CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

RESEACH SYMPOSIUM 2023

August 9, 2023

9:00 – 3:30

coffee at 8:30, lunch provided

Stocking Hall's PepsiCo Auditorium

Cornell University

8:30 – 9:00 Coffee and Pastries

9:05 – 9:15 Opening Remarks (Tobias Döerr, Microbiology)

9:15 – 9:45 Daniel Buckley (SIPS)
"Phenolic acid degrading bacteria prime soil organic matter degradation in the rhizosphere microbiome"

9:45 – 10:05 Joshua Kerkaert (CIHMID Postdoc)
"Identifying Novel Regulators of Carbon Utilization in Fungi"

10:05 – 10:25 Megan Keller (Microbiology)
"Sugar accumulation from disrupting central metabolism leads to bacterial cell-wall stress"

10:30 – 11:00 Coffee Break

11:00 – 11:20 Camille Holmes (Population Medicine & Diagnostic Sciences, CVM)
"Characterizing mucosal T cells and their role in Equine herpesvirus type 1 (EHV-1) infection"

11:20 – 11:40 Brian Wasik (CVM)
"Influenza in Horses and Dogs: When can we say a lineage is 'extinct'?"

11:40 – 12:10 Jaehee Kim (Computational Biology)
"Computational methods for genetic epidemiology of tuberculosis"

12:15 – 1:30 Lunch

1:30 – 2:00 Tory Hendry (Microbiology)
"Should I stay or should I glow? Bacterial epiphytes and aphid behavior"

2:00 – 2:30 Tobias Doerr (Microbiology)
"Multiple resistance factors contribute to Enterobacter cloacae survival in the presence of host-produced antimicrobial peptides"

2:30 – 3:30 Poster Session, Reception

ABSTRACTS – TALKS:

Phenolic acid degrading bacteria prime soil organic matter degradation in the rhizosphere microbiome

Daniel H. Buckley

Soil & Integrative Plant Sciences, Cornell University

Plants acquire essential nutrients from soil organic matter (SOM), but they lack the enzymatic ability to degrade SOM. Plants alter microbial activity within the rhizosphere microbiome, through root exudation and rhizodeposition, in order to optimize nutrient acquisition. This plant priming of microbial activity has a major impact on plant fitness and the terrestrial carbon cycle. Despite the importance of plant-microbe interactions on SOM turnover and plant nutrient acquisition, mechanisms by which plants prime SOM degradation remain poorly described. We hypothesize that phenolic acid degrading bacteria are critical mediators of this priming effect. We show that phenolic acid degrading bacteria prime SOM degradation in response to phenolic acids commonly detected in root exudates. We show that these bacteria are widespread in soils and that changes in their activity have significant impacts on SOM degradation. We propose that phenolic acids might be important mediators of plant-microbe interactions within the rhizosphere microbiome.

Sugar accumulation from disrupting central metabolism leads to bacterial cell-wall stress

Megan Keller, Tobias Döerr

Department of Biomedical and Biological Sciences, Cornell University

Like many gram-negative pathogens, *V. cholerae* utilizes a wide range of energy sources and pathways. This metabolic freedom enables gram-negative pathogens the ability to infiltrate the host and thrive in niche environments. Gram-negative bacteria are also common evaders of antibiotic efficacy, posing an increasing threat to public health. While the energy state of a bacterium has been linked to how effective an antibiotic is, *V. cholera*'s central metabolism remains unexplored in its connection to drug susceptibility, particularly towards the cell wall targeting β-lactams. Using a cell wall stress sensing system and a transposon mutagenesis genetic screen, we found that when central carbon metabolism is disrupted at the stage of early glycolysis (phosphoglucose isomerase, encoded by the *pgi* gene), the bacterial cell wall is subsequently damaged and *V. cholerae* becomes more susceptible to cell wall-active antibiotics, such as the β-lactams. Additionally, we observed severe morphological defects and sensitivity to low osmolarity conditions, further supporting the idea that the *pgi* mutant has a damaged cell wall. Suppressor mutations within the phosphotransferase system (PTS) restored all defects associated with the *pgi* mutation, suggesting that excess sugar phosphates are deleterious in a *pgi* mutant. We propose that these sugar phosphates act as a competitive inhibitor of the cell wall precursor enzymes, GlmMU, displacing its natural substrate, glucosamine-6-phosphate. With GlmMU's activity decreased the bacterial cell wall struggles to maintain efficient cell wall synthesis and thus is more susceptible to β-lactams. The external addition of the cell wall precursor GlcNAc surprisingly rescued the observed defects. GlcNAc is converted to glucosamine-6-phosphate by the enzyme NagA, which likely relieves competitive inhibition of GlmMU. This work demonstrates the need for further research into bacterial metabolism's ability to enhance antibiotic susceptibility, and the link between carbon metabolism and cell wall synthesis.

Characterizing mucosal T cells and their role in Equine herpesvirus type 1 (EHV-1) infection

Camille Holmes, Susanna Babasyan, Bettina Wagner

Cornell University, College of Veterinary Medicine, Population Medicine and Diagnostic Sciences

Equine herpesvirus type I (EHV-1) is an alphaherpesvirus that infects horses early in life and can establish latency in its host. Like the related alphaherpesviruses found in humans, EHV-1 infects via mucosal surfaces, in particular, the upper respiratory tract (URT). Viral replication at the site of entry leads to respiratory disease, and susceptible horses will establish cell-associated viremia. Systemic spread can lead to more severe disease manifestations including abortion in pregnant mares, and the neurological disease, equine herpesvirus myeloencephalopathy (EHM). A robust mucosal immune response could prevent viral replication and viremia, limiting the occurrence of these devastating outcomes. Here, we explore the presence and role of mucosal-derived T cells in the context of EHV-1 infection. First, mucosal and peripheral cells were collected from horses who showed serum antibody values consistent with protection (n=6) or susceptibility (n=6) to EHV-1. Mucosal cells were collected by three different methods to target different localized populations. Nasal washes collected lumenal cells, nasal swabs collected mucosal surface cells, and nasal brushes collected mucosal cells. Cells were characterized for surface expression of LFA-1, CD4, and CD8 to quantify the T cell population at each location. $CD4^+$ T cells were consistently represented in both mucosal and peripheral samples, while $CD8^+$ T cells were found in significantly higher numbers in mucosal ($p=0.0002$) and mucosal surface ($p=0.0120$) samples, compared to the periphery. Interestingly, a significantly higher percentage of $CD4^+$ $CD8^+$ T cells were present in all three nasal samples ($p<0.002$) compared to the periphery. Next, mucosal and peripheral cells were infected *in vitro,* to determine responsiveness to EHV-1. Cells were stained for co-expression of CD4, CD8, and IFN-γ, and the supernatant was collected for quantification of cytokines. Peripheral cells collected from horses protected from EHV-1 produced IFN-γ during viral stimulation, however, nasal T cells did not. Interestingly, with a general T cell stimulant, both peripheral and nasal T cells produced IFN-γ. Together this demonstrates that nasal T cells are mature and can become activated, but do not respond to the virus under the same conditions as peripheral cells. Work is ongoing to determine the origin of the T cell population that is present in the upper respiratory tract during infection.

Influenza in Horses and Dogs: When can we say a lineage is 'extinct'?

Brian R Wasik, Colin R Parrish

Cross-species virus transmission events can lead to dire public health emergencies in the form of epidemics and pandemics. The emergence of Influenza A viruses (IAV) into mammalian species is a rare occurrence, often limited to few subtypes that cross host barriers from waterfowl reservoirs. Further, both the necessity of mammalian host adaptation and stochastic host ecological structures limit the ability of spillover lineages to establish onward transmission conditions for respiratory epizoonosis. Yet, these phenomena occur. It is less clear how stable these new lineages will remain, or if they reside on a precarious path that may include extinction. The H3N8 equine influenza virus (EIV) is one of the longest continually circulating non-human IAVs known. EIV was first isolated in 1963 in Miami, Florida, USA, after emerging among horses in South America. In the early 21st century the American lineage of EIV diverged into two 'Florida' clades that persist today, while an EIV transferred to dogs around 1999 and gave rise to the H3N8 canine influenza virus (CIV), first reported in 2004. We compared CIV in dogs and EIV in horses to reveal their host-specific evolution, to determine the sources and connections between significant outbreaks, and to gain insight into the factors controlling their different evolutionary fates. H3N8 CIV only circulated in North America, was geographically restricted after the first few years, and went extinct in 2016. Of the two EIV Florida clades, clade 1 circulates widely and shows frequent transfers between the USA and South America, Europe and elsewhere, while clade 2 was globally distributed early after it emerged, but since about 2018 has only been detected in Central Asia. Any potential zoonotic threat of these viruses to humans can only be determined with an understanding of its natural history and evolution. Our comparative analysis of these viral lineages reveals distinct patterns and rates of sequence variation yet with similar overall evolution between clades, suggesting epidemiological intervention strategies for possible eradication of H3N8 EIV. We seek to identify the key conditions that determine influenza lineage success over extinction.

Should I stay or should I glow? Bacterial epiphytes and aphid behavior

Tory Hendry

Aphids, plant pest insects, encounter diverse bacterial communities on leaves. These microbes can impact aphid survival and behavior. Our previous work found that some *Pseudomonas syringae* strains could infect and kill aphids at high rates. We also found that bacterial virulence to aphids was correlated with another trait: fluorescent emission of blue light. Remarkably, highly virulent *P. syringae* strains create more blue fluorescence and aphids avoid feeding on plants when these strains are present. Here we tested a higher diversity of *Pseudomonas* strains, which vary significantly in their fluorescence, for their effects on aphid survival and behavior. We find that variation in bacterial fluorescence can lead to very different changes in aphid feeding behavior, and that these changes do not appear to be adaptive to aphids. Aphids are common vectors of plant diseases and can be difficult to control in agricultural systems; this work has implications for novel pest control methods for aphids.

"Multiple resistance factors contribute to *Enterobacter cloacae* survival in the presence of host-produced antimicrobial peptides"

Tobias Döerr

Department of Biological and Biomedical Sciences, Cornell University

In eukaryotic innate immunity, Antimicrobial peptides (AMPs) are part of the first line of defense against pathogens. In turn, bacterial pathogens have evolved multiple AMP defense systems that promote their survival during infection. Here, we genetically dissected the response of the important opportunistic pathogen Enterobacter cloacae to the insect AMP cecropin B. We find that AMP resistance is semi-redundantly mediated by both known and novel AMP resistance factors, and displays marked population heterogeneity at low AMP concentrations. Our data suggest that AMP resistance is multi-tiered, perhaps to optimize resistance against the large diversity of AMPs observed in nature.

SUBMITTED ABSTRACTS - POSTERS:

The role of cell wall remodeling in innate immunity of early divergent Mucoromycotina Fungi

Hana Barrett[1], Maria Laura Gaspar[2] and Teresa E. Pawlowska[3]

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Some strains of early divergent fungus *Rhizopus microsporus* (Mucoromycotina) form endosymbiotic relationships with *Mycetohabitans* spp. bacteria, while others have an antagonistic interaction. Due to the similarity between the symbiotic and antagonistic partners, this system is an excellent model for the study of the mechanisms of the innate immune response in early divergent fungi, which are not well understood. We use the antagonistic interaction between the bacteria from the symbiotic partner and the non-host fungus to investigate fundamental questions about the innate immune response. We also partnered with Dr. Victoriano Garre and his PhD candidate Carlos Lax (University of Murcia, Spain), to generate CRISPR/Cas9 disruption mutants of two adenylyl cyclase genes that encode receptors associated with immune activation and cell death in other fungi to identify their role in this system. Previous work from our lab found significant differential gene expression in non-host fungi in coculture with bacteria vs fungi alone. Notably, genes associated with cell wall components chitin, fucose, glucan, mannose, and galactose were differentially expressed in the antagonistic interaction. We are currently working to quantify and visualize cell wall remodeling in the antagonistic interaction between fungal germlings and *Mycetohabitans* bacteria by fluorescently probing cell wall components and using microscopy and flow cytometry for visualization of the stains. We are looking for any change in the form or composition of the cell wall, which would be expected based on the gene expression results. Preliminary results show increased exposed chitin content in the wildtype fungus in coculture vs the fungus alone, as expected from gene expression data. No change in mannose content between the two conditions has been seen. In the adenylyl cyclase mutants, disruption of adenylyl cyclase 1 had increased chitin in the fungus alone and disruption of adenylyl cyclase 2 had dramatically increased chitin in the coculture. This supports our hypothesis that adenylyl cyclase 1 is an immune activator and 2 is an immune suppressor.

Investigating the relationship between burying beetles (*Nicrophorus* spp.) and associated microbial symbionts

Kathryn Herr

Department of Microbiology, Cornell University

What governs the structure and function of our microbiota? Symbiosis with beneficial microbes can make available new frontiers for their host. It has long been known that a relationship with microbiota can be key in enabling host exploitation of new niches. However, the origin of microbiota which allow for such niche exploitation is often unclear. Are microbiota picked up by the host from the environment and through repeat transmission evolve to become symbionts? Or, is the host able to select for microbes which confer an evolutionary benefit to the host? Burying beetles (*Nicrophorus* sp.) provide a model system in which to study the evolutionary origins of symbiosis between host and microbiota. Through beetle-regulated manipulation of carcass microbial communities, burying beetles are able to 'preserve' small vertebrate carcasses for larval consumption. This manipulation is achieved through the application of the beetle's microbial-laden oral and anal secretions. Previous research into this system has worked to identify several possible *Nicrophorus*-associated symbionts, including *Yarrowia*-like yeasts (YLY), which may have roles in carcass microbiome manipulation and nutrient biosynthesis. However, few of these studies agree on a core set of symbionts and none have distinguished between benefits provided by symbionts as opposed to benefits provided by secretion fluids alone.

More information is needed to determine which microbes are consistently associated with *Nicrophorus*, and what role symbionts have in exploitation of this unique niche. Here, I investigate the relationship between *Nicrophorus* and *Yarrowia* symbionts. I further examine whether these symbionts have co-diverged with their hosts, or if this association could be the result of host-filtering of environmental microbes. To do this, I utilize metagenomic sequencing of guts and secretions from 5 *Nicrophorus* species as well as genomic sequencing of YLY isolates cultured from these *Nicrophorus* secretions.

Metabolic Costs of Chronic Bacterial Infection in *Drosophila melanogaster*

Ananda Kalukin, Drea Darby, Scott Keith, Brian Lazzaro

To effectively respond to pathogens, animals must mount an immune response at a significant metabolic cost. When challenged with infection, *Drosophila* individuals divert energetic resources to produce antimicrobial peptides (AMPs), phospholipids, and other molecules involved in immune defense. Our lab previously found that chronic, sub-lethal infection with a pathogenic strain of *Providencia rettgeri* decreases starvation resistance in flies, suggesting that sustained activation of the innate immune system in chronically infected flies may deplete energy stores. To further test this hypothesis, we assayed starvation sensitivity in flies sustaining chronic, systemic infection with six different bacterial strains that vary in host mortality and chronic bacterial burden. We show that increased starvation susceptibility correlates to higher chronic bacterial load, but not necessarily increased infection-induced mortality. Because starvation sensitivity can reflect reduced lipid stores, we analyzed how infection with two strains of the gram-negative bacterium *Serratia marcescens* affect triglyceride levels. Flies infected with S.m.-2698B develop a higher chronic bacterial burden than those infected with S.m.-BPL. Relative to S.m.-BPL infection, S.m.-2698B infection triggers a significant decrease in triglyceride levels 5 days post-infection. To investigate the molecular mechanisms behind this phenotype, we examined how infection impacts expression of genes involved in triglyceride and phospholipid storage levels, ER stress, glycogen breakdown, and innate immunity. We find that S.m.-2698B infection appears to upregulate transcripts of AMPs including *diptericin*, *drosocin*, and *drosomycin*, as well as lipase-encoding *brummer*, when compared to S.m.-BPL infection. Our data suggest a correlation between chronic infection load and the extent of stored energy diversion. Future work will continue to investigate the exact metabolic changes induced by chronic infection, how these changes vary across pathogens, and the host and bacterial mechanisms underlying these effects.

Mechanisms of immune regulation by Ecdysone and Juvenile Hormone

Scott Keith, Vanika Gupta, Dana Vargas Solivan, Brian Lazzaro

Department of Entomology, Cornell University

Circulating hormones simultaneously impact varied physiological functions through effects on gene expression and cell biology. In Drosophila, 20-hydroxyecdysone (20E) and juvenile hormone (JH) regulate development, metabolism, and reproduction. These insect-specific hormones and their cognate receptors have reciprocal effects on immunity, with 20E potentiating and JH suppressing innate immune responses. Yet little is known about the molecular bases of these effects or the scope of additional microbe-responsive physiologies controlled by these hormones. We are investigating both of these endocrine systems with the ultimate goal of understanding how they might interact to modulate host physiology and immune responses during infection. JH mediates an immune-reproduction tradeoff wherein mated females exhibit decreased infection resistance in a JH-dependent manner. We found that JH signaling persists long-term post mating. Our previous work showed that mating increases infection susceptibility in part by overwhelming the protein synthesis capacity of the fat body, the primary immune tissue, leading to E.R. stress. Future work will determine whether these mating-induced changes to fat body physiology during infection are mediated by JH signaling. In parallel, 20E activates immunity in developmental and infection contexts, but the exact regulatory mechanisms of this activation remain unclear. We hypothesize that 20E-EcR signaling controls a hierarchical gene regulatory network (GRN) that sustains immune activation. To test this hypothesis and begin to construct a GRN, we have utilized RNA-seq to characterize: 1) EcR-dependent gene expression changes in the fat body during infection, and 2) fat body tissue-autonomous transcriptional responses to exogenous 20E. This analysis has identified genes involved in metabolism, stress response pathways, and immunity that exhibit transcript-level changes in response to 20E signaling both in tissue culture and *in vivo*. Ongoing work will utilize CUT&RUN analysis to determine which 20E-response genes identified by RNA-seq represent direct versus indirect EcR targets. Through this work, we ultimately aim to better understand hormone-mediated regulation of the physiological balance of development, metabolism, reproduction, and immunity in the context of host-microbe interactions.

Genetic basis for *S. marcescens* infectivity and virulence to *D. melanogaster*

Brian P. Lazzaro[1], Ashley M. Frank[1], Jung-Ho Shin[2], Tobias Doerr[2]

[1]Department of Entomology
[2]Department of Microbiology

Serratia marcescens is an common opportunistic bacterial pathogen of insects and other animals, including humans. *S. marcescens* strains exhibit tremendous variability in infectivity and virulence to hosts, including *Drosophila melanogaster*. The present work characterizes *S. marcescens* virulence to *D. melanogaster* after systemic infection across a panel of 21 clinical and environmental isolates and seeks to implicate genes that underly differences among them. Combined use of host mutant and bacterial mutant strains reveals mechanisms that enable or prevent infection. The *S. marcescens* strains vary in virulence to infected *D. melanogaster* with 16 of the strains causing 100% host mortality within 18 hours, one strain causing intermediate mortality progressively over a week, and two pairs of closely related strains causing 10-20% mortality within 48 hours with no subsequent host death. All surviving hosts carry chronic infections, although the chronic burden varies across *S. marcescens* strains. *S. marcescens* strains vary in their capacity to proliferate within wildtype hosts but not immune-compromised hosts, indicating that differences among them are due to differential sensitivity to the host immune system and not due to differences in capacity to use the host as a nutrient source. Host control of infection by the lower-virulence strains depends on the Imd pathway, and to a lesser extent the Toll pathway, primarily due to production of Attacins with minor contributions from other antimicrobial peptides (AMPs). Reciprocally, bacterial capacity to proliferate in the host depends on anti-AMP defense mechanisms. Draft genomes have been generated for each of the *S. marcescens* strains and are being compared to identify additional genes and mechanisms that could contribute differences in virulence.

Intra-host variation in the spike S1/S2 region of a feline coronavirus type-1 in a cat with persistent infection

Ximena A. Olarte-Castillo [1,4,#], Beth. N. Licitra [1,3], Nicole M. André [1], Maria A. Sierra [6], Christopher E. Mason [6], Laura B. Goodman [2,4] and Gary R. Whittaker [1,2,5 #]

Departments of Microbiology & Immunology [1], Public & Ecosystem Health [2] and Clinical Sciences [3], James A. Baker Institute for Animal Health [4] and Feline Health Center [5], Cornell University College of Veterinary Medicine, Ithaca NY, USA;
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Feline coronavirus type 1 (FCoV-1) is widely known for causing feline infectious peritonitis, a systemic infection that is often fatal. Subclinical infection also occurs, with cats shedding the virus in the feces. In this study, we followed a cat for six years from 2017 to 2022, when the animal was euthanized. The cat was clinically diagnosed with inflammatory bowel disease, chronic rhinitis, and cardiac problems. Using hybridization capture targeting the spike (S) gene of FCoV-1 followed by NGS, we screened 27 clinical samples. We detected FCoV-1 in 4 samples taken in 2017 (intestine and nasal tissue, feces, and conjunctiva), and 3 in 2022 (feces, and intestinal and heart tissue), but not in samples taken in 2019 and 2020. Next, we focused on the S1/S2 region within S, which contains the furin cleavage site (FCS), a key regulator of viral transmission and pathogenesis. We show that most of the non-synonymous changes in the S1/S2 region occur within the FCS. In the heart, we found two variants that differed in one amino acid in their FCS motif, one of which is predicted to down-regulate spike cleavability. The variant from the conjunctiva (2017) had 2 additional amino acids that resulted in a longer and more exposed S1/S2 loop, predicted to be more accessible to the furin protease. Our studies indicate that FCoV-1 can independently persist in and outside the gastrointestinal tract of a cat over a long period of time and that most of the genetic changes observed are within the FCS.

High throughput discovery of protein-mediated interactions between host and microbiota with functional metagenomic surface display

Sarah E Post, Ilana L Brito

Biological and Biomedical Sciences, Cornell University, Meinig School of Biomedical Engineering, Cornell University

The intestinal microbiome influences a multitude of host health and disease states. However, the molecular mechanisms underlying this host-microbiome communication remain largely unknown. In recent years, a handful of proteins derived from commensal bacteria have demonstrated significant therapeutic potential in multiple murine disease models. While only a handful of examples of protein-mediated host-microbiota signaling have been described, we hypothesize that many proteins from the microbiome are capable of signaling with host tissue, and these host interacting proteins can significantly influence host cellular processes and overall organism health. In order to identify novel host-interacting proteins in a massively parallel scale, I have developed a system based on bacterial surface display technology which expresses protein fragments derived from human gut bacteria and intestinal microbiomes on the surface of an easy-to-culture “surrogate” organism, lab-strain *Escherichia coli.* This system, termed functional metagenomic surface display, allows screening of millions of proteins simultaneously for association with mammalian tissue. Here, we show that the functional metagenomic display system successfully identifies bacterial proteins that bind to and internalize into human tissue, and can be applied for discovery of novel host-binding proteins from the gut microbiome. Ultimately, the goal of this work is to identify novel signaling molecules between the gut microbiota and human intestine that can be leveraged as therapies or therapeutic targets to treat microbiome-associated diseases.

Characterizing regulated cell death of the early-divergent fungus Rhizopus microsporus in the innate immune response to antagonistically perceived Mycetohabitans

Delia Tota

Like plant and animal cells, fungi employ innate immunity mechanisms to perceive and regulate interactions with microbes, including bacteria. Regulated cell death (RCD) is one of the many innate immune mechanisms with functional similarities shared by plants and animals. However, the surveillance system and means of innate immune defense in fungi, including the use of RCD, is not well understood. The symbiosis between the mucormycete *Rhizopus microsporus* and the bacterial endosymbiont *Mycetohabitans* presents a valuable system in which to investigate the innate immune response of early-divergent fungi. In contrast to the accommodations for bacteria made by host *R. microsporus* strains, strains that naturally do not harbor endosymbionts perceive *Mycetohabitans* antagonistically upon bacterial interaction, launching a reactive oxygen species (ROS) response accompanied by the death of a subpopulation of fungal cells. Here, I present preliminary evidence of *R. microsporus* employing RCD as a defense against *Mycetohabitans* being initiated, at least in part, by a fungal adenylyl cyclase (AdCy) gene. I cocultured a nonhost *R. microsporus* strain (ATCC11559) with *Mycetohabitans* and quantified viability and lipid peroxidation using flow cytometry. Fungus cocultured with bacteria experiences elevated lipid peroxidation and elevated cell death with morphology consistent with hallmarks of regulated cell death. I repeated this approach using nonhost *R. microsporus* AdCy mutant strains and identified a putative AdCy gene involved in initiating the RCD in response to bacteria. Future experiments will be aimed at discerning the specific type of regulated cell death through differential staining in flow cytometry assays.

A mouse model of maternal antibody interference with rotavirus vaccination

Katherine Woodyear

Rotavirus causes gastroenteritis in young humans and animals, although widespread vaccination has thankfully reduced the global burden of disease. However, vaccine efficacy in low-middle income countries is significantly poorer than in the USA. Reasons are likely multifactorial, but the presence of high levels of maternal antibodies have been associated with vaccine failure in multiple clinical studies. The mechanisms underlying this phenomenon of maternal antibody interference are currently unclear and it is essential we understand these in order to develop strategies that can overcome this major vaccine issue.
To study the role of maternal antibodies in rotavirus vaccine efficacy, we have developed a mouse model of neonatal rotavirus vaccination +/- maternal antibodies. Whereas oral vaccination with attenuated murine rotavirus in pups with no maternal antibodies induced a robust neonatal antibody response, we found that vaccination in the face of maternal antibodies resulted in no detectable pup antibody production.

A leading hypothesis for how maternal antibodies block neonatal immune responses is neutralisation of vaccine particles. We showed that maternal antibody titres are reduced following vaccination, suggestive of clearance after interaction with vaccines. However, increasing vaccine dose results in more profound inhibition of pup immune responses, with significantly reduced germinal centre formation in draining lymph nodes. This suggests additional mechanisms of inhibition are involved. The impact of maternal antibodies on direct B cell activation is the focus of ongoing investigations.

We are also using our mouse model to test new vaccine strategies that evade maternal antibodies interference. Whereas interference is not readily overcome by vaccinating pups with a higher dose of vaccine, oral administration of recombinant viral protein induced a robust IgG response in 50% pups. Unexpectedly there was no IgA response in any mice, so we are exploring the biology underpinning this effect to determine the potential value of oral protein vaccines.

The following abstracts are from the CIHMID URE (Undergraduate Research Experience) students, and this summer's cohort of the NSF-funded REU (Research Experience for Undergraduates), "Microbial Friends & Foes."

***URE student**
+REU student

Congenital Cytomegalovirus Reprograms Dendritic Cells in the Cervical Lymph Node

+Taha Ali, Dr. Zachary Hilt, Dr. Brian Rudd,

Congenital Cytomegalovirus (CMV) is the most common cause of childhood disabilities and birth defects in the United States. Congenital CMV infects the brain and requires CD8+ T cells to control viral replication. During the immune response, CD8+ T cells are recruited to the brain to promote viral clearance by the chemokine receptor CCR9. Recent research has shown that dendritic cells in the gut produce retinoic acid to upregulate CCR9. This led us to our question: what is the mechanism by which CD8+ T cells are being primed to enter the brain? Using a mouse model of congenital cytomegalovirus, we found that dendritic cells in the cervical lymph node (CLN) produced retinoic acid. Conventional dendritic cell 1s (cDC1) were highly expressed in the cervical and inguinal lymph nodes leading us to investigate environmental factors that induced retinoic acid production in the CLN. We found that Aldh1a2 as well as Il13 and Csf2 were significantly expressed in the dendritic cells of congenital CMV mice. Collectively, we show that dendritic cells in the CLN prime CD8+ T cells for recruitment to the brain.

Investigating Binding Site and Oligomeric Arrangement of Hepatitis C Virus E1E2 Glycoprotein with HEPC108 Broadly Neutralizing Antibody

Xander E. Wilcox, +Kassandra Arias-Parbul, Marty Schoenle, Benedito Melito, Katherine McKane, Andrew I. Flyak

Hepatitis C virus (HCV) infects over 70 million people worldwide and kills more people in the United States annually than HIV. The biggest impediment to HCV vaccine development is HCV's immense genetic diversity. Structural studies of broadly neutralizing antibodies (bNAbs) isolated from HCV-infected individuals can inform HCV vaccine development. BNAb HEPC108 can neutralize multiple HCV strains by binding to the conserved epitopes on E1E2 heterodimer, which presumably forms trimers on the viral surface. Using a comprehensive E1E2 alanine scanning mutagenesis, previous studies have mapped the HEPC108 epitope to the back layer of E2 glycoprotein. However, the HEPC108 neutralization profiling indicated that the HEPC108 might bind to the E2 front layer. Information about the binding site of HEPC108 can inform its neutralizing mechanism as well as the oligomeric arrangement E1E2 on the native virus. Here we determined a crystal structure of HEPC108 bNAb in complex with full-length E2 ectodomain. We validated the HEPC108 epitope using antibody competition binding experiments and ELISA binding assays with a panel of E2 single-amino-acid, E2 front layer, and E2 back layer knockout mutants. In contrast to previously reported alanine scanning mutagenesis data that mapped the HEPC108 epitope to the back layer of E2, the crystal structure of HEPC108-E2 indicated that HEPC108 primarily binds to the front layer of E2. HEPC108 competed with other E2 front layer-specific bNAbs and failed to bind to an E2 front layer knockout, further supporting structural data placing conserved residues in the E2 front layer as HEPC108 epitope. Interestingly, in the crystal structure, HEPC108 makes several contacts with an adjacent E2 monomer targeting regions outside the E2 front layer. While single point mutations outside the E2 front layer have subtle effects on the binding of HEPC108, these same mutations have a profound impact on E2 recognition by HEPC108 germline precursor. These data indicate that HEPC108 bNAb simultaneously interacts with two distinct antigenic regions on two E2 monomers. This information might inform a potential oligomeric arrangement of E1E2 on the viral surface facilitating E1E2 immunogen design efforts. Furthermore, the identification of single point mutations that modulate HEPC108 germline precursor binding to E2 might inform the affinity maturation process necessary for HCV bNAb generation.

HUMIDITY... A FACTOR IN MALARIA SPREAD?

*Anna Asamoah, Joel Brown, Courtney Murdock

Malaria is an infectious disease that affects millions around the world. Many ecological types of research, therefore, target environmental factors that enhance malaria. Many findings have been that temperature affects malaria spread. Our experiment explored another factor in malaria spread that has not been extensively experimented on- humidity levels. We predicted that humidity levels are just as important indicators as temperature in the factors of malaria spread. In our experiment, we measured the wing size of mosquitoes– which correlates with the body size of mosquitoes. Body size is significant in mosquito control as it determines the egg production of the mosquitoes. Larger mosquitoes will produce more eggs, and smaller mosquitoes will produce fewer batches of eggs. We grouped our mosquitoes in different temperature and humidity combinations. Our experiment contained 40 combinations, acquired from 8 temperature settings – 12, 16, 20, 24, 28, 32, 35, 38, and 5 humidity levels– 30%, 45%, 60%, 75%, 90%. Overall, we found that temperature showed greater variances in wing sizes across our various combinations compared to humidity levels.

Metagenomic Analysis of Gut Microbiome in Lizards: Exploring Eukaryotes and Parasites

*Anamaría Páez Capador, +Kira O'Brien, Iris Holmes, Tory Hendry

The gut microbiome plays a vital role in animal well-being and evolution, yet its study often focuses solely on bacteria, ignoring the importance of eukaryotic microorganisms. This research aims to shed light on the gut microbiome of Brown anoles (Anolis sagrei), its implications for lizard health and ecology, and the interactions with Plasmodium parasites. About half of all described Plasmodium species infect lizards and in fact, it is hypothesized that they were the original host, opening the door for infecting other vertebrates. By using metagenomic shotgun sequencing, assembling, and classifying the reads, we identified different eukaryotic taxa within the gut microbiome, including pathogenic parasites like Haemosporidia, which may have some interactions with Plasmodium, the parasite responsible for lizard malaria. This finding calls for further investigation into potential ecological interactions between these taxa, with implications for understanding lizard malaria and its effects on the gut ecosystem diversity, function, and dynamics.

Assessing Osmotic Adaptation in Vibrio cholerae: Revealing the Impact of Point Mutations on the Endopeptidase ShyA

+Jamiya Chandler, Kelly Rosch, Tobias Dörr

Vibrio cholerae is a human pathogen that causes diarrhea and can be fatal; cholera disease is a leading cause of death in developing countries. Vibrio cholerae lives in an aquatic environment (oceans, coastal areas) and must adapt to changes in osmolarity as it infects the human host. Vibrio cholerae has a rigid cell wall that helps keep the cell's shape and protects the cell from osmotic lysis. The endopeptidase ShyA is an enzyme in Vibrio cholerae that cuts the bacterial cell wall in order for new cell wall material to be inserted. ShyA has an open conformation that is active and a closed conformation that is inactive; it remains unknown how ShyA switches between the active and inactive conformation. ShyA contains a LysM domain that binds carbohydrates; since the bacterial cell wall closely resembles a carbohydrate, we expect that the LysM domain in ShyA binds to the cell wall. This LysM domain is not a requirement for endopeptidase activity; not all endopeptidases have LysM domains. Our hypothesis is that the LysM domain helps to activate ShyA during osmotic stress. To assess the importance of the ShyA LysM domain in adaptation to osmotic stress, we first mutated each conserved reside in the LysM domain to alanine, then replaced the wild-type copy of ShyA with our mutated copies, and finally performed a plating assay in which we compared the growth of cells missing ShyA, cells expressing ShyA, and cells expressing a ShyA LysM domain mutant on both regular growth medium and salt-free medium. Cells missing ShyA have a growth defect on salt-free medium but not on regular growth medium. Surprisingly, the LysM domain mutants managed to grow just as well as the wild type did on salt-free medium. These results indicate that the amino acid residues that we mutated are not actually important for adaptation to osmotic stress. Future directions for this project include repeating the plating assay using a higher concentration of arabinose to potentially increase the expression of the mutants and using chitin to introduce the mutants into the chromosome while keeping the native promoter for ShyA.

Physiological consequences of ribosomal protein deletions in Bacillus subtilis.

+Joel Gonzales, Heather Feaga

One of the most abundant molecular machines found in all organisms is the ribosome, an enzyme responsible for protein synthesis. Ribosomes are made up of RNA and proteins, and only some ribosomal proteins are essential. Using Bacillus subtilis as a model organism, we hope to further understand the role that nonessential proteins play when it comes to fitness of the strains. Here we present data that demonstrate the deletion of “nonessential” proteins from diverse genetic loci (CTC, RpmI, RplO) exert a strong effect on physiology. Additionally, we investigate the physiological consequences of ysdA deletion, which is a gene of unknown function that is in an operon with rpmI. We observe growth defects among mutant strains when compared to wild-type cells in a liquid media. Accordingly, mutants lacking certain nonessential ribosomal proteins are also more susceptible to antibiotics that target both the large and small subunits of the ribosome. Additionally, we show that ribosomal mutant cells are deficient for sporulation. Sucrose density gradient centrifugation reveals aberrant ribosomal composition and subunit abundance patterns specific to different ribosomal protein deletions that may explain the observed physiological defects. Throughout this study using B. subtilis, we have been able to understand how fast-growing, genetically tractable bacterial models provide a convenient system to ask fundamental questions about ribosomes that are relevant to all branches of life. Ultimately, nonessential for cell proliferation is not without consequence.

Within-Host Evolution of Feline Coronavirus: The Effects of M1058L Mutation in Development of Feline Infectious Peritonitis from Feline Enteric Coronavirus

*Emily Jones, Annette Choi, Dr. Gary R. Whittaker, Ph.D.

Feline Coronavirus (FCoV) is a virus that affects a significant portion of the cat population in the United States. It has two biotypes, Feline enteric coronavirus (FeCV) and Feline Infectious Peritonitis (FIP). While FeCV typically causes an infection with mild symptoms or is completely asymptomatic, it can evolve into FIP, which is deadly. The mutations that cause FeCV to evolve into FIP are not fully understood. However, there have been mutations linked to changes in spike protein activation by the Furin Cleavage Site (FCS). In order for the virus to enter the cell, the spike protein needs to be activated by furin. One FCS is the S1/S2 site, and a mutation in the spike protein that has been associated with the evolution of FeCV into FIP is the M1058L mutation. This research project aimed to investigate the effects of the expression of the M1058L mutation on protein stability and virus entry into cells. To generate the mutation, we performed site-directed mutagenesis followed by a cell-cell fusion assay to test the function of the spike protein with the mutation. We plan to make pseudoparticles in order to test the effect the mutation has on the virus's ability to infect.

Systematic evaluation of computational methods for inferring transmissibility of Mycobacterium Tuberculosis with epi-evolutionary simulation

*Shenni Liang, Perry Xu, Ben Haller, Philipp Messer, Andrew Clark, Jaehee Kim

Tuberculosis (TB) is a major global public health concern, with multiple lineages exhibiting varying phenotypes. Genomic surveillance has been instrumental in understanding the transmission dynamics of TB and the evolution of Mycobacterium Tuberculosis (Mtb). Several methods exist for inferring TB transmissibility from genetic data, but most ignore epidemiological and evolutionary complexities, such as population structure, latent infection, and drug resistance. Here, we systematically evaluate the performance of inference methods and investigate the effects of potential confounders in simulated TB outbreaks. We developed a new simulation framework that captures the realistic evolutionary and epidemiological history of TB transmission based on SLiM, a flexible and powerful forward-in-time evolutionary software with Eidos scripting language. Using our simulator, we then generated synthetic data with known ground truth for genes associated with transmissibility and transmission history. We examined five computational methods for inferring transmissibility. We applied these methods to the synthetic data generated under various epi-evolutionary scenarios and evaluated their performance in recovering the true value from which the simulated data was generated. Our results indicated that, in the absence of any confounders, the inferred transmissibility generally exhibits positive correlations across all methods considered. However, the accuracy of the methods is influenced by confounders to varying degrees. Overall, our study highlights the importance of considering confounding factors when inferring transmissibility. Further, our newly developed simulation framework and numerical experiment provide a foundation for benchmarking current and future methods for transmissibility inference and can facilitate the development of more accurate and robust computational tools for TB control and prevention.

Characterizing the Interactions between Lentilactobacillus parabuchneri and Drosophila melanogaster

*Jailyn Loor, Kate L. Browning, Brian P. Lazzaro

In summer 2021, a contaminant was observed across Drosophila labs in the Cornell fly food, later identified as Lentilactobacillus parabuchneri. During this time, members of the Lazzaro lab also noticed higher mortality rates when D. melanogaster were systemically infected with bacterial pathogens, but the cause for this spike in mortality was never identified. Here, we sought to characterize the relationship between D. melanogaster and L. parabuchneri and hypothesized that this bacterium may have caused the previously observed increase in mortality. To test this, we first orally infected D. melanogaster and measured mortality and bacterial persistence of L. parabuchneri. We then measured mortality of flies first infected with L. parabuchneri, followed by a systemic infection with Providencia rettgeri 24 and 72 hours after their primary oral infection. We find that L. parabuchneri is rapidly cleared from D. melanogaster and that no mortality is observed from oral infection alone. In contrast to our initial hypothesis, our early results suggest there may be some benefit to consuming L. parabuchneri. Our preliminary findings from these experiments have not only provided us with information on the interactions between microbes found in the fly food but may also provide a better understanding of innate immunity in D. melanogaster.

Investigating the efficacy in solid media of a copper-zinc fungicide on Rhodospordium toruloides and Neurospora crassa

+Andres Lopez, Renato Carvalho, Lori Huberman

Fungi, bacteria, viruses, and nematodes are the most common plant pathogens and are responsible for generating massive amounts of crop losses worldwide. To control the spread of fungal plant diseases, fungicides are traditionally used. However, the current supply of fungicides lack different modes of action and novel or enhanced formulations. Many of the currently used fungicides heavily rely on copper. Although copper is effective in controlling plant pathogens, it can trigger environmental issues. Repeated use of copper-based fungicides also increases the likelihood of fungicide resistance. Farmers and growers worldwide need to expand their portfolio of fungicides to increase their options with novel materials. To mitigate this problem, we are investigating a novel fungicide called Curezin formulated with a mixture of copper and zinc. To determine the efficacy of this fungicide, trials in liquid media were conducted on the model basidiomycete Rhodosporidium toruloides and the model ascomycete Neurospora crassa, both closely related with important plant pathogens. Although the efficacy of Curezin in liquid has been determined, the efficacy in solid media remains unknown. To determine the efficacy in solid media, we grew Rhodosporidium toruloides and Neurospora crassa on plates and slants containing various dilutions of fungicide. Serial dilutions were made, and spotting technique was used to plate the fungi. Using both wildtype and mutants that showed sensitivity in fungicide in liquid media, we found that higher dilutions of fungicide dilutions such as 1:20 and 1:30 inhibited growth entirely in both Rhodosporidium and Neurospora. We observed efficacy of the fungicide at the dilutions higher than 1:70 where the spots showed less growth than untreated controls. In Neurospora crassa, slants treated with fungicide showed growth inhibition starting at the fungicide dilution 1:60. These results combined with liquid showed the efficacy of the fungicide in different scenarios. Moreover, we performed competition assays exposing different mutants of R. toruloides to the fungicide and watched the growth on solid media. We hope that the results of this research can increase the understanding of the Curezin in different scenarios and improve the information for better formulations and applications of the fungicide.

Assessing Activation/Exhaustion of CD8 T-cells in "Humanized" CD28tg Mice

+Alexandria Lydecker, Alex Brady, Mandy McGeachy

CD28 is a costimulatory molecule thought to be critical for activation of CD4 and CD8 T cells, with many functions including enhancing pro-inflammatory cytokines, proliferation and survival. Human and mouse studies of CD28 have given disparate results, most dramatically shown by the catastrophic clinical trial of a CD28 superagonist resulting in near-fatal cytokine storms in healthy volunteers. To study this signaling difference, the McGeachy lab have created a CD28 A210P mouse strain with a 'humanized' cytoplasmic signaling domain. They have shown CD28 A210P CD4+ T cells have enhanced responses. The objective of this project is to provide perspective on the outcomes of enhanced (humanized) CD28 signaling in CD8 T-cell activation. CD28 signaling differences between CD28 A210P and WT controls were assessed in vitro through the use of Flow Cytometry. We found increased activation and exhaustion markers and increased IFN-γ production in CD28 A210P CD8+ T cells. However, CFSE staining on CD28 A210P CD8+ T cells did not demonstrate significant increased proliferation observed in the CD28 A210P CD4+ T cell enhanced response. Currently, we are assessing initial CD28 signaling differences in vitro using Phospho-Flow Cytometry. Lymphocytic Choriomeningitis virus (LCMV) was used to evaluate CD28 signaling differences between CD28 A210P and WT in vivo through four-week chronic infection. The CD8+ T cell response to LCMV in WT mice has been well-characterized, allowing us to directly assess the impact of enhanced CD28 signaling during an inflammatory viral infection. We found increased weight loss, decreased survival rate, and increased cytokine production in CD28 A210P mice. In conclusion, we have found enhanced CD28 signaling increases the activation, exhaustion, and cytokine production of CD8+ T cells.

How New England Oyster farming beds impact marine sediment microbial communities

+Evelyn Martinez, Dr. Marian Schmidt

Oyster populations were decimated by human activity due to overfishing. However, oyster farming has recently become more prevalent in coastal areas as a sustainable source of protein. Oysters play an important role in coastal ecosystems by stimulating the microbial community in the sediment. The sediment microbial community is essential to biogeochemical cycling by aiding in nitrogen removal through denitrification, therefore reducing the effects of eutrophication. The biogeochemical effects of the presence of new oyster aquaculture have been extensively researched, but the effect that this practice has on sediment microbial communities has not been thoroughly addressed. Our study aims to characterize the sediment microbial communities found within four different New England oyster farms to analyze the effects of oyster farming on marine sediment microbes. Sediment samples and control samples were collected from four different New England Oyster farms and underwent DNA extraction and Illumina sequencing of the V4 hypervariable region of the 16S rRNA gene to assess microbial biodiversity and community composition. When compared to the control, we predicted that microbial communities from oyster farms would display higher microbial richness and evenness due to the availability of more organic matter provided by the oysters. Understanding how oyster farming impacts the microbial sediment community can lead the way to discover more about how microbes cycle nutrients in coastal ecosystems and how they contribute to the health of their ecosystem via biogeochemical cycling.

A geographic comparison of gut microbial flexibility in tree swallows (Tachycineta bicolor)

*NJ Morris, JL Houtz, C Zimmer, BK Trevelline, AH Moeller, MN Vitousek

Organisms can flexibly shift their phenotype to match environmental demands. In the face of both unpredictable challenges like severe weather, and predictable challenges like the energetically demanding periods of reproduction, the fitness of organisms may be influenced by phenotypic flexibility. Microbial flexibility, the ability to dynamically restructure the gut microbiome in the face of environmental change, may mediate phenotypic flexibility in fitness related traits such as body mass. To explore whether the gut microbiome can act as a mediator of phenotypic flexibility, I analyzed the gut microbial diversity and flexibility of breeding female tree swallows (Tachycineta bicolor) during incubation and nestling provisioning across a geographic range in Alaska, Wyoming, Tennessee, and New York. In the New York population, earlier breeders, which were heavier at the incubation capture, had more diverse microbiomes. Birds that laid earlier lost more mass and decreased more in microbial diversity from mid incubation through early provisioning. Cloacal microbiome samples are currently being sequenced for the Alaska, Wyoming, and Tennessee populations. A better understanding of the role of the gut microbiome in phenotypic flexibility of traits, such as body mass, can provide insight into how organisms are able to rapidly shift their phenotypes to cope with changing environments.

Metagenomic approaches to identify bacterial community of the small intestine in brown anoles

+Kira O'Brien,*Anamaría Páez Capador, Tory Hendry, Iris Holmes

The brown anole, Anolis sagrei, commonly faces malaria infection caused by Plasmodium parasites. In non-reptile model systems, gut bacterial communities have been shown to provide a protective defense against infection, however, the mechanism is mostly unknown. This research aims to characterize the relationship the native gut bacteria have on host health and parasitic burden. It is hypothesized that after examining metagenomic data that malaria infection will show to interact with gut bacteria causing one of two effects: (1) certain genes will be upregulated or downregulated or (2) the upregulation of such genes will make the host more or less susceptible to malaria. To examine this relationship, samples were taken from the small intestine and total DNA was extracted and sequenced using Illumina. Taxonomy of gut bacteria was characterized using the taxonomic classification program Kaiju and reads were run through the metaSPAdes metagenomic analysis pipeline. Gut bacterial taxonomy showed at the phylum level that Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes were the most prevalent in infected individuals while Proteobacteria and Chlamydiae was prevalent in one uninfected individual. Studies in monkeys, canaries, snakes, other lizards, chickens, and mice found overlapping taxonomic breakdowns at the phylum level for malaria infection and other parasitic infections. Identifying the pattern between gut microbiota and malaria risk will allow us to better understand the metabolic role of the gut microbiome during malaria infection and identify ecological and functional predictors. Because there is limited information about bacterial metabolic function during infection available in reptiles, investigating this experimental data set may allow for this pattern to be generalizable across a broad range of host taxa.

Fungal Metabolism in Mixed Nutrient Environments

+L. Vaughan Poland, Joshua D. Kerkaert, Lori B. Huberman

As the climate warms, scientists turn to biofuels as a potential replacement for fossil fuels. Filamentous fungi can degrade non-food plant biomass, essential for second-generation biofuel production. While fungal utilization of single carbon and nitrogen sources have been studied, fungal metabolism in mixed nutrient environments such as those found in industrial fermentation processes remains understudied. CRE-1 is a transcription factor that regulates carbon catabolite repression by repressing the genes necessary to metabolize non-preferred carbon sources when a more desirable source is accessible. To determine carbon source preferences we utilized the model filamentous fungus Neurospora crassa, as well as a cre-1 null strain to determine the role of cre-1 in mixed carbon environments. After a 24 hour pre-growth, N. crassa was transferred to a mixed medium of cellulose, a non-preferred nutrient, and a second carbon source of unknown preference for three days. We then determined cellulase production via an azo-carboxymethylcellulase assay. We observed variation in repression of cellulase production across a diverse panel of carbon sources in wiltype N. crassa. The cre-1 null strain remained more repressed than the cellulose only controls, suggesting that catabolite repression is possible without cre-1. We also observed derepression of cellulase activity on the nonpreferred nitrogen source, NO_3^-, compared to preferred nitrogen source NH_4^+ for both the wildtype and cre-1 deletion strains. This indicates that carbon and nitrogen repressing systems may be connected and invites further study on the genetic mechanisms intertwining them. Finally, we established a complementary system by transforming N. crassa with a luciferase gene under the control of the cbh-1 cellulase promoter. This will allow for a luciferase assay with a more sensitive measure of cellulase transcription on a shorter timescale to mitigate the effect of varying growth speed and isolate the effects of transcription itself. These data provide insight into the mechanisms used by N. crassa to metabolize in mixed nutrient environments, relevant for the bioengineering of fungi for more efficient biofuel production.

Unearthing the biodiversity of the Mucoromycota fungi within the Caesarea Mediterranean Scrub and the Be'er Sheva Negev Desert in Israel

+Edmarie Rivera Sánchez, Dr. Nicole Reynolds, Teresa E. Pawlowska

The phylum Mucoromycota is a poorly understood fungal group that encompasses globally distributed species of significant ecological value. Mucoromycota holds three subphyla: Mortierellomycotina, Glomeromycotina, and Mucoromycotina. All three subphyla of Mucoromycota, Mortierellomycotina, Glomeromycotina, and Mucoromycotina, can harbor endosymbiotic bacteria (EB) within their hyphae and these relationships also are still understudied. Using soil samples from Israel's Desert and Xeric Shrubland and Mediterranean Scrub biomes, the project had the following goals: (1) survey the diversity of Mucoromycotina fungal species present, (2) screen fungal isolates for the presence of putative EB, and (3) compare fungal/EB species composition and relationships across sites and plant hosts. To isolate fungi, soil samples were sprinkled onto two different media types and incubated at room temperature for several days. Fungal isolates were obtained in pure culture using sterile technique and mycelia were harvested for DNA extraction and PCR amplification. Cultures were screened with both fungal and bacterial primers. A variety of fungal species were found, whose morphology suggested that they belong to the genera Actinomucor, Cunningmella, Mucor, and Rhizopus. From the 29 isolates, four yielded sequences of 16S rRNA gene indicating putatitve EB. The most recurrent fungal species was from the genus Rhizopus in the Mediterranean scrub, while in the desert the most common species was Cunninghamella. Regarding the results of the desert, this finding does not support the initial hypothesis formulated based on the preliminary data from the Sonoran Desert in California, that Rhizopus would be the dominant genus. It was also clear that the Mediterranean scrub soil showed a more uniform fungal composition along a longitudinal gradient compared to the desert and that the most diverse plants were the Asteraceae hosts (Artemisia monosperma and Artemisia herba-alba) at the Mediterranean Scrub and Desert and Xeric Shrubland, respectively. These results are important in the context of human and plant health, as some of the focal species are opportunistic pathogens, and their distribution is likely to be affected by global change.

Different clones of the cotton aphid with different vector competence: Investigating transmission rate of cotton leafroll dwarf virus (CLRDV) using different aphid clones and acquisition access periods

*Hayk Shakhzadyan, Alejandro Olmedo-Velarde, Michelle L. Heck

Cotton leafroll dwarf virus (CLRDV) is an aphid-borne virus that infects cotton (Gossypium spp.). In the past, CLRDV has been known to cause massive reductions in cotton yield in South America. In 2017, CLRDV was detected in Alabama and since then the virus has been found already spread into most of the states of the cotton belt region in the US. CLRDV is transmitted by Aphis gossypii, commonly known as the cotton aphid. Not much is known about CLRDV and how it will affect cotton production in the United States, which warrants investigation into the virus and the mechanisms through how it is transmitted and operates between host and vector. This project aims to evaluate the transmission efficiency that different clones of the cotton aphid collected from various locations in four states in the US (New York, Alabama, Mississippi, and Tennessee), would present when exposed to CLRDV-infected cotton for two different lengths of time of virus acquisition access period (AAP): 3 and 7 days. After the AAP, aphids were transferred to 8 receptor cotton leaf discs for 4-5 days in order for the aphids to inoculate the virus (inoculation access period or IAP). After IAP and aphids were removed, 4-5 additional days were provided as virus replication time, after which virus presence was evaluated using PCR-based assays. We found that between the different strains of A. gossypii, the transmission rate between 3 days and 7 days were not significantly different, except for the Mississippi strain, which had twice the transmission rate between 3 days and 7 days. This suggests that there might be co-adaptation between the virus and the aphid, as the infected cotton plant used for this experiment was also sourced from Mississippi.

Localization of Chromatin Remodeling Complexes in HSV-1 Infection

*Lukas Vera, Sarah Saddoris, and Luis Schang

Herpes Simplex Virus 1 is a prevalent virus, infecting 60-70% of the global population. The latent nature of Herpes results in lifelong infection and reactivates upon stress. Our lab has been studying the epigenetics of herpes infection, specifically the chromatin dynamics. Lytic infection is associated with active dynamic chromatin while latent infection is associated with condensed silenced chromatin. The project I am working on is researching the role of chromatin remodeling complexes in HSV-1 infection. Immunofluorescence experiments in HeLa cells have been done and found that some subunits of these complexes localize with the viral protein ICP4 during infection, while others do not. I have been repeating these immunofluorescence experiments in Human Foreskin Fibroblasts to present a different model in human primary cells. The objective of these experiments was to characterize the localization of chromatin remodeling complexes in HSV-1 infection. The results have been the same as the previous experiments in HeLas where certain subunits of chromatin remodeling complexes are localizing with viral protein ICP4, while others are not.

CD73-driven cross protection in heterogeneous breast tumors

+Brenda L Ramos Villanueva and Anushka Dongre

The epithelial to mesenchymal transition (EMT) is a cellular process in which epithelial cells acquire quasi-mesenchymal characteristics, gain metastatic potential and drive resistance to anti-CTLA4 immune-checkpoint blockade therapy (ICB). While epithelial carcinomas are infiltrated by CD8+ T-cells and respond well to anti-CTLA4 ICB, quasi-mesenchymal tumors recruit immunosuppressive cells to their tumor microenvironment and are resistant to anti-CTLA4 ICB. Most importantly, in mixed tumors comprised of both cell types, a minority fraction of quasi-mesenchymal cancer cells can cross-protect their epithelial neighbors and drive resistance to anti-CTLA4 ICB in a CD73-dependent manner. CD73 is an adenosine-generating ectoenzyme. However, the underlying mechanism by which it drives cross protection remains unclear. Given that epithelial cancer cells express adenosine receptors, whether they themselves express CD73 to drive such cross-protection is elusive. To address this, we asked whether paracrine factors secreted by quasi-mesenchymal cancer cells could alter the immune-modulatory properties of nearby epithelial cancer cells. Epithelial cells were incubated in control media or conditioned media (CM) derived from quasi-mesenchymal cells. Strikingly, epithelial cells showed an increase in the expression of immune-suppressive CD73 only when cultured in conditioned media from quasi-mesenchymal cancer cells while also remaining epithelial. These results suggest that cross- protection observed in heterogeneous breast tumors may likely be due to the ability of epithelial cancer cells to gain CD73 expression in response to signals received from mesenchymal cancer cells.

Exploring the interactions of two component systems and carbapenem tolerance in Klebsiella pneumoniae

+Chaniya Wigfall, Facundo Torres & Tobias Döerr

Klebsiella pneumoniae is a gram-negative bacterium that causes infections such as UTIs, pneumonia, and sepsis. To treat infections caused by K. pneumoniae, -lactams such as penicillin are used to target cell wall synthesis by binding to PBPs (penicillin bonding proteins), which causes the cell to lyse. Following the widespread overuse of -lactam antibiotics, the emergence of antibiotic resistance has become a global health concern. When a bacterium becomes resistant to -lactams, it will continue to proliferate even in the presence of the antibiotic. Tolerance is a distinct phenomenon where bacteria survive in the presence of an antibiotic for an extended period of time without being fully resistant. In K.pneumoniae, -lactam tolerance is enabled by Two Component Systems (TCS). These systems are composed of two parts; a histidine kinase that senses changes in the cell and a response regulator which modulates gene expression. In our study, we are focusing on deleting then complementing four TCS (RcsBF, OmpRZ, PhoPQ, cpxRA) to examine their influence on tolerance to -lactams. Our results show that the four TCS collectively promote optimal survival, suggesting that cell envelope stress management is key to counteracting the detrimental consequences of antibiotic exposure.

Optimizing selectable marker genes in Bacillus subtilis for experiments on ribosome binding protein

*Letian Wu, Hye-Rim Hong, Heather Feaga

One commonly used selectable marker in Bacillus subtilis research is the MLS resistance gene (ermC), which provides antibiotic resistance to the MLS group of antibiotics. However, our lab has observed in the past that the introduction of the MLS resistance gene can lead to growth defects when certain genes involving ribosome-binding proteins are knocked out. This complicates researchers' ability to discern whether the observed growth defects are caused by the knocked-out gene or the introduced MLS resistance gene. The primary objective of this research is to investigate the role of the ermC gene in inducing growth defects in Bacillus subtilis and to explore whether specific mutants of Bacillus subtilis exhibit exacerbated growth defects. Through plate reader experiments, we demonstrate that wildtype Bacillus subtilis strains experience increasingly exacerbated growth issues as the level of MLS resistance expression is elevated, particularly during the log phase. Notably, the ΔmutS2 strain, when tested, does not exhibit increased growth defects when the MLS resistance gene is expressed. Based on our existing data, we have established that the MLS resistance gene does cause growth defects in wildtype Bacillus subtilis. To gain a more comprehensive understanding of the gene-knockout interactions, further experiments should be conducted on additional mutants. This will help identify specific gene knockouts that exhibit increased growth defects when the MLS resistance gene is present in Bacillus subtilis. By elucidating these interactions, researchers can make informed decisions when choosing selectable markers, thereby enhancing the accuracy and reliability of future experiments.