Trainee Reports

Sarah Cooper

Mentor: Brendan Podell, DVM, PhD, DACVP, Assistant Professor, Dept Micro-, Immuno, and Pathology, CVMBS, CSU

Research Project Title: The role of the granuloma in TB disease

Description of the trainee/scholar's research project and progress:
My research project focuses on elucidating the complexities of the canonical pathology lesion, the granuloma, which forms in response to infection with Mycobacterium tuberculosis. My work aims to identify what constitutes a protective granuloma versus one that is permissive to TB disease. To do so, I have optimized assays such as multiplexed fluorescent immunohistochemistry, in situ hybridization, and spatial transcriptomics as tools to identify host and bacterial responses in Mtb granulomas of BCG vaccinated and unvaccinated mice. Additionally, I have developed computational algorithms in image analysis software to quantitatively measure lesion burden, host immune cell phenotypes, and Mtb organisms within each granuloma. I have also built upon published work with collaborators to establish the RS index as a measure of bacterial replication in relation to granuloma size and bacterial load. Finally, I completed courses in coding and spatial statistics to quantify the heterogenous cellular organization that we qualitatively observe between granulomas. To accompany in situ experiments and further explore immune correlates of protection, I performed a 23-color immune cell flow cytometry panel and enumerated bacterial burden from lung homogenates. This first authorship manuscript for this study is currently in progress and will be submitted in Summer 2022.

Relevant Coursework:

- MIP 525 Flow Cytometry for Immunology, MIP700 Topics in Microbiology, NR 512 Spatial Statistical Modeling, and MIP 784 Supervised College Teaching (Course coordinator)

Conference presentations

- **Cooper S,** Gary H, Harris M, DiLisio J, Fox A, Karger B, Henao-Tamayo M, Podell B. Spatial patterns of immune protection reveal granuloma heterogeneity among BCG vaccinated, Mycobacterium tuberculosis-infected mice. Poster Presentation. College of Veterinary Medicine and Biomedical Sciences Research Day. Colorado State University, Fort Collins, CO. January 2022
- **Cooper S,** King K, Gary H, Harris M, DiLisio J, Fox A, Karger B, Henao-Tamayo M, Podell B. Spatial patterns of immune protection reveal granuloma heterogeneity among BCG vaccinated, Mycobacterium tuberculosis-infected mice. Poster Presentation. Keystone Symposia: A Research Reboot of Tuberculosis on the Keystone Symposia 50th Anniversary. Breckenridge, CO. January 2022, postponed until August 2022

Publications

- (Planned) Dutt T, Karger B, Fox A, Youssef N, Ali M, Dhadhwal R, Creissen E, **Cooper S**, Podell B, Gonzalez-Juarrero M, Obregon-Henao A, Henao-Tamayo M. Mycobacterium avium via oral route boosts BCG's protective efficacy against pulmonary tuberculosis: An essential role for B-cell mediated immunity. Role: Analyzed histological data and created corresponding figures.
- (Planned) Spatial patterns of immune protection reveal granuloma heterogeneity among BCG vaccinated, Mycobacterium tuberculosis-infected mice. Role: Performed animal experiments, sample collection, prepared and plated samples for CFU bacterial enumeration, performed pathology and spatial transcriptomic experiments, collected, and analyzed data, prepared figures and writing draft as first author.

Workshops attended:

- Rigor and Reproducibility Workshop 2/24 and 3/31/2022, Colorado State University
- Coding & Cookies: Data Visualization using ggplot2. Colorado State University. Spring 2022
- Coding & Cookies: Tidy Data in R. Colorado State University. Spring 2022

Career development activities:

- Vectra Polaris Imaging System Training. Colorado State University. Spring 2022C4MInD OVPR Liaison (July 2021-Current)

- Networking lunch, seminar, and dinner with Tim Errington. Colorado State University. Spring 2022
- Data Management Meetings: Immune Mechanisms of Protection Against M. tuberculosis Center – Colorado State University branch. Spring 2022
- Course coordinator for MIP seminar. Colorado State University. Spring 2022

Emily Fitzmeyer

Mentor: Gregory Ebel, PhD, Professor, Dept, Dept Micro-, Immuno, and Pathology, CVMBS, CSU

Research Project Title: Using barcoded West Nile virus to examine the impact of tissue and cellular bottlenecks on virus populations in enzootic and bridge vectors of West Nile

Description of the trainee/scholar's research project and progress:

The proposed project aims to establish how both whole-tissue and intracellular bottlenecks, within enzootic and bridge vectors of West Nile virus, alter virus populations and influence virus fitness, transmission potential, and emergence, with insight into the specific cell types involved in these bottlenecks throughout infection. Using a molecularly barcoded WNV (bcWNV) we have quantitatively measured population bottlenecks in three biologically relevant WNV vectors; *Culex tarsalis*, *Culex quinquefasciatus*, and *Aedes aegypti* and determined that population richness and complexity is reduced by tissue-level bottlenecks at comparable rates between our species of interest, indicating that bottleneck severity does not vary between vectors of different competencies. Additionally, we determined that barcodes present in the bloodmeal at the highest frequencies are most frequently maintained in midgut infection. In addition to characterizing tissue-level bottlenecks we will use single-cell sorting and sequencing of bcWNV infected mosquito tissues to characterize polyinfection (infection of a single cell with multiple unique genomes) in mosquitoes. We have optimized protocols to dissociate mosquito tissues into single cell suspensions and successfully identified infected single cell populations from the midguts and salivary glands of orally infected *Culex* mosquitoes. A pilot polyinfection study in *Cx. tarsalis* is underway and expected to be completed by May ‘22.

Conference presentations

- Title and author list to be determined. ASV annual meeting. Oral presentation. July 2022. Madison, Wisconsin

Publications

- (Planned) Tick-borne flaviviruses escape tripartite motif containing protein 5α restriction through a single mutation in the NS3 helicase. Role: Identified key mutations in Langat virus that confer resistance to TRIM5. Spearheaded the use of a circular polymerase extension reaction (CPER) cloning method in the Best Lab and generated the mutant clones used for subsequent experiments. Performed early characterization of the resistance phenotype
- (Planned) Using barcoded West Nile virus to quantify intrahost bottlenecks in enzootic and bridge vectors of WNV. Role: Performed all experiments and analyses included in the manuscript

Workshops attended:

- Rigor and Reproducibility Workshop 2/24 and 3/31/2022, Colorado State University

Career development activities:

- VPR Fellows & Anschutz Fellows Networking Reception (virtual), 01/25/22
- CSU Flow Core, lunch and learn - Wolf Cell Sorter, Microbiology building room C116, 02/24/22
- Anschutz Thematic Programming: pandemic preparedness guest speaker – Richard Wilder, OneHealth conference room in Johnson Hall, 04/20/22

Darby Gilfillan

Mentor: Zaid Abdo, PhD, Professor & Associate Dept Head for Graduate Education, Dept Micro-, Immuno, and Pathology, CVMBS, CSU and Gregg Dean, DVM, PhD, DACVP, Professor & Dept Head, Dept, Dept Micro-, Immuno, and Pathology, CVMBS, CSU

Research Project Title: Assessing host-microbiome interactions during recombinant *Lactobacillus acidophilus* vaccination against rotavirus

Description of the trainee/scholar's research project and progress:

My research through co-mentorship in the Abdo and Dean labs blends immunological techniques with computational tools to evaluate efficacy and off-target impacts of vaccination with an engineered probiotic bacterium. I helped construct a recombinant *Lactobacillus acidophilus* (rLA) vaccine containing two rotavirus antigens (VP8 protein, VP8 peptide) and adjuvants (flagellin, adhesion) with expression confirmed using flow cytometry and western blot. I vaccinated mice using this rLA strain followed by rotavirus challenge. I evaluated protection during infection using antigen shedding ELISA and measured antigen-specific IgA and IgG immune system responses during vaccination using ELISA and at necropsy with FluoroSpot. I am currently conducting a follow-up study to evaluate neutralizing antibody production and differences between vaccination using strains with or without adjuvants. I contributed to a multi-lab, systems-immunology project combining metagenomics, metatranscriptomics, and metabolomics. Mice were vaccinated with the same rLA strain and host-microbiome-immune system interactions assessed at an immune inductive site (Peyer's patches). I helped extract DNA/RNA from the collected tissue and am processing metatranscriptomic sequencing results using pipelines including BWA and SAMSA2 with statistical analysis in R. I have also investigated differential adjuvant expression during exponential rLA growth. I took a diverse course load to supplement my understanding in the lab.

Relevant Coursework:

- DSCI511 Genomics Data Analysis in Python and MIP700 Topics in Microbiology

Conference presentations

- **Gilfillan D,** Ecton K, Islam N, Vilander A, Eklund B, Belisle J, Dean G, Abdo Z. "Probiotic Puzzle: Host-Microbiome Response to Rotavirus Vaccination using Recombinant *Lactobacillus*." CVMBS Research Day. Fort Collins, CO. Oral Presentation. January 22, 2022.
- **Gilfillan D,** Ecton K, Islam N, Vilander A, Eklund B, Belisle J, Dean G, Abdo Z. "Arranging the Probiotic Puzzle: you, your gut microbes, and a Lactobacillus vaccine for rotavirus." Front Range Microbiome Symposium. Colorado State University, Fort Collins. Poster Presentation. April 14-15, 2022

Publications

- (Planned) **Gilfillan D*,** Vilander A*, Shelton K, LaVoy A, Abdo Z, Dean G. "Probiotic Puzzle: Host Immune-Microbiome Response to Rotavirus Vaccination using a Next-Generation Recombinant *Lactobacillus acidophilus* Vaccine Platform." In-progress with planned submission to *Vaccines*.
- (Planned) **Gilfillan D,** Chiang E, Skarlupka J, Merrell E, Allyn Tori, Carey H, Suen G, Florant G, Abdo Z. "Alpha and Beta Microbial Diversity in the Cecum of the Golden-Mantled Ground Squirrel." In-progress with planned submission to *BMC Microbiome*.
- (Planned) Hopken M, **Gilfillan D,** Gilbert A, Piaggio A, Pierce J, Samsel M, Abdo Z. "Oral Rabies Vaccination Causes Shifts in Striped Skunk (*Mephitis mephitis*) Gastrointestinal Microbiome." In-progress

Workshops attended:

- Rigor and Reproducibility Workshop 2/24 and 3/31/2022, Colorado State University

Career development activities:

- Center for Metabolism and Infectious Disease (C4MInD) Group Trainee Develop Club Co-Leader (June 2021-Current)
- C4MInD OVPR Liaison (July 2021-Current)
- Front Range Microbiome Symposium Colorado State University (April 14, 2022)
- Planned: American Society for Microbiology – Microbe 2022 (June 9-13, 2022)

Katherine Kokkinias

Mentor: Kelly Wrighton, PhD, Associate Professor, Dept Soil & Crop Sciences, CSU

Research Project Title: Weathering the Inflammatory Storm: a multi-omics analysis of the murine microbiome in response to *Salmonella* infection and diet-induced inflammation over time

Description of the trainee/scholar's research project and progress:

Inflammation caused by pathogens and the diet alters gut chemical resources, which in turn modulates the intestinal microbiome. Despite a relevance for human health, there are a paucity of

studies exploring the intersection of poor diet and pathogen colonization. In the CBA/J mouse model, we show a high fat diet decreased the fecal microbial diversity and increased inflammation relative to a high fiber diet. Additionally, mice fed a high fat diet had enhanced susceptibility when challenged with *Salmonella enterica* serovar Typhimurium, a model pathogen that colonizes the gut by taking advantage of redox changes from inflammation. We recruited time series metatranscriptomes to a database of 124 metagenome assembled genomes derived from mice treated with and without *Salmonella* under two dietary regimes. We discovered active taxa that responded positively (*Enterococcus)* and negatively (*Clostridia)* to *Salmonella* regardless of diet, while other taxa had diet-specific *Salmonella* responses (*Lactobacillus*). Consistent with findings derived from pure-culture experiments, metatranscriptomics validated that *Salmonella* respired nitrate and oxygen to outcompete commensal fermentative microorganisms, with greater gene expression of respiratory genes detected in the inflamed, high fat diet relative to the high fiber diet. Ongoing efforts are using genome-resolved gene expression networks between *Salmonella* and the commensal microbiome elucidated how the overall community metabolism changed during infection. Our findings uncovered commensal microbes and their metabolisms that weather *Salmonella* infection under diet-induced inflammation. These pathogen and inflammation resistant strains may offer new avenues for therapeutic interventions.

Within the past year, we have completed all necessary experimentation for the project and have completed the data processing. As of this spring, we are wrapping up data analysis and have refined numerous figures that will be used for publication. By the end of the spring will plan to begin the writing process and plan to have a manuscript submitted by the end of the summer. At the end of the summer, I will present my findings at the International Symposium of Microbial Ecology (ISME) in Lausanne, Switzerland.

Relevant Coursework:

- BZ 565 Next Generation sequencing platforms, MIP 700 Topics in Microbiology: Biochemistry of Infectious Disease

Conference presentations

- Poster Presentation, Front Range Microbiome Symposium, April 15, 2022

Workshops attended:

- Rigor and Reproducibility Workshop 2/24 and 3/31/2022, Colorado State University

Career development activities:

- A Vision, A Goal, A Plan: Improve Your Teaching Effectiveness, Lory Student Center, CSU, Jan 2022
- Collecting Evidence for Teaching effectiveness, Lory Student Center, CSU, Jan 2022
- Integrating Augmented and Virtual Reality into Instruction, Teaching and Integrative Learning Training, Mar 2022
- Strategies for Assessing Higher Order Thinking Skills Without Essays or Hand Grading, Dr. Andrew West, April 2022
- Congress on Gastrointestinal Function, Virtual Conference based out of University of Illinois-Champagne, April 10-13, 2022

Terry, James

Mentor: Brian Geiss, PhD, Associate Professor, Dept Micro-, Immuno, and Pathology, CVMBS, CSU

Research Project Title: Determining the Protein-Protein Interactions in the Flavivirus Replication Compartment

Description of the trainee/scholar's research project and progress:

My research is focuses on evaluating the proteome and protein-protein interaction of the flavivirus replication compartments found in the infected cell endoplasmic reticulum (ER). The first objective is to develop an optimized pipeline for isolating replication compartment-containing ER microsomes from infected cells. A

differential ultracentrifugation method has been validated and optimized further as the project has progressed. These efforts are ongoing and nearing completion. The second objective focuses on determining protein interactions within replication compartments through the use of cross-linking mass spectrometry. With the purification process optimization from the first objective nearing completion, the roadmap protein isolation and sample preparation for mass spectrometry has been developed. First, a reference database for the present proteins in microsome samples will be created by using nontargeted mass spectrometry on infected and uninfected microsomes. Next, samples will be crosslinked and rough structural interactions between all present proteins will be mapped. Last, purification steps of cross-linked flavivirus nonstructural proteins will be optimized/used prior to targeted mass spectrometry for further improving the resolution of present structural interactions. The preliminary steps for objective 2 are beginning with protein extraction and sample preparation optimization. These steps will be taken in within two weeks.

Relevant Coursework:

- MIP 654 Research Policies and Regulations and MIP700 Topics in Microbiology

Publications

- (Published)Samper IC, McMahon CJ, Schenkel MS, Clark KM, Khamcharoen W, Anderson LBR, Terry JS, Gallichotte EN, Ebel GD, Geiss BJ, Dandy DS, Henry CS. Electrochemical Immunoassay for the Detection of SARS-CoV-2 Nucleocapsid Protein in Nasopharyngeal Samples. Anal Chem. 2022 Mar 22;94(11):4712-4719. doi: 10.1021/acs.analchem.1c04966. Epub 2022 Mar 9. PMID: 35263100; PMCID: PMC8982495. Role: Provided sample validation and testing in addition to device blocking optimization. Developed and performed ELISA standardized assays for determining comparative efficacy of device against a standard industry assay.
- (Submitted)Carrell C, Link J, Jang I, Terry J, Scherman M, Call Z, et al. Point-of-Need Disposable ELISA System for COVID-19 Serology Testing. ChemRxiv. Cambridge: Cambridge Open Engage; 2020. Role: I provided consultation on ELISA's optimization of buffers and blocking solution, and ran samples by ELISA parallel to the device using as a comparison against a standardized assay

Workshops attended:

- Rigor and Reproducibility Workshop 2/24 and 3/31/2022, Colorado State University

Annika Weber

Mentor: Elizabeth Ryan, PhD, Associate, Dept Environmental Health & Radiological Sciences, CVMBS, CSU

Research Project Title: Investigation of the safety, acceptability, and efficacy of dietary rice bran in childhood malnutrition prevention and treatment

Description of the trainee/scholar's research project and progress:

Children with severe acute malnutrition (SAM) have dysbiotic gut microbiota that are not recovered with current malnutrition treatment options, leaving them susceptible to recurrent infection and malnutrition relapse. Rice bran is a prebiotic with demonstrated microbiome-directed health benefits that merits attention for its use in malnutrition treatments. We will begin a clinical trial to 1) determine the effect of RUTF + 5% rice bran on gut microbiota composition in the treatment of SAM in children ages 6-59 months when compared to RUTF alone and 2) examine and establish blood metabolite profiles associated with RUTF + 5% rice bran in the treatment of children with SAM compared to RUTF alone. I will travel to Indonesia this June (2022) to begin working on the food product for the clinical trial. The clinical trial will begin enrollment in October 2022, at which point I will likely travel back to Indonesia to help with sample collection. Thus far, I have organized and led team meetings across 3 international institutions with collaborators, helped prepare and obtain CSU IRB approval for the study, prepare NIH grants to help obtain additional funding, register the trial on clinical trials.gov (NCT05319717), present the aims and background information at various conferences and symposiums.

Relevant Coursework:

- FSHN 640 Nutritional Epidemiology, ANEQ 505 Microbiome and Animal Systems, CIVE 527 Tools for Food-Energy-Water Systems Analysis, FSHN 650C Recent Developments in Human Nutrition

Conference presentations

- Weber AM, Barbazza S, Soekarjo D, Wieringa F, Ryan EP. Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition. Front Range Microbiome Symposium. Colorado State University, Fort Collins, CO. 15 April 2022. [Poster].
- Weber AM, Barbazza S, Soekarjo D, Wieringa F, Ryan EP. Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition. College of Health and Human Sciences Research Day. Colorado State University, Fort Collins, CO. 1 March 2022. [Poster]
- Weber AM. Improving health outcomes with prebiotics. Vice President of Research Fellowship Three Minute Challenge Competition. 15 February 2022. [Oral Presentation]
- Weber AM, Barbazza S, Soekarjo D, Wieringa F, Ryan EP. Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition. College of Veterinary Medicine and Biomedical Sciences. Colorado State University, Fort Collins, CO. 22 January 2022. [Oral Presentation]

Publications

- Pfluger BA, Smith HV, Weber AM, Ibrahim H, Doumbia L, Bore A, Cissoko A, Douyon S, Kone K, Sangare L, Maiga A, Koita O, Goodman K, Evans AM, Ryan EP (2022) Non-Targeted Dried Blood Spot-Based Metabolomics Analysis Showed Rice Bran Supplementation Effects Multiple Metabolic Pathways during Infant Weaning and Growth in Mali. Nutrients. 14:3. Role: Data Analysis
- Seyoum Y, Humbolt C, Baxter BA, Nealon NJ, Weber AM, Ryan EP (2022) Metabolomics of Rice Bran Differentially Impacted by Fermentation With Six Probiotics Demonstrates Key Nutrient Changes for Enhancing Gut Health. Front Nutr. 8:795334. Role: Data analysis.

Workshops attended:

- Responsible Conduct of research workshop, Colorado State University, Spring 2022

Career development activities:

- Colorado State University CVMBS Research Day Conference Session Co-chair, January 2022