CORNELL CENTER FOR
IMMUNOLOGY

CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

RESEACH SYMPOSIUM 2022

March 1, 2022

9:00 – 5:00

coffee at 8:30, lunch provided

Stocking Hall's PepsiCo Auditorium

WEBINAR LINK

ID: 939 4702 4504 Passcode: 8971

Cornell University

8:30 – 9:00 **Coffee and Pastries, poster set up**

9:00 – 9:10 Opening Remarks (Brian Lazzaro, CIHMID Director; Gary Koretzky, CCFI Director)

9:10 – 9:35 David Russell (Microbiology & Immunology, CVM)
"Tuberculosis: Realizing the value of in vivo single cell RNA-seq data"

9:35 – 9:50 Megan Keller (Microbiology, CALS)
"Investigating the Induction Pathway of VxrAB, V. cholerae's antibiotic tolerance response system"

9:50 – 10:05 Shaun Cross (CIHMID Postdoc)
"Implementation of spatial transcriptomics to resolve splenic responses in mice infected with Type 1 Lang reovirus"

10:05 – 10:20 Brandon Hollingsworth (CIHMID Postdoc)
"Data-driven models of Aedes movement and control across heterogeneous landscapes"

10:20 – 10:35 Megan Greischar (EEB, CALS)
"The elusive when and why of synchrony in malaria infections"

10:45 – 11:25 **Coffee Break and presentation of ODD posters**

11:25 – 11:40 Zachary Hilt (Microbiology & Immunology, CVM)
"CD8+ T Cells Infiltrate the Brain Through Gut Homing Receptors During Congenital MCMV Infection"

11:40 – 11:55 Camille Holmes (Population Medicine & Diagnostic Sciences, CVM)
"Quantification of antileukoproteinase in nasal secretions during equine herpesvirus type 1 (EHV-1) infection utilizing newly developed equine specific monoclonal antibodies"

11:55 – 12:10 Elisabeth Larson (Population Medicine & Diagnostic Sciences, CVM)
"IgE-binding monocytes promote allergic inflammation through IL-8 production"

12:10 – 12:35 Gary Whittaker (Baker Institute, CVM)
"SARS-CoV-2 and COVID-19: how four amino acids changed the world"

12:35 – 1:45 **LUNCH**

1:45 – 2:10 Clare Casteel (SIPS)
"The potyviral protease NIa-Pro cleaves MEDIATOR SUBUNIT16 to decrease host resistance"

2:10 – 2:25 Vivianna Sanchez (Microbiology, CALS)
"Genomic diversification of Acinetobacter in floral nectar environments"

2:25 – 2:40 Daniel Sprockett (Ecology & Evolutionary Biology, CALS)
"Local adaptation drives deterministic assembly of host-species specific gut microbiota"

2:40 – 2:55 Catherine Kagemann (MBG, CALS)
"Assessing the interactions between W. pipientis *genotype and titer on the bag of marbles partial loss of function mutant (hypomorph) in* Drosophila melanogaster*"*

2:55 – 3:35 **Coffee Break and presentation of EVEN posters**

3:35 – 3:50 Madhav Mantri (BME/Computational Biology, CALS)
"Spatiotemporal transcriptomics reveals pathogenesis of viral myocarditis"

3:50 – 4:05 Chih-Chun Janet Lin (MBG, CALS)
"Yeast prions regulate host physiology"

4:05 – 4:30 Kelsi Sandoz (Population Medicine & Diagnostic Sciences, CVM)
"Survival lessons from a misfit bacteria; how unusual cell envelope architecture is key to Coxiella burnetii longevity"

SUBMITTED ABSTRACTS

Talks

Investigating the Induction Pathway of VxrAB, *V. cholerae's* antibiotic tolerance response system

Megan Keller

Weill Institute of Cell and Molecular Biology, Cornell University, Ithaca, NY

Bacterial antibiotic resistance is an ongoing epidemic that contributes to over 700,000 deaths annually worldwide. The bacterial cell wall is a vital feature of many pathogens. Various antibiotics are designed to target components of the cell wall, interrupting functionality, and eventually killing the bacteria. β-lactams are a class of cell wall targeting antibiotics that are widely used to treat many clinical infections. However, some species of bacteria, such as Vibrio cholerae, the causative agent behind the diarrheal disease Cholera, carry antibiotic resistance genes, as well as antibiotic tolerance-the ability to withstand bactericidal antibiotics for extended periods of time. V. cholerae has the ability to form spheroplasts, metabolically inactive states that can be reversed when the stressor is removed from the environment. Understanding the mechanisms behind bacterial antibiotic tolerance and resistance is key in mitigating the rising health concern. Recent studies have shown two-component stress response systems (TCS) play a role in increased tolerance to β-lactam antibiotics in V. cholerae. The cell wall two-component system VxrAB senses cell wall damage and initiates a massive cellular response, promoting tolerance. The goal of this project is to determine the induction pathway for VxrAB. VxrAB has been previously shown to be induced through penicillin G (penG) exposure, overexpression of cell wall autolysins, and other cell wall targeting antibiotics, however, the exact mode of induction is still unclear. I present the following data that suggests that when MurJ, the lipid II flippase, is depleted, VxrAB is induced, creating spheroplasts and becoming tolerant to PenG. Additionally, without a functional VxrAB system, the cells are unable to recover MurJ depletion, indicating this system is required for survival. This adds to the growing hypothesis that VxrAB senses the flux of cell wall fragments since without lipid II, the peptidoglycan cannot grow, and through the unchecked activity of autolysins, cell wall fragments accumulate. To find other cellular pathways that hyper-induce VxrAB independent of antibiotics, I conducted transposon mutagenesis. There I found numerous cellular pathways and metabolic cycles that contribute to VxrAB induction, as well as a putative cell membrane protein VCA0040. VCA0040 has been found to be important for cell wall homeostasis during stationary growth and seems to play a role in spheroplast formation.

Implementation of spatial transcriptomics to resolve splenic responses in mice infected with Type 1 Lang reovirus.

Shaun T. Cross[1,2,3], Madhav Mantri[2], Meleana M. Hinchman[1], David W. McKellar[2], Iwijn De Vlaminck[2,3], John S. L. Parker[1,3]

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[2]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University
[3]Cornell Institute for Host-Microbe Interactions and Disease (CIHMID), Cornell University

Mammalian orthoreoviruses (reovirus) are segmented, dsRNA viruses that have been a tractable virus system for studying viral pathogenesis. The Type 1 Lang (T1L) reovirus serotype has been particularly useful for understanding viral infection of the intestinal tract and subsequent systemic spread to secondary tissues. Although systemic spread from the gastrointestinal tract is well described, questions still remain regarding which cell types become infected within individual organs and the host responses that are elicited upon systemic spread. Within secondary tissues, we had a particular interest in the spleen to identify the cell tropism of T1L reovirus in this organ, characterize immune responses responsible for viral clearance at this site of infection, and capture changes of early adaptive responses that may be occurring. We performed spatial transcriptomic sequencing on fresh frozen tissue sections of infected tissues spleens at multiple time points in mouse pups infected with T1L reovirus. To enhance the resolution of spatial transcriptomics, we performed single cell RNA sequencing (scRNA-seq) in parallel to deconvolve the transcriptional information to allow better identification of the cell types infected by T1L reovirus and to identify host transcriptional patterns across various cell types.

Data-driven models of Aedes movement and control across heterogeneous landscapes

Brandon Hollingsworth[1], Michael H Reiskind[2], Alun L Lloyd[3],

[1]Department of Entomology, Cornell University
[2]Department of Entomology, North Carolina State University
[3]Biomathematics Program, North Carolina State University

Aedes albopictus is a disease vector and nuisance mosquito with a wide distribution world-wide, including much of the United States. Its propensity for utilizing small containers (pots and buckets) as larval habitat combined with its short dispersal distance results can significant variation in population densities between neighboring houses. This variation in densities can have a major impact on the effectiveness of mosquito control, but also provides opportunity for improving our current techniques through targeted control. Using a combination of field experiments and modeling, we estimated both the efficacy of common mosquito control strategies and mosquito movement rates between yards. We then simulated mosquito control across a heterogeneous neighborhood, showing that targeting yards with high population density can vastly improve the efficacy of mosquito control programs. Importantly, we found that this result does not require precise targeting and that in many cases nuisance reporting would likely be sufficient.

CD8+ T Cells Infiltrate the Brain Through Gut Homing Receptors During Congenital MCMV Infection

Zachary T. Hilt, Norah L. Smith, Wisler C. Charles, Megan Steinhilber, Brian D. Rudd

Department of Microbiology and Immunology, Cornell University, Ithaca, NY

Congenital cytomegalovirus (CMV) infection occurs in up to 2.2% of births, making it the most common congenital infection in the United States. Brain abnormalities are a frequent clinical outcome in congenital CMV infections that can lead to vision impairment, hearing loss, and motor/cognitive defects. Previous studies have shown that CD8+ T lymphocytes infiltrate the brain in great numbers during congenital CMV infection and have an active role in the resolution of this infection. However, there is still a large gap in the field as to how these CD8+ T cells enter the brain during infection. CD8+ T cells home to the intestine through the CCR9-CCL25 chemokine axis. CCR9 is highly expressed in CD8+ T cells found in congenitally CMV infected brain leading us to hypothesize CD8+ T cells utilize this trafficking mechanism.

Our lab utilizes a mouse model of congenital CMV to model the CD8+ T cell recruitment dynamics and the role CD8+ T cells play during disease progression. Wild-type (WT) and CCR9 knock-out (CCR9-/-) CD8+ T cells were adoptively transferred into MCMV congenitally infected mice. CCR9-/- CD8+ T cells had reduced trafficking into the brain compared to WT mice. Inhibition of CCL25 using an blocking antibody reduced trafficking of CD8+ into the brain. Histology sections of the brain showed that CD8+ T cells were found readily in the thalamus and hippocampus of WT infected mice; however, in CCR9-/- animals CD8+ T cells were not observed in the hippocampus.

These findings indicate that CD8+ T cells utilize a redundant physiological gut-homing mechanism to enter the brain.

Quantification of antileukoproteinase in nasal secretions during equine herpesvirus type 1 (EHV-1) infection utilizing newly developed equine specific monoclonal antibodies

Camille M. Holmes, Susanna Babasyan, and Bettina Wagner

Population Medicine & Diagnostic Medicine, Cornell University, Ithaca, NY

Equine herpesvirus type 1 (EHV-1) is a highly prevalent respiratory pathogen of the horse, which infects the mucosa of the upper respiratory tract (URT) leading to respiratory disease. Entry of the virus into nasal epithelial cells, followed by infection of local lymphoid tissues allows for the establishment of cell-associated viremia. Viral replication in the blood vessels of the placenta and central nervous system, can lead to abortion or neurologic disease respectively. A robust mucosal immune response in protected horses can prevent viremia and viral dissemination, limiting the occurrence of severe disease manifestations. To investigate differences in the mucosal immune response upon infection, nasal transcriptomic profiling during early EHV-1 infection was conducted and identified heightened antileukoproteinase expression in EHV-1 protected compared to susceptible horses. Antileukoproteinase is secreted at mucosal surfaces where it has multiple roles in maintaining homeostasis including inhibition of pathogens, serine proteases, and inflammation. In humans it is seen at high levels in both the respiratory and genital tract, where it has been shown to inhibit viral success. To further characterize the role of this protein during EHV-1 infection, we developed equine specific murine monoclonal antibodies (mAbs) against antileukoproteinase utilizing standard hybridoma technology. This resulted in 11 clones capable of recognizing recombinant protein. An ELISA assay was developed, and three different mAb pairs could detect native protein in mucosal secretion samples. One pair was selected and transitioned onto the Luminex platform for validation. Using this newly developed assay, antileukoproteinase can be detected in various equine derived samples including saliva, serum, nasopharyngeal secretions, and vaginal secretions. Here we identified antileukoproteinase as a potential biomarker during EHV-1 infection and developed mAbs and a Luminex assay to quantify the protein in secretion samples and further investigate its role at the nasal mucosa of the horse.

IgE-binding monocytes promote allergic inflammation through IL-8 production

Elisabeth Larson, Susanna Babasyan, Bettina Wagner

Department of Population Medicine and Diagnostic Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY

Following allergen exposure, individuals with allergies experience a cascade of events orchestrated by the immune system. Innate and adaptive immune cells are activated and recruited to the site of allergen exposure, aided by chemokine production and signaling. One of these chemokines, IL-8, is a pro-inflammatory molecule. We identified IgE-binding monocytes as an important source of IL-8 in allergic individuals. We explored the role of IL-8 in allergy using a large animal allergy model: horses with an IgE-mediated allergy called *Culicoides hypersensitivity.* First, we developed a novel equine IL-8 monoclonal antibody to study IL-8 signaling in allergy. Second, we characterized IgE-binding monocytes by flow cytometry and gene expression. IgE-binding monocytes comprise about 6% of peripheral monocytes in both allergic and healthy horses. Third, we activated cells through IgE crosslinking, which activates the same signaling pathway as an allergen, and found that allergic horses had significantly higher percentages of IL-8+ IgE-binding monocytes in peripheral blood than healthy horses. These results demonstrate how the initial innate immune response following allergen exposure is connected and leads to IgE-mediated clinical allergy. IL-8 production by IgE-binding monocytes provides a link between allergen-specific IgE and innate immune cell-mediated inflammation.

Genomic diversification of *Acinetobacter* in floral nectar environments

Vivianna Sanchez, Tory Hendry

Department of Microbiology, Cornell University, Ithaca, NY

In the anthosphere, transient floral nectar habitats can harbor high densities of microbes that are adapted to thrive in stressful conditions. The microbes present in floral nectar must withstand the environment's high osmolarity, nitrogen scarcity, and plant defense molecules. How these environmental stresses affect microbial ecology and evolution is unknown. *Acinetobacter* is one of the few bacterial genera that dominate the highly selective floral nectar environment, having made an ecological switch from soil to nectar dwelling. This ecological shift has driven dynamic genomic changes, including an overall reduction in gene number, and gains of genes hypothesized to be adaptive in nectar-associated habitats. Phylogenomic analysis of 15 novel *Acinetobacter* isolates and related strains shows that nectar-associated *Acinetobacter* species form a distinct clade that are likely specialized to these habitats. Ancestral reconstruction analyses reveal numerous gene losses, as well as gains of genes presumably beneficial in accessing nutrients in a nutrient poor environment like nectar. Because these common and culturable bacteria experienced an extreme change in selective pressures, they make a useful system in which to understand how ecological switches influence bacterial diversification.

Local adaptation drives deterministic assembly of host-species specific gut microbiota

Daniel D. Sprockett[1], Jeff Price[2], Amanda Ramer-Tait[2], Andrew H. Moeller*[1]

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The composition of the mammalian gut microbiota is strongly associated with host evolutionary history, but the ecological forces generating this pattern remain poorly understood. Here we show that local adaptation of bacterial lineages to hosts drives assembly of host-species specific gut microbiota in house mice. Wild-derived inbred lines of multiple *Mus* species harbored compositionally distinct gut microbiota after multiple generations in a common laboratory environment. Inoculating balanced mixtures of microbiota from the different host species into germ-free house mice revealed consistent competitive dominance of native bacteria. Native competitive dominance was enhanced in germ-free RAG1-/- mice, indicating that a functional adaptive immune system increases permissiveness of hosts to non-native bacteria. This study finds that bacterial adaptation to host species underpins patterns of host-species microbiota specificity.

Assessing the interactions between *W. pipientis* genotype and titer on the *bag of marbles* partial loss of function mutant (hypomorph) in *Drosophila melanogaster*

Catherine Kagemann

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

Wolbachia pipientis are maternally transmitted endosymbiotic bacteria commonly found in arthropods and nematodes. *W. pipientis* have complex interactions with their hosts, and many of these interactions serve to increase transmission. *W. pipientis* commonly manipulate reproduction of the host via cytoplasmic incompatibility, resulting in embryonic mortality. However, there are other known interactions between *W. pipientis* and its host. For example, *W. pipientis* rescues the *bag of marbles (bam)* partial loss of function (hypomorph) fertility phenotype in female *Drosophila melanogaster*. *Bam* is an important germline stem cell (GSC) gene involved in GSC renewal and cystoblast differentiation. GSCs are required for the production of egg and sperm, making the genetic interaction between *W. pipientis* and GSC genes such as *bam* of great evolutionary interest to us. While we understand that *W. pipientis* contributes to the rescue of the *bam* hypomorph phenotype, we have yet to determine the functional mechanisms that are behind this interaction. Therefore, I aim to elucidate 1) whether variation in *W. pipientis* variant genotype and titer influence the rescue of the mutant *bam* phenotype at different ages in adult females and 2) whether *W. pipientis* variants cause differential rescue of the *bam* hypomorph phenotype at the transcriptional level as the host fly ages. Results show that rescue of the mutant *bam* phenotype does in fact depend on the genotype of *W. pipientis* as the flies age and the magnitude of rescue is dependent on the age of the female fly. Relative quantification of *W. pipientis* titer via qPCR shows that titer increases in all *W. pipientis* genotypes at the peak rescue of the *bam* hypomorph phenotype. Our RNA-seq analysis revealed that *W. pipientis* infected *Drosophila* differentially express many of *bam's* genetic and physical interactors in the *bam* hypomorph genotype. RNAi will be used to determine whether any of these candidate genes identified from our RNA-seq analysis are responsible for aiding in the rescue of the *bam* hypomorph phenotype at the transcriptional level.

Spatiotemporal transcriptomics reveals pathogenesis of viral myocarditis

Madhav Mantri[1], Meleana M. Hinchman[2], David W. McKellar[1], Michael F. Z. Wang[1], Shaun T. Cross[1,2,3], John S. L. Parker[2,3*], Iwijn De Vlaminck[1,3*]

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A significant fraction of sudden death in children and young adults is due to myocarditis, an inflammatory disease of the heart, most often caused by viral infection. Here we used integrated single-cell and spatial transcriptomics to create a high-resolution, spatially resolved map of reovirus-induced myocarditis in neonatal murine hearts. We assayed hearts collected at three timepoints after reovirus infection and studied the temporal, spatial, and cellular heterogeneity of host-virus interactions. We further assayed the intestine, the primary site of reovirus infection to establish a full chronology of molecular events that ultimately lead to myocarditis. We implemented targeted enrichment of viral transcripts to establish the cellular targets of the virus in the intestine and the heart. Our data give insight into the cell-type specificity of innate immune responses, and into the transcriptional states of inflamed cardiac cells that recruit circulating immune cells, including cytotoxic T cells which induce pyroptosis in the myocarditic tissue. Analyses of spatially restricted gene expression in myocarditic regions and the border zone around those regions identified immune-mediated cell-type specific injury and stress responses. Overall, we observe a dynamic and complex network of cellular phenotypes and cell-cell interactions associated with viral myocarditis.

Yeast prions regulate host physiology

Chih-Chun Janet Lin[1], Maushmi D. Chitale[1], Jessica Y. Jiang[1], Elissa J. Cosgrove[1], Alexandria C. Van Elgort[2], Asha M. Jain[1], Julia C. Kelso[1], Xinyue Cui[3], Nilay Yapici[3], Danial F. Jarosz[2], Andrew G. Clark[1]

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Prions, misfolded proteins best known for causing mad cow disease, can help yeasts survive harsh environmental conditions. The prion-directed beneficial traits can also be passed down to their offspring for >100 generations. In addition, more than one-third of wild yeast isolates are found to harbor various kinds of prion proteins. Forming prion proteins seems to be a common strategy for microbes to endure physiological challenges. However, whether and how these yeast prions play a role in host-microbe interaction is entirely unknown. Fruit flies routinely encounter yeasts in the wild and the lab. Yeasts are an essential member of the fly mycobiome. They not only serve as a protein source, but yeasts also provide significant signaling cues regulating fly physiology, reproduction, and behavior. Here we use *Drosophila melanogaster* and *Saccharomyces cerevisiae* as a model to investigate the mechanism by which mycobiome-derived prions impact host health. After testing a battery of prion proteins, we found yeasts with the prion protein [MRPL10+] significantly promote cold tolerance in flies. We also discovered a wide range of cold tolerance levels across global diversity lines (GDLs), a collection of fly lines with different genetic backgrounds. By harnessing the power of large-scale GDL screening, GWAS, functional genomics, and RNAi screening, we identified a list of candidate fly genes functionally crucial in responding to yeast-derived prion protein [MRPL10+]. Following up on several candidate genes, we uncovered the neuronal cause of the cold tolerance phenotype in flies. Our results reveal the novel link between fungal prion proteins and host physiology. This appears to be the first systematic analysis of the genetic interaction of fungal prions in the host. Our approach will facilitate the discovery of beneficial prions in microbes and provide mechanistic insight into the mycobiome field.

SUBMITTED ABSTRACTS

Posters

Remodeling of the gut microbiome over the annual cycle of a long distance migratory bird

Catherine Andreadis

Department of Ecology and Evolutionary Biology, Cornell University

Host-associated microbiota (the archaeal, bacterial, fungal, and viral communities of the gastrointestinal tract) have been shown to have increasingly relevant roles in developing host physiological processes. However, most of these discoveries have been isolated to mammalian or human models. Migratory birds represent a powerful system to study dynamic shifts in the microbiome that could have potentially beneficial implications on host physiology and ecology. Here, we plan to show the role of the microbiome in avian models undergoing the process of migration, specifically that of the Blackpoll Warbler (*Setophaga Striata*), and how shifts in microbiome composition could be connected to biological changes that are essential for Blackpoll migratory physiology.

POSTER presentation

Upasana Basu

Department of Microbiology, Cornell University

The peptidoglycan (PG) cell wall, surrounding the bacterial cell is a heteropolymer, consisting of glycan strands made of alternating of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues, crosslinked via short peptide stems to form a robust yet elastic mesh-like sacculus. This critical structure is a ubiquitous feature found in most bacteria that enables it to withstand high internal turgor pressure and maintain cell shape. Though it is indispensable for survival in different conditions, it interferes with important life processes like cell division, growth, etc. Therefore, the cell wall has to be a highly dynamic structure, constantly undergoing degradation, recycling, and synthesis to maintain the integrity of the PG sacculus. The unregulated activity of either the PG synthases or the 'autolysins' can have catastrophic effects leading to compromised PG and ultimate lysis. Though various steps of the biosynthesis pathways have been attractive antibiotic targets, we lack a clear understanding of the underlying fundamental mechanisms.

The term 'autolysins' refers to the collective set of diverse enzymes that degrade different bonds within the PG. One of those enzymes, in the class of lytic transglycosylases (LTGs), catalyzes the non-hydrolytic cleavage of the glycosidic bonds between the NAM and NAG residues. Although they have been biochemically well-characterized, little is known about their physiological roles and the reason behind their highly redundant occurrence in bacteria. To address this gap in knowledge, we generated multiple gene knockouts of the LTGs, i.e., the Δ6LTG, and Δ7LTG mutants, and performed Tn-seq to determine genetic interactions, that were either conditionally essential or lethal during LTG insufficiency. Out of the many potential candidates, came up with two novel response regulators that have never been connected to cell wall turnover in any organism before. The absence of these response regulators in the LTG knockout mutant background affects the fitness of cells, leading to elongation of cells and lysis, thus, validating the importance of these genes.

A Role for Stimulator of Interferon Genes in Toll-Like Receptor Signaling

Karla García-Martínez, Jingyi Chen, and Cynthia Leifer, PhD

Department of Microbiology and Immunology, Cornell University, Ithaca, NY

The innate immune system is equipped with multiple receptors to detect microbial nucleic acids and induce type I interferon (IFN) to restrict viral replication. Yet, these same receptor pathways induce inflammation in response to host nucleic acids and promote development and persistence of autoimmune diseases like Systemic Lupus Erythematosus. Crosstalk between some innate receptor pathways is known, but crosstalk between nucleic acid sensing receptor signaling pathways is poorly understood. IFN production is regulated by the Interferon Regulatory Factor (IRF) transcription factor family downstream of several innate immune receptors such as Toll-like receptors (TLRs) and Stimulator of Interferon genes (STING). Both TLRs and STING activate TANK-binding kinase I (TBKI), which subsequently phosphorylates and activates IRFs. The pathway by which TLRs and STING activate TBK1 are considered independent. However, here we provide evidence that STING plays a previously unappreciated role in human TLR8 signaling. Using THP-1 cells encoding reporters for IRF and NFkB activity, the two major signaling pathways downstream of TLRs, we show that the TLR8 ligand TL8-506 increased IRF and NFkB activity. When STING was knocked out or inhibited with the inhibitor H151, IRF, but NFkB, activity was reduced. TL8-506 stimulation induced time-dependent phosphorylation of TBK1, which was absent in STING-deficient cells. Moreover, lack or inhibition of STING reduced TL8-506-induced IL-6 production. Additionally, RNA sequencing data identified multiple TL8-induced STING dependent genes. Absence of cGAS, a major sensor of cytoplasmic DNA and the upstream activator of STING, had no effect on TLR signaling or direct activation of STING by its ligand cGAMP suggesting an alternate mechanism of STING activation. TLR8 stimulation of THP-1 cells and primary human monocytes resulted in phosphorylation of STING in a time-dependent manner. STING inhibition significantly reduced IL-6 production in human primary monocytes. Together, these data demonstrate that TLR signaling leads to STING phosphorylation and that STING is a previously unappreciated component of the human TLR signaling pathway. Our studies expand our understanding of TLR signaling and regulation and build a new framework for crosstalk between cytosolic and surface/endosomal innate immune receptors that could be used to treat autoimmune diseases mediated by high IFN.

Application of mathematical modeling and economic approaches to improve the control of multidrug-resistant *Salmonella* Dublin in heifer-raising operations

Llanos-Soto, S.[1], Wiedmann, M.[2], Adalja, A.[3], Henry, C.[1], Ivanek, R[1]

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[2]Department of Food Science, Cornell University
[3]School of Hotel Administration, Cornell University

This study aimed to develop a model to predict multidrug-resistant (MDR) *S.* Dublin transmission dynamics in a heifer-raising operation under different mitigation strategies and determine the cost-effectiveness of those mitigation strategies. A preliminary Susceptible-Infected-Recovered (SIR) model was developed to describe the spread of MDR *S.* Dublin in a heifer-raising operation in the Northeastern USA. The model accounted for persistently infected heifers (i.e., carriers) and the influence of seasonal variation in ambient temperature on MDR *S.* Dublin decay rate in the environment. Stochasticity was introduced into the model through Monte Carlo simulations. Vaccination effectiveness (assessed through their effectiveness in reducing susceptibility, shedding levels, and duration of the infectious period) and testing & culling (i.e., removal of a carrier after identification through two ELISA tests applied in series), was evaluated in small- (200 heifers), medium- (500 heifers), and large-sized (1000 heifers) operations in terms of a predicted reduction in the proportion of carriers, number of *S.* Dublin-related deaths, and culled heifers by the end of a three-year period of simulation. Earnings from prevented losses and costs of these interventions were also evaluated and used to determine the operation's profit (i.e., difference between earnings and costs). Based on model predictions for all three tested herd sizes, testing & culling would result in a 3-times lower proportion of carriers compared to doing nothing, but the costs would increase 2.9 to 4.5 times. Furthermore, using a vaccine with 25% effectiveness prevented 68%, 24%, and 10% of the *S.* Dublin-related deaths predicted in the doing nothing scenario for small-, medium-, and large-sized operations, respectively. Meanwhile, using a vaccine with 50% effectiveness increased profit 1.3 to 1.4 times for all three operation sizes. We conclude that the application of imperfect vaccines can control and prevent infection and decrease mortality due to MDR *S.* Dublin in heifer-raising operations.

Viral capsid, antibody, and receptor interactions: Understanding the binding, neutralization, antibody escape, and receptor binding sites of parvoviruses

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Viruses circulating in nature evolve and frequently emerge as new variants due to evolutionary forces that can result from mutation and selection – including key interactions with receptors and antibodies. Here we study the evolution of canine parvovirus (CPV) incubated with neutralizing antibodies to reveal how those are controlled by the dynamics of binding, neutralization, antibody escape, and overlap with the receptor binding site. CPV is a non-enveloped virus with a single-stranded DNA genome, and it causes serious disease in animals worldwide. The original strain (CPV-2) emerged as a new dog pathogen during the late-1970s due to a host jumping event. The subsequent natural evolution of the virus in dogs and other hosts has altered both receptor- and antibody-binding, with some single changes affecting both functions.

Our study reveals that only a small number of mutations arise within the viral capsid (VP2) under the *in vitro* selection with each of two neutralizing antibodies which bind distinct sites on the capsid, and which show different levels of overlap with the transferrin receptor (TfR) attachment site. Mutations occurred primarily within the antibody footprints, and few changes occurred in the TfR binding footprint. Remarkably, 54% of the antibody-selected mutations identified were also present in natural circulating variants, showing the connections between our *in vitro* study and natural infection, and also the potential for emergence of variants in nature due the host-humoral immune response. To understand escape mutation dynamics, we also engineered the capsid-binding sites of the two antibodies. The *in vitro* selection experiments with those engineered antibodies also showed similar mutations to those selected by the wildtype, despite the alternative interactions with the capsid. This study suggests potential mechanisms by which these variants emerged in nature and provides a better understanding of the coordinated interactions between antibodies and receptors.

Functional characterization of the glycoproteins from a novel strain of Hendra virus

Andrew Z. Ma[1]*^, Yao Yu Yeo[1,2*], Colin M. Kim[1], Viraj Upadhye[1], Carolina Menchaca[1], David W. Buchholz[1], and Hector C. Aguilar[1], *Authors contributed equally

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^CIHMID Undergraduate Research Experience student

A novel Hendra virus strain (HeV-2) was recently discovered in a deceased horse from Australia. Phylogenetic analyses, glycoprotein domain mappings, and sera cross-reactivity assays were performed to demonstrate the antigenic similarity of HeV-2 to original Hendra virus strain (HeV-1). Basic glycoprotein processing and receptor binding were assessed via Western blot and flow cytometry, and presence of cell-cell and virus-cell fusion in human cells altogether suggest the ability of HeV-2 to infect human hosts. Furthermore, cells expressing heterologous combinations of HeV-1, HeV-2, and Nipah virus (NiV) glycoproteins resulted in large differences in fusion phenotypes, suggesting an intrinsic fusion capacity of each of these glycoproteins. Because of the zoonotic ability of Henipaviruses, it is crucial to determine spill-over dynamics, host pathogenicity, evolutionary patterns, and other characteristics of this novel Hendra virus.

How do Th1 cells and moDCs Cooperate in the Formation of CXCL10+ Peripheral Activation Niches?

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T cell activation is one of the main means through which the body defends itself against foreign pathogens. Initial antigen (Ag) presentation and differentiation occurs in the lymph node, but peripheral reactivation is required for proper microbial clearance. All of these processes rely on precise spatial and temporal cues to efficiently colocalize T cells with antigen presenting cells (APCs). We have found that antigenic challenge in the dermis generates perivascular clusters of CXCL10+ cells enriched for MHC-II+ CD11c+ monocyte-derived dendritic cells (moDCs). These perivascular chemokine expressing clusters were found to be preferred sites of entry for effector Th1 cells and serve to promote peripheral activation by facilitating Th1-APC encounters. To determine the origin and functional requirements for innate and adaptive immune cell subsets in establishing these activation niches we are utilizing a combination of photoactivation tools, to assess immune cell recruitment, and target depletion of immune subsets. Initial studies have found that many of the monocyte/macrophage and moDC CXCL10+ populations are actively recruited from the blood during the inflammatory response. Future experiments will test the role of these newly recruited cells in the establishment of the activation niche by targeted deletion of CCR2.

CXCL10 expression is upregulated by IFNg, prompting us to test the role of Th1 cells themselves in niche development. Deletion of CD4+ T cells led to a marked reduction in the number of CXCL10+ myeloid cells, in particular a loss in the CXCL10+ moDC subset. Furthermore, CD4 depletion had a profound impact on the activation niche itself, with a reduced frequency and range of these CXCL10+ clusters in the inflamed ear dermis. These findings support a model whereby the initial interaction of Th1 effectors and APCs in these perivascular niches, drives a positive amplification loop that boosts Th1 activation in peripheral tissues through the further recruitment of CXCL10+ moDCs and establishment of additional niches that optimize Th1 activation.

This flower ain't big enough for the both of us: In vitro competition between different strains of nectar-associated Acinetobacter spp.

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Floral nectar is known to harbor a rich microbial community. The microbial diversity and abundance in nectar is greatly affected by the movement of microbes by pollinators. To better understand this exchange, it is necessary to examine how well equipped microbial taxa are to persist in floral nectar environments. Many species of Acinetobacter bacteria have frequently been found in floral nectar in various locations globally. I examined how different strains of floral associated Acinetobacter spp. interact and compete for resources among themselves or other community members. I hypothesized that different strains of Acinetobacter spp. would contribute to the inhibition of other strains of Acinetobacter spp., due to competition for resources in the same niche. To test this hypothesis, I performed competitive inhibition assays using six different overlay strains of Acinetobacter and competing strains and tested for growth or inhibition of each drop/overlay combination. In future experiments, I will examine what genetic factors could be contributing to inhibitory effects of certain Acinetobacter strains as well as whether they could potentially inhibit pollinator pathogens. Using this knowledge of the microbial community in nectar environments can benefit our understanding of pollinator health and illuminate more details of this mutualistic pollinator-plant relationship.

Optimization of spatial transcriptomics to characterize the transcriptional landscape of arbuscular mycorrhizal symbiosis in *Medicago truncatula*

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Arbuscular mycorrhizal symbiosis is an association that forms between host plant roots and soil fungi. The underground relationship takes place within plant root cells, and the colonization of these plant cells enables both plants and fungi to exchange and acquire critical nutrients. Through the use of model plant species amenable to genetic manipulation, key plant genes central to the symbiotic program have been identified; however, the broader regulation and timing of the transcriptional landscape requires further characterization. To detect differences between colonized and noncolonized plant roots, we adopted a spatial transcriptomic approach with the objective to map the plant and fungal transcriptomes along a spatiotemporal gradient of colonization. Optimization for plant root tissue was necessary prior to constructing a spatial RNAseq library using the 10X Genomics Visium platform. A modified tissue optimization protocol was used which incorporated fluorophore-conjugated nucleotides during reverse transcription to visualize the cDNA footprints prior to sequencing. Tissue optimization experiments show that complete removal of plant root tissue is necessary to prevent strong autofluorescent signal from root vascular tissue. After complete removal of tissue, the cDNA footprint can be seen after either 24 or 30 minutes of permeabilization. Further optimization of permeabilization timing should produce more consistent spatial capture of transcripts and enable the progression of plant root spatial transcriptomic experiments.

Characterization of *Legionella* lysine palmitoyltransferases

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Legionella pneumophila is the causative agent for Legionnaire's disease, a potentially fatal form of pneumonia. *L. pneumophila* naturally infects freshwater protists but can accidentally infect patients who breath in contaminated aerosol. Upon phagocytosis by alveolar macrophages, *Legionella* establishes a replicative niche within the host cell by injecting more than 300 different bacterial effector proteins to hijack and subjugate conserved eukaryotic pathways. Despite considerable efforts over the past decades to study these effectors, the biological functions of many remain elusive, due to low sequence homology to most known bacterial toxins. Here, I have identified a *L. pneumophila* lysine-palmitoyltransferase, lpg1387, which specifically localizes to F-actin and palmitoylates a diverse cohort of eukaryotic substrates. The biophysical and biochemical characterization of lpg1387 will hopefully shed light on an under-studied class of enzymes that are critical for the evolutionary fitness of not just *Legionella*, but many other pathogens as well.

Manipulation of host actin cytoskeleton by the *Legionella* effector MavH

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Legionella pneumophila, the etiological agent of Legionnaires' disease, replicates intracellularly in protozoan and human hosts. Successful colonization and replication of this pathogen in host cells requires the Dot/Icm type IVB secretion system, which translocates approximately 300 virulence proteins into the host cell. These virulence proteins, called effectors modulate distinct host cellular processes to create a ER like membrane-bound niche called the *Legionella* containing vacuole (LCV) supportive of bacterial growth.

In this study, we identified MavH(Lpg2425) as a *Legionella* effector that targets actin cytoskeleton and serves as an actin nucleator to promote actin polymerization around PI3P positive organelles. MavH harbors a PI3P binding motif which targets MavH to early endosomes when overexpressed in mammalian cells. More importantly, MavH interacts with actin via N terminal actin binding motif and recruits CP proteins by CPI motif to control F-actin density around PI3P positive organelles in living cells. We recapitulate the function of MavH by in vitro pyrene actin polymerization assay and liposome imaging. We found that MavH regulates F-actin formation by promoting actin nucleation around PI3P positive liposomes and CP can be recruited on liposomes to regulate F-actin density. Furthermore, MavH has caused dysregulation of endosomal trafficking. Together with earlier studies, these results reveal that multiple *L. pneumophila* effectors target host cytoskeleton with distinct mechanisms, highlighting the importance of modulating cellular processes governed by the actin cytoskeleton in the intracellular life cycle of this pathogen.