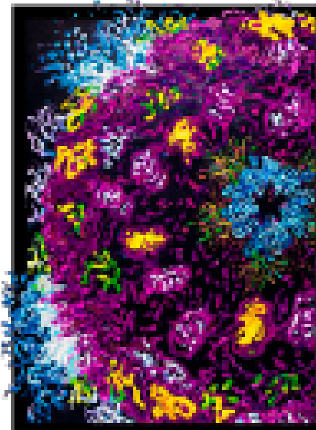


ALL-IOWA
VIROLOGY
SYMPOSIUM

13TH BIENNIAL / MARCH 6-7 2026 / IOWA STATE UNIVERSITY

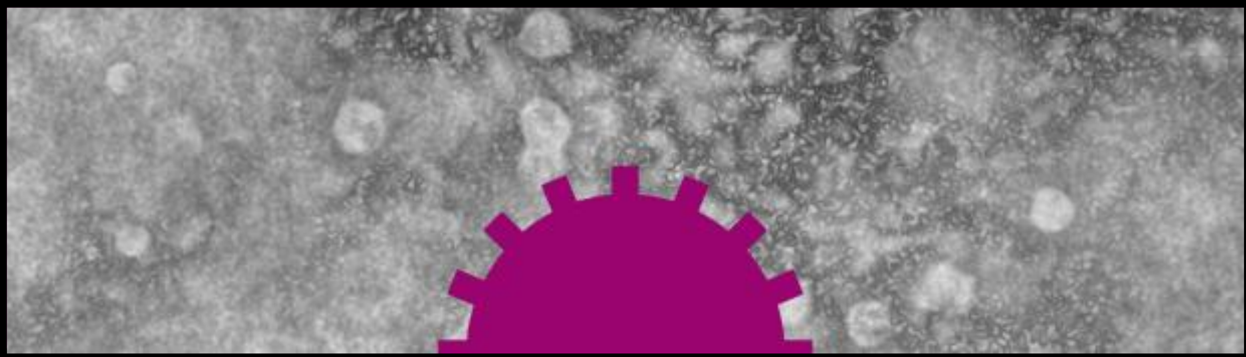
ALL-IOWA VIROLOGY SYMPOSIUM

Symposium Program

13th Biennial | March 6–7, 2026

Iowa State University

Alumni Center
429 Alumni Lane,
Ames, Iowa 50011



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ALL-IOWA VIROLOGY SYMPOSIUM

All-Iowa Virology Symposium

March 6-7, 2026

Friday, March 6

9:00am-9:55am — ***Registration***
Continental Breakfast

10:00am-10:55am — ***Plenary Speaker:*** **Peter Nagy, PhD**
Deep insights into biogenesis of tombusvirus replication organelles
Department of Plant Pathology
University of Kentucky
Introduction: Dr. Allen Miller

Session 1: Virus:Host Cell Interactions—Convenor Allen Miller

11:00am-11:15am — **Angela Liu**, Dr. Jin Pan, Dr. Brajesh Singh, Dr. Patrick Sinn
Impact of Environmental Stressors on the Survival of Cell-Associated versus Cell-Free Measles Virus
Microbiology, University of Iowa

11:15am-11:30am — **Sheila Gonzalez**, Jessica M Tucker
The RNA kinase, Clp1, supports murine gammaherpesvirus infection
Microbiology & Immunology, University of Iowa

11:30am-11:45am — **Dah Hyun Jung**, Richard Roller
HSV-1 Induces Stable Associations Between the Golgi and Endosomes Within the Cytoplasmic Viral Assembly Compartment of Neuronal Cells
Microbiology, University of Iowa

11:45am-12:00pm — **Jahanara Akter Sonia**, Xuping Xie, Andrew Severin, Rick Masonbrink, David C. Walker, Peng Liu, Pei-Yong Shi, W. Allen Miller
Accumulation of Zika virus noncoding sfRNA alters translation dynamics in placental trophoblast cells
Molecular, Cellular & Developmental Biology, Iowa State University

12:00pm-1:00pm — ***Lunch***

Session 2: Virus replication—Convenor Todd Bell

1:00pm-1:15pm — **David Price**
Structure Prediction of Complexes Controlling Beta- and Gamma-herpesvirus Late Transcription using AlphaFold 3
Biochemistry and Molecular Biology, University of Iowa

1:15pm-1:30pm — **Olufemi Fasina,** Lisa Uhl, Aidan Saxton
Parvoviral RNA processing: modulating the ends for productive viral replication, efficient gene therapy and oncolytic applications
Veterinary Pathology, Iowa State University

1:30pm-1:45pm — **Samir Mehanovic**, Cathy Miller
Development of a Novel RNA Interaction Mapping Assay Reveals S1-Dependent Genome Assortment Networks in Mammalian Orthoreovirus
Veterinary Microbiology and Preventive Medicine, Iowa State University

1:45pm-2:00pm — **Alex Kleinpeter,** Kaho Matsumoto, John-Mark Miller, Ella Morgan, Sydney Winecke, Sarah Gulick, Emilia Bolinski
Elucidating the molecular mechanisms underlying HIV-1 assembly, maturation, and resistance to capsid inhibitors
Microbiology & Immunology, University of Iowa

2:00pm-2:15pm — **Elizabeth Wagstaff**, Jessica Phillips, Dawn Dudley, Heather Simmons, and Chen S. Tan
Novel Rhesus Macaque Model for JC Polyomavirus Infection and Role of the Rearranged Noncoding Control Region During Infection
Microbiology, University of Iowa

2:15pm-2:30pm — **Joaquin Caceres**, Nayrmaliz Marrero, Eli Blay, Gulnara Brixius, Jennifer Groeltz-Thrush, Rachel Phillips, Rahul K. Nelli, Silvia Carnaccini, Todd M. Bell
Influenza A (H5N1): Molecular Approaches to Understanding Expanded Host Range
Veterinary Microbiology and Preventive Medicine, Iowa State University

2:30pm-2:45pm — **Trevor Arunsiripate**, C. Schmidt; M. Kroeger; L. Keeler; A. Hentz Gris; E. R. Burrough; P. E. Piñeyro
Porcine hemagglutinating encephalomyelitis virus (PHEV) entry receptor gene profiling and viral strand-specific detection for viral replication using suspension fluorescent in situ hybridization
Veterinary Pathology, Iowa State University

2:45pm-3:00pm — ***Break***

Session 3: Virus Evolution and Detection—Convenor Jessica Tucker

3:00pm-3:15pm — **Silvia Carnaccini**, Lucas Goble, Han Yang Wang, Caitlyn Middleton, Dikshya Regmi, Flavio Cargnin Faccin, Lisa Stabler, Garrett Cribbs, Samara Wartella, L. Claire Gay, Juliana Calil Brondani, Teresa D Mejias, Daniel Perez, Laura-Isobel McCall
Comparative Pathogenesis of a Surrogate LPAIV H5 Clade 2.3.4.4b in Ducks and Chickens
Veterinary Diagnostic and Production Animal Medicine, Iowa State University

3:15pm-3:30pm — **Lu Yen**, Juan Carlos Mora-Díaz, Ning-Chieh Twu, Bala Mounika Reddi, Apoorva Saxena, Jesús Horacio Lara Puente, Daniel Gustavo Rocher Maliachi, Ángel David Cota Valdez, Mario Palma Zepeda, Rahul Kumar Nelli, Luis Gabriel Giménez-Lirola
Feasibility of Porcine Enteroids as an Alternative to Cell Culture and Pig Bioassay for Assessing Porcine Deltacoronavirus Viability US VIABILITY
Veterinary Diagnostic and Production Animal Medicine, Iowa State University

3:30pm-3:45pm — **Pearl Chan**, Ajit Vikram, Stanley Perlman
Vascular Dysfunction in Mouse Aorta Following SARS-CoV-2 Infection
Microbiology & Immunology, University of Iowa

3:45pm-4:00pm — **Carter Casey**, Randall Vos, Adriana Larrea Sarmiento, Alejandro Olmedo Velarde
Virus Prevalence in Iowa Soybeans and Grapes
Plant Pathology, Entomology & Microbiology, Iowa State University

4:00pm-4:15pm —**Veeraya Bamrung**, Phillip C. Gauger, Michael C. Rahe, Ganwu Li, Rachel J. Derscheid, Michael A. Zeller
Divergent porcine astrovirus 4: Distinct respiratory and gastrointestinal clades with genetic differences
Bioinformatics and Computational Biology, Iowa State University

4:15pm-4:30pm — **Hillel Haim**, Rohith Rao Vujjini, Cassian M Birler, Madeline M Broghammer, Aaron N Gillman, Samuel A McCarthy-Potter
Modeling HIV-1 Env Evolution Across Scales to Predict Antibody Efficacy and Escape
Microbiology & Immunology, University of Iowa

4:30pm-4:45pm — **Vaishnavey Srinivasan Raghunath**, Supantha Dey, Sakib Ferdous, Riza Danurdoro, Dr. Michael A. Zeller, Dr. Ratul Chowdhury
Structure aware evolutionary fitness learning provides robust surveillance screening for PRRSV-ORF5 variants
Chemical and Biological Engineering, Iowa State University

4:45pm-5:00pm — **Eduarda Braga**, Michael A. Zeller, Fernando Leite and Pablo E. Piñeyro
Mapping the genotypic landscape of PCV2 in the U.S. patterns
Veterinary Diagnostic and Production Animal Medicine, Iowa State University

5:00pm-5:15pm— **Jeff Meier**, Ming Li, Qiaolin Hu, Arya Zandvakili, and David Price
A multimolecular pUL84-IE2-TBP-nucleosome complex at the human cytomegalovirus major immediate-early promoter blocks antisense transcription
Internal Medicine/Infectious Diseases, University of Iowa

5:15pm-7:00pm — ***Poster Session***
Drinks and Light Snacks

7:00— Dinner

8:00pm — ***Plenary Speaker:*** **Joseph Bondy-Denomy**
Elaborate mechanisms for immune nuclease avoidance by a bacterial virus
Department of Microbiology & Immunology
University of California San Francisco
Introduction: Dr. Wendy Maury

Saturday, March 7

9:00am — Continental Breakfast

9:30am-10:30am — ***Plenary Speaker:*** **Anice Lowen**
Influenza A virus population dynamics through the transmission event
Department of Microbiology and Immunology
Emory University School of Medicine
Introduction, Dr. Cathy Miller

Session 4: Viral Immunology—Convenor Cathy Miller

10:45am-11:00am — **Caroline Plescia**, José Alberto Aguilar-Briseño, Wendy Maury
Interferon beta sensitivity differentially restricts entry of diverse filovirus glycoproteins in mammalian hosts
Microbiology, University of Iowa

11:00am-11:15am — **Fatemeh Hassanpour**, Raymond Larsen, Santanu Raychaudhuri, Reza Montazami, Michael W. Cho
Development of a Vaginal Film for Sustained, Controlled Delivery of Prophylactic Antibodies Against Sexually Transmitted Infections
Chemical and Biological Engineering, Iowa State University

11:30am-11:45am — **Senthamizharasi (Arasi) Manivasagam**, Julianna Han, Boopathi Sowndhirarajan, Boyang Cheng, Jasmine T. Perez, Priya Issuree, Balaji Manicassamy
Host transcriptional repressor Capicua regulates virus-driven cytokine storm during respiratory viral infection
Microbiology & Immunology, University of Iowa

11:45am-12:00pm — **Pak Hin Hinson Cheung**, Pearl Chan, Hua Yang, Krisztina Ambrus, Shravya Honne, Baek Kim, Stanley Perlman, Li Wu
Loss of SAMHD1 restricts SARS-CoV-2 infection by downregulating HNF1-mediated ACE2 expression in lung epithelial cells
Microbiology & Immunology, University of Iowa

12:00pm-12:15pm — **Amanda Dudek**, William N. Feist, Sofia E Luna, Kaya Ben-Efraim, Elena J Sasu, Alex TH Cocker, Peter Parham, Mathew H Porteus
Harnessing cell-type specific gene editing for a combinatorial HAART cell therapy
Microbiology & Immunology, University of Iowa

Closing Remarks

Oral Presentations

Angela Liu, Dr. Jin Pan, Dr. Brajesh Singh, Dr. Patrick Sinn

Impact of Environmental Stressors on the Survival of Cell-Associated versus Cell-Free Measles Virus

Microbiology, University of Iowa

Rationale and goal: Recent widespread measles virus (MeV) outbreaks have led to the loss of elimination status for several countries worldwide. MeV is so contagious that eradication requires a vaccination and booster rate of >95%, the highest of any human virus. Our long-term goal is to understand why MeV is the most contagious human virus.
Background and hypothesis: We previously demonstrated that MeV uniquely forms foci of infection in primary cultures of well-differentiated human airway epithelia. These foci dislodge en masse from the epithelial layer days after infection. Most apically shed virus remains within the cell-associated form. These shed foci remain metabolically active and can further infect susceptible cells. We hypothesize that cell-association confers environmental stability to MeV which contributes to its contagiousness.
Methods: Here we investigate the stability of cell-free (CF) and cell-associated (CA) MeV in H358 cells. We use slow speed centrifugation, then cell lysis to prepare CF virus. After undergoing an environmental stressor, the viral titer is determined.
Results: We found that CF and CA MeV retain infectivity at -80°C,4°C and 25°C for at least 24 hours. The infectivity of both CF and CA MeV declined significantly at 37°C, with most CF MeV samples falling below the limit of detection. Relative humidity (RH) affects the viral decay rate and droplet morphology. The impact of RH differed for CF or CA MeV.
Conclusion: CA MeV has an advantage in protecting virions against temperature variation and desiccation as compared to CF MeV. Intermediate RH levels are likely the critical threshold for MeV to overcome in the environment. Now that these methods are established, in future work we will pivot from H358 cells to the more precious primary cultures. This information can be used to make informed policy decisions to reduce transmission in schools and health care environments during outbreaks.

Sheila Gonzalez, Jessica M Tucker

The RNA kinase, Clp1, supports murine gammaherpesvirus infection

Microbiology & Immunology, University of Iowa

Gammaherpesvirus infection drives widespread changes in host gene expression, in part due to viral hijacking of host translational machinery. We previously demonstrated that murine gammaherpesvirus 68 (MHV68) infection enhances the expression of host pre-tRNAs. We have evidence that mature tRNA pools do not significantly change, suggesting that viral infection is associated with a block in pre-tRNA maturation. Instead, pre-tRNAs undergo cleavage during infection, resulting in the accumulation of tRNA fragments (tRFs) in infected cells. Here, we show that the RNA kinase and tRNA splicing factor, Clp1, regulates tRF production from pre-tRNAs. Furthermore, knockdown of Clp1 decreases viral replication. Clp1 has two identified cellular functions, participating in both tRNA splicing and mRNA 3’ processing. To determine

which function is involved with Clp1's proviral role, we knocked down tRNA ligase, Rtcb, as well as Clp1's mRNA 3'-end processing partner, Pcf11. Pcf11, but not Rtcb, is required for MHV68 replication and infectious titer. While Rtcb knockdown resulted in increased levels of tRFs, it did not impact viral replication. This preliminary data suggests that Clp1's proviral activity is related to its role in mRNA 3'-end processing. Future work is aimed at mechanistically dissecting how Clp1 promotes MHV68 replication and the extent to which 3' end processing of viral and/or host mRNAs contributes to viral replication.

Dah Hyun Jung, Richard Roller

HSV-1 Induces Stable Associations Between the Golgi and Endosomes Within the Cytoplasmic Viral Assembly Compartment of Neuronal Cells

Microbiology, University of Iowa

Assembly of mature herpesvirus particles requires extensive remodeling of cytoplasmic membranes. In neuronal cells, herpes simplex virus type 1 (HSV-1) infection induces a distinctive cytoplasmic viral assembly compartment (cVAC) in which rearranged host membranes and viral proteins cluster at the perinuclear microtubule-organizing center (MTOC). Although both the trans-Golgi network (TGN) and endosomal compartments have been implicated as membrane sources for cytoplasmic envelopment, how secretory and endosomal pathways are reorganized within the cVAC remains unclear. Here, we treated HSV-1–infected neuronal cells with nocodazole at a post-entry stage to disperse cVAC membranes and identify microtubule-independent membrane associations using immunofluorescence imaging. We found that the Golgi apparatus became fragmented during infection but retained a mini-stack organization, as GM130, GalT, and GRASP65 remained colocalized regardless of nocodazole treatment. We also observed that HSV-1 infection preserves microtubule-independent association between the Golgi and Rab6-positive exocytic vesicles and strongly promotes association with Rab5 early and Rab11 recycling endosomes, resulting in Golgi–endosome super-mini-stacks. Viral capsid, glycoprotein, and tegument proteins localized to these super-mini-stacks in both the presence and absence of microtubules, suggesting that this membrane complex serves as a cytoplasmic envelopment site independent of clustering at the MTOC. Formation of these stable Golgi–endosome associations did not require viral capsid, and neither actin filaments nor acetylated tubulin colocalized with the structures, indicating that cytoskeletal elements do not mediate the association. Together, our data support a model in which the cVAC arises through the reorganization of Golgi and endosomal membranes, which facilitates the recruitment of viral proteins for cytoplasmic assembly and the export of mature virions.

Jahanara Akter Sonia, Xuping Xie, Andrew Severin, Rick Masonbrink, David C. Walker, Peng Liu, Pei-Yong Shi, W. Allen Miller

Accumulation of Zika virus noncoding sfRNA alters translation dynamics in placental trophoblast cells

Molecular, Cellular & Developmental Biology, Iowa State University

Zika virus (ZIKV) is a mosquito-borne flavivirus that can remodel host gene expression both at transcriptional and translational levels. A key determinant of this remodeling is the noncoding

subgenomic flavivirus RNA (sfRNA) that has been implicated in antagonizing innate immune pathways and cellular antiviral responses. How sfRNA influences host translation programs during ZIKV infection remains incompletely defined. To assess host translation regulation, we used ribosome profiling (ribo-seq) in placental trophoblast cells (JEG-3) infected with wild type (WT) ZIKV Cambodian strain (FSS13025) as well as with an sfRNA-deficient ZIKV mutant (MUTII). To understand the function of ZIKV sfRNA in the context of virus infection, mutant virus was generated by introducing three base changes that disrupt the xrRNA2 (exoribonuclease resistant RNA2) structure required for generation of sfRNA2 via host XRN1 exonuclease. From our RNA-seq and ribo-seq analysis, we found wide range of changes in gene expression due to ZIKV infection were at the mRNA level, and these levels differed little in response to WT vs MUTII virus infection. However, at the translational level the sfRNA-deficient mutant led to markedly fewer changes in host gene expression. Although infection by both WT and MUTII increased translation efficiency of genes involved in antiviral responses, presence of sfRNA win the WT infection affected translation of many other genes, including those involved in various stress responses and cell cycle pathways. Together, these data support a model in which ZIKV sfRNA alters host responses at the level of translation. To our knowledge, this is the first genome-wide study that highlights translational control of ZIKV sfRNA as a key layer of ZIKV host interaction in placental cells. Understanding this response of placental cells to ZIKV infection may shed light on how the virus causes fetal abnormalities.

David Price

Structure Prediction of Complexes Controlling Beta- and Gamma-herpesvirus Late Transcription using AlphaFold 3

Biochemistry and Molecular Biology, University of Iowa

All beta- and gamma-herpesviruses utilize a set of six viral proteins to facilitate transcription from specific promoters that become active late in the viral life cycle. These proteins form a complex that interacts with a TA rich sequence upstream of the late transcription start sites and recruits RNA polymerase II (Pol II). The structure of any of the late transcription factors (LTFs) alone or in complexes has not been solved by standard means yet, but a fair amount is known about how the proteins interact and where the complex is positioned over the late promoters. Here, AlphaFold3 was used to predict and analyze the LTF complex using proteins from the beta-herpesviruses, HCMV, MCMV, HHV6, and HHV7 and from the gamma-herpesviruses, EBV and KSHV. The predicted structures had high levels of confidence and were remarkably similar even though there is little sequence conservation in the LTFs across the viruses. The results are consistent with most of the previously determined information concerning the interaction of the factors with each other and with DNA. A conserved threonine phosphorylation in one of the subunits that is critical to the function of the LTFs is predicted to be at the junction of five subunits. AlphaFold 3 predicts seven metal ion binding sites in each of the four beta-herpesviruses and either five or six in gamma-herpesviruses created by conserved residues in three of the subunits. The structures also provide insights into the function of the subunits and which host general transcription factors (GTFs) may or may not be utilized during initiation.

Olufemi Fasina, Lisa Uhl, Aidan Saxton

Parvoviral RNA processing: modulating the ends for productive viral replication, efficient gene therapy and oncolytic applications

Veterinary Pathology, Iowa State University

Posttranscriptional mRNA regulation is a critical cellular homeostatic node that is often hijacked by viruses to support a productive life cycle and has recently been used to develop vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Parvoviruses are linear single-stranded DNA viruses that infect animals and humans and represent an excellent tractable model for understanding virus-host cell interactions. They are currently utilized for gene therapy and oncolytic virotherapy. Parvoviruses have compact genomic organizations with overlapping open reading frames and thus utilize alternative RNA processing strategies, including alternative splicing, alternative polyadenylation, and alternative translation mechanisms, to generate a range of diverse proteins. Alternative RNA processing strategies, including alternative splicing and alternative polyadenylation, are modulated by parvoviruses to generate a diverse proteome, including the capsid protein, which determines the tissue specificity for gene therapy applications. We plan to present a comprehensive review, new insights, and advances on the diverse mRNA processing mechanisms used by members of the Parvoviridae family to expand their protein repertoires and maintain their replicative advantage in infected host cells.

Samir Mehanovic, Cathy Miller

Development of a Novel RNA Interaction Mapping Assay Reveals S1-Dependent Genome Assortment Networks in Mammalian Orthoreovirus

Veterinary Microbiology and Preventive Medicine, Iowa State University

Mammalian orthoreovirus (MRV) is a segmented double-stranded RNA (dsRNA) oncolytic virus. Following infection, MRV undergoes a tightly ordered packaging pathway that includes transcription and translation, segment assortment, single-layer core formation, replication of single-stranded RNA (ssRNA) into dsRNA, and assembly of mature virions. How the assortment step consistently selects one copy of each gene segment for packaging remains unresolved. Increasing evidence suggests that specific viral ssRNA interactions and RNA secondary structures mediate the assortment stage of this pathway. The MRV small (S) 1 segment encodes the attachment protein σ1, which is located on the virion surface. Our previous work identified an essential RNA region within S1 required for S1-dependent genome packaging. However, due to assay limitations, it was unclear which step of the packaging pathway was impacted. To directly interrogate the assortment step, we developed a method termed Enrichment of Crosslinked RNA using Antisense Oligonucleotides (ECRASO). This approach, coupled with RT-qPCR, enables capture of in vivo crosslinked ssRNA complexes interacting with the S1 segment. Targeted capture using an S1 antisense oligonucleotide resulted in >100-fold enrichment of MRV segments S2, S3, L1, and L3 relative to an Act b control. In contrast, enrichment of S4, M2, and Hist1h2ai did not reach statistical significance. Fragmentation and proximity ligation of isolated crosslinked complexes generated chimeric RNAs, which were sequenced to map intra- and intermolecular RNA interactions. The most abundant sequence-resolved interactions were observed between S1 and S3 and between S1 and M1. Additional lower-frequency interactions

involved other genome segments. Collectively, these data define a sequence-level RNA interaction network linking S1 to multiple MRV genome segments. These interactions identify candidate RNA elements that may contribute to S1-dependent genome assortment and packaging.

Alex Kleinpeter, Kaho Matsumoto, John-Mark Miller, Ella Morgan, Sydney Winecke, Sarah Gulick, Emilia Bolinski

Elucidating the molecular mechanisms underlying HIV-1 assembly, maturation, and resistance to capsid inhibitors

Microbiology & Immunology, University of Iowa

The assembly of nascent HIV-1 virions at the plasma membrane of an infected cell is driven by the formation of a spherical protein lattice called the immature Gag lattice. The immature Gag lattice is composed primarily of hexamers of the viral Gag polyprotein precursor, which contains the matrix (MA), capsid (CA), nucleocapsid (NC), and p6 domains. Concomitant with virion budding and release, the viral protease (PR) proteolytically cleaves Gag, dismantling the immature Gag lattice and separating its individual domains to produce the mature forms of MA, CA, and NC. The newly liberated CA protein then assembles into a closed, conical structure called the capsid, which encloses the viral genome and enzymes reverse transcriptase (RT) and integrase (IN). The capsid and its contents are known as the viral core. This morphological conversion from the membrane-associated immature Gag lattice to a stable core is termed maturation and is required for particle infectivity. Utilizing a diverse array of virological, biochemical, and structural approaches, the Kleinpeter lab aims to define the host and viral factors that drive proper HIV-1 assembly and maturation, with a particular emphasis on elucidating the roles played by the host metabolite inositol hexakisphospahate (IP6) in these processes. Our lab also investigates the genetic, molecular, and structural mechanisms underlying HIV-1 resistance to inhibitors targeting the capsid and the impact of acquired resistance on viral fitness. The long-term goal of our research is to facilitate the optimization of current therapies and expose new vulnerabilities as targets for the development of antiretroviral compounds.

Elizabeth Wagstaff, Jessica Phillips, Dawn Dudley, Heather Simmons, and Chen S. Tan

Novel Rhesus Macaque Model for JC Polyomavirus Infection and Role of the Rearranged Noncoding Control Region During Infection

Microbiology, University of Iowa

JC Polyomavirus (JCPyV) infects 40-60% of the adult population as a benign kidney infection. However, in a subset of immunocompromised individuals it causes a rapid and often fatal infection known as Progressive Multifocal Leukoencephalopathy (PML). Currently there are no cures for PML and those that survive the initial infection are left with severe lifelong complications. Much of the process the virus must undergo to travel to the brain and establish infection is currently unknown. Previous research suggests rearrangements in the non-coding control region (NCCR) are necessary for the virus to change cell tropism from the kidney to the central nervous system (CNS). Research in this area has long been held back due to a lack of animal and in-vitro models. Our lab has employed several novel techniques to characterize the

infection of primary human cells with wildtype JCPyV. Using these methods we have discovered that rearranged NCCR significantly increases the virus's ability to infect astrocytes while minimizing its response to inflammatory cytokines such as IFN-γ, TNF-a, and IL-1β. This response appears to be highly cell specific and provides new insight into a potential driving mechanism of rearrangement as well as the interaction between cytokine pathways and the NCCR. Additionally, we have developed a novel non-human primate model in rhesus macaques' infection with JCPyV. Two monkeys were successfully infected intravenously or intrathecally with rearranged wildtype JCPyV. The virus was detected in the CNS and increased in quantities over several months. The development of this models allows us to better understand the role of the NCCR in JCPyV infection and neuropathogenesis and provide foundation to test antivirals and immunotherapies in the future.

Joaquin Caceres, Nayrmaliz Marrero, Eli Blay, Gulnara Brixius, Jennifer Groeltz-Thrush, Rachel Phillips, Rahul K. Nelli, Silvia Carnaccini, Todd M. Bell

Influenza A (H5N1): Molecular Approaches to Understanding Expanded Host Range

Veterinary Microbiology and Preventive Medicine, Iowa State University

Highly pathogenic avian influenza virus (HPAIV) of the H5N1 subtype poses a significant threat to animals and humans due to its inherent pandemic concern. Since 2022, more than 180 million poultry have been affected, including over 30 million in Iowa. In addition, in March 2024, HPAIV H5N1 was first identified in dairy cows, raising new concerns about its negative consequences for livestock and its expanded host range. Moreover, a record number of human cases were reported since 2024 in the U.S., including the first fatal case in the U.S. associated with HPAIV H5N1 in January 2025, reinforcing the pandemic concern associated with HPAIV H5N1. Therefore, there is a significant need to comprehensively assess the biology of HPAIV H5N1 in avian and mammalian models, evaluate the safety of livestock-derived products, and better understand the viral host range. Here, we examine the efficacy of pasteurization-like conditions for inactivating HPAIV H5N1 in milk, showing that the virus is successfully inactivated after treatment. We also show that H5N1 can bind multiple respiratory and non-respiratory tissues across multiple animal species, with host- and strain-specific differences. Taken together, our results provided novel insights into the expanded host range of HPAIV H5N1, together with relevant information regarding food safety.

Trevor Arunsiripate, C. Schmidt; M. Kroeger; L. Keeler; A. Hentz Gris; E. R. Burrough; P. E. Piñeyro

Porcine hemagglutinating encephalomyelitis virus (PHEV) entry receptor gene profiling and viral strand-specific detection for viral replication using suspension fluorescent in situ hybridization

Veterinary Pathology, Iowa State University

Porcine hemagglutinating encephalomyelitis virus (PHEV) is a betacoronavirus with tropism for the nervous and respiratory systems, producing vomiting and wasting disease and necrotizing bronchitis in commercial pigs. Neural entry has been associated with NCAM-1 (CD56), and recent evidence suggests DPEP-1 and DPP-4 may also interact with the spike receptor-binding

domain. However, the receptor(s) mediating respiratory infection remain undefined. PHEV replication follows the conserved Nidovirales strategy, involving a positive-sense genomic RNA, a negative-sense replicative intermediate, and subgenomic RNAs for structural protein translation. Detection of the negative-sense strand provides strong evidence of active replication. Here, we developed a suspension fluorescent in situ hybridization (SUS-FISH) assay to detect both positive and negative strands of PHEV RNA in cell culture and clinical specimens. We also evaluated expression of NCAM-1, DPEP-1, and DPP-4 in permissive cell lines (SPK, PK-15, HRT-18G) and not previously reported as permissive for PHEV, MARC-145 infected with 1.75 MOI of a field PHEV isolate. A consensus spike gene sequence derived from five respiratory isolates was used for probe design with Oligominer. Probes (36-41 nt; 20–80% GC content) were screened to eliminate cross-reactivity with other porcine viruses and the swine transcriptome. Strand-specific probe sets incorporated a universal FLAP sequence for fluorescent detection using FAM or Cy3-labeled secondary probes. SUS-FISH identified strong perinuclear cytoplasmic fluorescence in experimentally infected cell lines and in PCR-positive lung tissues, with higher signal intensity for the positive strand compared to controls. Similar localization was observed in respiratory epithelium and interstitial macrophages. Receptor gene expression differed significantly among cell lines, with SPK showing the highest and PK-15 the lowest expression levels. Notably, we demonstrate for the first time that MARC-145 cells support PHEV infection and replication. These findings establish SUS-FISH as a reliable method to confirm active PHEV replication and a platform adaptable to other animal viral pathogens.

Silvia Carnaccini, Lucas Goble, Han Yang Wang, Caitlyn Middleton, Dikshya Regmi, Flavio Cargnin Faccin, Lisa Stabler, Garrett Cribbs, Samara Wartella, L. Claire Gay, Juliana Calil Brondani, Teresa D Mejias, Daniel Perez, Laura-Isobel McCall

Comparative Pathogenesis of a Surrogate LPAIV H5 Clade 2.3.4.4b in Ducks and Chickens

Veterinary Diagnostic and Production Animal Medicine, Iowa State University

Highly Pathogenic Avian Influenza Virus (HPAIV) continues to circulate globally in wild waterfowl, leading to repeated outbreaks of severe disease and high mortality in poultry. To study virus–host interactions, we generated a low-pathogenic surrogate derived from a contemporary HPAI H5 clade 2.3.4.4b strain (A/turkey/Poland/H1289/2021) that was modified to include a stable monobasic cleavage site from an H6 subtype. Specific-pathogen-free 3-week-old Leghorn chickens and Mallard ducks were inoculated with 10^4 or 10^6 $TCID_{50}$ per bird and monitored daily for clinical signs and mortality. Viral shedding, tissue distribution, and pathology were evaluated and combined with spatial metabolomic analyses. Chickens required higher infectious doses compared to ducks and generally exhibited self-limiting, localized infection restricted to the upper respiratory tract. By contrast, ducks showed higher and more prolonged viral shedding, stronger serological responses, and systemic virus dissemination, despite the absence of clinical disease. These results indicate increased viral tropism for duck tissues and more effective evasion of duck innate immune responses. Metabolic disturbances were identified in both infected and distal tissues, demonstrating systemic effects arising from localized infection. Ducks displayed fewer and less pronounced metabolic changes than chickens, even with higher viral replication, suggesting greater tissue permissiveness in the natural host. In conclusion, this surrogate model reflects host-specific differences in viral

replication dynamics, tissue susceptibility, and metabolic responses between the natural host (ducks) and the accidental host (chickens). Although future pairwise comparisons with the wild-type phenotype are needed to identify differences, this model appears to offer a safe alternative for studying HPAIV pathogenesis in ways that are not feasible with fully pathogenic viruses.

Lu Yen, Juan Carlos Mora-Díaz, Ning-Chieh Twu, Bala Mounika Reddi, Apoorva Saxena, Jesús Horacio Lara Puente, Daniel Gustavo Rocher Maliachi, Ángel David Cota Valdez, Mario Palma Zepeda, Rahul Kumar Nelli, Luis Gabriel Giménez-Lirola

Feasibility of Porcine Enteroids as an Alternative to Cell Culture and Pig Bioassay for Assessing Porcine Deltacoronavirus Viability US VIABILITY

Veterinary Diagnostic and Production Animal Medicine, Iowa State University

This study evaluated the feasibility of porcine enteroids cultured on transwell inserts (PETCs) as a diagnostic platform for detecting viable porcine deltacoronavirus (PDCoV) by (1) determining the minimum infectious dose of PDCoV required to infect PETCs and swine testicular (ST) cells, and (2) assessing the infectivity of field samples using ex vivo (PETCs), in vitro (ST cells), and in vivo (neonatal piglet) models.

Firstly, PDCoV (Michigan/8977/2014; 3.16×105 TCID50/mL) was two-fold serially diluted (21 to 214) and inoculated into PETCs derived from duodenum, jejunum, and ileum of 7-day-old piglets (n = 3), as well as into ST cells. Infection was assessed by CPE, IFA targeting nucleocapsid (N) protein, and RT-qPCR targeting the N-gene. Secondly, field samples (feeders, rubber teats, pen floor, and sewage) collected from a PDCoV-affected farm post-disinfection were tested (Cq 30-34). These PCR-positive samples were inoculated into jejunum-derived PETCs, ST cells, and piglets. ST cells and PETCs were assessed using CPE, IFA, and RT-qPCR, while piglets were monitored for clinical signs, histopathology, and viral shedding.

In PETCs, CPE and IFA were minimal or absent, whereas RT-qPCR detected PDCoV RNA at dilutions between 21 to 210 (Cq 24-35). Jejunum-derived PETCs were most susceptible to PDCoV, justifying their use for field sample testing. On the other hand, ST cells displayed more pronounced CPE and IFA signals across all dilutions. However, no evidence of infection was detected in PETCs, ST cells, or piglets following inoculation with field samples.

While culture optimization is ongoing to enhance sensitivity and diagnostic performance, PETCs represent a promising ex vivo platform that aligns with the 3R animal welfare principles, with greater complexity than in vitro culture and closer relevance to in vivo conditions. This system can be extended to other enteric viruses of clinical importance to the swine industry.

Pearl Chan, Ajit Vikram, Stanley Perlman

Vascular Dysfunction in Mouse Aorta Following SARS-CoV-2 Infection

Microbiology & Immunology, University of Iowa

Endothelial injury and cardiovascular impairment are observed in COVID-19 patients, regardless of disease severity. Furthermore, increasing evidence suggests that the risks associated with COVID-19-related vascular dysfunction persist not only during the acute phase but also after the respiratory infection has cleared. The exact underlying causes are still unclear, thus more investigations are needed to clarify the mechanisms involved. This study primarily aimed to

understand COVID-19-related vascular impairment using a mouse model that could mimic SARS-CoV-2 infection in humans. Middle-aged (four to ten months-old) C57BL/6 mice were intranasally infected with a sublethal dose of mouse-adapted SARS-CoV-2. To understand the transcriptional phenotype, mouse aorta tissues were harvested at 4 days post infection (dpi) and processed for RNA-seq. Pathway enrichment analysis revealed significant upregulation of type I and II interferon signalling and downregulation of epithelial-mesenchymal transition genes. These results indicate vascular inflammation and mesenchymal maintenance. In addition, we identified the upregulation of numerous endothelial activation genes, including Vcam1, Icam1, Selp, Edn1 and Esm1. These findings suggested endothelial inflammation in the mouse aorta without being directly infected. Functionally, through the performance of vascular reactivity assays, mouse aortas harvested at 4 and 30 dpi showed inadequate relaxation, in comparison to samples from age-matched uninfected control mice. Supportingly, western blot revealed suppressed phosphorylation of endothelial nitric oxide synthase (eNOS, Ser1177), that suggested reduced nitric oxide production, an important vasodilator produced by endothelial cells that regulates arterial stiffness. Notably, no significant differences were observed in mice infected with PR8, a mouse-adapted H1N1 influenza, that suffered from a similar degree of respiratory illness. Together, these results have highlighted the potential development of vascular impairment induced by COVID-19 and the need for effective therapeutic approaches.

Carter Casey, Randall Vos, Adriana Larrea Sarmiento, Alejandro Olmedo Velarde

Virus Prevalence in Iowa Soybeans and Grapes

Plant Pathology, Entomology & Microbiology, Iowa State University

Soybeans and grapes are distinct crops, yet both contribute meaningfully to Iowa’s agricultural economy. Iowa is the second-largest soybean-producing state in the United States, and although viral diseases are not currently considered a major constraint, increasing virus pressure poses a potential risk to statewide production. Therefore, establishing baseline data on virus prevalence is therefore critical for proactive management. On the other hand, grapes, while a smaller specialty crop, play an important role in Iowa’s agritourism through a growing wine industry. Midwestern vineyards rely primarily on cold-hardy hybrids derived from European cultivars and native grape species, which are well adapted to regional climatic conditions. However, the virology of these hybrids remains poorly characterized, particularly in Iowa. This study presents virus indexing results from soybean (2024–2025) and cold-hardy grape surveys conducted across Iowa. By identifying the viruses currently present in these crops, this work establishes a baseline of virus prevalence and diversity. These data provide critical information for growers, researchers, and extension personnel, supporting informed decision-making and future disease management strategies.

Veeraya Bamrung, Phillip C. Gauger, Michael C. Rahe, Ganwu Li, Rachel J. Derscheid, Michael A. Zeller

Divergent porcine astrovirus 4: Distinct respiratory and gastrointestinal clades with genetic differences

Bioinformatics and Computational Biology, Iowa State University

In 2016, porcine astrovirus 4 (PoAstV4), historically associated with gastrointestinal infections, was first reported in piglets with acute respiratory disease in Oklahoma. Cases of PoAstV4 with consistent respiratory epitheliotropic viral lesions were later confirmed in 2023. Analyses of 38 whole genomes indicate that respiratory-associated PoAstV4 strains are genetically distinct from those found associated with gastrointestinal disease, with a divergence of approximately 22.5-26%. A total of 36 distinct nucleotide mutations, differing between the two clades, were identified: 17 in ORF1ab and 19 in ORF2. A consistent pattern of 11–12 amino acid differences in the C-acidic region was observed. Bayesian phylogenetic methods suggested that the most recent common ancestor for the respiratory clade was identified in 2003 (90% HPD: 1994.5-2010). Further experimental validation is needed to determine whether the identified genetic differences affect respiratory tropism. However, this study provides a foundation for investigating potential tissue-specific adaptations and may enhance diagnostic accuracy.

Hillel Haim, Rohith Rao Vujjini, Cassian M Birler, Madeline M Broghammer, Aaron N Gillman, Samuel A McCarthy-Potter

Modeling HIV-1 Env Evolution Across Scales to Predict Antibody Efficacy and Escape

Microbiology & Immunology, The University of Iowa

The “kick-and-kill” approach to HIV-1 cure seeks to eliminate the latent reservoir by inducing viral reactivation and then clearing infected cells. Clearance is mediated by broadly neutralizing antibodies (BNAbs), which bind the HIV-1 envelope glycoproteins (Envs) on infected cells and activate antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADCP). However, the diversity of BNAb epitopes across and within hosts poses a major barrier to this strategy. Selection of optimal antibody combinations for specific populations and patients requires a deep understanding of HIV-1 evolutionary patterns within and between hosts. To address this challenge, our lab develops computational learning frameworks to model and forecast evolutionary trajectories of HIV-1 Env across biological scales.

Using extensive sequence data from diverse HIV-1 clades, we identify patterns of genomic divergence and convergence that impact BNAb epitopes. The selection pressures that drive these changes are characterized using molecular tools. Together, our analyses reveal a central role for the host humoral immune response in shaping Env antigenicity among circulating variants. This work is complemented by machine learning models we developed to estimate the resistance profiles of HIV-1 strains to therapeutics based on amino acid sequence.

By integrating population-level evolutionary forecasting with genotype-phenotype prediction, this framework provides a strategy for rational BNAb selection in cure-directed interventions and identifies the antibodies with the highest likelihood of viral suppression in each patient.

Vaishnavey Srinivasan Raghunath, Supantha Dey, Sakib Ferdous, Riza Danurdoro, Dr. Michael A. Zeller, Dr. Ratul Chowdhury

Structure aware evolutionary fitness learning provides robust surveillance screening for PRRSV-ORF5 variants

Chemical and Biological Engineering, Iowa State University

Porcine Reproductive and Respiratory Virus (PRRSV) poses a formidable challenge to animal health and antibody-based therapeutic interventions, because of its ability to exploit alternative infection pathways, and its rapid evolution, which enables immune escape through evasion of antibody recognition. Antibody escape is primarily conferred by means of subtle mutations that alter the chemical charge distribution and shape complementarity of the variant towards binding to porcine receptors. Currently, no predictive tools exist that to mechanistically quantify the mutational effects on the fitness and escape propensity for evolving viral proteins beyond human-tropic viruses. Following our extensive structural characterization of the PRRSV epitope atlas (Dey et al., CSBJ, 2024, https://doi.org/10.1016/j.csbj.2024.08.029), we introduce Esca, a toolchain framework that scores for fitness and antibody escape propensities on the complete mutational landscape of any viral protein. We demonstrate Esca on the well-studied, hyper variant GP5 glycoprotein encoded by *ORF5* gene of PRRSV-2. (EscaPRRS-ORF5), by training a Bayesian Variational Autoencoder on ESM-2 embeddings of 32,146 PRRSV-2 GP5 sequences recorded from 2015 to 2021 across 140 sub-lineages. Conventional viral fitness-scoring tools heavily rely on sequence-only information, and necessitate explicit integration of up-to-date sequences to capture mutational patterns, and hence fail to capture the associated host biology. High escape propensity regions identified by EscaPRRS-ORF5 recover 85.7% of the key interfacial hotspot residues for porcine receptor binding, previously inferred from GP5 docked complexes with seven porcine receptors (CD151, CD209, CD163, Heparansulfate, Sialoadhesin, Vimentin and MYH-9), without being explicitly trained on host receptors or antibody information. Esca follows a mutation sensitive scoring scheme that extends beyond raw mutation counts (e.g., edit distances), while preserving seasonal and lineage specific patterns inherent to the sequencing data, providing a structurally grounded, evolution- aware framework for rapid surveillance of whole genome PRRSV strains.

Eduarda Braga, Michael A. Zeller, Fernando Leite and Pablo E. Piñeyro

Mapping the genotypic landscape of PCV2 in the U.S. patterns

Veterinary Diagnostic and Production Animal Medicine, Iowa State University

Porcine circovirus 2 (PCV2) has a high evolutionary hate, resulting in multiple genotypes. The capsid protein (Cap) is the main immunogenic structure and defines viral genotype. This study provides a comprehensive genetic, phylogenetic, selection pressure, and hydropathy analysis of PCV2 Cap sequences circulating in the United States (U.S.). After quality filtering, 3,133 sequences from the Iowa State University Veterinary Diagnostic Laboratory and GenBank were analyzed. Sequence alignment was performed with MAFFT and genotyping inferred using RAxML. Additional phylogenetic reconstructions used BEAST, and selection pressure was assessed using FUBAR, FEL, SLAC, and MEME on the Datamonkey server. Hydropathy profiles were evaluated using the Kyte–Doolittle method. Four genotypes were identified: PCV2d (67.9%, n=2,127), PCV2a (20.2%, n=634), PCV2b (8.5%, n=266), and PCV2e (3.4%, n=106). Since

2012, all four genotypes have remained present in the population. Temporally, from 2010, initially, PCV2b was predominant, with PCV2a also detected. Over time, there was a clear shift to genotype PCV2d, which became dominant in 2014. Intra-clade distance analysis indicated no major divergence among genotypes. Positive selection analysis, considering sites detected by at least three of four methods, identified codon 134 in PCV2a; codons 30, 131, and 134 in PCV2b; and codons 133, 134, and 169 in PCV2d. All positively selected sites are located on the external surface of the capsid. Notably, position 134, found across all genotypes, maps to immunodominant regions associated with reduced neutralization, implying potential impacts on vaccine efficacy. Structural and hydropathy analyses revealed amino acid differences, including polarity changes between genotypes, which may affect protein folding, stability, and immune recognition. In conclusion, this study highlights extensive genetic diversity of PCV2 in the U.S. Genetic distance, selection, and hydropathy patterns suggest potential antigenic variation with implications for disease outcomes. Continuous molecular surveillance is crucial to monitor emerging variants and inform vaccine strategies.

Jeff Meier, Ming Li, Qiaolin Hu, Arya Zandvakili, and David Price

A multimolecular pUL84-IE2-TBP-nucleosome complex at the human cytomegalovirus major immediate-early promoter blocks antisense transcription

Internal Medicine/Infectious Diseases, University of Iowa

The human cytomegalovirus (HCMV) major immediate-early promoter (MIEP) and its gene products are central to activation and maintenance of viral gene expression and replication. From the opposite DNA strand, UL124 transcription terminates immediately downstream of the MIEP. Here, we identify a previously unrecognized role for the viral protein pUL84 as a core component of a prominent multimolecular complex assembled at the MIEP. Using double-stranded DNA fragmentation factor-ChIP sequencing, we show that pUL84 co-occupies the MIEP with viral immediate-early 2 protein (IE2), host TATA-binding protein (TBP), and a precisely positioned H3K4me3-marked nucleosome. This complex assembles on viral genomes as early as 24 h after infection, is more prevalent in late-stage infection, and is conserved across fibroblasts and neural lineage cells. A two-base substitution in the TATA box nearly eliminates the complex, indicating dependence on TBP-TATA interactions. This substitution, as measured by precision run-on sequencing, causes defective termination of antisense UL124 transcription with readthrough across the MIEP, likely due to disruption of the complex. In contrast, it does not reduce MIEP-driven IE1/IE2 RNA and protein expression under high-multiplicity infection. These findings support a model in which the MIEP-associated complex functions primarily as a transcriptional boundary rather than a global repressor of MIEP output during viral replication. We propose two populations of viral genomes: one bearing the pUL84-IE2-TBP-nucleosome complex, in which UL124 antisense transcription occurs and terminates at the complex while the MIEP is inactive, and a second lacking the complex, in which UL124 antisense transcription is absent and the MIEP is active.

Caroline Plescia, José Alberto Aguilar-Briseño, Wendy Maury

Interferon beta sensitivity differentially restricts entry of diverse filovirus glycoproteins in mammalian hosts

Microbiology, University of Iowa

In 2025, there were multiple outbreaks of Ebola virus (EBOV) and the related filovirus Marburg virus (MARV). While there is an FDA-approved vaccine for EBOV, it offers no cross-protection against MARV. MARV outbreak frequency is rapidly increasing with >30% or outbreaks occurring since 2021; still, there remains no FDA-approved vaccines or therapeutics for MARV. Macrophages are consequential targets of filovirus infection and are responsive to type I interferons (IFN) such as IFN-β. Despite encoding IFN antagonists, EBOV infection is restricted in IFN-β-pretreated macrophages. In primary macrophages and immortalized murine Kupffer cells (ImKCs), substitution of the EBOV-glycoprotein (GP) with MARV GP within infectious rVSV and EBOVΔVP30 significantly diminished virus sensitivity to IFN-β. Viral entry studies in pretreated ImKCs using virus-like particles (VLPs) demonstrated that IFN-β potently inhibited EBOV-VLP fusion in a dose-dependent manner while MARV-VLP fusion was not inhibited. Entry studies using VLPs with GPs from diverse filoviruses revealed two distinct groups: (1) EBOV-like IFN-β sensitive GPs (EBOV, Lloviu virus), and (2) MARV-like IFN-β insensitive GPs (MARV, Měnglà and Déhóng viruses). Sequence-harmony analysis of EBOV-like and MARV-like GPs predicted residues involved in IFN-β sensitivity and they map to regions of EBOV GP which support membrane coordination for viral fusion. Biochemical studies provided evidence that IFN-β pretreatment restricts entry of EBOV GP at a post-cathepsin-proteolysis, pre-fusion step. EBOV GP-fusion was rescued by membrane-relaxing small molecules in IFN-β pretreated macrophages suggesting an IFN-β stimulated gene (ISG), such as IFITMs, mediate EBOV restriction by a membrane stiffening mechanism. Consistent with this, RNA-seq studies identified IFITM3, but not IFITM1 or 2, as upregulated by IFN-β in ImKCs (log2FC>1). These data highlight two divergent fusion requirement profiles of Filoviridae and identify a novel mechanism of immune evasion in mammalian hosts by MARV and MARV-like filoviruses.

Fatemeh Hassanpour, Raymond Larsen, Santanu Raychaudhuri, Reza Montazami, Michael W. Cho

Development of a Vaginal Film for Sustained, Controlled Delivery of Prophylactic Antibodies Against Sexually Transmitted Infections

Chemical and Biological Engineering, Iowa State University

Many pathogens are sexually transmitted. While protective antibodies have been identified against many of these pathogens, their therapeutic potential has not been fully exploited due to the lack of suitable delivery methods to the site of transmission. Immunotherapeutic strategies for preventing sexually transmitted infections (STIs) would greatly benefit from user-administered vaginal delivery platforms that remain structurally stable and continuously release protective levels of antibodies over extended periods. Gelatin is an attractive material for mucosal delivery due to its biocompatibility and protein miscibility. However, its thermoreversible gelation and rapid dissolution near body temperature limit prolonged use without chemical stabilization. Here, a vaginal film based on a multilayer, gelatin-based

interpenetrating polymer network (IPN) is developed using glyceraldehyde as a mild, biocompatible crosslinker. The platform features an asymmetric trilayer architecture consisting of two crosslinked outer layers surrounding a non-crosslinked, immunoglobulin G (IgG)–loaded reservoir, enabling independent regulation of hydration and diffusion. Polymer concentration, reflected by film thickness, governed hydration kinetics, microstructural persistence, and mechanical integrity, resulting in controlled swelling and enhanced structural retention under simulated vaginal fluid conditions. This architecture supported diffusion-mediated IgG release over extended durations while preserving multilayer integrity, capable of providing month-long protection between female menstruation. Together, these results demonstrate that chemically stabilized gelatin IPN films decouple rapid mucosal hydration from long-term structural stability, enabling a tunable platform for sustained vaginal delivery of antibody-based immunotherapeutics.

Senthamizharasi Manivasagam, Julianna Han, Boopathi Sowndhirarajan, Boyang Cheng, Jasmine T. Perez, Priya Issuree, Balaji Manicassamy

Host transcriptional repressor Capicua regulates virus-driven cytokine storm during respiratory viral infection

Microbiology & Immunology, University of Iowa

Severe respiratory viral infections often trigger excessive inflammatory responses that drive tissue damage and disease severity. Many highly pathogenic respiratory viruses induce markedly elevated levels of pro-inflammatory cytokines, a response commonly referred to as a cytokine storm, which is closely linked to severe disease and poor clinical outcomes. Although immunomodulatory therapies are frequently used to control hyperinflammation, approaches that focus on blocking individual cytokines have shown limited clinical benefits, emphasizing the need to better understand how inflammatory responses are broadly regulated during viral infection. In previous work from our laboratory, we used a genome-wide CRISPR/Cas9 screen to identify Capicua (CIC), a DNA-binding transcriptional repressor, as a key regulator of cell-intrinsic inflammatory responses during respiratory viral infection. Under homeostatic conditions, CIC binds to an 8-bp CIC-binding site (CBS) near inflammation-associated gene loci, preventing aberrant gene expression. Our studies show that CIC broadly represses inflammatory genes, including interferons (IFNs) and interferon-stimulated genes (ISGs) such as IFN-β, MX1, and ISG15, as well as pro-inflammatory cytokines and chemokines including IL-6, TNF-α, IL-1β, and CXCL1. During respiratory viral infection, CIC-mediated repression is rapidly removed through activation of the MAPK pathway and subsequent proteasome-dependent degradation of the CIC repressor complex. In addition to respiratory viral infection, pro-inflammatory cytokines such as type I IFN, IL-6, TNF-α, and IL-1β promote CIC degradation, creating a feed-forward loop that amplifies inflammation. Pharmacological inhibition of the MAPK pathway stabilizes the CIC repressor complex and reduces expression of ISGs and pro-inflammatory cytokines during H5N1 infection in mice. Taken together, our work identifies CIC as a critical regulator of inflammatory gene expression during severe respiratory viral infection and suggests that blockade of CIC degradation may be exploited as a therapeutic strategy to prevent virus-induced cytokine storm.

Pak Hin Hinson Cheung, Pearl Chan, Hua Yang, Krisztina Ambrus, Shravya Honne, Baek Kim, Stanley Perlman, Li Wu

Loss of SAMHD1 restricts SARS-CoV-2 infection by downregulating HNF1-mediated ACE2 expression in lung epithelial cells

Microbiology & Immunology, University of Iowa

Sterile-alpha motif histidine-aspartate domain-containing protein 1 (SAMHD1) is a deoxynucleotide triphosphohydrolase (dNTPase) that regulates dNTP pools, restricts virus replication, and suppresses innate immune responses. Not all viruses are restricted by SAMHD1 in cells. Some pathogenic viruses exploit SAMHD1 to facilitate their replication. SARS-CoV-2 is the causative agent to COVID-19. Loss of SAMHD1 suppresses SARS-CoV-2 infection in HEK293T cells, macrophage-like THP-1 cells and human macrophages by alleviating the restriction on type I interferon (IFN-I) response. These cell types, however, lack the viral receptor angiotensin-converting enzyme 2 (ACE2) necessary for productive SARS-CoV-2 replication. To further investigate this key question in a physiologically relevant cell model, we employed a lung epithelial cell line Calu-3, which endogenously expresses ACE2. Through CRISPR/Cas9 editing, we established SAMHD1 knockout (KO) Calu-3 cells. We found that SARS-CoV-2 replication was hindered in the KO cells independent of IFN-I antiviral responses. Instead, SARS-CoV-2 spike protein-mediated viral entry was suppressed in the KO cells. Transcriptomic and molecular assays suggested ACE2 mRNA and protein expression was downregulated in the KO cells compared to control cells, which suppressed SARS-CoV-2 infection. Mechanistically, we found that hepatocyte nuclear factor 1 (HNF1)-mediated ACE2 expression was repressed in the KO cells. SAMHD1 knock-in and deoxynucleoside supplementation experiments indicated that SAMHD1 expression, proinflammatory cytokine levels, and dNTP pool balance collectively regulated HNF1-mediated ACE2 expression in Calu-3 cells. Our findings identify a novel IFN-I independent proviral mechanism of SAMHD1 to SARS-CoV-2 infection via HNF1-mediated ACE2 expression in lung epithelial cells.

Amanda Dudek, William N. Feist, Sofia E Luna, Kaya Ben-Efraim, Elena J Sasu, Alex TH Cocker, Peter Parham, Mathew H Porteus

Harnessing cell-type specific gene editing for a combinatorial HAART cell therapy

Microbiology & Immunology, University of Iowa

Hematopoietic stem cell transplant therapy for HIV-1 can be curative, but any unmodified cells remain susceptible to infection, and highlight the difficulty of translating this option to the majority of people living with HIV. We have designed a multi-target gene editing strategy to combat HIV-1 in a cell-type or lineage-specific manner to harness the cells' natural funcation to combat the virus. We recently demonstrated that combinatorial CCR5 locus knockin of two restrictive proteins (C46 V2o and hRhTRIM5 alpha) in primary CD4+ T cells isolated from healthy donor PBMCs blocks replication of CCR5-tropic virus, and decreases CXCR4-tropic replication over 2,000-fold. In CD19+ B cells isolated from healthy human donors, knockin of protein-linker engineered broadly-neutralizing antibodies (bNAbs) expressed from a B cell promoter allows antibody secretion which effectively neutralizes HIV-1 in a TZM-bl assay, with the same tropism and IC50 as unmodified bNAb. Supernatant from combinatorial knockin of multiple antibodies

provides protection against all targeted Envs. We additionally repurposed RBCs as viral traps by knockin of a truncated CD4 receptor at the HBA locus for RBC-specific expression. Edited HSPCs differentiated into RBCs ex vivo neutralize HIV-1 in a modified TZM-bl assay at RBC concentrations roughly 100-fold lower than they exist in the blood. Additionally, we have deleted the HLA-E locus from T cells, allowing improved recognition of infection by Natural Killer cells. HLA-E knockout in CD4+ T cells allows up to an 83-fold drop in supernatant virus concentration when donor-matched NK cells are co-cultured with infected HLA-E knockout CD4+ T cells, compared to a maximum 6-fold drop when T cells are edited at a control locus. We have therefore edited HSPCs and their terminally differentiated progeny to provide 5 or more viral targets of intervention, using only 3 genetic loci, while harnessing the cell's natural functions to help combat infection.

Poster Presentations

1.) Kota Nakasato, Onias Castellanos-Collazo, Alejandro Olmedo-Velarde

An infectious clone and Nextstrain workflow for Soybean dwarf virus enable genomic surveillance and functional follow-up in Iowa

Plant Pathology, Entomology and Microbiology, Iowa State University

Soybean dwarf virus (SbDV; Luteovirus glycinis) is an aphid-transmitted virus of legumes and can cause severe yield losses in soybean. It is a recurring problem in regions such as Japan and Australia and has also been detected in Iowa and other Midwestern states. Although reported incidence in the Midwest is currently low, proactive development of genomic surveillance and experimental systems is critical to rapidly detect emergent variants and respond to potential future outbreaks. To enable rapid detection, tracking, and functional characterization of SbDV in Iowa, we established complementary molecular and bioinformatics foundations for real-time surveillance and hypothesis generation. We report (1) development of an infectious clone of an Iowa SbDV isolate (SbDV-IA-2017) and (2) a reproducible Nextstrain-based phylodynamic workflow built from publicly available SbDV genome sequences and associated metadata.
A pJL-SbDV-IA-2017 infectious clone delivered to plants through agroinfiltration showed high infectivity in Nicotiana benthamiana (100%), supporting its use as a tractable system for downstream biology assays in a model host. The Nextstrain workflow provides an interactive, standardized framework for integrating new sequences, visualizing phylogenetic relationships, and monitoring genetic change over time in a form that can be readily updated as additional Iowa genomes become available. Together, these resources establish a scalable platform for genomic surveillance and experimental follow-up, enabling timely mutation tracking and biological testing to support outbreak readiness. Future work will expand genomic surveillance in Iowa to capture additional SbDV diversity. We will then identify and test candidate mutations linked to vector specificity and symptom development by combining comparative genomics, protein structure-guided prioritization, and infectious clone-based functional assays.

2.) Stephanie Clark, Justin W. Kaufman, Kalpana Yadav, Roberto Cattaneo, Brajesh K. Singh, Patrick L. Sinn

The Measles Virus Membrane Fusion Apparatus Precedes the Replication Complex to Promote Rapid Cell-to-Cell Spread in Airway Epithelium

Microbiology, University of Iowa

Measles virus (MeV) is the most contagious human respiratory pathogen. To understand how it rapidly spreads in the airways, we infected well-differentiated primary cultures of human airway epithelial cells (HAE). We discovered that the MeV attachment protein (hemagglutinin, H), the fusion (F) protein, and the assembly organizer protein (matrix, M) reach newly infected cells before the replicative complex proteins. To characterize the mechanisms of MeV spread, we generated a replication-deficient adenoviral vector expressing the MeV M, F and H proteins in a doxycycline-inducible manner (Ad-MFH). This virus, upon induction of MeV protein expression, formed infectious centers like those formed during MeV infection. To further dissect this process, we generated Ad vectors expressing individual MeV proteins under the

control of an inducible promoter. These vectors were termed Ad-M, Ad-F, and Ad-H. Transduction of HAE with single, double, and the triple Ad vector combination revealed that Ad-F and Ad-H are necessary and sufficient to form MeV-like infectious centers. Since in the airway MeV spread depends on H protein binding to nectin-4, an adherens junction protein that serves as the MeV epithelial receptor, we introduced the point mutation HY543A, previously shown to interfere with nectin-4 binding, in the Ad-H vector. Co-transduction of Ad-F and Ad-HY543A did not result in MeV-like infectious center formation. Finally, since the MeV-related paramyxovirus Nipah (NiV) enters cells through another cell adhesion protein expressed in HAE, ephrin-B2, we assessed whether the NiV attachment and fusion proteins (G and F, respectively) are operative in the context of Ad-based transduction. Co-transduction of HAE with Ad-NiV-G and Ad-NiV-F did not result in infectious center formation, suggesting that decoupling of the membrane fusion apparatus function from replication is a unique feature of the MeV envelope glycoproteins.

3.) Paige T Richards, Maryam Fakhimi, Kelly Messingham, Wendy Maury

Marburg Virus Glycoprotein-Mediated Infection and Trafficking in Skin

Microbiology & Immunology, University of Iowa

Nearly one-third of all recorded Marburg virus (MARV) outbreaks have occurred in the last five years and the World Health Organization classifies MARV and related filoviruses, such as Ebola virus (EBOV), as pathogens with pandemic potential. Despite this threat, no licensed vaccine or therapeutics against MARV are available, underscoring the need to better understand this virus' cellular tropism and the transmission pathways that drive disease progression. Clinical reports indicate that MARV infection is more frequently associated with cutaneous rash than EBOV, and MARV antigens and viral particles have been detected in bat skin and in skin from a fatal human case, suggesting that skin—the largest organ in the human body—supports MARV infection. Here, using human skin explants and mouse models, we define MARV glycoprotein (GP)-mediated trafficking into and through the skin. Within human skin explants maintained at the air/liquid interface in a transwell culture, MARV GP mediates productive viral infection. We provide evidence that multiple morphologically distinct cell populations support MARV GP-dependent infection of these explants. Over the course of infection, increasing amounts of infectious virus were released from the explants into the basal media in a time-dependent manner. Like EBOV infections, infectious virus was detected on the apical epidermal surface of the explants, indicating that virus traffics to the surface of the skin and provides a potential mechanism of person-to-person transmission. In mouse studies, infection of skin distal to the site of injection was evident at late times following intraperitoneal administration of virus bearing MARV GP; however, unlike EBOV, infection was uniformly distributed across the skin, suggesting the MARV GP-mediated mechanism of viral spread within skin is distinct from that observed with EBOV GP. Collectively, these data indicate that skin infection represents a critical and potentially conserved component of filovirus pathogenesis.

4.) Siyu Huang, Li Wu

HIV-1 infection enhances m6A modification of host mRNA and induces METTL3 phosphorylation in CD4+ T cells

Microbiology & Immunology, University of Iowa

N6-methyladenosine (m6A) is the most prevalent cellular mRNA modification and plays an important role in RNA stability, localization, and gene expression. m6A modification is dynamically regulated by cellular methyltransferases and demethyltransferases (termed m6A writers and erasers, respectively), with the methyltransferase-like 3 and 14 (METTL3/METTL14) complex serving as the core m6A writer in cells. Several studies reported that HIV-1 infection upregulates m6A of cellular RNA in CD4+ T cells. Our lab also reported that HIV-1-induced m6A upregulation occurs without changes in the expression levels of m6A writers or erasers in CD4+ T cell lines and primary CD4+ T cells. However, the functional significance and the mechanism by which HIV-1 upregulates cellular mRNA m6A levels remains unclear. Here we show that HIV-1 infection of CD4+ T cells promotes METTL3/METTL14 interaction and regulates phosphorylation of METTL3 at multiple sites. Using co-immunoprecipitation, we found that HIV-1 infection of CD4+ T cells promoted METTL3/METTL14 interaction compared to mock controls. Our mass spectrometry analysis revealed that the phosphorylation level at S358 site of METTL3 was increased during HIV-1 infection of Jurkat CD4+ T cells. Our ongoing studies focus on defining the mechanisms by which HIV-1-induced METTL3 phosphorylation enhances the assembly of the METTL3/METTL14 complex and drives m6A upregulation of cellular RNA in CD4+ T cells. These studies will clarify how HIV-1 infection reshapes host RNA modification by regulating the m6A writer complex in CD4+ T cells.

5.) Ke Hu, Michael Zeller, Gustavo Silva, Daniel Linhares, Tavis Anderson, Amy Baker, Phillip Gauger

Longitudinal surveillance of influenza A virus at the human-swine interface in Midwest swine farms in the United States

Veterinary Diagnostic and Production Animal Medicine, Iowa State University

A three-year active surveillance study (2022-2025) was conducted to characterize the diversity and transmission of influenza A virus (IAV) at the human-swine interface on 30 Midwest U.S. swine farms. The project monitored 189 farm employees through monthly self-collected nasal swabs, as well as pigs within the corresponding production systems. The swine farms included 15 breeding herds that were sampled using udder and nasal wipes, and 15 downstream nurseries that were sampled with nasal wipes and oral fluids. The samples were tested for IAV RNA by PCR-based assays using the VetMAX™-Gold SIV and Subtyping Detection Kit (Thermofisher Scientific). Among 1,484 human samples, we detected 7 (0.47%) IAV-positive samples from 4 farms, yielding 5 hemagglutinin sequences, including 2 H3N2 G.2 human-seasonal (22-23 season), and 1 H1N1 D.3.1 human-lineage pandemic strain (24-25 season). Notably, we detected 2 swine-lineage H1 1A.1.1.3 in farm employees from different states (23-24 and 24-25 seasons), with phylogenetic evidence supporting on-farm swine-to-human transmission. In contrast, 29.8% of swine samples (n=7,665) were IAV-positive, with more detections in nursery (36.20%) versus pre-wean (25.09%) piglets. Three endemic swine IAV

subtypes (H1N1, H1N2, H3N2) and H3N1 were identified, composed of 7 contemporary swine-lineage hemagglutinin clades (H1 1A.1.1.3, 1A.3.3.3, 1B.2.2.2, 1B.2.1, and H3 1990.4, 1990.4.a, and 2010.1) with some mixed infections. No human-seasonal IAV strains were found in swine during surveillance. These findings highlight the distinct viral ecology at this interface, characterized by the frequent endemic IAV circulation in swine and the sporadic detection of human- and swine-lineage IAV in farm employees. These results underscore the crucial need for longitudinal surveillance that involves both humans and swine to comprehend IAV circulation at the human-swine interface, as well as to develop effective control measures.

6.) Balaji Manicassamy

Regulation of HIV-1 transcription by the conserved cellular transcription repressor Capicua

Microbiology, University of Iowa

Viruses depend on host cellular machinery for replication, while hosts deploy complex regulatory networks to defend against infection. Our lab previously identified Capicua (CIC) as a key regulator of antiviral responses, as CIC knockout cells showed increased innate immune responses and restricted replication of multiple RNA viruses. CIC is a highly conserved DNA-binding transcriptional repressor and suppresses target gene expression by binding to specific CIC binding sites (CBS) near promoters. In this context, CIC binds to the CBSs of interferon (IFN) and several interferon-stimulated genes (ISGs) to repress expression. Beyond its role in immunity, CIC regulates tissue growth during Drosophila development and is implicated in cancer progression and neurological disorders.

Given that many DNA viruses and retroviruses utilize host factors to control their life cycles, and CIC is a potent global transcriptional repressor, we hypothesized that CIC may directly regulate viral transcription. We posited that CIC could function as a host-imposed restriction factor to limit viral production or a hijacked repressor used by the virus to maintain latency and evade immune detection. To test this, we screened the genomes of several DNA viruses and retroviruses for the consensus CIC binding motif and identified CBSs across several viral families, including human polyomaviruses, papillomaviruses, and herpesviruses.

To investigate whether CIC binds these sites to regulate transcription, we utilized HIV-1 as our primary model, which has three conserved potential CBSs. In HEK293T cells co-transfected with CIC-expressing plasmids and 5’LTR-driven luciferase/GFP reporter plasmids, CIC repressed reporter gene expression in a dose-dependent manner. In J-Lat latency models, CIC overexpression decreased HIV-1 transcription during reactivation, while CIC knockout increased both basal reactivation and response to stimuli. Furthermore, CIC deficiency led to elevated viral gene expression during active infection in Jurkat cells. Collectively, these findings uncover a novel role for CIC as a host regulator of the HIV-1 life cycle.

7.) Allison Stolte, Paige Richards, Mary Lynn Trawick, Kevin G. Pinney, Wendy Maury

Identification of Novel Small-molecule Inhibitors of Filovirus Entry

Microbiology & Immunology, University of Iowa

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 tubulin polymerization inhibitors 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 IC50 values of ~8 nM and 190 nM, respectively. Similar IC50 values were observed in assays that measured entry events of Ebola virus-like particles (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.

8.) Boopathi Sownthirarajan, Katarina Kulhankova, Paul B. McCray, Sriram Neelamegham Balaji Manicassamy

Assessment of influenza virus replication in differentiated human airway cells lacking sialylglycoconjugate receptors

Microbiology & Immunology, University of Iowa

Influenza A viruses (IAV) infect epithelial cells in the upper respiratory tract by binding to cell-surface sialylglycoconjugate receptors. Specifically, the viral envelope glycoprotein hemagglutinin (HA) binds to terminal sialic acids (SIA) on these receptors. Host cells express a variety of surface sialylglycoconjugates, including N-glycans, O-glycans, and glycolipids, which display diversity in both structure and carbohydrate composition. We established an in vitro air-liquid interface (ALI) culture model using immortalized human airway basal cells (BCi-NS1.1). To investigate the requirements for individual glycoconjugate types during IAV infection, we generated BCi-NS1.1 cells with truncated N-glycans ([N]-), O-glycans ([O]-), or both glycoconjugates ([NO]-) using CRISPR/Cas9-mediated knockout (KO) and assessed IAV replication in both submerged and ALI cultures. Lectin staining confirmed truncation of N-glycans and O-glycans in the respective KO cells. In submerged cultures, IAV strains from avian, human, and swine hosts replicated efficiently in control, [N]-, and [O]- cells, whereas viral replication was significantly impaired in [NO]- double knockout (DKO) cells, indicating that IAV can utilize either N- or O-glycans for entry under these conditions. In contrast, ALI cultures revealed a dominant role for N-glycans: control and [O]- cells supported robust viral replication, while [N]-and [NO]- cells significantly reduced replication. Together, these findings demonstrate that extended N-glycans serve as the primary entry receptors for most IAV strains in differentiated human airway epithelium and highlight the flexibility of glycan receptor usage in distinct cellular contexts.

9.) Aidan Saxton, Olufemi Fasina

Elucidating the role of N-acetyltransferase 10 (NAT10) in Canine Hepatocellular Carcinoma and Minute Virus of Canines (MVC) infection

Veterinary Pathology, Iowa State University

Hepatocellular carcinoma is the most common hepatic neoplasm in dogs. mRNA modifications, including N4-acetylcytidine (ac4C) and N6-adenosine methylation (m6A), have been associated with hepatocellular carcinoma pathogenesis and prognosis in humans and mice. N-Acetyltransferase 10 (NAT10) is an RNA modification enzyme that acts as a "writer" for the addition of ac4C. Increased mRNA stability and translation efficiency have been correlated with ac4C modifications. The role of mRNA modifications has not been explored in canine hepatocellular carcinoma. This is a retrospective study to examine NAT10 expression in canine hepatocellular carcinoma. We have selected 60 cases of canine hepatocellular carcinoma for necropsy and biopsy to explore the role of ac4C-modified RNA. The Labrador Retriever is the predominant breed in the cohort, accounting for approximately 22% of the examined cases. Similarly, it has been reported that NAT10 is modulated by viral infection, and recently it was shown that Parvovirus Minute Virus of Canines (MVC) transcript is modified by mRNA modifications including m6A and ac4C. Parvoviruses are linear, single-stranded DNA viruses that infect animals and humans and represent an excellent tractable model for understanding virus-host cell interactions; they are currently used for gene therapy and oncolytic virotherapy. Alternative RNA processing strategies, including alternative splicing and alternative polyadenylation, are modulated by parvoviruses to generate a diverse proteome, including the capsid protein, which determines the tissue specificity for gene therapy applications. This study is designed to elucidate the role of NAT10 in canine oncology and virology, using canine hepatocellular carcinoma and MVC infection as a model system for evaluation.

10.) Tyler Simpkins, Joshua Beck, Bradley Blitvich

Proximity-Based Mapping of the NS3 Protein Interactome: Comparing Dual-Host and Insect Specific Flaviviruses

Veterinary Microbiology and Preventive Medicine, Iowa State University

Flaviviruses have diverse host specificities, with some capable of replicating in both vertebrate and mosquito hosts while others are restricted to a single host type. Although these viruses share similar genomic organizations, the determinants of why some viruses can infect multiple hosts remain poorly understood. This project seeks to identify vertebrate host proteins that interact with the viral nonstructural protein NS3. NS3 is essential for the formation of the viral replication complex and suppression of the host immune response. To accomplish this, we employed TurboID biotin-based proximity labeling to identify host proteins that interact with NS3 from representative dual-host, insect-specific, and vertebrate-specific flaviviruses in both mosquito and mammalian cell lines. Labeled host proteins were isolated, identified using LC-MS/MS, and analyzed using proteomic approaches to identify conserved and unique host-specific interactions. By defining these interaction networks, this work aims to provide insight into how different flaviviruses interact with host proteins and to determine protein interactions essential for multi-host infection and those that are specific to single-host infection.

Additionally, by identifying host proteins that are involved in viral replication and immune evasion, we can better understand the determinants of flavivirus host range restriction.

11.) Damilare Dabiri, Warren B Rouse, Walter N. Moss

Identification of conserved RNA secondary structures in human Respiratory Syncytial Virus A (hRSV)

Genetics and Genomics, Iowa State University

Respiratory Syncytial Virus (RSV) is a non-segmented, negative-sense, single-stranded RNA virus, belonging to the Family Pneumoviridae and the order Mononegavirales. It is a primary cause of lower respiratory tract infections, particularly affecting infants, the elderly, and immunocompromised individuals. The RSV genome is approximately 15 kilobases long and encodes eleven proteins through ten genes. As an RNA virus, RSV utilizes RNA as its genetic material, and the secondary structures of these RNAs are likely crucial for various RSV biological processes, including transcription, translation, and genome packaging. This study aims to computationally identify RNA secondary structures within the RSV genome that exhibit unusual structure and conservation, suggesting a high probability of functional importance.
To achieve this, four complementary computational approaches were employed to analyze the genome and antigenome of RSV for the presence of RNA secondary structures. A total of 123 thermodynamically stable structures were identified in the positive sense (antigenome) and 113 in the negative sense (genome) with 104 and 76 of these structures, respectively, demonstrating some level of structural conservation. Further analysis of the identified structures uncovered biases in the transition over transversion (ti/tv) mutation rate in stem vs. loop regions, indicating an RNA structural constraint on RSV genome evolution further emphasizing the likely importance of our identified structures.
In summary, this study reveals RNA secondary structures within the RSV coding strand (positive sense) likely to be functionally important and genome (negative sense) that are of interest, identifying potential targets for further analysis and, potentially, antiviral drug development. Most of these structures were localized in the L (RdRp), Fusion gene (F), Nonstructural gene I and 2, Matrix 2 gene (M2), Matrix (M) gene and 3’ UTR (155nt Trailer sequence), emphasizing their biological relevance and therapeutic potential. These findings provide foundation for research aimed at developing effective antiviral strategies against RSV.

12.) Lucas Muta, Seema Rani Raychaudhuri, Jahanara Sonia, W. Allen Miller

Agroinoculation of Zea Mays with Maize associated Rimosavirus (MaRV) and a Translational Conundrum

Plant Pathology, Entomology and Microbiology, Iowa State University

We used metagenomics to discover plant virus genomes in maize, including maize-associated rimosavirus (MaRV), which belongs to the Tombusviridae (Lappe et al BMC Genomics 2022). Its uniqueness prompted the creation of the genus Rimosavirus to accommodate it and other viral genomes from Genbank (Lozier et al. Front Virol. 2024). To determine (i) if the MaRV genome is infectious, (ii) its host, and (iii) any symptoms, we used Agroinoculation to attempt to infect maize (Golden Bantam) (Beernink et al. J. Vis. Exp. 2021). We injected maize coleoptyls with Agrobacterium transformed with plasmid pJL89-MaRV, carrying the full MaRV genome driven

by a 35S promoter, and plasmid pJL89-P19. Inoculated leaf tissue was sampled seven days post-inoculation and fourteen days post-inoculation for the noninoculated leaves. RT-PCR and PCR were performed on the samples' RNA extract to detect RNA containing portions of open reading frames 1 and 2 (ORF1-2) and portions of open reading frame 3 (ORF3). After gel electrophoresis, many samples inoculated with pJL89-MaRV and P19 produced bands of sizes associated with ORF1-2 and ORF3. However, MaRV's translational mechanism remains unknown. Hypothesizing the 5' untranslated region (UTR) contained an internal ribosome entry site (IRES), we translated a transcript of MaRV 5' UTR and 3' UTR surrounding a luciferase ORF (MatLucMat). In wheat germ extract (WGE), capped and uncapped MatLucMat produced no luciferase in comparison to a similar construct containing the UTRs of Barley yellow dwarf virus (BYDV) that has a 3' cap-independent translation element (CITE). Looking for internal translation elements, we translated capped and uncapped MaRV full-length transcripts in WGE, which both produced low levels of a protein approximately the expected size of the coat protein. As translation of ORF1 was not detected, there may be a weak IRES upstream ORF3 but downstream ORF1-2. Yet, ORF1-2 is required for replication, creating a translational conundrum.

13.) Gayathri Loganathan, Yan Lib, Senthamizharasi Manivasagama, Boopathi Sownthirarajana, Balaji Manicassamy

Genome-wide CRISPR/Cas9 Screen to Identify Host Factors Essential for the Replication of Respiratory Syncytial Virus

Microbiology & Immunology, University of Iowa

Respiratory syncytial virus (RSV) is a major cause of bronchiolitis and pneumonia in young children and older adults, and there are currently no approved therapeutics for treatment of RSV infection. Identifying host dependency factors and pathways required for RSV replication could reveal potential therapeutic targets. In comparison to other respiratory viruses, only a limited number of genome-wide siRNA screens or unbiased proteomic studies have been performed to identify host factors important for replication of paramyxoviruses. We performed a genome-scale CRISPR/Cas9 knockout (KO) screen in A549 cells to identify host factors essential for RSV replication. Our screen identified 366 common hits for both RSV A2 and RSV 2-20 strains, enriched in signaling pathways including PI3K-AKT, EGFR, TNF, TLR, WNT, and MAPK. Validation experiments in individual CRISRP KO cells confirmed the importance of these pathways in early RSV replication. Interestingly, several other respiratory viruses such as H5N1, H7N7, H1N1, and HPIV3 showed reduced replication in these CRISPR KO cells. These findings highlight key host factors and pathways that could be targeted for the development of broad-spectrum antivirals.

14.) Jinhua Xiang, McKinley Sanders, Qing Chang, James McLinden, Wendy Maury, Jack Stapleton

Dengue virus serotype-specific envelopes alter cell receptor usage in vitro

Internal Medicine/Microbiology & Immunology, University of Iowa

Background and Methods: TIM-1 and Axl contribute to flavivirus binding and entry. We previously identified serotype-specific differences in dengue virus (DV) T cell receptor (TCR)

signaling. DV-1/-4 inhibited T cell receptor (TCR)-signaling while DV-2/-3 did not. DV-1 env amino acids (aa) V55/S66 and DV-4 L55/S66 were responsible for the TCR-signaling inhibition. Replacement of those aa's with DV-2 and DV-3 T55/T66 abrogated TCR inhibition in human T cells and altered viral replication in Vero cells. To determine if these aa's influenced virus cell interactions, wild type (wt), single and double envelope mutants of DV serotypes 1-4 were used to infect Vero-E6 cells with or without TIM-1 and/or Axl expression (CRISPR). Mutant viruses were generated using CPER methods. Results: All 16 viruses were sequenced to verify wt and mutant env sequences. DV-1, -2, -3 required TIM-1 or Axl expression for replication. DV-4 replication was reduced (3 log10) in TIM-1/Axl double KO cells, but substitution of DV4 env L55T or S66T replicated without TIM-1 and Axl, suggesting an alternative receptor interaction. Further, DV-1/-2 wt and DV-4 L55T viruses replicated to higher levels in Axl KO cells compared to wild type VeroE6 cells. DV-4 cell binding at 4°C was significantly reduced for wt and L55T DV4 in wt Vero-E6 cells; however, the env mutant viruses containing L55T and S66T bound significantly more than wt (> 2 log10).
Discussion: We confirm that TIM-1 and Axl are involved in early steps of DV-1,-2,-3 replication. DV-1,-2,-3 env residues involved in TCR signaling inhibition (aa 55 and 66) influenced replication in TIM-1 KO cells, particularly in the presence of Axl. Further, these residues improved DV-4 replication in the absence of TIM-1/Axl. Further characterization of the entry pathways involved and additional DV serotypes binding are underway.

15.) Ke Hu, Tavis Anderson, Amy Baker, Jianqiang Zhang, Phillip Gauger, Michael Zeller

Establishment of Human H3 G.1.1 Clade Influenza A Virus in Swine

Veterinary Diagnostic and Production Animal Medicine, Iowa State University

Influenza A virus (IAV) is a major respiratory pathogen causing disease in both humans and swine. Swine serve as the unique "mixing vessel" in IAV ecology. It raises a One Health concern that human-to-swine transmission increased viral genetic diversity through reassortment with the endemic swine lineage. The human seasonal H3 lineage of IAV, has continuously evolved each season and has exhibited multiple reverse zoonotic spillovers and establishment events. Since 2022, an increased number of H3 human-to-swine spillover cases have been detected through passive surveillance at the Iowa State University Veterinary Diagnostic Laboratory. Through sequencing, we detected repeated spillovers of 19 H3 G.1.1 (formerly 2a.1) clade into swine from 2023 to 2025. This study recognized repeated spillover of G.1.1 clade into swine across 6 states of the United States (U.S.). G.1.1 is no longer detected in the human population since 2024. However, we continued to detect 12 human seasonal G.1.1 virus in swine samples, indicating that this clade is now spreading endemically within U.S. swine. Although G.1.1 detections remain human-origin H3, the genetic similarity continues to decrease over time, with a maximum of 42 nucleotide differences observed in 2025 compared with the 2022 human ancestor. Eleven whole genomes were recovered, and reassortment carrying swine N2.2002B neuraminidase and internal gene cassettes has been established since 2024. All polymerase genes are from the swine triple reassortant lineage, with the nucleoprotein and matrix genes from the 2009 pandemic lineage. The NS genes from 2024 cases were from the pandemic 2009 lineage, while those from 2025 cases were from the triple reassortant lineage, suggesting further reassortment. Repeated reverse zoonosis events of G.1.1 have been identified since

2022, and its establishment represents the first endemic H3 clade in the U.S. swine in this decade.

16.) Yuchu Wang, Katarina Kulhankova, Alexandra C. Loren, Kari Thrasher, John D. Lueck, Colin Hemez, David R. Liu, Ashley L. Cooney, Paul B. McCray Jr, Patrick L. Sinn

Three models of the CFTR R553X mutation corrected by an adenine base editor delivered with HDAd

Microbiology, University of Iowa

Introduction: Cystic fibrosis (CF) is a lethal genetic disease that results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Approximately 10% of individuals with CF carry nonsense, frameshift, or splice site mutations that prevent them from benefiting from currently available CFTR modulator therapies. Adenine base editors (ABEs) have shown promise in correcting the CFTR R553X nonsense mutation; however, in vivo demonstration of efficacy has been limited by the lack of an efficient delivery. The goal of this study was to evaluate the genetic and functional correction of the CFTR R553X mutation using a versatile helper dependent adenoviral (HDAd)-based vector (HDAd ABE8e R553X). Here we demonstrate efficacy in 3 genetic models: transduced reporter constructs, immortalized airway epithelial cells, and primary airway epithelial cells for human CF lung donors.

Methods:

1) CFTR R553X reporter construct. To evaluate the base editing capacity of HDAd ABE8e R553X, we employed an EF1α driven reporter construct (EF1α–NLS mVenus secNanoLuc mScarlet3) that includes a human CFTR exon 11 fragment containing the R553X mutation inserted between the split NanoLuc luciferase subunits. Restoration of the full NanoLuc enzyme and mScarlet3 occurs only when the premature stop codon is corrected. Primary HAE cells were transduced with the lentiviral vector encoding the reporter system and subsequently transduced with HDAd ABE8e R553X. Expression of mScarlet3 and NanoLuc was measured.

2) In vitro correction of CFTR R553X. 16HBEo cells homozygous for the CFTR R553X mutation (16HBEge-R553X) were transduced with HDAd ABE8e R553X at multiplicities of infection (MOI 50, 100, 250, and 500). High-throughput sequencing (HTS) was used to measure the correction of the R553X site.

3) Correction of primary human CF airway epithelia (HAE). Primary cells carrying the R553X mutation were differentiated at an air–liquid interface (ALI) to model physiological airway epithelium. HTS was utilized to determine the editing of R553X site.

Results:

1) CFTR R553X reporter construct. Robust expression of NanoLuc and mScarlet3 indicated successful editing in the HAE reporter system.

2) In vitro correction of CFTR R553X. Editing efficiencies in 16HBEge-R553X cells ranged from ~20% to ~90% in an MOI dependent manner.

3) Correction of primary human CF airway epithelia (HAE). HDAd ABE8e R553X transduction resulted in 10–20% editing. These levels have previously shown to restore CFTR function in ALI cultures, suggesting physiological relevance.

Conclusion: These studies demonstrate that an HDAd vector delivering an adenine base editor can achieve robust correction of the CFTR R553X mutation across four complementary models:

a reporter system, an in vitro airway epithelial cell line carrying the R553X mutation, and primary human airway epithelial cultures. HDAd ABE8e R553X shows strong potential as a therapeutic platform for individuals with CF caused by nonsense CFTR mutations.

17.) Leo Flores, Bryan S. Kaplan, Amy L. Baker, Meghan Wymore Brand

Swine Influenza A infection-induced antibody responses to homologous H1N2 (clade 1B2.2) and heterologous H1N1 (clade 1A.3.3.2) virus

ORISE-USDA

Influenza A virus (IAV) is a significant respiratory pathogen of pigs. Multiple subtypes (H1N1, H1N2, and H3N2) circulate in swine with diverse antigenic variation in the viral hemagglutinin (HA) and neuraminidase (NA). Inactivated whole influenza virus (WIV) vaccines provide reasonable protection against viruses with homologous HA but limited protection against viruses with heterologous HA and NA. Additionally, cross-protective immunity induced by natural infection is poorly understood. To further study infection-induced immunity, we assessed antibody reactivity to homologous and heterologous viruses. Five pigs were exposed to an H1N2 (clade 1B.2.2), while five IAV-naive pigs served as controls. At 21 days post-infection (DPI), the pigs were necropsied to collect serum and bronchoalveolar lavage fluid (BALF), and antibody reactivity was evaluated by hemagglutinin inhibition (HI), neuraminidase inhibition (NI), and ELISA to homologous virus and a heterologous H1N1 (clade 1A.3.3.2).
All exposed pigs were confirmed to have been infected via viral shedding in nasal swabs, with elevated temperature readings further confirming infection. Lesion scores at necropsy indicate recovery post-infection. In serum, there was no cross-reactivity by HI or NI, but serum whole virus-specific IgG ELISA showed cross-reactivity between the two viruses. Additionally, BALF whole virus-specific IgG and IgA were also cross-reactive between viruses by ELISA.
In conclusion, we demonstrate that infection with IAV can induce cross-reactive whole-virus-specific antibodies in serum and the mucosa, even with divergent HA and NA. Ongoing studies are evaluating cross-reactivity between individual IAV proteins and assessing cross-reactive T cell responses. Further studies are necessary to understand how infection-induced cross-reactive antibodies may play a role in immune protection from divergent IAV infection.

18.) Lauren Bloodgood, Breigha Boyle, Todd Bell

High throughput screening of RVFV antivirals

Veterinary Pathology, Iowa State University

Rift Valley Fever virus, in the Bunyavirales order and Phenuiviridae family, is an emerging zoonotic pathogen and public health threat. We have previously published that combination kinase inhibition suppresses the pathogenesis of Rift Valley fever virus (RVFV) through diminished ability for viral replication. Globally, there is a lack of available antiviral treatment options for this family of viruses. We are now working to repurpose FDA-approved modulators to create a multimodal treatment option for RVFV. We analyzed cell signaling changes in response to infection in mouse and human hepatocytes via western blots and reverse phase protein microarray (RPPA) to uncover antiviral targets. In order to validate our in vitro models for drug development, we evaluated differences in cellular responses between human and mouse liver cells enabling translation to human medicine. We found several pathways activated

early during infection with the most consistent upregulation happening in translational control pathways and cell cycle control pathways. We then sourced FDA-approved inhibitors for the signaling pathways most active during infection. Using luminescence-based viability bioassays, we defined the cytotoxicity and efficacy concentration of these inhibitors in vitro. This was conducted for individual drugs followed by drug combinations to mimic findings from our earlier paper. We confirmed a significant reduction in viral replication of the vaccine strain of RVFV, rMP12, in vitro using combination kinase modulation from readily available FDA-approved kinase modulators. Additional testing showed the singular and combination drugs used in vitro were safe and tolerable in our BALB/c mouse model. Future aims include efficacy testing of these drugs and drug combinations in vivo as well as investigating how these drugs are modulating the immune system to prevent pathogenicity.

19.) Breigha Boyle, Rachel Phillips, Supantha Dey, Abraham Adeyemo, Caitlin Woodson, Lauren M. Bloodgood, Kylene Kehn-Hall, Todd M. Bell

Spatial Transcriptomic Characterization of Rift Valley Fever Virus rMP12 Infection in BALB/c Mice

Veterinary Pathology/Immunobiology, Iowa State University

Rift Valley Fever virus (RVFV), in the order Bunyavirales and Phenuviridae family, is a negative-sense ssRNA virus labeled by the World Health Organization (WHO) and the National Institute of Health (NIH) as a potential pandemic pathogen. RVFV, an arthropod-borne virus, poses a threat to both public health and agriculture. Despite its discovery in 1931, an accessible BSL-2 animal model has yet to be characterized for RVFV, a BSL-3 virus. In collaboration with Virgina Tech University, we have developed and characterized a BALB/c mouse model of RVFV using vaccine strain, rMP12. This model recapitulates the meningoencephalitis phase of wild-type Rift Valley Fever. Upon receiving and acclimating a group of 10 BALB/c mice, the mice were infected intranasally with low serum grown rMP12. Mice were weighed and observed 1-2 times a day for 9 days, followed by euthanasia and brain tissue collection on day 8 and 9 post-infection. Here, we use formalin-fixed paraffin embedded (FFPE) BALB/c mouse brain tissue to investigate the spatial transcriptomic profile of rMP12 induced neurologic disease in comparison to control tissue via the 10x Genomics Visium Spatial Gene Expression platform. Through this probe-based assay, we visualized gene expression of the whole mouse transcriptome in the context of infected brain tissues. Additionally, RVFV custom viral probes were used to colocalize changes in gene expression with viral nucleic acid. In RVFV rMP12 infected brain tissue, we saw viral concentration in neuron rich regions. These findings provide groundwork for characterization of rMP12 infection in BALB/c mice. Further studies will help to elucidate the genetic mechanisms used by RVFV to replicate and induce pathology and may provide information for future investigation of potential therapeutic targets for this emerging viral threat.

20.) Daniel I. Sands, Tarun Mishra, Stacia Phillips, and Li Wu

HIV-1 infection upregulates m6A reader protein YTHDC1 expression in CD4+ T cells

Microbiology & Immunology, University of Iowa

N6-methyladenosine (m6A) RNA modifications regulate cellular RNA through nuclear export, stability, splicing, and translation. HIV-1 infection increases cellular m6A-RNA levels in CD4+ T cells. m6A modifications of HIV-1 RNA regulate viral splicing, gene expression, and genomic RNA packaging. m6A-specfic RNA-binding proteins, or “m6A readers”, are cellular proteins that bind modified RNAs and alter their processing. The m6A reader YT521-B homology domain containing protein 1 (YTHDC1) regulates cellular RNA nuclear export, splicing, and transcription. YTHDC1 binds HIV-1 transcripts, and its overexpression can regulate HIV-1 transcript levels and splicing in HeLa or HEK293T cells. However, the effect of HIV-1 infection on YTHDC1’s expression and YTHDC1’s role in HIV-1 infection of CD4+ T cells are unknown.
We demonstrated that HIV-1 infection of the CD4+ Sup-T1 cell line and activated primary CD4+ T cells enhanced YTHDC1 protein expression relative to uninfected cells. HIV-1 infection of Sup-T1 cells treated with a reverse transcriptase inhibitor also increased YTHDC1 protein levels. However, HIV-1 infection of CD4+ T cells did not alter YTHDC1 mRNA expression, suggesting HIV-1 infection post-transcriptionally upregulates YTHDC1. Activation of primary CD4+ T cells increased YTHDC1 protein expression without changing its mRNA levels. Furthermore, we observed that YTHDC1 knockdown in Sup-T1 cells increased HIV-1 infection levels, suggesting that YTHDC1 negatively regulates HIV-1 infection of CD4+ T cells. Our results indicate that HIV-1 infection and CD4+ T cell activation can post-transcriptionally upregulate YTHDC1 levels. Further evaluation of YTHDC1’s role during HIV-1 infection in CD4+ T cells will enhance our understanding of m6A-mediated regulation in HIV-1 replication.

21.) Kaho Matsumoto, Ella R Morgan, Emilia Bolinski, John-Mark Miller and Alex B Kleinpeter

Investigating the role of the HIV-1 capsid C-terminus in particle infectivity and viral replication

Microbiology & Immunology, University of Iowa

The HIV-1 capsid (CA) protein plays two key structural roles in the formation of infectious virions. First, as an internal component of the Gag polyprotein, CA promotes the oligomerization of Gag into the immature Gag lattice, driving particle assembly and release. Second, following protease-mediated cleavage of Gag during virus maturation, the liberated CA protein assembles into the mature viral capsid, which encloses the viral genome and is required for productive infection. While the C-terminus of CA plays a crucial and well-defined structural role in immature Gag lattice formation and particle assembly, its role in capsid assembly and infection remains poorly characterized because it is flexible and unstructured in the mature lattice. However, a recent study demonstrated that a mutation in the CA C-terminus (G225R) promotes structural stabilization of the CA C-terminus by facilitating interactions at hexamer-hexamer interfaces in the mature capsid lattice.
To investigate the function of the C-terminus in the context of the mature capsid, we performed alanine-scanning mutagenesis of selected residues in the CA C-terminus and G225R contact-residues (D152, R154, and K199). Mutant viruses were propagated iteratively in T-cell lines, and emerging secondary mutations were identified by sequencing of proviral DNA. These

mutations were introduced into infectious molecular clones, and their effects on viral infectivity and replication kinetics were evaluated.
Alanine substitutions throughout the CA C-terminus, including contact residues of G225R, exhibited a range of infectivity phenotypes, demonstrating that several positions cooperatively modulate viral fitness. Notably, secondary mutations that emerged during viral propagation localize near a dimer interface that forms between CA monomers from neighboring hexamers, suggesting that they restore CA interactions disrupted by primary alanine substitutions.
Together, our results suggest that the CA C-terminus and its interaction with the dimer interface within the CA lattice are crucial in supporting the function of CA after particle release.

22.) Hia Kalita, Juliana Osse de Souza, Carter Casey, Adriana Larrea-Sarmiento, Olufemi Alabi, Ashleigh M. Faris, Maira Rodrigues Duffeck, Ivair Valmorbida, Alejandro Olmedo-Velarde

Genetic Diversity and Microbe Prevalence of the Corn Leafhopper (Dalbulus maidis) Across Four States of the United States

Plant Pathology, Entomology and Microbiology, Iowa State University

The corn leafhopper, Dalbulus maidis (Hemiptera: Cicadellidae), is a key vector of multiple plant pathogens that threaten corn production in the United States. As D. maidis has only recently expanded into the U.S. Corn Belt, characterizing the genetic background of invading populations alongside their virome is essential for understanding virus emergence, maintenance, and transmission risk. In this study, we investigated mitochondrial genetic diversity and microbe prevalence in D. maidis populations collected from Iowa, Missouri, Oklahoma, and Texas. Median-joining network analysis of concatenated mitochondrial genes (NadH1, COI, and CytB) revealed 18 haplotypes, including a dominant haplotype shared across all regions, consistent with recent expansion and gene flow among populations. In parallel, we conducted targeted screening for insect-specific RNA viruses and corn stunt–associated pathogens of this vector. The virus panel included a beny-like virus (ChMBLV), a bunya-like virus (ChMBV), two iflaviruses (ChMIfV-1 and -2), an orthomyxo-like virus (ChMOMLV), and two flaviviruses (ChMFV-1 and -2) recently characterized by our group, in addition to Maize Bushy Stunt Phytoplasma and Corn Stunt Spiroplasma. Insect-specific viruses (ISVs) were widespread across all states, with ChMIfV-2 and ChMFV-2 reaching prevalences of up to 0.6 and 0.9, respectively, in IA and MO samples. Notably, females exhibited higher prevalence of most ISVs, whereas males showed higher prevalence in corn stunt–associated pathogens. Collectively, these results highlight a rapidly expanding vector population harboring a diverse and sex-structured virome, underscoring the potential role of insect-specific viruses and host population dynamics in shaping pathogen transmission and disease emergence in U.S. corn agroecosystems.

23.) Biyun Xue, Benjamin E. Nilsson-Payant, Pengfei Li, Nicholas Schnicker, Jian Zheng, Stanley Perlman, Paul B. McCray, Jr., Alejandro Pezzulo

Role of lipocalin-type prostaglandin D synthase on respiratory virus replication and innate antiviral immune response

Microbiology, University of Iowa

SARS-CoV-2 is the major causative pathogen of COVID-19 which poses a threat to global public health and economies. Recent studies revealed that eicosanoid pathway signaling increases in the elderly and contributes to SARS-CoV-2-induced inflammation. Interestingly, the transcription of some eicosanoid-related genes including lipocalin-type prostaglandin D synthase (PTGDS) is regulated by NF-κB signaling pathway which is known to be required for SARS-CoV-2 replication.
We recently carried out an siRNA screen of NF-κB targets in SARS-CoV-2-infected A549-hACE2 cells and found that knockdown of PTGDS caused a decrease in nucleocapsid protein detection. We therefore hypothesized that PTGDS may act as a pro-viral factor and increase susceptibility to SARS-CoV-2 infection.
We showed that SARS-CoV-2 infection increases the mRNA and protein expression levels of PTGDS in human epithelial cells. Additionally, overexpression of PTGDS promotes SARS-CoV-2 replication and suppresses the activation of type I and type III interferons (IFN-I/IIIs), while PTGDS knockout impairs the virus replication and enhances the IFN-mediated innate immune response. Interestingly, we found that PTGDS knockout has a broad-spectrum activity against infection of multiple respiratory viruses, including SARS-CoV-2, MERS-CoV, influenza A, HCoV-229E, and RSV. Meanwhile, inhibiting PTGDS activity by AT-56 reduces replication of the above viruses in HAE cells and restores IFN-I/IIIs transcript production in a dose-dependent manner.
To evaluate the antiviral effect of AT56 against SARS-CoV-2 in vivo, we established a mouse-adapted SARS-CoV-2-infected model. Middle-aged C57BL/6J mice were intranasally inoculated with SARS-CoV-2 strain SARS2N501MA30 and were intraperitoneally administered with AT56 (30 mg/kg) at 1 hour before infection and on days 1-5 after infection. AT56 treatment resulted in approximately 60% reduction of mortality and less body weight loss after SARS-CoV-2 infection.
Collectively, our findings reveal a preliminary unrecognized function for an eicosanoid metabolism protein and highlight how multiple respiratory viruses can exploit a host innate immune response for their own survival.

24.) Shraddha Tripathi, Tarun Mishra, and Li Wu

m6A-mediated control of HIV-1 replication through HGF/c-MET signaling in CD4+ T cells

Microbiology & Immunology, University of Iowa

N6-methyladenosine (m6A) is the most abundant internal RNA modification and regulates gene expression, RNA stability, viral replication, and immune responses. HIV-1 infection increases m6A modifications of host RNA in CD4+ T cells in vitro and peripheral blood mononuclear cells from viremic individuals. The cellular mesenchymal-epithelial transition factor (c-MET) is a receptor tyrosine kinase that binds to hepatocyte growth factor (HGF) to drive cancer metastasis, making it a critical therapeutic target. Many cellular proteins contribute to HIV-1 replication and innate immunity. However, the role of c-MET during HIV-1 infection is unknown.

Here, we demonstrate that HIV-1 infection reduces c-MET mRNA and phosphorylated MET protein (p-MET) levels in CD4+ T cells, and inhibition of HIV-1 replication reduces p-MET levels. Knockout (KO) of c-MET in MT4 cells reduced HIV-1 Gag mRNA and protein levels as well as viral capsid release into the supernatant, indicating that c-MET supports HIV-1 replication. Notably, cell surface expression of the CD4 receptor and CXCR4 coreceptor also decreased in c-MET KO cells, suggesting that c-MET facilitates HIV-1 replication by regulating viral entry. Intriguingly, c-MET KO in MT4 cells reduces p38 mitogen-activated protein kinase, NF-κB activation, and signal transducer and activator of transcription (STAT) 1/3 phosphorylation, thus regulating multiple signaling pathways during HIV-1 infection. RNA immunoprecipitation and qPCR analysis reveal a significant increase in m6A enrichment at the c-MET mRNA during HIV-1 infection. Our results suggest new mechanisms by which HIV-1 hijacks the m6A machinery to regulate HGF/c-MET signaling to promote viral replication and cellular responses in CD4+ T cells.

25.) Sydney Winecke, Kaho Matsumoto, Alex Kleinpeter

In vitro evolution of lenacapavir-resistant HIV-1 reveals novel changes in the C-terminal domain of the capsid protein

Microbiology, University of Iowa

Lenacapavir (LEN) is an HIV-1 capsid inhibitor recently approved for use in the treatment of multidrug-resistant HIV and as a pre-exposure prophylaxis agent. LEN is remarkably potent and long acting, boasting an EC50 of ~50 pM and a six-month dosing interval. Although these qualities have branded LEN as a potentially revolutionary treatment option, the emergence of LEN-resistance remains a significant concern. Indeed, LEN resistance has been reported in clinical trials following the acquisition of mutations in the drug binding pocket that conferred up to 869-fold resistance. Of the identified amino-acid changes in the LEN-binding pocket that have emerged in response to treatment, substitutions at lysine 70 (K70) in the HIV-1 capsid (CA) protein are among the most common. Here, we characterized the LEN resistance conferred by four K70 mutants (K70A/H/R/N). Because mutations at K70 result in fitness defects, we assessed the evolution of these K70 mutants in a long-term propagation experiment that placed continuous pressure on viral replication via treatment with increasing LEN concentrations. To evaluate the accumulation of resistance and fitness-restoring mutations in CA, we sequenced PCR-amplified proviral DNA at discrete intervals. Mutations that arose in the clinical trials were recapitulated in our study, and 9 novel mutations in CA in response to LEN treatment were identified. Four of the novel mutations mapped to the C-terminal domain (CTD) of CA, distal to the LEN binding pocket. Structural modeling of the sites of these mutations suggests that these CTD residues may participate in interactions between capsid subunits within and between the CA hexamers and pentamers (capsomers) that comprise the mature HIV-1 capsid lattice. Our findings suggest that mutations in these CTD residues may alter inter- and intra-capsomer subunit interactions to offset perturbations in the fullerene capsid architecture imposed by the binding of LEN.

26.) Marla Shaffer, Richard Roller

Structure-Function Analysis of ICP22 in HSV-1

Microbiology & Immunology, University of Iowa

Infected cell protein 22 (ICP22) is a multifunctional viral protein found throughout the nucleus during herpes simplex virus type 1 (HSV-1) infection. Deletion of ICP22 results in a cell-type dependent viral replication and cell-cell spread defect, but it is unclear how these defects correlate to previously described functions of the protein or its structure. The predicted AlphaFold3 model of ICP22 is a dimer in which each monomer contains three distinct regions. The N- and C-terminal intrinsically disordered regions (R1 and R3) flank a conserved, ordered core and predicted dimerization domain (R2). We constructed ICP22 truncation deletion mutant viruses for each region (ΔR1, ΔR2, ΔR3) to assess alterations in known functions of ICP22. We found that ΔR3 had the greatest impact on the fitness of the virus and it more closely resembled ΔICP22, showing a significant decrease in plaque size and greater than 10-fold decrease in replication across cell types. We next correlated these defects with known nuclear functions of ICP22 such as formation of virus-induced chaperone-enriched (VICE) domains, incorporation into replication compartments and proper localization of the nuclear egress complex (NEC). All mutants displayed defects in VICE domain formation showing that this function is not correlated with spread or replication. ΔR1 and ΔR3 could not incorporate ICP22 into replication compartments and disrupted NEC localization. ΔR2 had no impact on replication compartment incorporation or NEC localization compared to WT. These data suggest that the ability of ICP22 to incorporate into replication compartments and to facilitate proper localization of the NEC is dependent on R1 and R3, but not R2. Finally, we analyzed HSV-1 nuclear egress of the mutants using transmission electron microscopy and confocal microscopy. We observed nuclear membrane reduplications in ΔICP22 and ΔR3, suggesting a potential novel role for ICP22 in regulation of nuclear envelope architecture during HSV-1 infection.

27.) Georgia Chaffin, Marla Shaffer, Carson Page, Richard Roller

The Functional Analysis of ICP22 Mutants During an HSV-1 Infection

Microbiology & Immunology, University of Iowa

All herpesviruses utilize nuclear egress to translocate viral capsids from the nucleus into the cytoplasm for further maturation. We engineered a nuclear egress-deficient Herpes Simplex Virus Type I (HSV-1) to identify otherwise unknown viral proteins important for this process. After passaging this virus, ICP22, encoded by US1, was determined to be a mutational hotspot across multiple isolated viruses via whole genome sequencing. ICP22 is a multifunctional protein but has only one previously identified function related to nuclear egress which is proper localization of the nuclear egress complex (NEC) at the nuclear rim. We engineered five ICP22 point mutations, from our initial study, on a WT background: R131W, L132P, G141V, R219H, and R347C. We hypothesized that, on a WT background, these mutations would impair known functions of ICP22, providing insight into its role in nuclear egress. We measured changes in viral replication and cell-to-cell spread, as well as known functions of ICP22 such as formation of virus-induced chaperone enriched (VICE) domains, incorporation into replication compartments (RC), and proper localization of the NEC. L132P, G141V, and R347C had no significant impact on

viral replication, spread, or any ICP22 function tested compared to WT. However, R131W and R219H showed a significant decrease in viral replication, like that of an ICP22 null virus. Interestingly, R219H produced significantly smaller plaques than WT while R131W did not. Additionally, R219H was defective in VICE domain formation and incorporating ICP22 into RCs. R219H and the ICP22 null virus had shown similar phenotypes in mislocalization of the NEC and disruption of the nuclear envelope. We hypothesize that R219H likely disrupts multiple functions of ICP22 due to the position of the mutation localizing to a predicted dimerization domain of the protein.

28.) John-Mark Miller, Kaho Matsumoto, Sarah Gulick, Alex Kleinpeter

Elucidating the role of the C-terminus of the HIV-1 capsid protein on capsid-like particle assembly

Microbiology, University of Iowa

The HIV-1 CA protein plays two key roles in HIV-1 replication. During particle assembly, as an internal domain of the viral Gag polyprotein, CA promotes the multimerization of Gag into a spherical, hexameric lattice called the immature Gag lattice, driving the production of immature virions. Concomitant with particle release, Gag is proteolytically processed by the viral protease, liberating CA, which assembles into the mature conical capsid. The capsid, which is required for particle infectivity, is composed of 200-250 CA hexamers and 12 CA pentamers that induce curvature and facilitate the closure of the capsid around the viral genome. The host metabolite inositol hexakisphosphate (IP6) promotes the assembly of closed capsids by facilitating CA pentamer formation. The C-terminus of CA plays a well-defined structural role during virus particle production by facilitating assembly of the immature Gag lattice; however, its role in capsid assembly and particle infectivity remains unclear due its flexibility and lack of structure in this context. A recent study demonstrated that mutations to the CA C-terminus enhanced capsid assembly, suggesting that the C-terminus may play an underappreciated role in this process. To test the importance of the C-terminus in capsid assembly, we expressed mature wild-type CA protein (WT) and a truncated CA missing its C-terminal 11 residues (Δ11). Using in vitro assembly assays, SDS-PAGE, and negative stain electron microscopy, we compared IP6-driven assembly of WT and Δ11 CA. We found that Δ11 required ~10-fold more IP6 to assemble in vitro. At lower IP6 concentrations, Δ11 assembles into long open-ended tubes instead of conical capsid-like particles. Tubular assemblies lack CA pentamers, suggesting that the CA C-terminus may promote CA pentamer formation. Future work will aim to isolate specific amino acids in the CA-C-terminus responsible for this assembly phenotype via the construction of several C-terminus mutants harboring shorter truncations or point mutations.

29.) Madeline M Broghammer, Aaron N Gillman, Cassian M Birler, Rohith Rao Vujjini, Samuel McCarthy-Potter, Alex Kleinpeter, Hillel Haim

Deep mutational scanning of the HIV-1 capsid protein

Microbiology & Immunology, University of Iowa

Lenacapavir (GS-6207, LEN) is an FDA-approved therapeutic that targets the HIV-1 capsid protein. This long-acting injectable is administered every six months and prevents infection by HIV-1 as well as reduces viral loads in infected individuals. Mutations in capsid that increase

resistance to LEN are not common; however, they have been detected on treatment. Of these, K70R/H/N and Q67H are frequently observed. To better understand the escape paths of HIV-1 from LEN, and those that may emerge, we used deep mutational scanning (DMS). The approach is based on production of virus libraries that contain all possible amino acids at sites of interest and assessing their effects on virus fitness and resistance to therapeutics. Our experiments focused on capsid position 70, which we examined in the absence or presence of Q67H. We discovered that LEN preferentially inhibits the K70 form (relative to other amino acid variants). However, the Q67H mutation altered the fitness profile of position 70, and increased the fitness of 70K in the presence of LEN. We also found that Arg, His and Asn are highly fit in the absence of LEN. In contrast to 70K, the fitness profiles of 70R/H/N were unchanged by LEN or by Q67H. Taken together, these findings suggest that the wild-type K70 is uniquely sensitive to LEN and that its fitness can be restored by mutations at other positions of capsid. We propose the use of DMS to characterize the mutational escape paths from LEN as well as the epistatic interactions across this protein.

30.) Prabuddha Sarkar, Sabrina Tan

Differential surface HLA-E stabilization during JC-polyomavirus (JCPyV) infection across human cell types

Internal Medicine, University of Iowa

Background: JC polyomavirus(JCPyV) is a ubiquitous double stranded DNA virus with seroprevalence of 40%-60% of healthy adults. JCPyV is the etiologic agent of a frequently fatal brain demyelinating disease progressive multifocal leukoencephalopathy(PML), in immunocompromised individuals with no treatment option. Recently, we have demonstrated NK cell response to JCPyV. NK cell functions are regulated by a complex and versatile repertoire of activating or inhibitory surface receptors. On healthy cells, non-classical HLA class Ib molecule HLA-E (Human leukocyte antigen-E) binds to HLA class I signal peptide sequences to engage the inhibitory NK cell receptor NKG2A. In virus-infected cells, HLA-E can bind to virus-derived peptides to engage either NKG2A or the activating receptor, NKG2C, to differentially modulate NK cell function. We hypothesize that HLA-E stabilization during JCPyV infections is diverse among different cell types, resulting in differential NK cell responses.
Method: We used human primary cells susceptible to JCPyV infection, such as kidney (HEK293T, RPTEC), brain (SVG-A, Astrocytes), unrelated cervical epithelial carcinoma cell (C33A) and immune cells (macrophages) were infected with different strains of JCPyV. Post infection viral proteins T-Antigen, VP1 and HLA-E expression were quantified by flow-cytometry and immunofluorescence. Multiple open reading frames (ORFs) from JCPyV genome were mapped in-silico to predict HLA-E binding 9-mer peptides.
Results: Magnitude and dynamics of infection were found to be distinct in different cell types. Expression of HLA-E increased in all cell types except for C33A upon infection with JCPyV. Mapping of ORFs across viral genome revealed several HLA-E binding 9-mer peptides. These peptides will be tested for HLA-E stabilization across all the cell types and modulation of NK cell functions in coculture.
Conclusion: JCPyV infection alters HLA-E expression on human primary cells relevant to infections associated with JCPyV. Increased HLA-E expression could modulate NK cell functions.

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

Characterizing Ebolavirus Localization and Neuroinflammation in the Murine Brain

Internal Medicine, University of Iowa

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. 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.

32.) Ian Holtz-Hazeltine, Prabuddha Sarkar, Sabrina Tan

Regulation of JCPyV Infection by Transcriptional Repressor Capicua (CIC)

Microbiology, University of Iowa

JC Polyomavirus (JCV) is an opportunistic virus that causes a persistent yet asymptomatic infection in the kidneys of an estimated 40-80% of the population. Severe immune suppression can lead to the development of Progressive Multifocal Leukoencephalopathy (PML), a rare and often fatal brain disease, characterized by demyelination. During immune suppression, the JCV Non-Coding Control Region (NCCR) rearranges, increasing the transcription and replication kinetics of the virus, while also altering the composition of the genome's noncoding regions. PML-type JCV strains have a heightened prevalence of Capicua (CIC) binding sites in these noncoding regions. CIC is a highly conserved transcriptional repressor, responsible for suppressing interferon-stimulated genes (ISGs), transcription factors, and cell-cycle regulatory factors. CIC's function in repressing gene expression suggests it is an innate immune repressor

of JCV replication, either through directly binding the genome or by reducing expression of essential pro-viral factors. Here, using multiple clinical isolates and lab-adapted JCV strains, along with CIC-KO HEK293T cells, we show that CIC prevents JCV infection potentially through pro-viral gene repression. Furthermore, we characterized the genotypes of clinical JCV isolates from hundreds of patients, finding a direct correlation between the immune suppression and the development of additional CIC binding sites.

33.) Stacia Phillips, Beck Fitzpatrick, Madeline Allen, Li Wu

Effects of individual N6-methyladenosine modifications in HIV-1 RNA on viral replication

Microbiology & Immunology, University of Iowa

N6-methyladenosine (m6A) is an abundant eukaryotic RNA modification that influences the fate of modified transcripts. HIV-1 RNAs are m6A-modified, and manipulating the expression of host cell m6A regulatory proteins alters virus replication. However, the effects of individual m6A modifications of HIV-1 RNA on viral replication remain largely undefined. We previously identified sites of m6A modification in HIV-1 RNA from infected CD4+ T cells with quantitative, single-nucleotide resolution. To assess the contribution of individual m6A to HIV-1 replication, we selected several abundant m6A sites within motifs that tolerate synonymous mutation. We then introduced individual mutations in the HIV-1 proviral plasmid DNA to ablate the DRACH m6A consensus motif by substituting the conserved adenine with guanine or cytosine with thymine. These plasmids were transfected into HEK293T cells and HIV-1 RNA, protein, and particle release were measured. The resulting virus stocks were then used to infect CD4+ target cells to determine viral infectivity. Our results demonstrated that eliminating a single m6A modification in HIV-1 RNA resulted in a significant increase in the levels of viral RNA and particle release from transfected cells. However, these mutations had a negative impact on virion infectivity compared to wild-type HIV-1. Further, mutations that had no effect on virus production in transfected cells still resulted in significantly lower virion infectivity in CD4+ target cells. Our results suggest that m6A modifications of HIV-1 genomic RNA regulate virus replication in a site-specific manner and at different stages of the viral life cycle.

34.) Tarun Mishra, Shraddha Tripathi, Jack T Stapleton, Li Wu

Elevated RNA m6A in people with HIV-1 and cancer

Microbiology & Immunology, University of Iowa

N6-methyladenosine (m6A) modifications of human immunodeficiency virus type 1 (HIV-1) and cellular RNA contribute to regulation of viral and host gene expression. RNA m6A dysregulation has been implicated in multiple cancers. However, the role of m6A modifications in HIV-1-associated cancers remains unclear. Here, we aim to address this important question using clinical samples. Using enzyme-linked immunosorbent assay (ELISA), RNA m6A levels were quantified in peripheral blood mononuclear cells (PBMCs) from 43 de-identified people living with HIV-1 (PLWH), comparing those with cancer (n=15) to those without cancer (n=28). Correlation analyses were performed to evaluate the associations between m6A levels and HIV-1 RNA copies, CD4+ T-cell counts, and age of PLWH. The mRNA and protein expression of m6A regulatory genes was measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and Western blot, respectively. Furthermore, quantitative transcriptomic profiling of

84 type I interferon (IFN-I)-responsive genes was performed using an RT-qPCR array. We observed that HIV-1 viral load in the cancer group was higher than the non-cancer group. m6A levels of PBMCs were 2.8-fold higher in the cancer group and correlated with the expression of some m6A regulatory genes and proteins. Higher m6A levels were positively associated with HIV-1 RNA copies and negatively associated with CD4+ T-cell counts and age. Transcriptomic analysis of 84 IFN-I-responsive genes revealed upregulation of many pro-inflammatory and interferon-stimulated genes, along with downregulation of some genes in PLWH with cancer.

35.) Kyle Rapchak, Lauren Johnson, Calyssa Huff, Rylie Elbert, Jessica M Tucker

Brf1 dependent RNA polymerase III activity limits MHV68 viral spread

Microbiology & Immunology, University of Iowa

Herpesvirus infection represents an ongoing competition between the host and virus to control the cellular environment. Key factors in this interplay are host immune responses and viral manipulation of host machinery. RNA polymerase III (pol III) makes non-coding RNAs involved in these essential cellular processes, and its activity is enhanced by several herpesviruses. Despite a conserved enhancement of pol III, the functional consequence for the host or virus remains unclear. Both Kaposi Sarcoma associated Herpesvirus and murine gammaherpesvirus 68 (MHV68) boost the activity of host pol III during infection, leading to increased expression of premature tRNAs (pre-tRNAs), which we have shown is due to enhanced transcription and blocked maturation. We hypothesize that enhanced pre-tRNA expression may preferentially benefit the host or virus during infection. To investigate this hypothesis, we knocked down the TFIIIB transcription factor, Brf1, to limit pol III transcription in murine NIH 3T3 fibroblasts. Brf1 knockdown increased viral titers at a low MOI (MOI=0.05), but not at a high MOI (MOI=5). Plaque assays revealed larger plaques in Brf1 knockdown cells compared to control cells, suggesting that Brf1 expression restricts cell-cell spread. Additionally, Brf1 knockdown enhanced viral protein production, contrary to the expectation that Brf1 knockdown would lead to decreased translation. To identify pathways linking pol III activity with enhanced viral protein production and spread, we performed RNA sequencing comparing WT and Brf1 knockdown conditions during MHV68 infection. interferon related genes showed minimal change upon Brf1 knockdown in MHV68-infected cells. Conversely, we observed an enrichment of differentially expressed genes involved in mRNA splicing and cellular growth. This suggests that dissecting the crosstalk between pol III and cellular metabolism may reveal new therapeutic angles to control MHV68 infection. Our future goals are to mechanistically dissect the role of Brf1-dependent pol III transcription in viral gene expression and spread of MHV68.

36.) Ming Li, Qiaolin Hu, Arya Zandvakili, David H. Price, and Jeffery L. Meier

A multimolecular pUL84-IE2-TBP-nucleosome complex at the human cytomegalovirus major immediate-early promoter blocks antisense transcription

Internal Medicine, University of Iowa

The human cytomegalovirus (HCMV) major immediate-early promoter (MIEP) and its gene products are central to activation and maintenance of viral gene expression and replication. From the opposite DNA strand, UL124 transcription terminates immediately downstream of the MIEP. Here, we identify a previously unrecognized role for the viral protein pUL84 as a core

component of a prominent multimolecular complex assembled at the MIEP. Using double-stranded DNA fragmentation factor-ChIP sequencing, we show that pUL84 co-occupies the MIEP with viral immediate-early 2 protein (IE2), host TATA-binding protein (TBP), and a precisely positioned H3K4me3-marked nucleosome. This complex assembles on viral genomes as early as 24 h after infection, is more prevalent in late-stage infection, and is conserved across fibroblasts and neural lineage cells. A two-base substitution in the TATA box nearly eliminates the complex, indicating dependence on TBP-TATA interactions. This substitution, as measured by precision run-on sequencing, causes defective termination of antisense UL124 transcription with readthrough across the MIEP, likely due to disruption of the complex. In contrast, it does not reduce MIEP-driven IE1/IE2 RNA and protein expression under high-multiplicity infection. These findings support a model in which the MIEP-associated complex functions primarily as a transcriptional boundary rather than a global repressor of MIEP output during viral replication. We propose two populations of viral genomes: one bearing the pUL84-IE2-TBP-nucleosome complex, in which UL124 antisense transcription occurs and terminates at the complex while the MIEP is inactive and a second lacking the complex, in which UL124 antisense transcription is absent and the MIEP is active.

37.) Marie Nguyen, Samiksha Patil, Kayli Liles, and Prajakta Pradhan

Antiviral effects of MST-312 on the lytic phase of HSV-1 replication in neuronal cells

Microbiology, Immunology and Pathology, Des Moines University

Herpes simplex virus 1 (HSV-1) infects approximately two-thirds of the United States population. HSV-1 can cause disease as minor as a cold sore or as severe as keratitis or encephalitis. To establish new, more effective HSV-1 treatments, a further understanding of how the virus life cycle can be manipulated is needed. HSV typically enters the body through mucosal epithelium, where its initial replication takes place. During this time, HSV enters sensory neurons servicing the site of infection. Most of the infected neurons eventually enter a latent state, however, in cases of HSV encephalitis in neonates, the virus can spread to the central nervous system and undergoes lytic replication without entering latency. Unfortunately, HSV-1 encephalitis in this population can be fatal, even with current antiviral treatment. Thus, identifying novel antiviral targets to block or reduce HSV replication in neurons would greatly reduce the disease burden of this virus. Our laboratory previously demonstrated that a cell permeable, reversible inhibitor of the telomerase enzyme, MST-312, inhibits the replication of HSV-1 in epithelial cells. The aim of this research was to determine the effect of MST-312 on HSV-1 infection in neuronal cells. We used Lund human mesencephalic (LUHMES) cells, which are human embryonic neuronal precursor cells that can be differentiated into post-mitotic neuronal cells as our model for neuronal infection. Initially, we used an MTT assay to determine the toxicity of MST-312 treatment of LUHMES cells. Concentrations between 20-100 µM did not lead to increased cell toxicity compared to controls. Next, we infected differentiated LUHMES cells with HSV-1 in the presence and absence of 20-100 µM MST-312. Plaque assays and immunoblots revealed that MST-312 treatment significantly reduced HSV-1 protein and virion production. Together, this shows that MST-312 can inhibit the replication of HSV-1 in neuronal cells.

38.) Maya Mason, Gayathri Loganathran, Elizabeth Wagstaff, Deb Paroma, Miranda Sturtz, and Balaji Manicassamy

Utilization of Alternative Host Pathways for Sialic Acid Independent Entry by Influenza Virus

Microbiology, University of Iowa

Influenza A viruses (IAV) are zoonotic pathogens that have the potential to cause a global pandemic and thus are a major threat to human health. The virus can move from a zoonotic reservoir to humans and the seasonal influenza vaccines are ineffective against these strains. More research is needed to prevent these spillover events. Typically, the influenza glycoprotein hemagglutinin (HA) binds to sialic acid to induce clathrin-mediated endocytosis and enter the cell. Previously in the lab, a loss of function screen identified SLC35A1 as a crucial gene important for IAV infection. SLC35A1 is the only gene that facilitates import of CMP-Sialic acid into the Golgi. A549 SLC35A1 CRISPR KO cells showed complete loss of cell surface sialic acid and were unable to support robust IAV replication; however, some IAV strains showed very low levels replication in SLC35A1 KO cells. This suggested that IAV may utilize alternative entry mechanisms in the absence of sialic acid. To select for an IAV population that could enter and replicate in cells independent of sialic acid, the lab passaged a low pathogenic H5N1 strain in SLC35A1 KO cells 10 times and isolated a mutant capable of efficient replication in these cells. The mutant virus had gained several mutations isolated in HA. Virion stability and mode of cell entry were measured to determine how these mutations allowed for sialic acid independent entry. The mutant virus is less stable than the parental strain, indicating that the pH threshold for membrane fusion has decreased. The mutant virus cannot enter A549 wild-type cells when macropinocytosis is inhibited. Additionally, both the parental and mutant virus are blocked when macropinocytosis is inhibited in the SLC35A1 KO cells. Macropinocytosis becomes a primary mode of cell entry when no sialic acid is present. This work expands our understanding of IAV evolution.

39.) Audrey Hake, Richard Roller

Biochemical Investigation of AlphaFold-Predicted Dimerization of ICP22

Microbiology & Immunology, 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. Since the ratio of monomeric to dimeric ICP22 might have been affected by the denaturation/renaturation preparation method, ongoing efforts are focused on producing a soluble form by fusion to

maltose binding protein and by production of truncated forms of ICP22 that lack some intrinsically disordered sequences. Determination of ICP22 oligomerization state will allow specific mutagenesis to determine the functional significance of the oligomer.

40.) Levi Rosenboom, Elizabeth Wagstaff, Sabrina Tan

Determining host and viral factors directing Human Polyomavirus JC cell tropism

Microbiology & Immunology, University of Iowa

The Human Polyomavirus JC (JCPyV) infects a large portion of the human population and resides in the kidney asymptomatically. In patients with severe immunosuppression, it is known to cause Progressive Multifocal Leukoencephalopathy (PML), an often fatal demyelinating disease of the central nervous system (CNS) that has no cure. JCPyV has a Non-Coding Control Region (NCCR) in its genome, which binds host factors and is involved in gene expression. It is possible that the NCCR responds to CNS cells differentially, which could explain some of the symptoms of PML. In clinical strains isolated from the brain of PML patients, the NCCR is rearranged, but it is unknown how the rearrangements affect the development of PML. We hypothesize that rearrangement of the NCCR is responsible for increased infection in brain cells. To test this, primary Oligodendrocytes, Astrocytes, and Renal Proximal Tubule Epithelial Cells (RPTEC) were infected with variants of the virus with either rearranged (MAD1) or non-rearranged (Archetype) NCCRs. The cells were also treated with different inflammatory cytokines (IFN-γ, TNF-α, and IL-1β) to characterize an interaction between the NCCR and the host cells in an inflammatory state. After infection, the cells and supernatant were collected, and the DNA was analyzed using digital nanoplate polymerase chain reaction (dPCR). Preliminary data suggests that inflammatory cytokines increase infection with the Archetype NCCR in Astrocytes. Infection in both Astrocytes and RPTEC with the MAD1 NCCR appeared to be unaffected by the inflammatory cytokines. Oligodendrocyte infection increased in inflammatory conditions regardless of the NCCR type. This suggests that NCCR rearrangement may aid in immune evasion in CNS cells. The results of this experiment will help to define how the NCCR is involved in the infection of CNS cells by JCPyV, and provide crucial knowledge on the mechanism of PML, which may lead to more effective treatment methods.

41.) Ella Morgan, Kaho Matsumoto, Alex Kleinpeter

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

Microbiology & Immunology, University of Iowa

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.

42.) Noah Schuster, Lu Tan, Abhishek Verma, & Stanley Perlman

Evaluating Olfactory and Neurological Dysfunction Following Infection with MERS-CoV

Microbiology, University of Iowa

Individuals who recover from COVID-19 frequently report a variety of symptoms that persist for weeks to months, or even years, after resolving the acute infection. Although substantial effort has focused on defining mechanisms that drive such complications, there remains less work examining if similar outcomes happen after infections with other CoVs. In a manner similar to our previous work with SARS-CoV-2, we utilized a mouse-adapted strain of MERS-CoV that induces severe respiratory disease without directly infecting the brain. In our model, mice exhibited severe lung disease; however, infection of the olfactory epithelium (OE) had occurred as well. Notably, viral infection was localized to sustentacular cells and reached peak titers of ~10^5 PFU/mL at 4 days post-infection (dpi), accompanied by a clear antiviral response and pathological changes. A further consequence of infection was a significant decrease in olfactory receptor expression, with variable recovery over time. Interestingly, the long-term upregulation of stress markers was observed at both 35 and 65 dpi, suggesting a persistent state of stress in the OE. Phenotypically, mice exhibited signs of anosmia, as assessed by a buried-food test at early and late timepoints post-infections. In the olfactory bulb (OB), altered expression of tyrosine hydroxylase (TH) occurred during and after infection, suggesting possible changes among resident dopaminergic neurons. We also observed adjusted TH expression changes within the substantia nigra (SN) at 35 and 65 dpi, suggesting impairments of dopaminergic neurons in this region as well. Collectively, these findings demonstrate that MERS-CoV infection can induce acute and long-term alterations in the olfactory and neurological systems of mice, paralleling observations made with SARS-CoV-2. Ongoing studies aim to define the consequences of these alterations using additional behavioral assays, clarifying the role of the immune response in driving neurological changes, and to identify determinants for enabling a robust MERS-CoV infection of the OE.

43.) Libby Nyberg, Kyle Rapchak, Calyssa Huff, Jessica M. Tucker

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

Microbiology & Immunology, University of Iowa

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). Excitingly, we observed increased MHV68 titers from cells knocked down for Brf1 compared to control cells treated with non-targeting siRNA. To substantiate these findings, we set out to validate the effect of Brf1 knockdown on Pol III transcripts in our experiment. There are three distinct promoter types that Pol III uses to make transcripts. Type I and type II promoters depend on Brf1 to recruit Pol III, and so we hypothesized that transcript levels for type I and type II promoters would be diminished by Brf1 knockdown. Type III promoters do not use Brf1 and thus transcript levels should be unaffected by Brf1 knockdown. As predicted, we saw decreased expression of type II transcripts, however, type I transcripts remained mostly unchanged, as measured by RT-qPCR of 5S and pre-tRNA-Leu. Surprisingly, type III transcripts, including 7SK and U6, were found to have increased expression levels over the course of MHV68 infection, which was unexpected, and suggests that perhaps Brf1 knockdown permits higher levels of recruitment to RNA polymerase III to Brf1-independent genes. To further investigate how different Pol III transcripts impact MHV68 replication in our system, we will overexpress individual noncoding RNAs and measure their impact on MHV68 viral titer. Understanding the role that Pol III transcripts play in gammaherpesvirus infection may help us to appreciate why viral infection results in an upregulation of Pol III activity, and how non-coding RNAs play a role in host-viral interactions.

44.) Olivia Heller, Sheila Gonzales, Jessica Tucker

Conformation of cell lines for rescue experiment of Tsen2/Clp1

Microbiology & Immunology, University of Iowa

Murine gammaherpesvirus (MHV68) is a large double-stranded DNA virus genetically related to human Kaposi sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV). Our previous research found significant dysregulation of transfer RNAs (tRNAs) in response to MHV68, suggesting that tRNA control may be important during infection. Specifically, host pre-tRNAs are significantly upregulated in their expression during an MHV68 infection. Furthermore, we demonstrated that several of these upregulated pre-tRNAs undergo cleavage, resulting in the formation of tRNA fragments (tRFs). The presence of these tRFs suggest that MHV68 not only influences tRNA transcription but also alters tRNA processing pathways within the host cell. We have explored the role of two host proteins that participate in tRNA processing, Tsen2 and Clp1. Tsen2 is an endonuclease that cleaves at the 5’ splice site of the pre-tRNA introns, and Clp1 is an RNA kinase with its overall role in tRNA processing unclear. We found that siRNA-mediated knockdown of Tsen2 and Clp1 have opposing effects on both tRF accumulation and viral replication. While Clp1 knockdown shows an increase in tRF

accumulation and a decrease in overall viral titers, Tsen2 knockdown shows decreased tRF accumulation and increased titers at a low MOI. Overall, we hypothesize the proviral effect of Clp1 and the antiviral effect of Tsen2 is linked to their roles in tRNA processing. To further test our hypothesis, we will perform rescue experiments which will test for off-targeting effects of siRNAs used to knockdown endogenous Tsen2 or Clp1. We are currently producing overexpression cell lines by transducing NIH 3T3 cells with a codon optimized (and thus siRNA-resistant) Tsen2 or Clp1 overexpression lentiviruses, or an empty vector as a control. The cell lines are being produced with or without a FLAG-tag on the C-terminal end of each protein. We will validate exogenous expression of Tsen2 or Clp1 by probing for the FLAG-tagged protein via a western blot. Next, we will treat cell lines with non-targeting or Tsen2-/Clp1- targeting siRNAs. We expect that cell lines overexpressing codon optimized Tsen2 or Clp1 will rescue the phenotype induced by Tsen2- or Clp1- targeting siRNAs. We will additionally use these cell lines to test whether there is an overexpression phenotype for Tsen2 and Clp1. Future work is aimed at mechanistically determining how Tsen2 and Clp1 influence MHV68 replication, and whether canonical tRNA processing is required for these effects.

45.) Harli Herrington, Calyssa Huff, Allison Didychuk, Jessica Tucker

Characterizing a MHV68 Dual Reporter Virus to Identify Viral Stages Associated With tRNA Fragment Biogenesis

Microbiology & Immunology, University of Iowa

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.

46.) Graham D Crippen, Alex TH Cocker, Elena J Sasu, Amanda M Dudek

Defining Host Genetic Contributions to NK Cell Regulation in HIV Using CRISPR-Cas9-mediated Allele Swapping

Microbiology & Immunology, University of Iowa

Although individuals with HIV who have access to antiretroviral therapy (ART) can have an essentially normal life expectancy, HIV remains incurable and those infected must rely on consistent and continuous ART. However, a small subset of individuals living with HIV are able to naturally suppress the virus without ART. Understanding how to induce the immune response seen in these individuals, termed Elite Controllers and Long-Term Non-Progressors (LTNPs), could inform immune-based therapies which reduce reliance on ART.

This project will focus on determining whether host genetic differences are responsible for immune regulation of HIV infection. HIV is able to manipulate immune recognition pathways through the regulation of HLA genes, making it difficult to determine whether immune control is mainly due to viral influences or host genetics. Currently in the field, there is major uncertainty surrounding the role of natural killer (NK) cells during immune control, as NK cells are typically regulated through HLA gene expression.

To address this, we will utilize CRISPR-Cas9 gene editing to swap specific HLA regions between donor T cells and measure NK cell activity. This isolated switching of donor alleles would provide insight into whether host immunogenetics alone or viral manipulation mainly dominates NK cell inhibition. Defining this relationship is an important step toward ascribing a genotype to Elite Controllers and LTNPs and building upon current infection models.