Student Research Day

March 17, 2023

University of Pennsylvania School of Veterinary Medicine

Vernon & Shirley Hill Pavilion
Program

11:15 a.m.  **Boxed Lunches & Registration**  H132 & Hill Lobby

12:00–12:15 p.m.  **Opening Remarks**  H131 Billhardt Auditorium
Andrew M. Hoffman, DVM, DVSc
The Gilbert S. Kahn Dean of Veterinary Medicine

12:15–2:20 p.m.  **Student Presentations**  H131 Billhardt Auditorium

12:15–12:20 p.m.  **Introduction of short-term project oral presenters—Dr. Mike May**

12:20–12:32 p.m.  **Rebecca Brisman: Identification and Functional Analysis of Dirofilaria immitis Products Secreted During Infection of Aedes aegypti Mosquitoes**  (Mentor: Dr. Michael Povelones)

12:32–12:44 p.m.  **Katherine Morucci: Peri-Urban Cave Dogs Threaten Rabies Elimination Programs in Arequipa, Peru**  (Mentors: Drs. Ricardo Castillo-Neyra and Michael Levy)

12:44–12:56 p.m.  **Nimisha Pattada: Does Disruption of FAP⁺ Stromal Cells by FAP-CAR T Cells Enhance the Efficacy of Immune Checkpoint Inhibitor?**  (Mentor: Dr. Ellen Puré)

12:56–1:08 p.m.  **Rachel Tevere: Chronic Social Defeat Stress in Group Housed Breeding Swine**  (Mentor: Dr. Thomas Parsons)

1:08–1:30 p.m.  **Break**  H132 & Hill Lobby

1:30–1:35 p.m.  **Introduction of long-term project oral presenters—Dr. Liz Lennon**  H131 Billhardt Auditorium

1:35–1:50 p.m.  **Jaclyn Carlson: Collagen III Deficiency Following Injury in Female Murine Tendons Alters Mechanical and Structural Tendon Properties**  (Mentors: Drs. Louis Soslowsky and Susan Volk)

1:50–2:05 p.m.  **Elisabeth Lemmon: Anakinra Reduces Inflammatory Pathways Activated in Canine Synovium after Cranial Cruciate Ligament Injury**  (Mentors: Drs. Robert Mauck and Kimberly Agnello)

2:05–2:20 p.m.  **Martha Stone: Resistance to Arousal State Transitions in Aged Mice**  (Mentor: Dr. Max Kelz)

2:20–3:35 p.m.  **Poster Session — Introduction by Dr. Elizabeth Woodward**  H131 Billhardt Auditorium

2:20–2:50 p.m.  **Poster Slam—A video presentation played on a loop**  H131 Billhardt Auditorium

2:50–3:25 p.m.  **Poster Crawl***  Hill Lobby

2:50–3:25 p.m.  **Session I: Odd-numbered posters**  Hill Lobby

2:55–3:25 p.m.  **Session II: Even-numbered posters**  Hill Lobby

3:35–4:30 p.m.  **The Class of 1966 Lectureship**  H131 Billhardt Auditorium

3:35–3:40 p.m.  **Keynote speaker introduction by Stephanie Sila**

3:40–4:30 p.m.  **Genetics of Cancer, Morphology and Aging in Dogs**  40 min talk with 10 min Q & A
Elaine A. Ostrander, PhD
Chief and Distinguished Senior Investigator
Cancer Genetics and Comparative Genomics Branch
National Human Genome Research Institute
National Institutes of Health

4:30–5:30 p.m.  **Awards and Reception**  H131 Billhardt Auditorium
Awards presented by Dr. Phillip Scott, Vice Dean for Research and Academic Resources, and Student Research Club representative Nimisha Pattada and Hill Lobby
Poster Slam Voting

Scan this QR code:

Or follow this link:
https://tinyurl.com/mwrtd4zk

Posting From the Event?

Use #ResearchAtPennVet

Scan this QR code:
In 1991, a gift from the Class of 1966 established an endowed fund to support keynote lectures at the School of Veterinary Medicine.

Elaine A. Ostrander, PhD

Cancer Genetics and Comparative Genomics Branch
National Human Genome Research Institute
National Institutes of Health

Genetics of Cancer, Morphology and Aging in Dogs

Dr. Elaine Ostrander is Chief and Distinguished Investigator of the Cancer Genetics and Comparative Genomics Branch at the National Human Genome Research Institute of NIH, and Head of the Section on Comparative Genetics. She received her PhD from Oregon Health Sciences University and did postdoctoral training at Harvard and UC Berkeley. Dr. Ostrander has published over 375 papers and won several awards including the Asa Mays Award, the International Canine Health Lifetime Achievement Award, and the 2013 Genetics Society of America Medal. She is an AAAS Fellow and was elected to the National Academy of Sciences in 2019. The Ostrander lab is interested in understanding the genomic factors that control the enormous amount of morphologic, disease susceptibility, and behavioral variation observed in canines across the world.
Acknowledgments

We acknowledge **Boehringer Ingelheim Animal Health** for their support of Student Research Day. Moreover, we wish to acknowledge the **NIH/Boehringer Ingelheim Summer Research Program** that is designed to expose students in their first or second year of veterinary school to all phases of biomedical research.

We are grateful to the sponsors, donors, and families of our patients, without whom our research would not be possible.

**Richard O. Davies Fund:** Established in 2007, by former students and colleagues of Dr. Richard O. Davies, this fund provides support for an award or fellowship to be given at the discretion of the Department of Biomedical Sciences.

**The Class of 1966 Endowed Lectureship:** A gift from the Class of 1966 established an endowed fund in 1991 to aid and support annual major lectures at the School of Veterinary Medicine.

*Photos: John Donges*
Special Thanks

Organizing Committee

Chair:

Jennifer A. Punt, AB, VMD, PhD

Faculty:

Elizabeth M. Lennon, DVM, PhD
Michael J. May, PhD
Phillip Scott, PhD
Elizabeth M. Woodward, PhD

Students:

Nimisha Pattada
Stephanie Sila

Abstract and Poster Judging

Abstract and Poster Judges:

Matthew J. Atherton, PhD
Elizabeth M. Lennon, DVM, PhD
Frank Luca, PhD
Michael J. May, PhD
Jennifer A. Punt, AB, VMD, PhD
Elizabeth M. Woodward, PhD

Heartfelt thanks to Associate Dean for Education Kathryn E. Michel for her foundational efforts in establishing, growing, and professionalizing Student Research Day.

We are grateful to Dr. Ellen Puré, Chair, Department of Biomedical Sciences, for generously offering the Richard O. Davies Fund to be used for student awards.

Our thanks to the Student Research Club, Steven Atchison, John Donges, Stephen Hawkins, Ashley Hinton, Lydia Melnyk, Penn Vet Facilities Services, and Penn Vet Information Technology, who provided much-needed organizational, technological, photographic, promotional, and logistical assistance.
Phi Zeta was originated in 1925 by a group of senior veterinary students in the New York State Veterinary College at Cornell University. With the assistance of a group of faculty members, including the Dean of the College, Dr. Veranus A. Moore, the Society was formally organized, and Dean Moore was elected as the first president of the Alpha Chapter. The Society of Phi Zeta was organized in 1929 at a meeting in Detroit, Michigan, and Dean Moore became the first president of the Society. Also, in 1929, a charter was granted to the School of Veterinary Medicine at the University of Pennsylvania, and the Beta Chapter was established. In 1931, the Executive Committee approved the petition of a group from Iowa State College, and the Gamma Chapter was established. Since then, twenty-four chapters have been chartered, bringing the total number of chapters to twenty-seven. Chapters of the Society may be formed at any recognized veterinary medical college or at any other institution of higher learning.

Name and Symbols of the Society: The organizers of the Society, when seeking a suitable name, sought the help of a learned Greek scholar, Professor George P. Bristol, of Cornell University. Professor Bristol suggested a Greek word, which in the Latin form is spelled PHILOZOI and means "love for animals." The abbreviation of Phi Zeta was adopted as the name of the society.

University of Pennsylvania Officers—Beta Chapter

Dr. Margret Casal, President

Class of 2024
Kara Anderson, Rebecca Brisman, Lauryn Cooper, Tryssa de Ruyter, Nicole DeRogatis, Alyssa Gargagliano, Suna Li, Kaitlin Murphy, Jacob Rabin, Katherine Reilly, Alyssa Marie Silverman, Jaclyn Soulas, Julia Supino, Bridgette Zerbe

Class of 2023

Inducted as Juniors (V23)
Nicholas Anderson, Alexandra Cabra, Leslie Concepcion, Margaret Duchene, Kay Malia Foos, Brent Gerbec, Isabella Rose Healy, Robert Maxwell Holzman, Jaesun Lee, Rachel Siskind, Josie Thal, Anna Tran, Alessandro Valle
Abstracts

Oral Presentations: Indicated by an asterisk *

Poster Presentations: Abstract numbers correspond to the poster board number.

1. Maria A. Bartolucci
2. Natalie Bauer
3. Estefania D. Benavides
4. Rebecca Brisman *
5. Dana Bubka
6. Jaclyn Carlson *
7. Bridget Cincotta
8. Megan Clark
9. Kaelea Composto
10. George DeMers
11. Jessica F. DiStefano
12. Andrew Dunlap
13. Kayla M. Even
14. Kay Foos
15. Elisse Friedman
16. Elizabeth Gregorio
17. Mary Kate Grubbs
18. Philip Hicks
19. Emerson Hunter
20. Antonina Kalkus
21. Elisabeth A. Lemmon
22. Elisabeth A. Lemmon *
23. Kate Marciano
24. Katherine Morucci *
25. Amanda Patev
26. Nimisha Pattada *
27. Mackenzie Pickford
28. Alexander Post
29. Corisa Y. Quincey
30. Brinkley Raynor
31. Juliana Reynoso
32. Daana Roach
33. Melissa Seiberlich
34. Stephanie Sila
35. Martha Stone *
36. Rachel Tevere *
37. Ashley Vanderbeck
38. Yucheng Wu
Abstracts

Abstracts are listed in alphabetical order by the presenting student's last name.

1. **Towards a Better Understanding of Meningoencephalitides of Unknown Origin.**

   **Maria A. Bartolucci**, Molly E. Church, and Jorge I. Alvarez.

   1 Delaware Valley University, Doylestown, PA; 2 Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

   Meningoencephalitides of unknown origin (MUO) is a broad term for a group of canine neuroinflammatory diseases with unknown etiologies. Diseases within this broad category include Granulomatous meningoencephalitis (GME), Necrotizing meningoencephalitis (NME), and Necrotizing leukoencephalitis (NLE). The purpose of this study is to summarize demographic and clinical findings in a large cohort of GME cases in an effort to identify patient, clinical, and environmental factors associated with outcome. This retrospective study was conducted by searching the archives of the Penn Vet Diagnostic Laboratories anatomic pathology database for GME cases and reviewing associated clinical records. Information evaluated included: patient signalment, lesion location, date of diagnosis, date of presentation, and date of death for each canine patient. A total of 28 cases were evaluated with a total of 16 males (3 intact males, 13 castrated males), 12 females (1 female intact, 11 females spayed). Survival time was determined as the length of time from the date of initial clinical signs to the date of death. It was found that altered females survived 27.4 days, altered males survived 54.1 days, females intact survived for 1 day and males intact survived for 7.7 days. While patients with only brain lesions had a 46.5-day average survival time, patients with both spinal cord and brain lesions had a 27.8-day average survival time and patients with spinal cord lesions had an average survival time of 12 days. The seasonality of this disease is impacted most in the fall and winter months of 16 months, versus the spring and summer months of 12 months. While our findings do not support previous reports of a propensity for GME in females relative to males, we show that average survival time is shorter for female patients relative to male. Interestingly, patients with brain lesions had a longer average survival time than patients with lesions in the spinal cord. Future studies will expand data collected to include cases of other MUO subtypes as well as cases from veterinary hospitals across the country.

2. **Detecting SARS-CoV-2 in White-Tailed Deer (Odocoileus virginianus) Lymph Nodes Using Real-Time RT-PCR.**

   **Natalie Bauer**, Erick Gagne, and Eman Anis.

   The Wildlife Futures Program, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

   SARS-CoV-2 is the causative agent of the COVID-19 pandemic. It has subsequently spilled over from people into multiple animal species due to the broad host range of this coronavirus along with its widespread distribution. Established infections in wild species could lead to accelerated evolution of the virus as well as novel variant strains with increased pathogenicity and the capacity to escape acquired or vaccine derived immunity in people.

   Studies have shown that white-tailed deer throughout the United States have high levels of SARS-CoV-2 infection. PCR testing of retropharyngeal lymph nodes in free-living and captive deer from Iowa found 94 of the 283 samples were positive with a total of 12 different SARS-CoV-2 lineages isolated. Based on this evidence, deer could be established as an expansive reservoir host with the risk of infecting other wild animals and possible reemergence back into humans.

   The purpose of this research is to determine the prevalence of positive COVID-19 cases in Pennsylvania deer over time by testing lymph node samples collected between 2019-2021 for the presence of SARS-CoV-2. Viral RNA will be extracted and identified through RT-qPCR. Having temporal samples available for genetic material detection can help provide valuable insight into the course of outbreak among the deer population. To effectively prepare for potential risks
associated with spillover infections, it is imperative to recognize the variant types and how long deer can shed the pathogen into the environment. Genome sequencing could then compare virus strains to determine shared lineages with humans and facilitate better understanding of the evolution of SARS-CoV-2.

3. The Correlation of Hippocampal Microgliosis and Astrogliosis with Severity of Heart Disease in Dogs.

Estefania D. Benavides, Margaret M. Sleeper, Molly E. Church, and Charles H. Vite.

Hypotension can result in brain hypoxia, resulting in fainting or syncope if acute. Cardiomyopathy places the brain at risk for these events when entering severe stages of heart failure. About 2% of the human population has congestive heart failure while 75% percent of these are individuals older than 65 years of age. As compared to the canine population, a very common degenerative process, myxomatous valve disease, occurs in about 80% of small dogs, 30% of which progress into mitral regurgitation. Mitral regurgitation places the patient at risk for developing cardiomegaly and potentially congestive heart failure, particularly in late stages; thus, increasing the risk for neurovascular malperfusion. It has been well established that hypoxic events, depending on the duration and severity, can result in cognitive dysfunction in humans, presenting as neurodegeneration in certain areas of the brain (i.e., hippocampus). Previous studies noted cognitive impairments such as anxiety, depression, and memory loss in post-coronary bypass and in heart failure. This study focused on the relationship between cardiac insufficiency and neurodegeneration in canines while the localizing lesions such as microgliosis and astrogliosis as indicators of chronicity within the brain. Using immunofluorescence, degenerative lesions were localized from the brain samples acquired from 14 deceased canine patients. This pool is comprised of patients diagnosed with mild, moderate, and severe heart disease and a form of idiopathic neuronal degeneration. A relationship between severity of heart disease and degree of astrogliosis was not detected in this study. However, there was a correlation between microgliosis and moderate to severe heart disease.

ORAL PRESENTATION

4. Identification and Functional Analysis of *Dirofilaria immitis* Products Secreted During Infection of *Aedes aegypti* Mosquitoes.

Rebecca Brisman, Abigail R. McCrea, Shane Denecke, Sutopa Dwivedi, and Michael Povelones.

The parasitic nematode *Dirofilaria immitis* causes heartworm disease worldwide in domestic dogs, cats, and ferrets. Since mosquitoes are required as both an intermediate host and vector for *D. immitis*, they provide a unique opportunity to disrupt the heartworm transmission cycle. In a competent vector, microfilariae ingested during blood feeding migrate from the midgut into the Malpighian tubules, the mosquito renal organ. Here, parasites develop into infectious third-stage larvae (L3) over a two-week period. L3 migrate through the body cavity to the proboscis where they can be transmitted during the next blood meal. Given that the hemolymph, the fluid filling the body cavity, contains high concentrations of immune proteins, it is not known how parasites can survive without being eliminated. The aim of this study is to investigate the interaction between L3 and *Aedes aegypti* mosquitoes. To begin, we sought to elevate the mosquito immune response to overcome the ability of larvae to modulate mosquito immunity. The Toll immune signaling pathway was activated through gene silencing using RNAi by injecting dsRNA 14 days after infection when L3 are present in the body. The ability of L3 to emerge from mosquitoes was then evaluated to determine whether they were. The results showed that enhancing immune signaling through gene silencing on day 14 of infection does not significantly impact the number of L3s that emerged infected mosquitoes ($p = 0.77$). Additionally, mosquito hemolymph was analyzed using bioinformatics pipelines for the presence of *D. immitis* proteins that could potentially modulate mosquito immunity. These analyses revealed several differentially expressed proteins of interest that could play a role in evading the mosquito
immune system. By learning more about these transcriptional changes and products that *D. immitis* secrete to evade the mosquito immune response, this data can be used to devise novel strategies to reduce heartworm incidence.

**Research Support:** Morris Animal Foundation Grant (D22CA-015)

**Student Support:** NIH T35 OD010919, Boehringer Ingelheim, and the University of Pennsylvania

5. **Impact of Common Bedbug Infestations on Laying Hen Health and Welfare.**

Dana Bubka¹, Meghann Pierdon¹, Kris Smith¹, and Koranda Walsh².

¹Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; ²Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Poultry experience a wide-ranging group of pests that can spread quickly throughout a flock, negatively impact their health and welfare, and can prove challenging to eradicate. In particular, the Common Bedbug (*Cimex lectularius*) is a hematophagous ectoparasite that shows no host specificity, and so can prove particularly insidious for both the workers and birds alike, and has a notorious reputation for being extremely difficult to exterminate. The objective of this study is to quantify the effects of a Common Bedbug infestation on laying hen health and welfare. Four flocks of approximately 500,000 commercial caged white layer hens were selected for this study: two bedbug-free control flocks, and two naturally infested flocks. Each flock was studied for 5 consecutive days. 500 hens from each flock were randomly selected to be evaluated via the Welfare Quality Assessment (WQA) tool, while a separate group of 50 hens was randomly selected from each flock for blood collection to determine PCV, lymphocyte to heterophil ratio, and corticosterone levels. Each day, four cameras were placed randomly throughout the flock to assess hen behavior over the course of 3 hours. Flock records were also collected to evaluate production, and photographs of bedbug groupings were taken at each house to determine infestation levels. Preliminary analysis of the WQA data and production records appears to show the infested flocks scored worse on two of the welfare indicators and maintained a lower feed conversion ratio than the control flocks. However, the PCV results have shown no difference between the control and infested flocks. Hopefully, the final results of this research will significantly enhance the current understanding of the impacts of the Common Bedbug on commercial laying hens and provide a stepping stone for further research that will lead to more successful methods of eliminating these infestations in poultry farms.

**Research Support:** Center for Livestock and Poultry Excellence

LONG-TERM PROJECT

ORAL PRESENTATION

6. **Collagen III Deficiency Following Injury in Female Murine Tendons Alters Mechanical and Structural Tendon Properties.**

Jaclyn Carlson¹, Stephanie N. Weiss¹, Susan W. Volk², and Louis J. Soslowsky³.

¹McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA; ²School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Tendon is composed of a highly aligned type I collagen-rich matrix, allowing it to withstand large loads. Following injury, matrix disruption results in a shift to a type III collagen (Col3)-rich matrix and susceptibility to excessive scarring and loss of structure and mechanical function. The objective of this study was to define the role of Col3 in response to injury through examination of fibril diameter and resultant changes in tendon mechanics. We hypothesized that a reduction in Col3 leads to an increase in fibril diameters, due to the regulatory role that Col3 plays in fibrillogenesis, resulting in a stiffer matrix with improved mechanical properties.

Expression of *Col3a1* was decreased at all time points post-injury in *Col3a1*⁻/⁻ tendons. Stiffness, failure stress and modulus were increased at both 1- and 3w post-injury, and failure load was increased 3w post-injury in *Col3a1*⁻/⁻ compared to WT tendons. Dynamic modulus was decreased 1- and 3w post-injury in WT tendons at 6% strain. Fibril diameters had an
increased population of larger fibrils in both uninjured and 3w post-injury Col3a1 +/- tendons when compared to WT distributions. Interestingly, Col3a1 +/- tendons 6w post-injury had a larger population of smaller diameter fibrils when compared to WT.

An increased population of larger fibrils 3w post-injury in Col3a1 +/- tendons likely influenced increases in mechanical properties. Genotypic differences in mechanics are no longer seen by 6w post-injury, potentially due to a distribution shift of WT fibrils to a larger population, while the Col3a1 +/- tendon fibril diameter distribution remains similar to 3w. This indicates that WT fibrils continue fibrillogenesis later post-injury, while Col3a1 +/- fibrils lateralize earlier in the healing process due to dysregulation of fibrillogenesis. The abundance of Col3 affects the structural and functional properties of the healing tendon, implicating Col3 as an important regulatory molecule of healing and a future therapeutic target.

Research Support: Penn Center for Musculoskeletal Disorders (AR069619), R01 GM124091 and R01 AR080029

7. Antibiotic Use for the Treatment of Lameness in Sows.

Bridget Cincotta and Meghann Pierdon.
School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Documented to be a common medical issue afflicting sows, lameness can have a severe negative impact on the welfare of animals and economic success of swine farms. To combat such effects, antibodies are often used to treat sow lameness. In this study, treatment records were collected from 3 sow farms using pen gestation, including a small farm with 258 sows and two larger farms with 5,232 and 5,273 sows respectively. Total antibiotic use was first calculated as the total milligrams of product used. Antibiotic use rate was then calculated as the total milligrams of antibiotics used per average head of inventory per day to standardize the measure across the farms with different herd sizes. Over a one-year period, 0.746 mg/sow/day of antibiotics were used across all farms for individual treatments. Lameness was responsible for more antibiotic use than any other treatment across all three farms, accounting for 72.7% of the total milligrams of antibiotics used. With regards to lameness specifically, 93.7% of the milligrams of antibiotic used was lincomycin and 6.2% were ceftiofur. These two antibiotics that were most used to treat lameness, are both classified by OIE as second level (of three) antibiotics. This indicates they should be used with care due to the potential threat of antimicrobial resistance. Given this level of concern, it is worth finding ways to prevent lameness to preclude their use.

LONG-TERM PROJECT

8. Remodeling the Electron Transport Chain in Macrophages by a Peptide-miRNA Axis.

Megan Clark1,4-3, Kamen Simeonov4,5, and Jorge Henao-Mejia1,3.
1Department of Pathology & Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Veterinary Medical Scientist Training Program, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 3Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 4Medical Scientist Training Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 5Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Macrophages are critical innate immune cells necessary for anti-pathogen immunity, cancer immunosurveillance and wound healing. Powering these diverse responses in macrophages is the dynamically regulated electron transport chain (ETC) within the mitochondria. An important regulator of ETC function is complex IV (CIV), which is critical for oxygen consumption, ATP generation, and cell survival. Intriguingly, CIV is the only respiratory complex which remodels its protein subunit composition in response to environmental stimuli, such as hypoxia, to modulate its function. This is achieved through the upregulation of subunit isoforms which replace highly homologous core CIV subunits that function to improve cellular fitness. However, whether immune cells remodel CIV subunit composition during immune responses and whether this remodeling is important for myeloid cell function is unknown. To this end, we identified a single transcript induced in pro-inflammatory macrophages which encodes a miRNA and a highly conserved peptide with striking homology to a core CIV subunit.
My data, in concordance with recent studies, indicate that this peptide/miRNA axis work in concert to replace the core CIV subunit with its co-encoded peptide homolog. Strikingly, while remodeling of CIV subunit composition by this peptide/miRNA axis was dispensable for ATP production, we showed that this subunit switch played a critical role in regulating measure of mitochondrial stress, such as mitochondrial morphology, membrane potential and mitochondrial ROS. To investigate the functional relevance of CIV subunit remodeling in macrophages by this peptide/miRNA axis, we examined tumor-associated macrophage (TAM) responses in mice lacking the peptide/miRNA axis, or those lacking the core CIV subunit which is replaced. To do so, we challenged these mice and wild-type counterparts with melanoma, thymoma or pancreatic ductal adenocarcinoma. Strikingly, tumor growth was drastically abrogated in mice lacking the core CIV subunit, whereas the growth rate of tumors was significantly augmented in mice lacking the peptide and miRNA. These results suggest that presence of the core CIV subunit in TAMs is critical for tumor growth, as its genetic deletion or its degradation by the peptide and miRNA dramatically restricts tumor progression. Intriguingly, we found that in tumors from mice deficient in the core CIV subunit, there was an accumulation of TAMs that were rich in interferon-stimulated genes and polarized towards pro-inflammatory macrophages, suggesting that remodeling CIV impacts TAM polarization and in vivo anti-tumor function.

Research Support: NIH R01 – NHLBI, Mark Foundation Center for Radiobiology and Immunology
Student Support: NIH F30AI154694


Kaelea Composto1, Jesse Miller2, Kasirajan Ayyanathan3, Kanuypriya Whig2, Brinda Kamalia5, David Schultz2, Andrew Vaughan4, Lenka Dohnalova1, Christoph Thiass2, and Sara Cherry1,2,3.

1Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; 2Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA; 3Department of Microbiology, University of Pennsylvania, Philadelphia, PA; 4School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

SARS-CoV-2, a positive sense, single stranded RNA virus has infected more than 555 million people and led to over 6 million deaths in the past two and a half years (WHO-https://covid19.who.int/). In the search for potential therapeutics, a library of approximately 3,000 compounds has been screened for antiviral activity against SARS-CoV-2 (ancestral strain WA1) infection in Calu-3 cells which are a respiratory epithelial cell line. Compounds that displayed antiviral activity without toxicity in this cell-based microscopy assay were further characterized. We identified 12 drugs that showed antiviral activity. Antiviral activity was further tested against the Omicron variant which represents more contemporary strains. In addition, we performed quantification of the viral RNAs in both Calu-3 cells and a second cell line, A549 transduced with human ACE2. UPNUC-001, -003, -006, -007 and -009 reduced Omicron infection up to 1,000-fold and Washington infection up to 10,000 fold in Calu-3 cells. UPNUC-009 reduced both Omicron and Washington infection ~100 fold in A549-ACE2 cells. The mechanism of action of these drugs is not known but should be explored as these compounds display promise as novel antiviral drugs. Additionally, a murine air-liquid interface (ALI) model is being developed as a physiologically relevant model to study drug efficacy and viral infection using genetically modified animals. Respiratory epithelial cells are isolated from murine nasal turbinates, lungs and trachea and selected. Basal cells, a respiratory epithelial stem cell, will be expanded prior to proliferation and differentiation on transwell inserts. This method will allow for us to test the role of genetically modified mice for susceptibility to infection as well as drug efficacy. Overall, these studies may contribute to the identification and characterization of novel antiviral agents and genes that impact susceptibility that could treat pandemic coronavirus infections.

Research Support: National Institute of Health, Penn Center for Precision Medicine, Mercatus, the Bill and Melinda Gates Foundation, Boehringer Ingelheim, and the University of Pennsylvania
Student Support: NIH/ Boehringer Ingelheim
10. The Effect of Dietary Zinc on *C. difficile* Colonization and Pathogenesis in Neonatal Piglets and Dairy Calves.

George DeMers, Joseph Zackular, Meghann Pierdon, and Laurel Redding.

Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

*Clostridioides difficile* is a significant enteric pathogen capable of causing severe and sometimes fatal diarrhea in both humans and livestock, including neonatal swine and cattle. Transition metals such as zinc play an essential role in gut microbiota ecology and alter susceptibility of the host to enteric infections. In mice, excess dietary zinc has been shown to increase susceptibility to and severity of *C. difficile* infection by modifying the structure and diversity of the gut microbiome. In this study, controlled feeding trials were performed to evaluate the effect of high levels of dietary zinc on carriage of *C. difficile* in preparturient cows and sows and in their offspring. Cows and sows were randomized to receive a standard or high level of dietary zinc 6 weeks and 1 week prior to parturition, respectively. Fecal samples were collected from the dam before the trial and after parturition, as well as from neonates within 3 days of birth. To detect *C. difficile* colonization, anaerobic culture was performed on all fecal samples and DNA was isolated for comparative genomics across isolated strains. Toxin-specific cytotoxicity assays for *C. difficile* was performed; growth dynamics, motility, and biofilm formation capacity of isolates was also assessed. Risk factors for carrying *C. difficile* other than diet (e.g., maternal age, parity, early calving/farrowing) was determined. Characteristics and composition of the gut microbiota was assessed and compared among treatment and control animals. Culture results are pending and final results will be shared at Student Research Day. The findings of this study will help further elucidate factors that shape gut health of piglets and dairy calves and the characteristics of pathogens that affect them. This work may serve to guide nutritional recommendations given by practitioners for pre-parturient swine and cattle considering the resulting health effects it may have on neonates.

**Research Support:** Pennsylvania Department of Agriculture

**Student Support:** NIH T35 OD010919, Boehringer Ingelheim, and the University of Pennsylvania


Jessica F. DiStefano, Lisa Mattei, Jalisa D. Zimmerman, Andrew Shulman, Alexander Berry, Meghann K. Pierdon, and Daniel P. Beiting.

Swine Teaching and Research Center, Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

The piglet gut microbiome has been linked to numerous aspects of swine health and productivity, including growth and development, fat content of tissues important in meat quality, diarrhea in neonates, and even swine welfare. Despite this association, little is known about the factors that contribute to the initial colonization of the neonatal gut. The goal of this study was to chart the assembly of the piglet gut microbiome in the three weeks following birth, while simultaneously sampling maternal and environmental microbes and using strain-tracking to identify potential sources of early life colonization. Ten farrowing units, each consisting of one sow and at least 6 piglets were housed in individual farrowing stalls in the same room. Sow feces and piglet rectal swabs were collected on days 2, 4, 7, 14, and 21 post-gestation, and sow vaginal swabs were collected pre-and post-gestation. Environmental samples, including sow feed, piglet feed from a creep-feeder, and floor swabs, were used to monitor the farrowing environment for microbes. To profile the microbiome in these samples, we extracted DNA using the Qiagen PowerSoil Pro kit, used this DNA to prepare sequence-ready libraries, and carried out shotgun metagenomic sequencing. Analysis of these data is currently underway and may provide valuable insight into the seeding and assembly of the neonate gut microbiome. This data may help identify ‘keystone’ bacteria that are beneficial for promoting healthy, fast-growing piglets, thus potentially setting the stage to develop probiotics that can improve animal health and production in swine farming.

**Research Support:** NIH T35 OD010919, Boehringer Ingelheim, Pennsylvania Department of Agriculture, and the University of Pennsylvania.

Andrew Dunlap¹, Anna Massie¹, Charles Bradley², and David Holt¹.

¹Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ²Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Osteosarcoma (OSA) is the most common primary bone tumor seen in dogs. Amputation is generally considered the standard of care in these patients but carries a poor long-term prognosis, with 90% of dogs succumbing to metastatic disease within a year of surgery. Limb-sparing procedures provide an alternative to amputation and facilitate recuperation to normal mechanical function. Near-infrared (NIR) imaging provides real-time intraoperative visualization of tumors and tumor margins. Various targeted near-infrared fluorophores have been developed for intraoperative NIR imaging, including several activated by cathepsins which osteosarcoma cells overexpress. This study assessed the correlation between tumor margins determined with MRI, NIR imaging, and histopathology and employed quantitative image analysis to detect OSA tumor margins. We found that using NIR with VGT-309 (NIR/VGT-309), a cathepsin fluorophore, localized a substantial intensity signal to the tumor-associated cortical bone destruction allowing for a sensitive assessment of canine appendicular osteosarcoma tumor margins compared to the standard of care, histopathology analysis. Similar tumor margins among in vivo and various ex vivo tissue presentations suggest effective intraoperative detection of canine osteosarcoma using NIR/VGT-309. The area of tumor-associated cortical bone destruction using NIR/VGT-309 was approximately 1.3-fold higher than MRI. Enhanced accuracy of canine osteosarcoma tumor margin detection using NIR/VGT-309 can provide an applicable, spontaneous, large animal translational model for human limb-sparing procedures.

Research Support: Companion Animal Research Fund at the University of Pennsylvania

Student Support: NIH T35 OD010919, Boehringer Ingelheim, and the University of Pennsylvania

13. Comparing the Protective Effects of Equine Extracellular Vesicles and BM-MSCs Cultured in FBS and Equine Serum on an Osteoarthritis Model.

Kayla M. Even, Angela M. Gaesser, and Kyla F. Ortved.

Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

Osteoarthritis (OA) can occur following joint trauma where global posttraumatic inflammation leads to progressive degradation of cartilage extracellular matrix (ECM). Mesenchymal stem cells (MSCs) have been proposed as a potential therapy for joint disease due to their potent immunomodulatory properties. Extracellular vesicles (EVs), produced from bone marrow derived (BM)-MSCs, have been shown to hold therapeutic potential due to their role in mediating intercellular communications and modulating inflammatory responses. Concerns have been raised regarding the immunogenicity of BM-MSCs traditionally cultured in fetal bovine serum (FBS) culture media prompting research into xenogen-free culture alternatives. Our lab has previously shown that BM-MSCs cultured in equine serum exhibit superior immunomodulatory properties compared to BM-MSCs cultured in FBS. The objective of this study is to compare the protective effects of BM-MSCs cultured and EVs produced from FBS and equine serum culture media on cartilage explants cultured in an OA model. We hypothesize that BM-MSCs and EVs produced from equine serum media will exhibit superior protective capabilities compared to FBS media. BM-MSCs will be isolated from six horses and cultured to passage 3 in equine serum or FBS culture media. BM-MSCs and EVs isolated from each culture condition will be co-cultured with cartilage explants with or without interleukin-1β/tumor necrosis factor-α (TNF-α) to drive in vitro inflammation as a model of OA. ECM degradation will be assessed by histological analysis of explants and a quantification of glycosaminoglycan in the supernatants and cartilage explants using a DMMB assay. Gene expression of IL-1β, TNF-α, IL-6, collagen type II, aggrecan, MMP13, and ADAMTS4 in cartilage explants will be determined. Preliminary data showed that equine serum
cultured BM-MSCs and EVs did not exhibit superior cartilage protection from ECM degradation. Results are currently pending.

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**LONG-TERM PROJECT**

14. **Tumor Infiltrating Lymphocyte Therapy for the Treatment of Canine Cancer.**

**Kay Foos**<sup>1,2</sup>, Veethika Pandey<sup>2,6</sup>, Nicola J. Mason<sup>3,4,5</sup>, and Daniel J. Powell, Jr<sup>2,3,6</sup>.

<sup>1</sup>Department of Cancer Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.; <sup>2</sup>Center for Cellular Immunotherapies, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.; <sup>3</sup>Parker Institute for Cancer Immunotherapy, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.; <sup>4</sup>Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.; <sup>5</sup>Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA.; <sup>6</sup>Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.

Immunotherapy based on the adoptive cell transfer of tumor infiltrating lymphocytes (TILs) has proven to be highly effective in treating human patients with metastatic melanoma, yielding an overall response rate of around 50%. TILs are a heterogeneous population of T cells that recognize and target a diverse repertoire of tumor antigens, leading to a polyclonal anti-tumor T cell response. Although TILs have the benefit of targeting numerous neo-antigens, T cell cytotoxic function can be limited due to the immunosuppressive nature of the tumor microenvironment. The activation of inhibitory pathways combined with insufficient costimulation limits TILs from achieving maximal anti-tumor activity. To overcome this limitation, our lab has engineered TILs sourced from high-grade serous ovarian cancer patient tumor digests to ectopically express a folate receptor alpha (FRα)-targeting scFv with intracellular CD28/CD40 costimulatory domains. The FRα-binding domain engages FRα expressed on tumor cells, leading to a strong costimulatory signal that can enhance T cell function. Collectively, we have shown that patient-derived TILs can be armored with costimulatory signals that enhance anti-tumor activity both *in vitro* and in an *in vivo* patient-derived xenograft (PDX) mouse model. While PDX models recapitulate the pathohistological and genetic features of original tumor tissue more closely than cell-line derived xenograft models, they still require the implantation of tumors in immunocompromised mice. Spontaneous tumors in client-owned dogs represent a valuable parallel patient population in which to investigate the efficacy of adoptive cell therapy for the treatment of solid tumors in an immunocompetent host. To explore the feasibility of translating TIL therapy to the veterinary sector for the treatment of canine cancers, we have developed an *ex vivo* canine TIL expansion protocol and have successfully expanded TILs from canine osteosarcoma and oral melanoma tumor digests. With successful TIL expansion protocols in place, this work paves the way for further *in vitro* and *in vivo* analysis of a TIL-based approach to target solid tumors in canines and provides a comparative tool to investigate the efficacy of engineered TILs in a spontaneous animal model of disease.

**Research Support:** InsTIL Bio, Mason Cancer Research Fund

**Student Support:** NIH MSTP Grant, Mindy and Andy Heyer Endowment, Armour-Lewis Foundation
15. Osteocalcin Is a Possible Biomarker for Detection of Musculoskeletal Injury in Racehorses.

Elisse Friedman1, Joanne Haughan1, Lauren Pittman1, Darko Stefanovski1, Kyla Ortved1, and Mary Robinson1,2.

1Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; 2Pennsylvania Equine Toxicology and Research Center, West Chester University, West Chester, PA.

Tools to predict and prevent catastrophic injuries in Thoroughbred racehorses are needed. Early-stage changes in fetlock joints, thought to lead to catastrophic injury, can be viewed using standing computed tomography (CT). To identify circulating biomarkers for early injury detection, blood samples were collected from 2-year-old Thoroughbred racehorses at 0 and 6 months during their first year of training, concurrent with standing CT imaging of the fetlock joints. Samples from 3 horses with minimal fetlock pathology (H) and 3 horses with severe fetlock pathology (P) at 6 months were selected for analysis. Markers of bone resorption (CTX-1), bone formation (osteocalcin), and inflammation (IL1α, IL1RA, and TGFβ) were evaluated with commercially available ELISA kits or by qPCR. There was a small significant difference between P and H horses at 6 months (p = 0.024) but no other statistically significant differences in CTX-1 levels. Osteocalcin levels were significantly lower (p < 0.0001) in P animals than H animals and were significantly increased in H animals at 6 months (p = 0.002). IL1RA gene expression was significantly higher in H animals at 6 months compared to 0 months (p = 0.009). TGFβ expression was significantly higher in P animals compared to H animals at 0 months (p = 0.002) and 6 months (p = 0.012). Similarly, IL1α expression was also significantly higher in P animals compared to H animals at 0 months (p = 0.001) and 6 months (p < 0.001). In conclusion, osteocalcin shows promise as a biomarker of early fetlock injury. Elevated levels of IL1α and TGFβ may indicate injury when analyzed in conjunction with osteocalcin. Further studies are needed for verification.

Research Support: University of Pennsylvania School of Veterinary Medicine, Pennsylvania Horse Breeders Association

Student Support: NIH T35 OD010919, Boehringer Ingelheim


Elizabeth Gregorio, Kara Brown, Danielle Piccone, and Kyla Ortved.

Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Autologous blood-based therapies, such as platelet-rich plasma (PRP) and autologous protein solution (APS), are frequently used intra-articularly and intra-lesionally for management of musculoskeletal injury in the horse. These substances have been shown to promote healing through their immunomodulatory and anabolic properties by concentrating leukocytes and platelets that release cytokines and growth factors after activation. These same horses with musculoskeletal injury are often also managed with non-steroidal anti-inflammatories (NSAIDs) to decrease inflammation and pain. It is possible that the administration of NSAIDs prior to obtaining blood samples for processing may result in a less effective product due to the documented effects of NSAIDs on platelet function. NSAIDs have been shown in both horses and humans to affect the concentrations of cytokines and growth factors in blood-derived autologous substances such as PRP. However, there is no peer reviewed literature evaluating the effects of administration of NSAIDs on PRP and APS in the horse. Therefore, the objective of this study is to determine the effects of commonly used NSAIDs, phenylbutazone and firocoxib, on the concentrations of growth factors and cytokines in PRP and APS preparations, and that these concentrations will return to normal values after a one-week washout period. This study will help to guide practitioners in optimal timing for obtaining and processing blood derived products after NSAID use in horses.

Mary Kate Grubbs¹, Joanne E. Haughan¹, Darko Stefanovski¹, Julie B. Englies¹, Mary A. Robinson¹,²

¹Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; ²Pennsylvania Equine Toxicology and Research Laboratory, West Chester, PA.

Exercise associated death (EAD) due to orthopedic injury (OI) or sudden death (SD) is a primary cause of Thoroughbred racehorse fatalities. A recent study showed age and training were associated with higher risk of SD in Australian racehorses. This study aimed to characterize risk factors for SD in a local population of racing Thoroughbreds. Post-mortem reports for Thoroughbred racehorses submitted to the Pennsylvania Animal Diagnostic Laboratory System at New Bolton Center 2016-2020 were analyzed. Horses that died or were euthanized during or directly after racing or training were selected. Cause of death was categorized by a veterinary pathologist. Horse sex, age and number of starts were verified in Equibase. Chi² test and logistic regression were used to compare SD in training versus racing and evaluate risk of racing/training, age, sex and number of starts for SD compared to OI.

There were 137 EADs: 31 SD (20 cardio-pulmonary arrest, 1 gastrointestinal, 2 vascular exsanguination and 8 undiagnosed) and 106 OI (99 primary fractures and 7 primary soft tissue injuries). 96 EADs occurred during racing and 41 in training. SD was the cause of 19% of racing and 32% of training associated EADs. The apparent increase in the percentage of SD during training when compared to racing trended towards but did not show statistical significance (P=0.97). There was no effect of age, sex or number of starts on risk of SD in this dataset. Risk of SD was higher in training relative to racing (Odds ratio: 2.0) but this difference was also not statistically significant (P=0.1). Larger numbers of cases are necessary to further evaluate risk factors for SD.

Research Support: Pennsylvania Horse Racing Commission

LONG-TERM PROJECT

18. Abolishment of an Intracellular Retention Signal in the Cytoplasmic Tail of the SFTSV Glycoprotein Gc Improves Its Incorporation onto the Vaccine Vector VSV.

Philip Hicks¹,², Tomaz Manzoni¹, Kendall Lundgreen¹, Alexander Jochmans³, and Paul Bates¹.

¹Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; ²Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ³Department of Biology, Rogers State University, Claremore, OK.

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging bunyavirus that carries a high case fatality rate in human and feline infections. Despite the public health risk posed by this and genetically related viruses, there is no approved vaccine for use in any patient population. Recently we demonstrated that a recombinant vesicular stomatitis virus (rVSV) expressing the SFTSV glycoproteins Gn and Gc in place of the cognate VSV glycoprotein (rVSV-SFTSV) protected mice from lethal SFTSV challenge. However, like many rVSVs encoding bunyavirus glycoproteins, rVSV-SFTSV was challenging to launch from a plasmid recovery system and grew inefficiently in cell culture relative to wild type VSV. The mismatch between the cell surface assembly site of VSV virions and the intracellular retention sites of SFTSV Gn/Gc complexes were responsible for this phenotype. To improve incorporation of SFTSV glycoproteins onto VSV particles, we pursued a genetic strategy to redistribute Gn/Gc complexes to the cell surface. We identified a lysine residue near the C-terminus of Gc through sequence homology that was predicted to function in the intracellular retention of Gn/Gc complexes. Substitution of this lysine with alanine (K-3A) redistributed a recombinant fluorescent protein chimera containing the transmembrane domain and cytosolic tail of Gc from the ER to the cell surface. In addition, K-3A increased expression of full length Gn/Gc on the surface of transfected A549 cells as measured by flow cytometry. K-3A also improved incorporation of fluorescent protein chimeras onto the surface of fluorescent VSV particles as measured by spectrophotometry. Finally, K-3A improved the recovery kinetics of infectious replication-deficient VSV particles pseudotyped with the full-length glycoproteins of SFTSV or the genetically-related Heartland bandavirus. These data suggest rVSVs expressing bunyavirus glycoproteins may be improved by genetically redistributing the glycoproteins to the cell surface. This change in biological behavior may translate to more efficacious vaccines.
LONG-TERM PROJECT

19. **ESPRESSO-TEA: A Long-Read RNA Sequencing Workflow to Study Locus-Specific Expression of Transposable Elements.**

Emerson Hunter\textsuperscript{1,2,3}, Robert Wang\textsuperscript{2,3}, Andrew Hu\textsuperscript{3}, and Yi Xing\textsuperscript{2,3}.

\textsuperscript{1}Veterinary Medical Scientist Training Program, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; \textsuperscript{2}Genomics & Computational Biology Graduate Group, School of Medicine, University of Pennsylvania, Philadelphia, PA; \textsuperscript{3}Center for Computational & Genomic Medicine, Children’s Hospital of Philadelphia, Philadelphia, PA.

Transposable elements (TEs) are mobile genetic elements that compose \( >45\% \) of the human genome, with major implications in health and disease. The expression of TEs is notoriously challenging to study with short-read sequencing technology due to mapping ambiguity attributed to a TE’s highly repetitive content, sequence length relative to read length, insertional polymorphisms, and age-related sequence divergence. Recent advances in long-read technology support more comprehensive TE detection and quantification. We developed ESPRESSO-TEA (Error Statistics Promoted Evaluator of Splice Site Options – Transposable Element Analysis), a workflow to measure locus-specific TE expression from long-read RNA-sequencing data. Using a long-read sequencing simulator, NanoSim, we assessed ESPRESSO-TEA’s performance in identifying and quantifying locus-specific TE expression. We simulated reads from TEs belonging to four major TE families (Alu, L1, MIR, L2) with varying ages and sequence lengths. We demonstrate that TE read mappability varies by TE family, with mapping accuracy of a younger family (Alu) more dependent on relative age than that of older families (L1, MIR, and L2), but TE read mappability impacted by sequence lengths in all families. The young age of Alu and shorter lengths (<150-200bp) of TEs belonging to any family contributes to TE expression underestimation, but overall mapping performance is as expected and ESPRESSO-TEA captures locus-specific TE expression. Moving forward, the ESPRESSO-TEA workflow will characterize TE-transcriptome landscapes, elucidating TE contributions as independent transcriptional units, novel exonizations in chimeric transcripts, and sources for multi-exonic full-length transcripts.

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20. **Evidence for Optogenetic Control of Manual Dexterity in a Macaque Model.**

Antonina Kalkus\textsuperscript{1}, Sebastien Tremblay\textsuperscript{2}, Kristin Gardiner\textsuperscript{1}, and Michael Platt\textsuperscript{2}.

\textsuperscript{1}Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; \textsuperscript{2}Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Neuropathologies involving manual dexterity are prevalent in human cases and well-represented in non-human primate models, but the means to manipulate and test manual dexterity in non-human primates in the face of neural lesions is still being refined. Currently, optogenetics have proven very successful in small animal models such as rodents and flies at precisely controlling behavior. However, there have been varied successes in the translation of this powerful neurostimulation technique to a non-human primate model. While the neuronal similarities between humans, mice, and flies can be applied to a certain extent, non-human primates represent an important missing link in the translation of novel neurological treatments to human clinical cases. The aim of this study is a proof of concept of the use of certain opsins in the macaque cortex as a means of manipulating neuronal activity. First, we verified the efficacy of C1V1, a previously injected opsin, in exciting neuronal activity in the left frontal cortex. Our recordings demonstrated visually responsive neurons with significant excitatory activity when a green laser was applied. In separate cortical columns, we injected SwiChr++, an opsin designed to inhibit neuronal activity when blue light is applied, and resume regular activity when a red light is applied. The results of the recordings after incubation of that vector will help validate the use of different opsin types to manipulate neuronal activity. The next steps will include a SwiChr++ injection to a larger area in the motor cortex in order to affect a basic reach-and-grab task and checking peripheral nerve effect with NCV testing. The ultimate goal is clinical application to neuropathologies such as epilepsy.

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**Student Support:** Boehringer Ingelheim, and the University of Pennsylvania
LONG-TERM PROJECT


Elisabeth A. Lemmon1,2,3, Ryan C. Locke1,2,3, Brendan D. Stoeckl1,2,3, Austin C. Jenk1,2,3, Michael W. Hast1,3, Robert L. Mauck1,2,3.

1Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA; 3Translational Musculoskeletal Research Center, Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA.

The meniscus, a crucial load-bearing structure in the knee, is commonly injured and heals poorly after surgical intervention. Pro-inflammatory cytokines (such as IL1β) in the wound environment impede migration of meniscus fibrochondrocytes (MFCs) and inhibit tissue repair. While IL1β receptor antagonist (IL-1Ra) can block these effects in vitro, application of localized and sustained biologics to meniscus injuries remains challenging. Here, we developed a hybrid meniscus tear augmentation strategy that incorporates a fibrin tissue adhesive with a novel drug delivery system termed mechanoactive microcapsules (MAMCs) containing biologically active IL-1Ra. We queried whether IL-1Ra delivered from MAMCs could rescue MFC migration following IL1β exposure and whether orthotopic delivery in fibrin would enable progressive release of microcapsule contents in a meniscus tear subjected to physiologic loading. To assess the efficacy of IL-1Ra MAMCs in rescuing migration, cells were exposed to IL1β prior to addition of soluble or IL-Ra from mechanically activated MAMCs. Notably, the supernatant of activated IL-1Ra-loaded MAMCs restored migration and % wound closure to control levels compared to treatment with soluble IL-1Ra. Results from mechanical testing showed that under uniaxial compression, MAMCs have a load dependent rupture profile with the presence of meniscus tissue segments protecting MAMCs from rupture with increasing levels of load. Further, when applied to cadaveric stifle joints in a physiologic loading rig, fibrin+MAMCs remained in meniscus tears and showed progressive rupture with increasing load cycles. These findings show that treatment with IL-1Ra derived from ruptured MAMCs had comparable efficacy in rescuing migration (vs. soluble IL-1Ra). Furthermore, IL-1Ra MAMCs can be mechanically activated when encapsulated within tissue adhesive hydrogels and delivered to meniscus tears under physiologic loading. This system has the potential to progress clinical treatment by delivering multiple, mechanoactivated biologics directly to the repair site to improve outcomes for otherwise irreparable meniscus tears.

Research Support: NIH (R01 AR056624 and T32 GM007170) and the VA (I01 RX003375 and IK1RX003932-01A1).

LONG-TERM PROJECT ORAL PRESENTATION

22. Anakinra Reduces Inflammatory Pathways Activated in Canine Synovium after Cranial Cruciate Ligament Injury.

Elisabeth A. Lemmon1,2,3,5, Kevin G. Burt1,2,5, Elizabeth Bernstein1, Emily E. Sharp1,2,5, Brendan D. Stoeckl1,2,5, Lorielle Laforest1, Carla R. Scanzello4,5, Robert L. Mauck1,2,5, and Kimberly A. Agnello3.

1Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA; 3School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 4Department of Medicine, Division of Rheumatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; and 5Translational Musculoskeletal Research Center, Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA.

Inflammatory cytokines associated with cruciate injuries across species, such as IL1β and its catabolic downstream signaling targets, contribute to persistent joint inflammation and poor healing of injured tissues. While a number of therapies have been developed to treat inflammation, there is no naturally occurring preclinical injury model routinely used to evaluate safety and efficacy of these treatments prior to human application. Here, we queried the inflammatory state of the canine knee in patients with naturally occurring CCL tears compared to healthy donors. Next, we established a canine synovial organ culture model and tested whether the human formulation of IL-1Ra (Anakinra) could rescue canine IL1β (C-IL1β) induced inflammation. Histologic scoring showed that synovium from dogs with CCL tears had increased subintimal fibrosis and hyperplasia compared to healthy knees. These histologic findings correlated with whole tissue RT-
qPCR gene expression, with CCL synovium having increased IL1β, IL6, and PTGS2 (gene encoding cyclooxygenase-2), as well as decreased PRG4 expression, compared to healthy knees. To query whether Anakinra could rescue C-IL1β induced inflammation, healthy canine knee synovium was cultured for 3 days either simultaneously with C-IL1β and IL-1Ra or with C-IL1β prior to delayed IL-1Ra treatment on day 3. While exposure to C-IL1β alone increased expression of MMP13, IL1β, IL6, and PTGS2 while downregulating PRG4 expression, this was significantly attenuated by both simultaneous and delayed treatment with Anakinra. Our findings show that human IL-1Ra treatment dampened expression of inflammatory markers MMP13, IL6, and PTGS2, known to contribute to cartilage degeneration after injury. Treatment with IL-1Ra also upregulated PRG4 (lubricin) expression, a glycoprotein that provides boundary lubrication and is necessary for preserving cartilage health. Overall, this work supports that the knee joint environment in canine patients with naturally occurring CCL tears is a promising preclinical testbed for interventions targeting inflammation prior to human application.

Research Support: NIH (R01 AR056624 and T32 GM007170) and the VA (IK6 RX003416 and I01 RX003988)


Kate Marciano1, Marisol Parada Sarmiento2, Rachel Tevere1, Carlin Hagmaier1, Jenni Punt3, and Tom Parsons2.

1Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2Swine Teaching and Research Center, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; 3Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Swine welfare in production environments is a broad issue with economic and ethical implications as consumers demand more thoughtfully produced animal products and are willing to pay a premium for them. Measuring animal stress is a demanding problem, however, and presently there is no widely accepted marker for chronic stress in swine. Human studies have suggested that indicators of chronic stress may be reflected in peripheral blood components, notably telomeres of white blood cells and in inflammatory cell populations. We devised two systems designed to mimic normal stressors for production sows and compared the blood of ‘stressed’ animals versus that of ‘unstressed’ animals. Our attempts to elucidate a relationship between stress status and peripheral blood mononuclear cells were unsuccessful, but we did uncover significant changes in T cell populations. These data show significant decreases of helper T cells and increases in double negative T cells in our stressed populations. This work provides the basis for using peripheral blood mononuclear cells as a biomarker for chronic stress and perhaps suggests a pathogenesis for chronic stress as a causative agent of disease.

Student Support: NIH/Boehringer Ingelheim

ORAL PRESENTATION


Katherine Morucci1, Virginia Micaela de la Puente Leon2, Brinkley Raynor1, Elvis Diaz Espinoza2, Michael Levy1, and Ricardo Castillo-Neyra1.

1University of Pennsylvania, Philadelphia, PA; 2Universidad Peruana Cayetano Heredia, Lima, Peru.

In 2015, public health officials in Arequipa city, Peru, reported the reintroduction of the canine rabies virus following a 15-year period of elimination in the country. Despite the implementation of city-wide disease surveillance and control strategies, hundreds of rabid dogs have since been diagnosed in the region with an average rate of one positive case per week. Here, we report quantitative and qualitative observations of potentially feral, cave-dwelling dog packs adjacent to the Arequipa city limits and discuss the threats that these populations posit to disease control programs and public health. Through a series of monthly pedestrian surveys conducted across a 3.91km² study region, we identified and geo-referenced 173 caves that were associated with feral dog populations. Living adult dogs were observed in the direct vicinity of these caves during each visit (mean: 24.5; C.I. 8.54 - 40.46) but the number of sightings varied across the study region. In addition, a significant number of deceased dogs were found during each of the surveys (mean: 10; C.I. 9.5 - 12.75). Finally, litters of puppies were found in two localities across the study region during each visitation. The presence of unvaccinated
and unsurveyed peri-urban feral dogs may undermine the efficacy of rabies elimination programs and pose significant health risks to local dog, wildlife and human populations alike. Establishing cave dog surveillance, sterilization and vaccination programs would not only minimize the harmful effects that these animals exert on the health and success of domestic and protected wildlife populations, but may also have significant implications for the elimination of dog-mediated human rabies by 2030.


Amanda Patev and Meghann Pierdon.

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Introduction: Lameness is a welfare issue for sows and a common reason for premature removal from the herd. This presents a significant economic issue for the producer. There is little data on the impact of hoof trimming in sows. There are, however, studies that indicate that preventative hoof trimming can improve the gait of the animals, which could correlate to decreased lameness. The objective of this study is to randomly allocate gilts to either receive hoof trimming or no hoof trimming to determine the impact of preventative foot maintenance on productivity, longevity, and foot health.

Materials and Methods: This study was conducted over the course of 6 weeks at a 5000-sow farm utilizing pen gestation with large pens and electronic sow feeding stations. Select gilts (n = 348) were allotted to either treatment (trimming n = 177) or control (no trimming n = 171). Enrolled gilts were brought into a single large pen in the gestation barn each week and treatment and controls allotted from that pen. Gilts were walked to a foot trimming chute (Zinpro Corp, MN, USA) and their feet scored prior to trimming using a modified version of the Zinpro® FeetFirst® scoring system receiving a 0 for no lesion, a 1 for mild to moderate lesion, and 2 for severe lesions. Lesions scored include heel overgrowth and erosion, heel-sole crack, white line cracks, and cracked walls. Toe length was also recorded. Lameness and any injuries or abnormalities to the hoof, dewclaws, or coronary band were also noted. Treatment animals were trimmed to correct heel overgrowth and any long toes. Control animals were scored and released without trimming. Sows are being scored again when they entered farrowing. Records for breeding, pregnancy, and herd removal were obtained for all enrolled gilts. Foot scores were added together to calculate a total score for each animal at entry and at farrowing. A t-test was used to look for differences between follow up scores in treated and control animals. Logistic regression was performed for each binary production outcome including treatment and lameness at allotment as fixed effects.

Results: Of the 177 gilts in the treatment group, 127 gilts had one or more hooves trimmed. The average total foot score at entry was 4.77 ± 2.08 and the average toe length was 37.4mm ± 3.2. The average total foot score at farrowing was 6.3 ± 3.6 for the controls and 5.5 ± 2.8 for the trimmed sows (p = 0.12) There was no impact of treatment on the odds of being bred (0.83, 95% CI 0.39 - 1.8, p = 0.63) with 92% of control animals bred and 93% of treatment gilts bred. There was also no impact on the odds of a gilt becoming pregnant (0.85, 95% CI 0.49 - 1.5, p = 0.57) with 83.0% of control gilts confirmed or presumed pregnant and 81.0% of treated gilts. There was no difference in the odds of removal for treated sows compared to control sows (1.1 95% CI 0.58 – 1.9, p = 0.86). There were 3 control sows and 5 treated sows removed for lameness.

Discussion: Overall, foot scores were low and average toe length was short relative to the trimming length recommended by the Zinpro® FeetFirst® scoring system which advises to trim toes once they exceed 55-60 mm. Preventative hoof trimming could be beneficial. However, it is important to consider hoof trimming will not prevent injury associated with the environment, inter-animal aggression, or existing issues, but may prevent future hoof health issues such as wall or white line cracks. Regular hoof trimming could help compensate for problems caused by conformation but will not fix conformational deformities. There was some difference in the follow up foot scores where the trimmed sows were numerically lower. Future follow up should include monitoring their foot scores for differences over time, future productivity, and eventual time to removal. Since the economic value of the gilt is regained as she farrows more litters, the economic benefit of foot trimming can be best ascertained by whether it improves her longevity and her productivity.
26. Does Disruption of FAP⁺ Stromal Cells by FAP-CAR T Cells Enhance the Efficacy of Immune Checkpoint Inhibitor?

Nimisha Pattada, Zebin Xiao, Li Huang, and Ellen Puré.

Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Immunotherapies, including both immune checkpoint blockade (ICB) and adaptive cell therapy (ACT) employing tumor antigen specific chimeric antigen receptor (CAR) T cells, have proven challenging in solid tumors, especially in pancreatic ductal adenocarcinoma (PDAC). PDAC’s immunosuppressive tumor microenvironment (TME) and extensive remodeling of tumor stroma contribute to the limited success of immunotherapies. A pro-tumorigenic subset of cancer associated fibroblasts (CAFs) that express fibroblast activation protein (FAP) are major deterrents of immune cell infiltration and potent mediators of immunosuppression in the TME. Using multiparametric flow cytometry and multiplex immunofluorescence, we showed that stromal cell targeted FAP-CAR T cells, effectively infiltrate and inhibit tumor growth in mouse models of PDAC due to their capacity to deplete stromal cells and ECM that otherwise present a barrier to adoptive cell therapies. More importantly, depletion of FAP⁺-CAFs resulted in more endogenous T cells trafficking to and infiltrating into tumor nest. We are now testing the hypothesis that disruption of FAP⁺ stromal cells by FAP-CAR T cells may enhance efficacy of subsequent treatment with ICB (anti-PD1) by enhancing the function of FAP-CART cells and endogenous T cell infiltration and functionality. To date, our findings established that FAP-CAR T cell-mediated ablation of immunosuppressive FAP⁺-CAFs and disruption of the desmoplastic stroma they generate, can enhance accumulation and functionality of endogenous T cells and ongoing studies will determine if in addition, treatment with FAP-CAR T cells can enhance the efficacy of ICB therapy in the context of highly desmoplastic solid tumors.

Research Support: This work was supported by NIH T35 OD010919, Boehringer Ingelheim, TMUNITY, and a grant from the PHS.

Student Support: NIH/BI Veterinary Research Scholars Program

27. Impact of Direct IL-27 Signaling on Regulatory T-cells.

Mackenzie Pickford¹, Zachary Lanzar², Joseph Perry², Anthony Phan¹, and Christopher Hunter².

¹School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ²Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Interleukin 27, an IL-12 family member, is a heterodimeric cytokine which has been shown to mediate pro- and anti-inflammatory responses. Previous work from this laboratory has shown the impact of IL-27 on various cell types, including the regulatory T-cells. Regulatory T cells (Tregs) function to mediate tolerance at steady state and quell aberrant inflammation during infection. The impact of IL-27 signaling on the Treg compartment at steady state has yet to be defined. Therefore, we have generated a mouse model which utilizes a Cre-lox system to cause specific genetic depletion of IL-27R on Treg cells. Using high-parameter flow cytometry, we were able to analyze the Treg compartment at the resolution of unique subsets. Our high-resolution approach shows that IL-27 signaling at steady state impacts the composition of the Treg compartment. Loss of IL27 signaling leads to a reduced frequency of effector Treg cells. Previous studies observed that IL27 signaling does not impact the Treg population as a whole, but our data suggests that IL27 may play a role in the development of the Treg compartment at steady state.

LONG-TERM PROJECT


Alexander Post, Shreya Suneja, Jianping Li, and Stewart Anderson.

Department of Child and Adolescent Psychiatry and Behavioral Sciences, The Children's Hospital of Philadelphia, Philadelphia, PA.
One of the most common genetic risk factors for schizophrenia is the 22q11.2 deletion syndrome. Occurring in approximately 1 in 3000 births, approximately 25% of individuals with 22q11DS develop schizophrenia (22q+Sy), a rate 25 times higher than that of the general population. Mechanistically, bioenergetics and mitochondrial function have long been posited to influence the development and course of schizophrenia. Past research from our lab demonstrated through gene expression and oxidative phosphorylation biochemical experiments that an increase in mitochondrial biogenesis and function was associated with the absence of schizophrenia in induced pluripotent stem cell (iPSC) neurons and lymphoblast lines (LCLs) from individuals with 22q11DS. This study aimed to examine the hypothesis that iPSC neurons and LCLs derived from individuals with 22qSz- will have increased mitochondrial turnover compared to 22qSz+, thus compensating for mitochondrial genetic deficits by maintaining a younger mitochondrial pool. To study this, we used iPSC and LCLs derived from 22qSz+, 22qSz-, and control individuals grown. iPSCs were differentiated into excitatory neurons and labeled using MitoTrackerFM Green and LysoTrackerFM Red, allowing the imaging of the movement and colocalization of mitochondria and lysosomes within neurons in real time for 2 hours using an Olympus FV10i microscope.

Further in-depth analysis of mitochondrial turnover and related lysosomal function in cell-based models will further highlight the mechanism behind the variable penetrance of schizophrenia in 22q11DS. Our goal is to determine whether and how FDA-approved medications that enhance mitochondrial biogenesis and/or lysosomal function might be used to prevent or treat schizophrenia in the context of the 22q11.2 deletion syndrome and quite possibly beyond.

29. Development of Type III Collagen Biomaterials to Improve Clinical Outcome of Canine Mammary Tumors.

Corisa Y. Quincey, Becky K. Brisson, and Susan W. Volk.

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Breast cancer is a leading cause of morbidity and mortality in veterinary and human medicine. Recent studies have focused on the tumor microenvironment (TME) as a promising target to decrease recurrence in patients with malignant mammary tumors. Collagen, a major component of the TME, plays critical roles in neoplastic and nonneoplastic cell behavior in cancer development. Type I collagen (Col1) expression in the breast cancer TME has been widely studied and is linked to a worse prognosis due to its tumor permissive effects on cancer and stromal cells. However, there has been less focus on the related fibrillar type III collagen (Col3). Our previous studies have shown that in contrast to the tumor permissive effects of Col1, Col3 reduced tumor growth, suppressed a tumor-permissive stromal matrix architecture, and decreased aggressive cancer cell behavior in human and murine models. We hypothesize that Col3 will decrease aggressive canine cancer cell behavior and may be used therapeutically to decrease local and distant recurrence in canine mammary tumor patients. To compare the effects of collagen types on canine cancer cell behavior, canine mammary carcinoma cells were grown in 3D culture supplemented with either Col1, Col3, or both. Immunofluorescence staining of e-cadherin and active caspase 3 was used to characterize cell phenotype associated with this adhesion molecule and apoptosis, respectively. The cell line tested, CAMAC2, formed more spheroids in Col3 than in Col1 (where colonies were less organized). Further supporting a role for Col3 in promoting a more benign phenotype, e-cadherin expression was increased in the presence of Col3. Additional assays will determine the impact of collagen type on proliferation and apoptosis. This study, in addition to previous studies in murine models and using human samples, will provide support for future clinical trials targeting cancer recurrence in canine and human patients.

Research Support: Canine Health Foundation

Student Support: NIH/Boehringer Ingelheim
LONG-TERM PROJECT

30. Staggered Mass Vaccination Campaigns: A Waste of Resources or an Effective Public Health Strategy?

Brinkley Raynor^1,2, Michael Z. Levy^3, and Ricardo Castillo-Neyra^3.

^1Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania, Philadelphia, PA; ^2School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Introduction: In an ideal world, mass vaccination would instantaneously confer immunity to the threshold proportion of the population required for herd immunity, thereby halting the transmission chain of an infectious disease. However, in reality, a population is often divided into subpopulations and vaccinated sequentially due to practical constraints of administering mass vaccination campaigns. Any manner of waning immunity levels leads to concern of infectious disease persistence through re-introductions due to interactions between subpopulations not yet vaccinated. For example, in Arequipa, Peru, a city with a population of over a quarter million dogs, a canine rabies mass dog vaccination campaign conducted in a single day is practically infeasible. In 2022, the campaign was administered by subdividing the population spatially at the sub-district level, and vaccination campaigns were conducted every weekend over a six-month period.

Objectives: We aim to quantify prospects of local canine rabies elimination given the current vaccination campaign scheme in Arequipa, Peru. More broadly, we aim to characterize theoretical transmission dynamics of infectious diseases with significant incubation periods with spatially asynchronous mass vaccination.

Methods: We constructed a stochastic compartmental patch model to explore transmission dynamics associated with spatially asynchronous mass vaccination campaigns. We parameterized our model to the dog population of Arequipa, Peru and simulated the current vaccination model to assess for probability of rabies elimination.

Results: We modeled infectious disease spread with spatially and temporally asynchronous mass vaccination campaigns and characterized generalized scenarios that successfully achieve regional elimination. Specifically, we examined the schematic of a mass canine rabies vaccination campaign spread over a six-month time period, as proposed and implemented in Arequipa, Peru in 2022. We simulated that a sustained campaign reaching recommended vaccination coverage targets (70% - World Health Organization recommendation, 80% - Pan-American Health Organization) theoretically eliminates canine rabies in the city within 5 years.

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Juliana Reynoso^1, Sara Kass-Gergi^2, and Andrew Vaughan^2.

^1School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ^2Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Mice represent a potentially powerful animal model to study the lung injury caused by SARS-CoV-2 using a newly generated Mouse-Adapted SARS-CoV-2 (MA-SARS-CoV-2) strain. In this project, 8-week-old C57BL6/J mice were infected with the MA-SARS-CoV-2 strain and lungs were collected at day 5 post-infection. Immunofluorescence imaging was used to identify infected cells (SARS-CoV-2 Nucleocapsid +). By multiplex staining with markers of various lung cell types, we will quantify the percent of infected alveolar type 1 cells (AT1, RAGE+) and alveolar type 2 cells (AT2, LAMP3 and/or SPC+). We observed infected (Nucleocapsid +) AT2s and AT1s, as well as a number of infected cells in the alveolar space expressing neither marker. These cells may represent immune cells (e.g., macrophages) that have engulfed infected epithelial cells or epithelial cells that have downregulated canonical marker expression in response to viral infection.

Onward we will stain for more biological markers such as CD45 that help identify leukocytes, such as monocytes/macrophages. We are interested in expanding the experiment to explore infection of airway cells, such as club cells, tuft cells, etc. Future experiments following will analyze comorbidities between metabolic diseases like Type 1 diabetes and SARS-CoV2 injury.

Research Support: Institute for Translational Medicine and Therapeutics (ITMAT)

Student Support: NIH T35 OD010919, Boehringer Ingelheim, and the University of Pennsylvania
32. Defining the Roles of SKIV2L & TTC37 in Colonic Epithelial Homeostasis and Disease.

Daana Roach¹, Noor Nema², Gloria E. Soto², Tatiana Karakesheva², Brooke E. Boyer², Judith R. Kelsen², and Kathryn E. Hamilton⁷,⁸

¹Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ²Division of Gastroenterology, Hepatology and Nutrition, Children’s Hospital of Philadelphia, Philadelphia, PA; ³Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Very Early Onset Inflammatory Bowel Disease (VEO-IBD) is a classification of inflammatory disorders that occur in children under 6 years of age. Similar to adult chronic IBD such as Crohn’s, the number of pediatric cases has increased over the past years. Tricho-Hepato-Enteric Syndrome (THES) is a rare, monogenic form of VEO-IBD with a heterogeneous clinical presentation. For example, alongside characteristic intractable diarrhea, some patients may present with cardiac defects, liver and hair abnormalities and facial dysmorphia. THES has been linked to mutations in either of the two genes, TTC37 and SKIV2L. These genes encode subunits of the Ski complex, a cofactor of the cytoplasmic RNA exosome. Current literature suggests that the Ski complex may not only be involved in post-transcriptional RNA processing but also in translation given its purported interaction with the ribosome. The contribution of these gene variants to THES pathogenesis, and particularly to epithelial barrier dysfunction or homeostasis, is poorly understood. Furthermore, current therapies for THES tend to only address immune dysregulation rather than primarily the impaired epithelial barrier. Therefore, a more concrete understanding of the role of these gene variants in the intestinal epithelial barrier during both disease and healthy states is necessary if we are to address THES and other monogenic VEO-IBD. The following study utilized 3D colonoids derived from patients with THES to model the disease in vitro and characterize how the gene variants described contribute to disease pathogenesis. Furthermore, the study sought to define the roles of SKIV2L and TTC37 in colonic epithelial homeostasis and disease, as well as expand our current understanding of the role of the Ski complex in RNA degradation and processing pathways. Preliminary data shows that colonoids from patients with THES grow at a reduced frequency compared to healthy control colonoids, and that there is altered gene expression observed in these in vitro models.

33. Understanding Myocardial Metabolic Changes in a Rodent Model of Stress Cardiomyopathy.

Melissa Seiberlich¹, Amit Iyengar², Noah Weingarten², Danika Meldrum², David Herbst², Jessica Dominic², Sara Guevara-Plunkett², Joyce Ho², Shahe Tchillingirian², Chaitanya Karimanasseri², and Pavan Atluri².

¹School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; ²Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Stress cardiomyopathy is characterized by acute contractile dysfunction in response to sudden physical or emotional distress. While chronic cardiomyopathies have linked significant metabolic alterations to contractile dysfunction, these mechanisms are unknown in acute cardiomyopathy. The aim of this study was to evaluate for metabolic changes associated with stress cardiomyopathy. A cohort of rats (N=133) were injected with 75 mg/kg IP isoproterenol and underwent hemodynamic assessments at prespecified time-points after treatment (Day 0, Day 1, Day 3, and Day 7). Animals were anesthetized, and transthoracic echocardiography and left heart PV loop catheterization were performed. Hearts were then rapidly harvested, frozen, and subject to additional analyses. Transthoracic echo revealed peak apical hypokinesia at Day 1 after injury that improved by Day 7 (0.45±0.27 vs. 0.21±0.26, p<0.05). Invasive hemodynamic assessments confirmed parallel reductions in stroke work, cardiac output, ejection fraction, and dP/dt that largely recovered by day 7. Metabolomic analysis via LC/MS demonstrated a significant increase in acylcarnitine and decrease in lactate concentrations that correlated with the pattern of injury. A colorimetric plated assay demonstrated markedly decreased intracellular triglyceride concentrations that did not recover by day 7. Fluoro-respirometry showed no difference in electron transport chain activity with disease (p=ns). In our rat model of stress cardiomyopathy, an apparent defect in fatty acid oxidation was correlated with reduction in function, without permanent deficit to the electron transport chain. Further assays are underway to better characterize this deficit and explore opportunities for treatment.

Research Support: NIH T35 OD010919, Boehringer Ingelheim

Student Support: NIH-BI Veterinary Scholars Program
34. Characterization of the Wing Microbiome in Bats Afflicted with White Nose Syndrome.

Stephanie Sila1, Greg Turner1, Amber Nolder2, Barrie Overton3, Clara R. Malekshahi4, Lisa M. Mattei4, and Daniel P. Beiting4.

1School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 2Bureau of Wildlife Management, Pennsylvania Game Commission, Harrisburg, PA; 3Department of Biological Sciences, Lock Haven University, Lock Haven, PA; 4Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

White Nose Syndrome (WNS) is a disease caused by the fungus Pseudogymnoascus destructans, which has resulted in massive population losses in bat species across the United States. WNS is transmitted by direct contact in a seasonal pattern, impacting bats during their hibernation period. Fungal growth on the wings of bats afflicted with White Nose Syndrome is not uniform. We hypothesized that the composition of the wing microbiome might contribute to the restriction of fungal growth in some areas, a notion supported by evidence from in vitro studies where bacteria isolated from bats showed inhibitory properties in co-cultures with P. destructans. Utilizing shotgun metagenomics and RNA-Seq analysis of bat wing biopsies, this project seeks to test this hypothesis by profiling the bat wing microbiome and characterizing the host immune response in both P. destructans affected and unaffected areas on the wing of individual bats. The data collected will help further our understanding of how the fungal infection develops with potential implications for future conservation and control efforts.

Research Support: This work was supported by NIH T35 OD010919, Boehringer Ingelheim, and the University of Pennsylvania.

LONG-TERM PROJECT
ORAL PRESENTATION

35. Resistance to Arousal State Transitions in Aged Mice.

Martha Stone1,2, Andrzej Wasilczuk4, Gabriela Cano4, Ashley Uppani5, Alexander Proekt1,3, and Max Kelz1,3.

1Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2VMD-PhD Program, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; 3Department of Anesthesiology and Critical Care, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 4Department of Bioengineering, University of Pennsylvania, Philadelphia, PA; 5Department of Biochemistry, University of Pennsylvania, Philadelphia, PA.

Most patients emerge from anesthesia without complications, yet the elderly are predisposed to postoperative delirium and cognitive dysfunction. Prevention and treatment strategies are lacking because the neurobiological mechanisms through which aging increases the prevalence of long-lasting cognitive impairment following anesthesia are unknown. Recent evidence suggests that anesthesia recovery is not simply the reverse of anesthesia entry. Rather, subjects consistently emerge at lower anesthetic doses than were required for anesthesia induction. This phenomenon can be explained by the brain’s inertial tendency to resist changes in arousal state. We hypothesize that elderly individuals display increased resistance to state transitions (RST) compared to younger individuals, which could help explain their vulnerability to delayed cognitive recovery after anesthesia. To begin to test how RST might change with age, we quantified RST in a convenience sample of male and female mice 18-30 weeks old (Young; n=20) and 48-61 weeks old (Aged; n=37). We exposed subjects to a constant, sub-hypnotic dose of isoflurane and assessed responsiveness via righting reflex every 3 minutes for 4 hours. Individuals fluctuated between responsive and unresponsive states trial-to-trial, and we quantified RST from the probabilities that each individual remained responsive, or unresponsive, on two consecutive righting reflex assessments. Despite our prediction, we found no significant difference in RST in Aged compared to Young mice, even when the dose of isoflurane used was adjusted to account for age-related increases in anesthetic sensitivity. As this pilot study is limited by its relatively small sample size, restricted age range, and between-subjects design, the data are likely insufficient to rule out RST as an age-dependent property that contributes to post-anesthesia cognitive recovery. Future longitudinal studies aim to assess RST as a function of age, and to correlate individual RST to cognitive performance following exposure to anesthesia, at multiple timepoints across the lifespan of individual mice.
ORAL PRESENTATION

36. Chronic Social Defeat Stress in Group Housed Breeding Swine.

Rachel Tevere, Thomas Ede, and Thomas D. Parsons.

Department of Clinical Studies—New Bolton Center, Swine Teaching and Research Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

The chronic social defeat stress (CSDS) model of major depressive disorder (MDD) in rodents has contributed greatly to our understanding of the role of chronic stress in psychological well-being. Here we test its applicability to swine housed in groups. Group housing precipitates conflict and aggression during the establishment and maintenance of a social hierarchy. This can result in chronic conflict and social stress for many animals, especially those lower in the hierarchy; those higher in the hierarchy are less likely to experience social stress. In this study, 16 naive gilts (“new” group, 29 wk old) were introduced to a pen housing ~60 pigs. 16 high ranking animals (selected based on daily feed order data) already residing in the pen were included in the “resident” group. Anxious behaviors and anhedonia were compared between groups for 4 weeks post-introduction. Anxious behaviors were examined with a combined open field/novel object test, and anhedonia was assessed using a sucrose preference test. We observed a group-by-time interaction effect for sucrose consumption as the new group consumed both increased sucrose compared to the resident group and their own baseline. Both groups decreased their water consumption with time. Skin lesions were assessed pre- and post-introduction by blinded observers. There was no significant difference in skin lesion scores at 1-2 days pre-introduction, however new animals had significantly higher skin lesion scores at both 1-2 and 5-6 days post-introduction compared to resident animals. Saliva samples were collected from animals at the end of the study to measure IgA concentrations and assess animal welfare. Applying the CSDS model in a group housing setting is a novel approach that has the potential to be a valuable tool for assessing animal welfare as well as furthering our understanding of MDD beyond what has been learned from the current rodent model.

Research Support: NIH T35 OD010919

Student Support: University of Pennsylvania NIH/Boehringer Ingelheim Summer Research Program

LONG-TERM PROJECT

37. Notch Signaling Regulates Allo-T Cell Gut-Trafficking by Altering Integrin Pairing Dynamics.


1Massachusetts General Hospital, Center for Transplantation Sciences, Boston, MA; 2Division of Hematology/Oncology, Boston Children’s Hospital and Department of Pediatric Oncology, Dana Farber Cancer Institute, Department of Pediatrics, Harvard Medical School, Boston, MA; 3Division of Hematology/Oncology, Department of Medicine, Perelman School of Medicine, Philadelphia, PA; 4Immunology Graduate Group and Veterinary Medical Scientist Training Program, University of Pennsylvania, Philadelphia, PA; 5Graduate Program in Cellular and Molecular Biology, University of Michigan, Ann Arbor, MI; 6Clinical Research Division, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA; 7Ben Towne Center for Childhood Cancer Research, Seattle Children’s Research Institute, University of Washington, Seattle, WA; 8EPFL, Lausanne, Switzerland; 9Medical Research Center, Kantonsspital St. Gallen, St. Gallen, Switzerland; 10Department of Laboratory Medicine and Pathology, Center for Immunology, Masonic Cancer Center, University of Minnesota School of Medicine, Minneapolis, MN; 11Division of Blood & Marrow Transplant & Cellular Therapy, Department of Pediatrics, University of Minnesota School of Medicine, Minneapolis, MN; 12Regeneron Pharmaceuticals Inc., Tarrytown, NY.

Intestinal GVHD is the major life-threatening complication of acute GVHD in allo bone marrow transplant (BMT) recipients. Prophylaxis with systemic Notch ligand anti-DLL4 mAb blockade prevents GI-GVHD and reduces T cell accumulation in the gut of both mice and non-human primates, indicating that this is a conserved feature of Notch inhibition. In order to
understand how Notch signaling regulates allo-T cell GI trafficking, we first assessed Notch-deprived allo-T cells for expression of classical gut-homing molecules such as \( \alpha 4 \beta 7 \) and CCR9. We find that Notch loss-of-function blunted \( \alpha 4 \beta 7 \) surface expression in Tconv 4 days post-BMT. However, Notch blockade does not appear to regulate overall retinoic acid signaling or gene expression of classical T cell gut-homing molecules, as RNA-seq did not show a reduced retinoic acid signaling or gut-trafficking signature. Interestingly, we find that Notch inhibition leads to increased \( \text{Itg} \beta 1 \) (integrin subunit \( \beta 1 \)) in Tconv. \( \text{Itg} \beta 1 \) overexpression was previously reported to reduce surface \( \alpha 4 \beta 7 \) in T cells by outcompeting \( \beta 7 \) for \( \alpha 4 \) binding. Thus, we hypothesized that Notch signaling alters allo-T cell GI trafficking by skewing the surface integrin repertoire. We find that allo Tconv cells express higher levels of surface integrin \( \beta 1 \), and co-expression of integrin \( \alpha 4 \) and \( \beta 1 \). Moreover, genetic inactivation of \( \text{Itg} \beta 1 \) in Notch deprived allo-T cells restores surface expression of \( \alpha 4 \beta 7 \), thus suggesting that Notch loss-of-function blunts \( \alpha 4 \beta 7 \) expression via upregulation of \( \text{Itg} \beta 1 \). Altogether, our data reveal a new potential mechanism of Notch immunomodulation in alloreactive T cell gut trafficking and bring a new perspective to the signaling pathways that underlie gut-homing programs in T cells, as Notch has not previously been implicated in regulating gut-tropism.


Yucheng Wu\(^1\), Brittany Watson\(^2\), Chelsea Reinhard\(^2\), and Stephen D. Cole\(^1\).

\(^1\)Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Antibiotic resistance is a rising global crisis and critically threatens the basis of modern medicine. Beta-lactam antibiotics are among the most commonly used antibiotics and the most important drugs in both human and veterinary medicine. In veterinary patients, extended-spectrum beta lactamases (ESBL) and carbapenemases present a huge concern for the treatment of Gram-negative bacterial infections. Although infections caused by these bacteria are traditionally viewed as of nosocomially acquired, increasing reports of ESBL-producing Enterobacterales (EPE) and carbapenemase-producing Enterobacterales (CPE) isolated from companion animals suggest the need for surveillance among a variety of animal populations to establish baselines and identify potential sources. Therefore, in this study, we will collect fecal samples from sheltered animals within 1 week of admission to 4 shelter facilities in the Philadelphia region. For each specimen, selective culture with phenotypic and PCR confirmation will be performed to identify EPE and CPE. 364 samples have been collected thus far and more samples are needed for more statistically valid results. As we have little knowledge on the prevalence of CPE and ESBL carriage among animals, the proposed research will lay a foundation for future surveillance projects such that we can develop proper control strategies to be implemented in veterinary settings to protect the health of animals and the people who care for them.

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