used to identify proteins in these samples. Proteins were screened for statistical significance with a p-value < 0.1, false discovery rate (FDR)-corrected, then ranked by fold change. Principal component analysis showed distinct PTOA and control groupings among both species; several proteins were similarly up- and down-regulated in dogs and humans. Periostin was the most upregulated protein in dog and human samples individually as well as the top-ranked protein overall in multispecies analysis. Other proteins were also conserved between species, including vimentin and α-2-macroglobulin. Similar trends in humans and dogs with analogous injuries provides support for dogs as a spontaneous animal model for human ACL injury as well as the detection of more robust biomarkers and therapeutic targets.

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Colloquium Planning Team

Eleanor Pressman
Student Coordinator

Emily Herring
Student Secretary

Juselyn Tupik
Student Colloquium Aide

Kate Williams
Student Communications Lead

Carley Dearing
Student Colloquium Aide

Daniel Regan
Faculty Director, CSU

Albert Mulenga
Faculty Director, Texas A&M

Renata Ivanek Miojevic
Faculty Director, Cornell

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