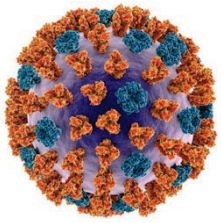
Department of Microbiology and Immunology
Undergraduate Research Poster Symposium



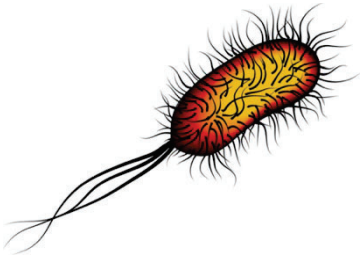
Thursday, April 30th, 2026

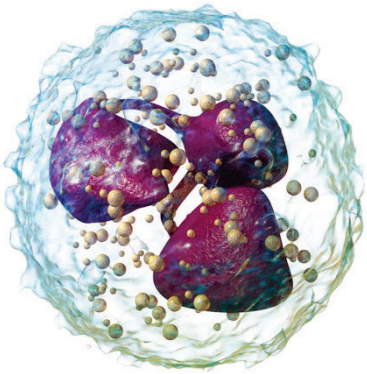
3:00-5:00 PM

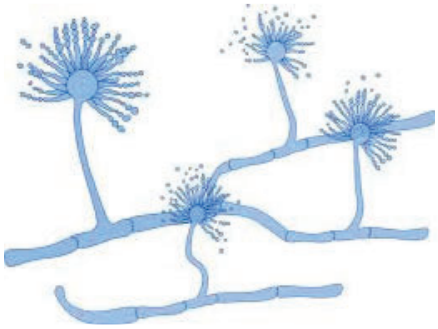
Odd-numbered: 3:00-4:00

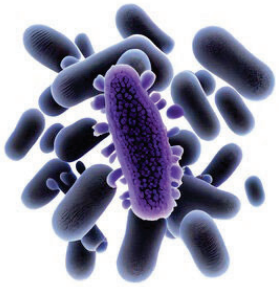
Even-numbered: 4:00-5:00

MERF Atrium









Poster Number	Student (last-first)	Lab
1	Aristizabal-Omar	Tan
2	Betts-Ty	Butler
3	Bhagwat-Armaan	Butler
4	Broghammer-Madeline	Haim
5	Chodur-Ethan	Bosch
6	Fitzpatrick-Beck	Wu
7	Frye-Logan	Korkmaz
8	Gulick-Sarah	Kleinpeter
9	Gutzman-Grace	Wilson
10	Hake-Audrey	Roller
11	Heller-Olivia	Tucker
12	Herrington-Harli	Tucker
13	Holbert-Haleigh	Zander
14	Kafka-Madison	Korkmaz
15	Kremer-Morgan	Butler
16	Morgan-Ella	Kleinpeter
17	Nyberg-Libby	Tucker
18	Pathuru-Venkat	Weber
19	Pechter-Shay	Gurung
20	Roberts-Emily	Johnson
21	Sajadian-Arya	He
22	Sanders-McKinley	Stapleton
23	Schroeder-Grace	Gebhardt
24	Stolte-Allie	Maury
25	Taylor-Kelsey	Johnson
26	Frisina-Josefina	Ellermeier

Characterizing Ebolavirus Localization and Neuroinflammation in the Murine Brain

Omar Aristizabal, Blake Bernauer, Jack Siemering, Hanora Van Ert, Paige Richards, Andrea Marzi, Wendy Maury, Sabrina Tan

Ebola Virus disease is rare, yet highly lethal. Infection is characterized by hemorrhage, systemic hyperinflammation, in part due to cytokine storm, and multiorgan failure. While the central nervous system (CNS) is not considered a primary site of early infection, survivors frequently experience long-term neurological sequelae, including headaches, memory loss, and encephalitis with the potential of relapse. However, the mechanisms by which Ebola Virus enters the brain and the specific cellular targets of infection remain poorly defined.

Interferon-α/β receptor knockout (IFNAR-/-) mice were used as a small-animal model of Ebola virus disease. These mice are highly susceptible to wild-type Ebola virus, develop robust systemic infection, and recapitulate key aspects of disease pathogenesis. Wild-type Ebola virus infection was performed under biosafety level-4 (BSL-4) conditions at Rocky Mountain Laboratories. Mice were euthanized at 5 days post-infection (DPI). RT-qPCR was used to quantify viral RNA and cytokine transcripts, while multiplex immunofluorescence (IF) combined with RNA in situ hybridization (RNAscope) enabled single-cell mapping of infected and activated brain cell populations.

Within the brain, Ebola Virus protein VP40 colocalized predominantly with microglia and macrophages. Neurons and astrocytes exhibited minimal direct infection, although astrocytes were frequently adjacent to infected cells. Infected brains exhibited reduced signal intensity in neurons and CD31+ endothelial cells. Multiple pro inflammatory mediators were upregulated in the brain, including IFN-α, IFN-β, CXCL10, ISG15, and GBP5. This profile is consistent with a strong antiviral interferon-stimulated gene response within the CNS

These results support a ‘Trojan horse’ model in which infiltrating macrophages carry Ebola virus into the CNS, seeding infection of resident microglia and triggering a local type I interferon–driven antiviral proinflammatory response. This work highlights innate immune pathways in the brain as potential targets to mitigate long-term neurological complications of Ebola virus disease.

ACLy Deletion in Germinal Center B Cells Confers no Change in Anti-Parasite Protective Immunity

Ty Betts, Jonathon Bernardi, Kaylee Norman, Noah Butler

Department of Microbiology and Immunology, University of Iowa

Plasmodium infection elicits systemic cell-mediated and humoral immune responses, with high-affinity class-switched antibodies being critical for control of malaria. A key mechanism of this antibody conversion process is germinal center (GC) formation. Within germinal centers, GC B cells undergo clonal expansion, rapidly proliferate, and upregulate metabolic processes to maintain growth. Acetyl-CoA is a critical metabolite for energy production, fatty acid/membrane synthesis, and protein/epigenetic modification. Acetyl-CoA can be synthesized from citrate through ATP citrate lyase (ACLy)-mediated catalysis. We hypothesize that GC B cell expressed ACLy is necessary for GC B cell responses and host protection against *Plasmodium* infection. To test this hypothesis, we genetically engineered a mouse model with an inducible GC B cell-specific deletion of *ACLy*. After infection with *Plasmodium*, mouse GC B cell *ACLy* deletion was induced beginning at germinal center formation on days 4-7 post-infection (p.i.). Parasite burden was measured, and sera/spleens were collected for antibody/cellular analyses. Unexpectedly, we found no differences in parasite burden when ACLy was deleted in mouse GC B cells. This observation was affirmed by flow cytometry analysis of GC B cells harvested from spleens at a late GC timepoint (day 21 p.i.), showing no change in frequency or count after *ACLy* loss. Antigen specific antibody titers and affinity for IgM/IgG were measured by ELISA using serum collected at early (day 14 p.i.) and late GC timepoints. Both antibody titer and affinity for IgM and IgG remain unchanged in *Plasmodium*-infected mice harboring ACLy-deficient GC B cells. Taken together, these data indicate that ACLy is dispensable for GC B cell differentiation, function, and protection against malaria. Additionally, the lack of phenotype in mice with ACLy deficient GC B cells supports that alternative pathways for acetyl-CoA production are predominant in GC B cells.

ACLY Enzyme and Citrate Transporter Slc25a1 are Dispensable for Metabolic Regulation of Germinal Center B Cells Responding to Blood-Stage Malaria

Armaan A. Bhagwat, Kaylee M. Norman, and Noah S. Butler
Department of Microbiology and Immunology, University of Iowa

Malaria is a deadly infectious disease caused by the *Plasmodium* parasite. *Plasmodium*-specific antibodies, a product of germinal center (GC) B cells, are fundamental to long-lasting humoral immune responses to mediate parasite clearance effectively. Yet, individuals in malaria-endemic regions lack long-lived immunity. Therefore, it is imperative to understand why this happens. Our lab has demonstrated that mitochondrial glutamate is vital for GC B cell survival and proliferation during blood-stage *Plasmodium* infections. However, the biochemical pathways fed by mitochondrial glutamate in GC B cells remain unknown. One possibility is that mitochondrial glutamate is utilized for citrate production, which is exported to the cytosol through the Slc25a1 transporter. Once in the cytosol, citrate is converted to acetyl-CoA through the ACLY enzyme. We hypothesize that during blood-stage *Plasmodium* infection, rapidly proliferating GC B cells require Slc25a1 or ACLY to mediate protective immunity. To test our hypothesis, we conditionally deleted Slc25a1 in GC B cells using the S1PR2-Ert2/Cre x Slc25a1$^{fl/fl}$ mouse line. Additionally, we deleted ACLY in GC B cells using the S1PR2-Ert2/Cre x ACLY$^{fl/fl}$ mouse line. We infected wild-type control and experimental mice (Slc25a1- or ACLY-deficient GC B cells) with *P. yoelii*, and measured GC B cell quantity, anti-*Plasmodium* antibody production, and protection against *Plasmodium* infection. We observed a significant decrease in GC B cell frequency when Slc25a1 was deleted from GC B cells but unexpectedly observed no changes in GC B cell quantity, antibody production, or protection when ACLY is deleted from GC B cells. Our findings support that mitochondrial glutamate in GC B cells responding to *P. yoelii* infection powers mitochondrial metabolism via mechanisms independent of cytosolic acetyl-CoA production. Our current and future studies will improve our understanding of the humoral immune response to *Plasmodium* and potentially identify new opportunities to boost malaria immunity.

Poster# 4

Deep mutational scanning of the HIV-1 capsid protein reveals genetically accessible and fit forms of the virus that increase resistance to Lenacapavir

Madeline M Broghammer, Aaron N Gillman, Cassian M Birler, Rohith Rao Vujjini, Samuel McCarthy-Potter, Hanxin Lyu, Alexander B Kleinpeter, and Hillel Haim

Department of Microbiology and Immunology, Carver College of Medicine, The University of Iowa, Iowa City IA 52242

Lenacapavir (GS-6207, LEN) is a first-in-class, FDA-approved inhibitor of Human Immunodeficiency Virus (HIV-1) capsid (CA). By targeting the viral capsid, this drug interferes with multiple stages of the virus life cycle, including capsid entry into the nucleus, reverse transcription, and viral capsid assembly. LEN is administered as a subcutaneous injection every six months and is used to prevent infection and treat HIV-infected individuals who harbor multi-drug-resistant HIV-1. Mutations in the viral capsid that confer resistance to LEN are not common among circulating strains; however, resistance-imparting mutations have emerged upon LEN treatment. To gain a better understanding of the resistance paths that HIV-1 may pursue in response to LEN pressure, we employed a deep mutational scanning (DMS) system. To this end, we produced HIV-1 libraries that contain all possible amino acid substitutions at sites of interest in the viral CA. The effect of each variant on viral fitness and resistance to LEN was assessed. We focused our initial studies on positions 66, 67, 70, 74, 105, and 107 of the viral capsid, which form the LEN-binding pocket. In addition, for all possible substitutions at these sites, we calculated their mutational accessibility based on the likelihood of acquiring the necessary nucleotide changes from the clade B ancestral form. Our studies recapitulated resistance mutations observed in clinical trials and identified additional mutationally-accessible and fit forms of the virus that increase resistance to LEN. Taken together, our studies demonstrate that the genetic barrier to the development of LEN resistance is low, suggesting that conservative administration and extra monitoring should be applied in the treatment of HIV-infected individuals by this long-acting therapeutic.

Secretion of DNAse Effectors Confers a Competitive Advantage to Bacillota in the Gut Microbiome

Ethan Chodur, Bishal Parajuli, Dustin E. Bosch

Department of Pathology, University of Iowa, Iowa City, Iowa

Background

Polymorphic toxins are antibacterial effector proteins that mediate interbacterial competition in diverse microbial communities, including the human gut. These effectors are paired with immunity proteins that prevent self- and kin-intoxication. The effector and immunity protein of *Bacillus thuringiensis* (Bt) form a pair, BtTde1-BtTdi, that contributes to competitive fitness. The mechanism of secretion is currently unknown. Dysbiosis is characteristic of many human diseases, with polymorphic toxins potentially playing a role in shaping disease-associated microbiomes through interbacterial antagonism.

Hypothesis

BtTdi1 neutralizes BtTde1 through an active site mimicry mechanism which promotes degradation of the effector toxin proteins. BtTde1 secretion occurs via a non-T7SS pathway and contributes to interbacterial antagonism and competitive intestinal colonization.

Methods

Bt strains were engineered to inactivate BtTde1 or T7SS, or express BtTdi1. Secretion was assessed with bioluminescent reporters and proteomics. The BtTde1-BtTdi1 structure complex was assessed by X-ray crystallography. In vitro competition assays assessed interbacterial antagonism against non-immune *Bifidobacterium longum* and *Bacillus thuringiensis* HD73. Competitive colonization of Bt and B. longum was modeled in pseudo-germ-free mice.

Results

Genomic analysis revealed Tde/Tdi loci adjacent to mobile elements and diverse secretion systems across Bacillota. BtTde1 secretion increased upon deleting the T7SS ATPase EssC, indicating a T7SS-independent mechanism, confirmed by secretome analysis and luminescence- assays. Crystallography revealed that BtTdi1 inserts into the core of BtTde1, mimicking the active site with a $P(\Phi)_4GG$ motif, and inducing conformational changes that disrupt the active site. Limited trypsin proteolysis confirmed increased protease sensitivity upon binding. Bt strains secreting active BtTde1 outcompeted non-immune *Bacillus* and *B. longum* in vitro, and in gnotobiotic mice, highlighting its role in shaping gut microbial composition.

Conclusion

Our findings reveal a non-conventional secretion pathway for BtTde1, and a novel neutralization mechanism via BtTdi1 active site mimicry. This toxin system drives microbial competition, suggesting a key role in shaping gut microbiome structure.

Poster# 6

Investigating the Effect of Site Specific *N*^{6}-methyladenosine Modifications on HIV-1 Infection

Beck Fitzpatrick, Stacia Phillips, and Li Wu

Department of Microbiology and Immunology, Carver College of Medicine, The University of Iowa, Iowa City, Iowa

N^6-methyladenosine (m^{6}A) is the most abundant epitranscriptomic modification in eukaryotic mRNA and is also present in HIV-1 RNA. Our lab has mapped the location of m^{6}A in the HIV-1 genome at single-nucleotide resolution in infected CD4+ T cells. Many of these m^{6}A sites are conserved in over 90% of HIV-1 RNA sequences. We hypothesize that site-specific m^{6}A's are evolutionarily conserved, and their removal negatively affects HIV-1 replication in cells. The DRACH motif (D = A, G or U, R = A or G, H = A, C or U) can be recognized by methyltransferases for m^{6}A modification and can be mutated to inhibit m^{6}A modification. One important consideration when mutating HIV-1 RNA is that amino acid sequences of viral proteins remain unchanged. Of the most abundant m^{6}A sites in the HIV-1 genome, we changed eight sites from an a to g nucleotide, or a c to t nucleotide without impacting their coding sequences. Mutations in HIV-1 proviral DNA plasmids corresponding to sites in the gag/pol (1193, 1969, 2296, 2626, 2749, 4396), vpu (5712), or nef genes (8660) of the HIV-1 RNA inhibits a single m^{6}A placement per plasmid. Mutants and wild type (WT) HIV-1 were produced by transfection of HEK293T producer cells with the proviral DNA plasmids. Compared to WT HIV-1, mutants c2749t, c4396t and a8660g have lower infectivity in TZM-bl luciferase reporter cells, suggesting that the site-specific removal of a single m^{6}A site from HIV-1 RNA reduces viral infectivity. Upon passaging of the three mutant viruses with lower infectivity, a8660g, c2749t and c4396t all saw increased infectivity relative to WT after 2 passages and retained this phenotype for the remainder of passaging. Sequencing may reveal reversion mutations that restore WT DRACH motifs, indicating that these m^{6}A sites are evolutionarily conserved modifications that are beneficial to HIV-1 infectivity.

Tissue-Specific Immune Regulation in Macrophages

Logan Frye, Rhett Ellerbroek, Devin C. Gatica, Natalia Estrada Hernandez, Ryan S. Illig, Matthew S. Dunn, Fernando William Moreira Santana, Madison Kafka, Filiz Korkmaz

Understanding tissue-specific immune regulation is important for developing treatments to fight infections and limit inflammation. Although LOX-1 is traditionally associated with pro-inflammatory signaling in the vasculature, new evidence shows it may also help control inflammation in the lung. We hypothesized that LOX-1 activation produces tissue-specific inflammatory responses that limit cytokine production in macrophages from tissues that are more sensitive to damage.

To test this, macrophages from lung, bone marrow, and spleen were stimulated with OxLDL, LPS, HMGB1, or PBS for 4 – 48 hours. Cytokine expression was measured by ELISA and qPCR. Due to a previously unknown TLR4 defect, spleen macrophages were only analyzed for OxLDL and PBS.

MHS lung macrophages showed the strongest activation, with a >1000-fold CXCL2 response to LPS at 4 hours and high transcription of TNF-α early in stimulation. Also, MHS showed a sustained IL-6 expression, transcription of IL-6 being elevated from 4 to 48 hours and IL-6 levels increasing between 4 and 12 hours. BMDMs showed modest early IL-6 induction that declined quickly, showing that there is a limited capacity for prolonged inflammatory signaling. Spleen macrophages showed a high baseline CXCL2, but the other data gathered was invalidated due to a TLR4 defect for the cell line used. This suggests that macrophage response to Gram-negative bacteria depends on the tissue it was derived from, and that specific tissues in the body may suppress inflammation to prevent self-damage. Further analysis of different time points and using alternative stimuli that do not rely as heavily on TLR4 will be done. This will help to accurately measure IL-6 and CXCL2 on more diverse cell lines and for understanding the differences in immune regulation for macrophages from distinct tissues

Poster# 8

Characterizing the effects of Lenacapavir resistance on the kinetics of HIV-1 post-entry functions

Sarah Gulick, Kaho Matsumoto, Alex Kleinpeter

Department of Microbiology and Immunology, University of Iowa

Lencapavir (LEN) is a first-in-class, highly potent antiretroviral medication used in the treatment of multidrug-resistant HIV-1. LEN targets the viral capsid, which plays multiple roles during viral replication. Previous studies have shown that LEN can interfere with both the early and late stages of the viral replication cycle, with its most potent effects occurring during the early post-entry phase of replication. However, as LEN continues to be used in the treatment of HIV-1, clinically relevant LEN-resistant strains of HIV-1 have the potential to become more prevalent. “HRN”, a clinically-relevant LEN-resistant HIV-1 variant, replicates at near wild-type (WT) levels in the presence and absence of LEN. The mutations that enable HRN to escape LEN occur at the interface where LEN interacts with the viral capsid. However, this interface is also functional in other steps of viral replication, raising questions about how HRN replicates as efficiently as WT HIV-1. Here, we have used product-enhanced reverse transcriptase (PERT) to measure viral reverse transcriptase (RT) content to quantify WT and HRN HIV-1 pseudovirus. Using these quantifications, we will conduct normalized infections of relevant cell lines to compare WT and HRN post-entry infection kinetics. We have demonstrated that HRN displays a similar level of infectivity as WT HIV-1 in the absence of LEN, and future experiments will quantify reverse transcription products in infected cells. These experiments are foundational for understanding how LEN-resistant variants like HRN maintain fitness despite harboring mutations in key functional regions associated with LEN escape.

Dietary effects on the course of Visceral leishmaniasis in a mouse model

Grace Gutzman[1], Natalie C. Jarvis[1], Yani Chen[2], Bayan Zhanbolat[2], Selma M. B. Jeronimo[3,4] and Mary E. Wilson[1,2,5]

[1]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA 52242, USA
[2] Department of Internal Medicine, University of Iowa, Iowa City, IA, USA
[3] Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, RN, Brazil
[4] Department of Biochemistry, Federal University of Rio Grande do Norte, Natal, Brazil
[5] Veterans' Affairs Medical Center, Iowa City, IA 52252, US

Following recent shifts in dietary habits, our collaborators reported that obesity in infected Brazilians leads to a form of cutaneous leishmaniasis (CL) that is refractory to treatment. We hypothesized that diet-induced changes in host inflammatory profiles may also be determinants of visceral leishmaniasis (VL) outcome. We used a murine model of VL in which parasites expand first in the liver and subsequently the spleen. Groups of mice were maintained on a control, high-fat high-cholesterol (HFHC) or protein-energy malnutrition (LP) diet. After 4 weeks, half of the mice in each group were infected intravenously with 10^6 *Leishmania infantum*, the primary cause of VL in Brazil. qPCR and histological analyses of tissue samples demonstrated an increase in parasite loads in livers and spleens caused by the LP diet, whereas parasites bypassed liver and grew primarily in the spleens of HFHC fed mice. The changes in parasite trafficking prompted evaluation of the hepatic environment. Like other intracellular infections, granulomas are essential for parasite containment and clearance in the liver. Well-formed granulomas were observed in the livers of control-fed *L. infantum* infected mice. *L. infantum-infected* LP-fed mice formed smaller but more abundant granulomas, whereas a robust inflammatory infiltrate in *L. infantum* infected HFHC-fed mice was not organized. Spatial transcript analysis with RNAScope revealed parasites were confined to granulomas in control diet mice, but parasites were found throughout liver parenchyma in LP-fed mice suggesting their granulomas were ineffective at parasite containment. Nanostring analysis suggests that both the HFHC and LP diet differentially prime the host immune and inflammatory environment resulting in altered infection courses of *L. infantum*. Our findings suggest diet influences host immune responses to *L. infantum* infections. This data may improve the understanding of the influence of diet-induced metabolic and immune changes on the course of VL and other infectious diseases.

Poster# 10

Title: Biochemical Investigation of Dimerization of HSV-1 ICP22

Authors: Audrey Hake, Richard Roller

Affiliations: Department of Microbiology and Immunology, The University of Iowa

ICP22 is an immediate early protein necessary for optimal viral replication and spread in Herpes Simplex Virus type I (HSV-1). ICP22 has many reported activities, but no thorough structure-function analysis has been published, making it difficult to determine the significance of each activity. Though no experimental structure has been determined for the protein, AlphaFold3 models suggest that it is more likely to be found as a homodimer rather than a monomer. To determine the validity of this model, Flag- and HA-tagged forms of ICP22 were co-expressed in mammalian cells for co-immunoprecipitation analysis. Immunoprecipitation of FLAG-tagged ICP22 also precipitated the co-expressed HA-tagged ICP22 indicating association and possible dimerization. Because association in mammalian cells could be indirect due to ICP22 binding to cellular factors, a His-tagged ICP22 construct was then expressed in *E. coli* for mass photometry analysis. The full-length ICP22 was insoluble, necessitating denaturation and refolding. ICP22 appeared in both monomeric (2/3) and dimeric (1/3) forms in mass photometry, confirming propensity for dimerization. Analysis of the AlphaFold model revealed amino acids likely to stabilize the ICP22 dimer. Ongoing efforts are focused on mutating these amino acids to disrupt dimerization. This information will allow genetic studies to determine the significance of ICP22 dimer formation for viral replication and spread.

Characterizing the Role of tRNA Processing Enzymes TSEN2 and CLP1 in Viral Infection Using Overexpression Cell Line Models

Olivia Heller and Jessica Tucker

Department of Microbiology and Immunology, Carver College of Medicine , University of Iowa

Murine gammaherpesvirus (MHV68) is a double-stranded DNA virus closely related to the human pathogens Kaposi sarcoma-associated herpesvirus and Epstein–Barr virus. Previous work from our lab identified widespread dysregulation of transfer RNAs (tRNAs) during MHV68 infection, suggesting that tRNA regulation may play an important role in the viral life cycle. In particular, host pre-tRNAs are significantly upregulated during infection, and several of these transcripts undergo cleavage to generate tRNA fragments (tRFs). The accumulation of these tRFs indicates that MHV68 not only alters tRNA transcription but also perturbs tRNA processing pathways within the host cell.

To further investigate this phenomenon, we examined two host proteins involved in tRNA processing: Tsen2, an endonuclease responsible for cleaving pre-tRNA introns, and Clp1, an RNA kinase implicated in tRNA splicing regulation. Using siRNA-mediated knockdown, we found that these proteins have opposing effects on both tRF accumulation and viral replication. Specifically, Clp1 knockdown results in increased tRF levels and decreased viral titers, whereas Tsen2 knockdown leads to reduced tRF accumulation and increased viral titers. These results suggest that Clp1 has a proviral role, while Tsen2 functions in an antiviral capacity, potentially through their contributions to tRNA processing.

To validate these findings and control for potential off-target effects of siRNA, we generated rescue cell lines expressing codon-optimized, siRNA-resistant versions of Tsen2 or Clp1 in NIH3T3 cells. These models are expected to restore infection phenotypes to baseline and also allow us to assess the effects of gene overexpression.

Ongoing studies aim to define the mechanisms by which Tsen2 and Clp1 influence MHV68 replication and to determine whether canonical tRNA processing is required for these effects.

Characterizing a MHV68 dual reporter virus

Herrington, Harli A.[1], Huff, Calyssa J.[1,2], Didychuk, Allison L.[3], Tucker, Jessica M.[1]

[1]Department of Microbiology and Immunology, Carver College of Medicine, University of Iowa, Iowa City, IA

[2]ICARE Postbac program, Holden Comprehensive Cancer Center, University of Iowa, Iowa City, IA

[3]Department of Molecular Biophysics and Biochemistry, Yale School of Medicine, New Haven, CT

Transfer RNAs (tRNAs) play an important role during viral infection, enabling both cellular and viral gene expression. Studies have displayed increased expression of tRNA genes during infection with members from all three subfamilies of herpesviruses, including the murine gammaherpesvirus 68 (MHV68). In addition, our analysis of tRNA expression during MHV68 infection has revealed the accumulation of tRNA fragments, which are endonucleolytic cleavage products of tRNAs produced in response to viral replication. To determine the viral stage associated with the increased accumulation of tRNA fragments, we are characterizing an MHV68 dual reporter virus (MHV68-DR) containing both an early gene reporter (CMVp:mIFP) and late gene reporter (ORF52p:GFP). We first optimized the performance of MHV68-DR in the presence of phosphonoacetic acid (PAA), which inhibits the viral DNA polymerase, ultimately blocking viral DNA replication and subsequent late gene expression. To characterize the response of MHV68-DR to PAA, we used flow cytometry to compare NIH3T3 cells infected with MHV68-DR at both 6 and 24 hours post infection, with the latter representing peak titer. By comparing the percentage of cells in each sample that were mIFP- or GFP-positive, we confirmed that the GFP signal was diminished in PAA-treated cells compared to untreated. Because GFP was still detectable, though at a lower mean fluorescence intensity, we independently confirmed a block in late gene expression by RT-qPCR. Thus, we conclude that we can successfully use MHV68-DR to capture early and late phase replication stages of MHV68. We have also validated published results indicating that enhanced tRNA expression is dependent on early viral gene expression. Moving forward, we will apply these tools to pinpoint the viral stage required for the biogenesis of tRNA fragments during gammaherpesvirus infection.

Poster# 13

Transcription factor, RUNX1, promotes the formation of effector CD8 T cell populations in response to chronic viral infections.

Haleigh Holbert[1], Rebecca Bartell[2], Kayla Reisch[2], Mandy Scherer[1], and Ryan Zander[1,2]

[1]Department of Microbiology and Immunology, University of Iowa, [2]Interdisciplinary Graduate Program of Immunology

In response to persistent viral infection, $CD8^+$ T cells display a progressive loss of effector function, as exemplified by their impaired cytokine production, reduced cytotoxicity, and diminished proliferative potential. This deterioration in function, termed T cell “exhaustion” is further accompanied by the upregulation of multiple coinhibitory receptors and eventual clonal deletion. Although previously considered to be a homogenous population of dysfunctional T cells, emerging evidence indicates that the “pool” of exhausted T cells is comprised of multiple heterogenous populations, including a self-renewing progenitor subset that transitions through an intermediate state before bifurcating into either terminally exhausted cells or highly functional effector cells that are critically required for viral control. Despite these recent advances in our understanding of the lineage relationship between these subsets, the precise molecular circuits underpinning the differentiation of progenitor cells into either exhausted cells or protective effector cells remains to be determined. Through the use of single-cell RNA sequencing, it has been identified that the transcription factor, RUNX1, is selectively upregulated in the face of LCMV clone 13 infection, in effector-like subpopulation. To examine whether RUNX1 expression directly regulates $CD8^+$ T cell differentiation during chronic infection, we used a CRISPR Cas-9-mediated gene editing approach to delete *Runx1* in TCR transgenic P14 cells (which recognize the GP_{33-41} epitope of LCMV) prior to *in vivo* adoptive transfer and subsequent infection with LCMV CL13. The deletion of transcription factor RUNX1 showed a significant decrease in frequency of effector cells. Additionally, deletion of RUNX1 lead to a decrease in the ability of these cells to produce the effector molecule Granzyme B. Future studies will aim to quantify the viral titer of these LCMV clone 13 infection models and delve into the mechanisms behind the decreased effector populations.

LOX-1-dependent regulation of Dectin-1 may result in differential susceptibility to fungal pneumonia.

Madison R. Kafka, Madeline M. Chorazy, Natalia Estrada-Hernandez, Devin Gatica, Fernando Moreira-Santana, Lee J. Quinton, Filiz T. Korkmaz.

Rationale:
Pneumonia is the leading cause of infection-related mortality worldwide. Alveolar macrophages (AMs) are the first line of defense against lung pathogens. LOX-1 and Dectin-1 are both C-type lectin receptors expressed by AMs. LOX-1 protects against inflammation while Dectin-1 elicits an immune response to fungal pathogens. Previous work showed that inhibition of LOX-1 results in Dectin-1 downregulation. Therefore, the goal of this project is to determine if LOX-1 regulates the response to fungal infection through receptor co-regulation.
Methods:
MH-S (AMs) cells were treated with oxLDL and lipopolysaccharide (LPS) for 24h. Expression of LOX-1 and Dectin-1 were assessed by flow cytometry. MH-S and bone marrow derived macrophages (BMDMs) were stimulated with heat-killed *Candida albicans* for 24h to assess receptor and cytokine expression by RT-qPCR.
Results:
Treatment of MH-S cells with oxLDL and LPS led to downregulation of both intra- and extracellular LOX-1 and Dectin-1. IL-6 induction was measured in MH-S cells post-LPS, while HKCA led to an increase in IL-1 expression and a small increase in IL-6. However, LOX-1 is significantly induced in both AMs and BMDMs following exposure to HKCA, with no change in Dectin-1 expression, indicating that LOX-1 induction may need to occur prior to Dectin-1 activation.
Conclusion:
While LOX-1 and Dectin-1 have well-established roles during lung infection, our data suggest that LOX-1 and Dectin-1 are co-regulated. Future work will determine the specific pathways that lead to LOX-1-dependent regulation of Dectin-1 in macrophages and subsequent response to fungi.

Adenosine and IL-12 directly promote T_{FH1} differentiation during *Plasmodium* Infection.

Morgan Kremer, Manan Shah, and Noah Butler. University of Iowa, Iowa City, IA

Plasmodium, a parasite that causes malaria, infects over 250 million people and leads to nearly 600,000 deaths yearly worldwide. Robust humoral responses are essential in controlling the infection. T follicular Helper (T_{FH}) cells play a pivotal role in supporting B cells in germinal centers (GC) and promoting their differentiation into long-lived plasma cells and memory B cells. Emerging evidence reveals the presence of a distinct subset of helper CD4 T cells responding to *Plasmodium* infection, termed "type 1 T_{FH}" (T_{FH1}) cells, which exhibit phenotypic and functional characteristics of both T helper type 1(T_{H1}) and T_{FH} cells. To define T_{FH1} cells, we look at their cytokine profile, which is IFN-γ+ like T_{H1} cells and IL-21+ like T_{FH} cells. In human Plasmodium infection, it has been shown that T_{FH1} when compared to T_{FH} provide suboptimal B cell support, resulting in a suboptimal humoral response. Using *Plasmodium*-infected cytokine reporter mice, we found that T_{FH1} cells constitute approximately 50% of the total effector T_{FH} pool and comprise a substantial fraction of the total memory T_{FH} response. We also found that T_{FH1} cells exhibit elevated expression of adenosine and IL-12 receptors. Thus, we hypothesized that adenosine and IL-12 signaling individually promote T_{FH1} formation. To test this hypothesis, we administered an adenosine receptor agonist and anti-IL-12 blocking antibody and found that both adenosine and IL-12 promote T_{FH1} cell formation. To confirm that these compounds were acting directly through CD4 T cells, we performed a CRISPR-mediated deletion of both the A2aR adenosine receptor and the IL-12R individually. Consistent with the previous data, deletion showed a decrease in T_{FH1} cell formation. These results identify critical pathways that could potentially be targeted to modulate immunity to malaria.

Mutations in the HIV-1 CA C-terminus reduce the efficiency of virus production

Ella Morgan, Kaho Matsumoto, Alex Kleinpeter

The HIV-1 capsid (CA) protein plays distinct roles in particle assembly and maturation. During particle assembly, the C-terminus of the CA domain of the Gag polyprotein and the adjacent spacer peptide 1 (SP1) form a six-helix bundle (6HB) with five neighboring copies of Gag, stabilizing the hexameric immature Gag lattice and driving virus production. Upon particle release, the virion undergoes maturation, in which Gag is proteolytically cleaved into individual mature proteins. The newly liberated CA protein then assembles into the capsid, which conceals and protects the viral genome for subsequent infection. In this mature state, the CA C-terminus is unstructured and without a clear role in capsid formation. However, a recent study demonstrated that some CA C-terminus mutations result in a structurally defined C-terminus and enhance the efficiency of capsid assembly. Here we seek to elucidate the role of the CA C-terminus in capsid assembly. To study the effect of mutations in the CA C-terminus on capsid assembly, we first needed to identify mutants capable of immature Gag lattice formation and particle assembly. Because the CA C-terminus plays a crucial role in particle assembly, mutations in this region generally ablate virus production, preventing the study of capsid assembly. To circumvent this issue, we tested the inclusion of the T8I mutation in SP1 with several C-terminus mutants due to its stabilizing effect on the 6HB and ability to restore virus production to assembly-incompetent mutants. Importantly, the T8I mutation does not confound further analysis of capsid assembly due to its removal from CA during maturation. Our results showed that virus production efficiency was rescued by the addition of T8I only for the P224A mutant, underscoring the importance of the C-terminus in particle assembly and demonstrating that the ability of T8I to reverse virus production defects is not ubiquitous.

Knockdown of the transcription factor, Brf1, impacts Brf1-dependent and -independent Pol III transcript levels during MHV68 infection

Libby Nyberg[1], Kyle Rapchak[1], Calyssa Huff[1], Jessica M. Tucker, PhD[1].

1 The Department of Microbiology and Immunology, Carver College of Medicine

RNA polymerase III (Pol III), which transcribes many important non-coding RNAs, is known to be upregulated during gammaherpesvirus infection. How increased Pol III activity impacts infection is not known. To understand whether Pol III upregulation is important for the host or virus during infection, we used siRNAs to knock down an essential transcription factor for Pol III, Brf1, followed by infection with murine gammaherpesvirus 68 (MHV68). First, we validated the effect of Brf1 knockdown on individual Pol III transcripts, with representatives transcribed from each of three known Pol III promoter types. Type I and Type II promoters depend on Brf1 to recruit Pol III, thus, we predicted that these transcripts would be diminished by Brf1 knockdown. Following Brf1 knockdown, RT-qPCR analysis for type II pre-tRNAs showed decreased expression, as expected. However, the type II transcript, 7SL, and the type I transcript, 5S, appeared unaffected by loss of Brf1, likely due to their impressive cellular stability and abundance. An additional Pol III promoter type, called type III, uses Brf2 in place of Brf1 for transcription, and so we predicted that type III transcripts should be unaffected by Brf1 knockdown. However, the type III transcripts tested, 7SK and U6, showed increased expression levels over the course of infection, specifically when Brf1 is knocked down. Thus, Brf1 knockdown differentially impacts specific pools of Pol III transcripts in the cell. Surprisingly, we see enhanced MHV68 titers (up ~6-fold) from cells knocked down for Brf1 compared to control cells treated with non-targeting siRNA. We hypothesize that perhaps a Pol III transcript that is perturbed by Brf1 knockdown is responsible for this change in viral replication. To test how different Pol III transcripts impact MHV68 replication, we overexpressed individual noncoding RNAs and measured their impact on viral titer. Our results indicate that 7SK overexpression increases viral titer, possibly suggesting a pro-viral role of this transcript. Because 7SK was also elevated following Brf1 knockdown and MHV68 infection, it is possible that 7SK drives our Brf1-dependent antiviral phenotype, but this needs to be tested in future experiments. Overall, working towards an understanding of how pol III transcripts impact gammaherpesvirus infection may help us to understand why infection triggers enhanced Pol III activity, and how individual non-coding RNAs participate in host-viral interactions.

Identification and characterization of newly secreted effectors in *Orientia tsutsugamushi*

Department of Microbiology and Immunology

Venkat Pathuru, Paige McCaslin, Mary Weber

Orientia tsutsugamushi (*O.t.*) is a gram-negative, obligate intracellular bacterium and the causative agent of scrub typhus. This is a neglected, emerging disease that has no vaccines and infects about 1 million people annually. *O.t*. likely modulates its host environment using secreted effectors delivered by type 1 secretion systems and type four secretion systems. Predicted effectors in *O.t.* are categorized into two classes that contain common eukaryotic protein interaction motifs: Ankyrin repeat-containing proteins (Anks) and tetratricopeptide repeat containing proteins (TPRs). But identification of secreted effectors during native infection has been hampered by the lack of genetic tools for *O.t.* Anks have previously been shown to be secreted through a T1SS surrogate bacteria in *E. coli* and implicated in effecting host transcription, apoptosis and immune pathways. However, the role of tetratricopeptide repeat containing proteins (TPRs) is largely unexplored. We hypothesize that *O.t*. TRP proteins function as T1SS effectors..Using a *E. coli* hemolysin A secretion system, we demonstrated that several *O.t.* TPRs are secreted such as KARP_00126, KARP_00341, KARP_00529, KARP_01308 which were capable of being secreted. When ectopically expressed in HeLa cells, the secreted TPRs have unique localization patterns. Bioinformatics identified several domains that may bind with components of the anaphase promoting complex (APC/C), therefore we utilized co-immunoprecipitation and determined KARP_00126, KARP_00529, and KARP_01308 bind with the APC/C co-activator CDC20. Future research will determine if *O.t.* secretes TPRs to manipulate the host cell cycle and why this is beneficial for the bacteria.

Poster# 19

Absence of CD47 disrupts erythroid recovery after sublethal radiation exposure

Shay Pechter, Saini Saurabh, Prajwal Gurung

Acute radiation syndrome (ARS) results from exposure to ionizing radiation during medical treatment, nuclear accidents, & space travel. A common side effect of ARS is bone marrow damage & anemia. Healthy red blood cells (RBCs) highly express CD47. A previous study has shown progressive decrease in CD47 expression by RBCs following radiation exposure, suggesting RBC damage & death. Preliminary data from our lab shows Cd47-/- mice are highly susceptible to radiation. Clearance of damaged RBCs is typically mediated by SIRPa expressing phagocytes following decrease in CD47 expression by RBCs. Recent work has revealed RBCs can also undergo programmed inflammatory cell death known as spectosis. To this end, we evaluated RBC death & morphology, as well as anemia following radiation in the absence of CD47. We non-lethally irradiated C57BL/6, Cd47-/-, & Cd47-/-Sirpa-/- (DKO) mice. Mice were bled retroorbitally at multiple time points during the recovery period. We evaluated RBC morphology via blood smears, total white blood cells, RBCs, and complete blood counts. We observed slight anemia in Cd47-/- compared to controls, and even more exaggerated anemia in DKO. We also observed more morphological abnormalities in the absence of CD47 than in controls. This data suggests that CD47 is required for efficient recovery of the hematopoietic compartment following radiation. This is a potential mechanism mediating susceptibility of Cd47-/- to ionizing radiation.

The Role of Calprotectin in Nutritional Immunity: Zinc Sequestration During *Campylobacter* Infection

Emily Roberts, Lincoln Lewerke, Dr. Jeremiah Johnson

University of Iowa Department of Microbiology and Immunology

C. jejuni is a highly motile, foodborne pathogen that is a leading cause of bacterial gastroenteritis worldwide. *Campylobacter* infection is unique because pathology is tied to the host's innate immune response, as opposed to specific bacterial toxins or effectors. During Campylobacteriosis, neutrophils are recruited to the site of infection and significantly contribute to damage to the gastric epithelium. Neutrophils possess various mechanisms to eliminate bacterial invaders, one being the antimicrobial protein calprotectin. Calprotectin is a highly abundant, neutrophil-associated protein that sequesters transition metals from bacterial pathogens, limiting their ability to grow. After undergoing structural changes dependent on calcium concentration, two distinct metal-binding sites in calprotectin are formed that sequester zinc, copper, iron, manganese, and nickel. Given the link between Campylobacteriosis and neutrophils, we were interested in how calprotectin impacts *C. jejuni.* We started by identifying the calprotectin dose that inhibits wild-type *Campylobacter* growth by 50%. Next, we sought to explore which calprotectin-binding metal is most important and found that zinc supplementation restored *Campylobacter* growth during calprotectin treatment, suggesting zinc limitation as the mechanism of bacterial inhibition. We further found through RT-qPCR that *C. jejuni* upregulates *znuABC* during calprotectin-induced zinc limitation. We then used a mutant in the high-affinity zinc transporter to illustrate attenuated growth during calprotectin treatment. Fluorescent microscopy was used to correlate elevated calprotectin in tissue following *Campylobacter* infection, and RT-qPCR revealed that calprotectin transcripts are elevated during wild-type infection in mice. All together, this work highlights the importance of transition metal nutrients to bacterial virulence.

Identifying and categorizing antifungal tolerance in clinical *Candida glabrata* isolates

Arya Sajadian[1,2], Jessica Miller[2], Aloysius Klingelhutz[1], Bin Z. He[1,2]

[1]Department of Microbiology and Immunology, [2]Department of Biology, University of Iowa

Treating fungal infections in a clinical setting requires specific concentrations of antifungals. Even for resistant strains that have high minimum inhibitory concentrations (MICs), the administered concentration is well above the MIC, but still low enough to avoid harming patient cells. While resistant cells are defined by their ability to withstand higher concentrations of drug related stress, usually due to genomic variation, tolerant cells are a subpopulation that differ in phenotypic expression of stress responses. In a clinical setting, prolonged fungal infection despite low MICs can be the result of tolerant cells that exhibit slowed growth under drug induced stress. Based on the literature, we define tolerance as consistent growth at or above the MIC50, which is the concentration of the antifungal required to reduce the growth of the population to 50% of the drug-free control. In practice, tolerance is measured by Supra-MIC growth (SMG), calculated as the ratio of the mean of ODs at all wells at or above the MIC50 concentrations, divided by the OD of the drug-free well. The aim of this study was to determine the tolerance with rapamycin, a natural compound secreted by the gram positive bacteria *Streptomyces hydroscopicus* that has antifungal activities. The secondary goal was to determine the variation in tolerance against rapamycin among a panel of clinical and globally sampled strains of *C. glabrata*. The latter is significant as there is notable strain-to-strain variation in virulence and determining the spectrum of phenotypic variation among strains is crucial for clinical treatment. Data from the reference strains and one experimental strain were consistent and accurate in determining tolerance. Underestimation of resistance for the other experimental strains, however, led to overgrowth after 48 hours and an inability to accurately determine the capacity for SMG. Concentration adjustments will be made to test the tolerance of those strains.

Poster# 22

Human Pegivirus Type 1 Reduces Low-Level HIV-1 Replication and Viral Blips

Sanders MK, Pollock T, Xiang J, Bates M, Stapleton JT

The University of Iowa and Iowa City VA Healthcare

Combination antiretroviral therapy (cART) reduces viremic HIV-1 in people living with HIV-1 (PLWH) to a viral load (VL) of less than 200 genome equivalents (GE) of RNA per milliliter of blood—defined as viral suppression. PLWH on cART commonly have a VL of <20 GE/mL, but they can experience unexpected, transient increases in HIV-1 RNA in the bloodstream, viral blips. The occurrence of blips is associated with the efficacy of cART, vaccinations or infections that temporarily increase HIV-1 replication, random variation in viral replication, and immunologic status. Human Pegivirus type 1 (HPgV-1), discovered in 1995, is a member of the *Hepaciviridae* family and *Pegivirus* genus. HPgV-1 is highly prevalent; 20% of the world's population are infected. HPgV-1 is prevalent amongst PLWH and is associated with improved survival in those individuals. Following written informed consent, plasma samples and clinical data of PLWH were obtained from the UIHC Virology Clinic. Using real-time RT-PCR to detect HPgV-1 RNA, HPgV-1-positive PLWH were identified, and their likelihood and number of viral blips were compared with HPgV-1-negative PLWH. Using unpaired nonparametric tests with $p<0.05$ indicating statistical significance, PLWH coinfected with HPgV-1 had reduced likelihood of viral blips and a reduced number of low-level (20-50 GE/mL) viral blips. Specifically, the proportion of those with HPgV-1 infection who experience viral blips (48.2%; 41/85) was significantly lower ($p<0.01$) than people without HPgV-1 infection (63.2%; 276/337). Moderate (51 -200 GE/mL) to high-level (201-600 GE/mL) blips did not appear significantly influenced by HPgV-1 co-infection. Results demonstrated that HPgV-1 coinfection is significantly associated with the reduction of viral blips in PLWH. Sex, race, or antiretroviral treatment does not appear to significantly influence HPgV-1 interactions with HIV-1. However, those with HPgV-1 viremia were significantly younger than those without ($p<0.0001$).

“Ah FhuE! sRNA-50 Represses Siderophore-Receptor FhuE in *A. baumannii*”

Authors: Grace Schroeder, Mikaela Daum, Valerie Intorcia, Dr. Michael Gebhardt

Acinetobacter baumannii is a gram-negative opportunistic pathogen that thrives in the nosocomial environment due to its ability to resist desiccation, form biofilms, and resist antibiotic treatment. These “persist and resist” strategies can be regulated by multiple mechanisms, including post-transcriptional regulation mediated by small RNAs (sRNAs). sRNAs interact with target mRNA transcripts through limited base-pairing, which can influence the stability or translation of the mRNA. The Gebhardt lab has previously identified 22 targets of sRNA-50, many of which are involved with metabolism or heavy metal homeostasis. One particular target is *fhuE*, a TonB-dependent siderophore receptor. Siderophores tightly bind iron and are taken up by bacteria through outer membrane receptors, aiding survival in iron-limited environments. One goal of this project was to determine if sRNA-50 post-transcriptionally regulates *fhuE*, since previous literature has found that the antibiotic Rifabutin decreases *A. baumannii* viability in a FhuE-dependent manner. Transcriptional and translational *fhuE-lacZ* fusions were made in a wild type background and a Δ*sRNA-50* background. Through a beta-galactosidase assay, it was determined that sRNA-50 post-transcriptionally represses *fhuE*. An additional goal was to determine what conditions induce *sRNA-50* expression, since sRNAs are typically expressed under specific environmental conditions to maintain cellular homeostasis. We wanted to know if *sRNA-50* expression is influenced by iron availability since sRNA-50 represses the TonB-dependent siderophore receptor. A *sRNA-50-lacZ* promoter fusion was used to determine that increasing iron availability causes an increase in *sRNA-50* expression. Further exploration would focus on determining the novel mechanism of FhuE in Rifabutin resistance in *A. baumannii* and investigating whether sRNA-50 plays a role in regulating Rifabutin resistance through its effects on the *fhuE* transcript.

Poster# 24

Title: Novel Small-molecule Inhibitors Block Filovirus Glycoprotein-mediated Entry

Authors: Allison Stolte[1], Paige Richards[1], Mary Lynn Trawick[2], Kevin G. Pinney[2] and Wendy Maury[1]

[1]Department of Microbiology and Immunology, University of Iowa, Iowa City, IA, USA.
[2]Department of Chemistry and Biochemistry, Baylor University, Waco, Texas, USA

Ebola virus (EBOV) and Marburg virus (MARV) are members of the *Filoviridae* family and the causative agents of severe viral hemorrhagic fever, which can be fatal. Although an FDA-approved vaccine and monoclonal antibody therapeutics exist for EBOV, outbreaks continue to occur, highlighting the necessity for additional antiviral strategies. In contrast, no FDA-approved vaccines or therapeutics are currently available for MARV. Here, we identify robust anti-filoviral activity of two small-molecule inhibitors: an indole analog, OXi8006, and a benzosuberene analog, KGP18. These compounds have previously been characterized as inhibitors of tubulin polymerization and vascular disrupting agents in tumors. In Vero E6 cells and a murine Kupffer cell line, KGP18 and OXI8006 potently inhibited EBOV- and MARV-glycoprotein (GP)-mediated infection, with IC_{50} values of ~8 nM and 190 nM, respectively. Similar IC_{50} values were observed in assays that measured entry events of Ebola virus-like particles (VLPs) and MARV VLPs in the Kupffer cells, implicating virus entry inhibition as a primary mechanism of action. In human skin explants maintained at the air-liquid interface, KGP18 blocked EBOV- and MARV-GP-mediated infection at 33 nM over a 12-day infection with little to no cellular toxicity observed at that concentration. Ongoing mechanistic studies aim to define the entry step(s) targeted by these compounds. Collectively, these studies have identified novel, small-molecule inhibitors that inhibit filovirus entry at sub μM concentrations and represent promising candidates for the development of new filovirus antivirals.

Campylobacter jejuni Transposon Library (TnShrink)

Kelsey Taylor, Lincoln Lewerke, Dr. Jeremiah Johnson
University of Iowa Department of Microbiology & Immunology

Transposons are mobile genetic elements that insert themselves into regions of a bacterial genome and disrupt the genes they insert into. Transposon libraries are a collection of singular transposon insertions across the entire bacterial genome, allowing screenings to identify genes with specific functions, regulation, and survival. We started with 8,640 individual *Campylobacter* strains from a previous mariner transposon mutagenesis. We sought to confirm and organize these individual strains into an ordered library of less than 1,000 strains that can be easily utilized.

Annotation of each transposon hit was mapped to individual genes by comparing the position of the hit to the known sequences. We found 963 genes with transposon insertions, accounting for 58% of the *Campylobacter* genome, with an average of 4 insertions per gene. Intergenic regions, accounting for 10% of the hits, along with strains that had two transposons, were deleted. This left 905 unique hits and 3574 total insertions, representing 55% of the bacterial genome.

Isolations were done for two insertions of each gene. A universal primer and gene-specific primer were created, and genomes were PCR amplified. Gel electrophoresis was used to confirm the expected transposon hit, and the strain was organized into an ordered library for future screens.

We plan to use this transposon library to investigate genes required for neutrophil infection, cold/milk survival, nitric oxide stress, and more. These assays will hopefully help determine genes involved in the spread and pathogenicity specific to such environments, potentially having broader implications for the food and health industry.

Developing a Screen to Identify Phosphatidylglycerophosphate Phosphatases in *Clostridioides difficile*

Josefina Frisina, Elizabeth Warren, Craig Ellermeier

Department of Microbiology and Immunology, University of Iowa

Bacterial cell envelopes, and the enzymes required for their synthesis, are key targets for antibiotics. *Clostridioides difficile*'s membrane is largely made up of phosphatidylglycerol and is essential for *C. difficile* survival. The enzymes required for synthesis have largely been identified, except for the phosphatase that is responsible for converting phosphatidylglycerophosphate to phosphatidylglycerol, which until recently, was true of nearly all Gram-positive bacteria. It was recently discovered that PgpP (phosphatidylglycerophosphate phosphatase) is essential for phosphatidylglycerol synthesis and survival in *Bacillus subtilis*. Utilizing bioinformatics, we searched the *C. difficile* genome for this family of phosphatase and identified 16 candidate genes. We used a *B. subtilis* strain in which *pgpP* expression is dependent upon IPTG (in the presence of IPTG this strain grows normally; however, in the absence of IPTG it is not viable). We cloned the candidate *C. difficile* genes under the control of a xylose-induced promoter. Preliminary plating of these 16 constructs showed no restoration in the *pgpP B. subtilis* strain. This suggests either the phosphatases are not expressed or they are not sufficient for phosphatidylglycerol synthesis. We plan to confirm the expression of the putative phosphatases in *B. subtilis*. If we find they are indeed expressed, it will suggest that there is a distinct phosphatase *in C. difficile* that has not been identified. Future work would then require a genetic screen to identify the missing phosphate(s). Understanding the enzymatic pathway of this essential phospholipid in *C. difficile* could be important when discerning potential antibiotic targets.