

'Wisc-e-sota'

6th Joint UMN-UW Virology Training Grant Symposium



October 11th, 2018

Thursday evening: Reception, keynote lecture, & career development panel discussion

October 12th, 2018

Radisson Hotel, La Crosse, WI



NIH T32-supported virology training programs at:

University of Wisconsin-Madison

University of Minnesota-Twin Cities

Featuring talks and poster sessions by students, postdocs and
faculty

*Sponsored by NIH grants T32 AI083196, T32 AI078985, and the
Department of Microbiology at the University of Wisconsin-La Crosse*



*In memory of Dr. Michael P. Murtaugh, Professor, University of Minnesota-Twin Cities,
who sadly passed away on September 18th, 2018*



6th Annual **Wisconsin** Virology Symposium

Thursday-Friday, October 11-12, 2018

Radisson Hotel

La Crosse, Wisconsin

Oral Presentations

Talk abstract numbers in left column

October 11, 2018
(Thursday)

5:30-7:00pm Registration

6:55-7:00pm Welcome and opening remarks

1 7:00-8:00pm Opening talk – Elizabeth Wright (UW)

8:00-9:30pm Career Development Panel Discussion
9:30-11:00pm Evening Reception

October 12, 2018
(Friday)

Convener: Tony Dawson (UW)

7:00-8:30am Check-in and poster set-up

7:30-8:30am Breakfast

2 8:30am Julia Davydova (UMN)

3 9:00am Nick Van Sciver (UW)

4 9:15am Elizabeth Fay (UMN)

9:30-10:00am Morning break

Convener: Heather Hanson (UMN)

5 10:00am Amelia Haj (UW)

6 10:15am Yumeng Zhang (UMN)

7	10:30am	Megan Spurgeon (UW)
	11:00am	<p>“3-Minute Elevator Pitch”</p> <ul style="list-style-type: none"> • Lisa Koodie (UMN) • Tao Wei (UW) • Carlos Ribeiro (UMN) • Sanath Janaka (UW) • Morgan Brisse (UMN) • Emily Albright (UW)
	11:30am-12:30pm	Career Development Lunch Table Discussions
	12:30-2:00pm	<p>Poster Session and Group Photo</p> <p><i>Convener: Blue-Leaf Cordes (UW)</i></p>
8	2:00pm	Fang Li (UMN)
9	2:30pm	Anna Heffron (UW)
10	2:45pm	Nadine Shaban (UMN)
		<i>Convener: Lisa Fazzino (UMN)</i>
11	3:00pm	Gloria Larson (UW)
12	3:15pm	Allison Siehr (UMN)
13	3:30pm	Shannon Kenney (UW)
	4:00pm	Closing Remarks and Wisc-e-sota Prizes Announced!

Poster Presentations

1. “Differential Splicing of ANP32A in Birds Alters its Ability to Stimulate RNA Synthesis by Restricted Influenza Polymerase”
Steven F. Baker, Mitchell P. Ledwith, Andrew Mehle (UW)
2. “Exosomes Secreted by Microglia During (Virus-Induced Demyelinating Disease Contribute to Neuroinflammation”
Nhungoc Luong and Julie K. Olson (UMN)
3. “Ultrastructural Studies of RSV Assembly by Cellular Cryo-Electron Microscopy”
Jae Yang, Zunlong Ke, Rebecca S. Dillard, Tatiana Chirkova, Christopher C. Stobart, Cheri M. Hampton, Joshua D. Strauss, Martin L. Moore, Larry J. Anderson, Elizabeth R. Wright (UW)
4. “TCR Affinity Influences Helper T Cell Differentiation by Regulating Eef1e1 and Gbp2 Expression”
Dmitri I. Kotov, Jason S. Mitchell, Thomas Pengo, Christiane Ruedl, Sing Sing Way, Ryan A. Langlois, Brian T. Fife, Marc K. Jenkins (UMN)
5. “Characterizing the Putative Antiviral Factor SYNCRIP During Influenza Virus Infection”
Katherine Amato, Vy Tran, Andrew Mehle (UW)
6. “Efficient Systemic Treatment with Mesothelin-Targeted Oncolytic Adenovirus in Patient-Derived Xenograft (PDX) Model of Pancreatic Cancer”
Mizuho Sato-Dahlman, Yoshiaki Miura, Praveensingh Hajeri, Hideki Yoshida, Kari Jacobsen, Chikako Yanagiba, and Masato Yamamoto (UMN)
7. “Human APOBEC3B and HPV E6 and E7 Oncoproteins in Tumorigenesis in Vivo”
Cameron Durfee, Lindsay Larson, Emily Law, Reuben Harris (UMN)
8. “Human Cytomegalovirus Enters Latency through Macropinocytosis and Endocytosis”
Jeong-Hee Lee, Joseph R. Pasquarella, and Robert F. Kalejta (UW)
9. “Guinea Pig Cytomegalovirus Infects the Amnion and Induces Pathways Associated with Preterm Birth”
Dira S. Putri, Claudia Fernandez-Alarcon, Juan E. Abrahante, Mark R. Schleiss, and Craig J. Bierle (UMN)
10. “Human Cytomegalovirus UL138 Inhibits STING to Silence Viral Lytic-Phase Transcription and Counteract Host Innate Immune Defenses During Latency.”
Emily R Albright and Robert F Kalejta (UW)

11. “Bacteriophage Attack Can Break Metabolic Dependency and Indirectly Affect Non-Host Species”
Lisa Fazzino, Jeremy Anisman, Jeremy Chacón, Rick Heineman, William R. Harcombe (UMN)
12. “Hypoxia Mimics Target EBV-Positive Gastric Cancer Cells in Lytic Induction Therapy”
Blue-Leaf A Cordes, Richard J Kraus, Andrea Bilger, James C Romero-Masters, Parita A Patel, Paul F Lambert, Shannon C Kenney, and Janet E Mertz (UW)
13. “Role of Tuft Cells During Influenza A Infection”
Shanley Roach, Jessica Fiege, Ian Stone, and Ryan Langlois (UMN)
14. “The Influence of Keratin 17 on MmuPV1-Induced Pathogenesis”
Aayushi Uberoi, Wei Wang, Megan Spurgeon, Xiang-Yang Xue, Vladimir Majerciak, Pierre Coulombe, Zhi-Ming Zheng, Paul F. Lambert (UW)
15. “Development of a Pichinde Virus-Based Vaccine Vector for the Treatment of Tuberculosis”
Sophia Vrba, Wanjun Zhu, Vikram Verma, Anna Tischler, and Yuying Liang (UMN)
16. “Substitutions in SIV Nef that Selectively Impair Anti-SERINC5 Activity”
Sanath Kumar Janaka, Alexandra V Palumbo, Aidin Tavakoli-Tameh, David T Evans (UW)
17. “Characterization of Species-specific Molecular Determinants of Innate Immune Suppression in Pathogenic Arenavirus Infection”
Morgan Brisse, Junji Xing, Junjie Shao, Yuying Liang, Hinh Ly (UMN)
18. “Evidence for a Novel Species-specific Cellular Barrier to HIV-1 Gene Expression”
Sofia Romero, Edward L. Evans III, Nathan M. Sherer (UW)
19. “An Improved Oncolytic Adenovirus for Radioiodine Therapy and Imaging”
Lisa Koodie (UMN)
20. “Potential Interactions Between HIV-1 and HPV”
Soyeong Park, Denis Lee, Paul Lambert, Nate Sherer (UW)
21. “The Actin Cortex as a Physical Barrier in Retroviral Assembly”
C Isaac Angert, Morgan E Meissner, Louis M Mansky, Joachim D Mueller (UMN)
22. “HIV-1 Vif Induces a Prolonged Metaphase Cell Cycle Arrest Characterized by Pronounced Mitotic Spindle Defects and Centrosome Amplification”
Edward L. Evans III, Christopher Bastin, Jordan T. Becker, Laraine M. Zimdars and Nathan M. Sherer (UW)

23. “Subcellular Localization of APOBEC3 Proteins Regulate Interactions with and Restriction of HIV-1”
Jordan T. Becker, Nathan M. Sherer, & Reuben S. Harris (UMN)
24. “Elucidating HIV-1 RNA Interactomes”
Rachel A. Knoener, Jordan T. Becker, Edward L. Evans III, Bayleigh Benner, Mark Scalf, Lloyd M. Smith, Nathan M. Sherer (UW)
25. “Analysis of HTLV-1 RNA Packaging and its Interrelationship with RNA Dimerization”
Ruth J. Blower, Heather M. Hanson, Weixin Wu, Joshua Hatterschide, William A Cantara, Karin Musier-Forsyth and Louis M Mansky (UMN)
26. “An in vitro System for Studying Mouse Papillomavirus”
Simon Blaine-Sauer, Denis Lee, Andrea Bilger, and Paul F. Lambert (UW)
27. “Perturbing the HIV-1 Programmed Ribosomal Frameshift Site: Translational Regulation Dictates Transcript Fate and Gag-Pol Incorporation”
Bayleigh E. Benner, Jordan T. Becker, Pablo Garcia-Miranda, Samuel E. Butcher, and Nathan M. Sherer (UW)
28. “Development of a New Model for Papillomavirus-induced Head and Neck Cancer Using Mouse Papillomavirus”
Tao Wei, Andrea Bilger, Paul F. Lambert (UW)
29. “Influenza Virus Repurposes the Canonical Antiviral Protein IFIT2 as a Pro-Viral Effector to Promote Translation of Viral mRNAs”
Vy Tran, Mitchell P. Ledwith, Thiprampai Thamamongood, Christina A. Higgins, Shashank Tripathi, Max W. Chang, Christopher Benner, Adolfo Garcia-Sastre, Martin Schwemmle, Adrianus C. M. Boon, Michael S. Diamond, Andrew Mehle (UW)
30. “Polymorphisms in Rhesus Macaque Tetherin are Associated with Differences in Acute Viremia in SIV Δ Nef-Infected Animals”
Sanath Kumar Janaka, Aidin Tavakoli-Tameh, William J. Neidermyer Jr., Ruth Serra-Moreno, James A. Hoxie, Ronald C. Desrosiers, R. Paul Johnson, Jeffrey D. Lifson, Steven M. Wolinsky and David T. Evans (UW)
31. “The suitability of the porcine model for assessing performance of the species B adenoviruses.”
Matthew Robertson, Lisa Koodie, Malavika Chandrashekar, Kari Jacobsen, George Ruth, Michele Dunning, Praveensingh Hajeri, Richard Bianco, Julia Davydova (UMN)
32. “Phosphorylation Regulates Influenza Virus Polymerase Function”
Anthony R Dawson, Arindam Mondal, Elyse Freiburger, Joshua J. Coon, and Andrew Mehle (UW)

33. “A Novel Chemotype as Hepatitis B Virus Capsid Assembly Effectors”
Jing Tang, Carlos J. A. Ribeiro, Andrew D. Huber, Jayakanth Kankanala, Jiashu Xie, Stefan G. Sarafianos and Zhengqiang Wang (UMN)

34. “Type 2 EBV Induces Highly Lytic B-cell Lymphomas in a Cord Blood-Humanized Mouse Model”
James C Romero-Masters, Shane M Huebner, Makoto Ohashi, Elizabeth Barlow, Eric Johannsen, Shannon C Kenney (UW)

Abstracts - Oral Presentations

1. "Structural Studies of Measles Virus Assembly"
Elizabeth Wright

We use cryo-electron tomography (cryo-ET) to study the principles governing paramyxovirus assembly in MeV-infected human cells. The three-dimensional (3D) arrangement of the MeV structural proteins including the surface glycoproteins (F and H), matrix protein (M), and the ribonucleoprotein complex (RNP) were characterized at numerous stages of virus assembly and budding, and in released virus particles. The M protein was observed as an organized two-dimensional (2D) para-crystalline array associated with the membrane. A two-layered F-M lattice was visualized suggesting that interactions between F and M may coordinate processes essential for MeV assembly. The RNP complex associates with and in close proximity to the M lattice. In our model, the M lattice facilitates the well-ordered incorporation and concentration of the surface glycoproteins and the RNP at sites of virus assembly.

2. "Oncolytic adenovirus: progress and challenges of clinical development"
Julia Davydova

Oncolytic adenoviruses, designed to destroy cancer cells as a part of virus replication, are promising anti-cancer agents as they have the potential to overcome limitations of conventional therapies. The current interest in virotherapy reflects both the scientific progress in engineering novel therapeutic viral vectors and recent success in clinical development. Indeed, FDA approval in 2015 of the first oncolytic virus, Talimogene laherparevec (T-VEC), brings us into a new era of virotherapy. The first clinical successes have also highlighted the importance of the immune component in achieving a therapeutic effect. After a turbulent past the stigma related to gene therapy approaches has been replaced by the wide acceptance of the feasibility of *Oncolytic Virotherapy* and *Immunotherapy*. Here, the advances and complexity of oncolytic adenovirus therapies, as well as the challenges and hurdles which still need to be overcome, will be discussed.

3. "Np63 Negatively Regulates Epstein-Barr Virus Lytic Reactivation in Epithelial Cells"
Nick Van Sciver, Dhananjay Nawandar, Makoto Ohashi, Ahmed Ali, Denis Lee, Kathleen Makielski, Paul Lambert, Eric Johannsen and Shannon C. Kenney

Epstein-Barr virus (EBV) lytically infects normal differentiated oral epithelial cells, while EBV is predominantly latent in undifferentiated nasopharyngeal carcinomas (NPC). We have previously shown that lytic EBV protein expression is confined to the more differentiated cell layers in rafted EBV-infected normal oral keratinocyte cells (NOKs). We also demonstrated that the epithelial cell

differentiation factors, KLF4 and BLIMP1, synergistically induce lytic EBV reactivation by binding and activating both EBV immediate-early gene promoters (BZLF1 and BRLF1). Furthermore (due to the hypo-methylated state of the EBV genome in normal oral epithelial cells) we found that only BRLF1 (R) expression, but not BZLF1 (Z) expression, can induce lytic EBV reactivation in NOKs. However, cellular factors that repress EBV lytic reactivation in the basal layer of epithelial cells, where EBV remains latent, have not been identified. Np63 (a p53 family member) is required for proliferation and survival of basal epithelial cells and is overexpressed in NPC tumors. Here we have examined whether Np63 represses EBV lytic reactivation in epithelium. We show that Np63 depletion increases expression of lytic viral proteins in both EBV-infected NOKs and EBV-infected HONE cells. Additionally, we demonstrate that over-expression of Np63 reduces lytic viral reactivation in EBV-infected NOKs, as well as in EBV-infected gastric (AGS) cells, and find that this inhibitory Np63 effect is independent of cellular differentiation and does not require p53. Finally, we show Np63 blocks the ability of the immediate-early protein, R, to induce lytic reactivation in epithelial cells. These findings demonstrate that expression of Np63 in normal basal epithelial cells and in undifferentiated nasopharyngeal carcinomas promotes EBV latency. Conversely, the loss of Np63 expression that occurs during normal epithelial cell differentiation helps to promote lytic EBV reactivation in a differentiation-dependent manner.

4. “Distinct Antiviral Signatures Revealed by the Magnitude and Round of Influenza Virus Replication in Vivo”
Elizabeth Fay, Louisa E. Sjaastad, Stephanie L. Aron, Jessica K. Fiege, Marissa G. Macchietto, Ian A. Stone, Matthew W. Markman, Steven Shen, and Ryan A. Langlois

Influenza virus has a broad cellular tropism in the respiratory tract. Infected epithelial cells sense the infection and initiate an antiviral response. To define the antiviral response at the earliest stages of infection we used a series of single cycle reporter viruses. These viral probes demonstrated cells in vivo harbor a range of virus replication. Transcriptional profiling of cells supporting different levels of replication demonstrated tiers of interferon-stimulated gene responses. Uninfected cells and cells with blunted replication expressed a distinct and potentially protective antiviral signature, while cells with high replication induced a unique reserve set of antiviral genes. We used these single cycle reporter viruses to determine the antiviral landscape during virus spread, which unveiled disparate protection of epithelial cell subsets mediated by interferon in vivo. We have also begun in vitro characterization of a single cycle virus that can only undergo primary transcription. We have demonstrated tiered activation of antiviral genes in response to varying levels of viral replication. This virus has shown potential as a tool to examine the early innate response to infection. Together these results highlight the complexity of virus-host interactions within the infected lung and suggest that magnitude and round of replication tune the antiviral response.

5. “High-Throughput Identification of MHC Class I Binding Peptides in SIV/SHIV with an Ultradense Peptide Array”

Amelia K. Haj, Meghan E. Breitbach, Mariel S. Mohns, Gage K. Moreno, Nancy Wilson Schlei, Victor Lyamichev, Jigar Patel, David H. O'Connor

Developing peptide vaccines against viruses such as HIV requires identifying and measuring the magnitude of epitope-specific CD8+ T cell responses. Conventional discovery methods have been used to identify several CD8+ T cell epitopes in simian immunodeficiency virus; however, these methods are labor-intensive and do not scale well. Here, we accelerate this process by using an ultradense peptide array as a high-throughput tool for viral epitope identification. In a single experiment, we directly assess the binding of four common rhesus macaque MHC class I molecules Mamu-A1*01, -A1*02, -B*08, and -B*17 to approximately 61,000 8-mer, 9-mer, and 10-mer peptides derived from the full proteomes of 82 simian immunodeficiency virus (SIV) and simian-human immunodeficiency virus (SHIV) isolates. Several CD8+ T cell responses restricted by these four MHC molecules have already been identified in SIVmac239, an SIV strain commonly used in research studies; 27-48% of these epitopes are found in the top 192 SIVmac239 peptides with the most intense MHC binding signals in our experiment. To assess whether our method identified putative novel CD8+ T cell epitopes, we are now validating the method by IFN- γ ELISPOT assay. We anticipate that this high-throughput identification of peptides that bind rhesus MHC class I molecules will enable more efficient CD8+ T cell response profiling for vaccine development, and in particular will be useful in streamlining the process of peptide vaccine development for understudied viruses and those with complex proteomes.

6. “Enhancing Hepatitis B Virus Mutagenesis: an Approach for Reducing Virus Infectivity?”

Yumeng Zhang, Steven E. Patterson, Louis M. Mansky

University of Minnesota-Twin Cities, Institute for Molecular Virology, Minneapolis, MN 2University of Minnesota-Twin Cities, Center for Drug Design, Minneapolis, MN About 1 million people die annually from hepatitis B virus (HBV)-associated liver cancer, which emphasizes the need of more effective treatments or a cure in the face of existing HBV vaccines. Reverse transcriptase (RT) is an essential enzyme for the replication of both human immunodeficiency virus type 1 (HIV-1, a retrovirus) and HBV (a hepadnavirus). Recent studies have shown two types of small molecules i.e., viral mutagens (i.e. 5-aza-dC) and ribonucleotide reductase inhibitors (RNRIs) (i.e. gemcitabine) possess anti-HIV-1 activity, mainly through targeting reverse transcriptase (RT) and extinguishing viral infectivity via enhanced viral mutagenesis. In this study, we sought to investigate whether such a therapeutic strategy could be deployed for treating HBV infection. We observed that HBV-producing cells treated with either 50 μ M 5-aza-dC or 50 nM gemcitabine led to a 90% reduction in viral infectivity. 5-aza-dC and gemcitabine were shown to inhibit HBV rcDNA synthesis without affecting cell viability. As predicted, the treatment of 5-aza-dC not only increased the HBV rcDNA mutation

frequency, but also significantly enhanced the level of G-to-C transversion mutations. Furthermore, gemcitabine was observed to potentiate 5-aza-dC in reducing HBV rcDNA synthesis and lead to a higher mutation frequency compared that of 5-aza-dC alone. These observations indicate that the antiviral effects of 5-aza-dC and/or gemcitabine correlated with a late step in HBV replication (i.e., reverse transcription). Intriguingly, we also observed that cccDNA formation and transcription were diminished by 5-aza-dC, but was associated with reduced synthesis and transcription of the nick/gap region rather than by enhanced viral mutagenesis. These observations are likely attributed to the still elusive cellular enzymes responsible for the conversion of rcDNA to cccDNA. Taken together, our results demonstrate that virus infectivity can be extinguished by viral mutagens, and this antiviral activity can be intensified by RNRIs. We propose a model in which viral mutagens like 5-aza-dC can induce mutagenesis during rcDNA formation, but diminish viral DNA synthesis during the conversion of rcDNA to cccDNA. To our knowledge, these observations represent the first demonstration of a therapeutic small molecule treatment possessing a dual mechanism to reduce HBV infectivity by enhanced viral mutagenesis as well as by reducing viral DNA synthesis.

7. “Development of an In Vivo Infection Model to Study Papillomavirus Infection and Neoplastic Disease in the Female Reproductive Tract”

Megan E. Spurgeon, Aayushi Uberoi, Stephanie McGregor, Tao Wei, and Paul F. Lambert

Papillomaviruses exhibit species-specific tropism and cause several forms of cancer in humans and other animals. The recent discovery of a murine papillomavirus (MmuPV1) in immunodeficient FoxN1nu/nu mice provides the opportunity to study papillomavirus infections in a tractable, in vivo laboratory model. MmuPV1 can infect and cause disease in the cutaneous epithelium, as well as the mucosal epithelia found in the oral cavity and anogenital tract. We have established a murine model of MmuPV1 infection and neoplastic disease in the female reproductive tracts of immunocompetent FVB/N (wild-type) mice. We observed low-grade lesions in the reproductive tract of wild-type animals infected with MmuPV1 for 4 months, and mice infected for 6 months developed significantly worse disease, including squamous cell carcinoma (SCC). Our previous studies identified estrogen as a cofactor for cervical carcinogenesis in HPV-transgenic mice, and we recently reported that ultraviolet radiation (UVR) makes wild-type mice highly susceptible to MmuPV1-induced cutaneous disease. We therefore tested the contribution of estrogen and/or UVR to cervicovaginal disease. Exogenous estrogen treatment significantly increased the severity of disease in the reproductive tracts of MmuPV1-infected mice compared to MmuPV1 infection alone, and 6 months of estrogen treatment was sufficient to promote SCC development. Nearly 70% of MmuPV1-infected mice treated with both UVR and exogenous estrogen developed cervicovaginal cancers. We have also used our MmuPV1 cervicovaginal infection model to establish an in vivo model of papillomavirus sexual transmission. These murine models will provide a

platform for fundamental studies in papillomavirus sexual transmission and cervicovaginal neoplastic disease.

8. “Structure, Function, and Evolution of Coronavirus Spike Proteins”

Fang Li

The coronavirus spike protein is a multifunctional molecular machine that mediates coronavirus entry into host cells. It first binds to a receptor on the host cell surface through its S1 subunit and then fuses viral and host membranes through its S2 subunit. Two domains in S1 from different coronaviruses recognize a variety of host receptors, leading to viral attachment. The spike protein exists in two structurally distinct conformations, prefusion and postfusion. The transition from prefusion to postfusion conformation of the spike protein must be triggered, leading to membrane fusion. We have extensively investigated the structures and functions of coronavirus spike proteins, illustrating how the two S1 domains recognize different receptors and how the spike proteins are regulated to undergo conformational transitions. Our research has also illustrated how coronavirus spikes have achieved structural and functional diversity through evolution to guide viral entry into host cells.

9. “Profiling Antibody Responses to Zika Virus Proteins in Macaques”

Anna S. Heffron, Emma L. Mohr, David Baker, Amelia K. Haj, Connor R. Buechler, Adam Bailey, Dawn M. Dudley, Christina M. Newman, Mariel S. Mohns, Michelle Koenig, Meghan E. Breitbart, Mustafa Rasheed, Laurel M. Stewart, Jens Eickhoff, Richard S. Pinapati, Erica Beckman, Hanying Li, Jigar Patel, John C. Tan, David H. O’Connor

The specificity of the antibody response against Zika virus (ZIKV) is not well-characterized. This is due, in part, to the antigenic similarity between ZIKV and closely related dengue virus (DENV) serotypes. Since these and other similar viruses co-circulate, are spread by the same mosquito species, and can cause similar acute clinical syndromes, it is difficult to disentangle ZIKV-specific antibody responses from responses to closely-related arboviruses in humans. We used high-density peptide microarrays to profile anti-ZIKV antibody reactivity in pregnant and non-pregnant macaque monkeys with known exposure histories and compare these results to reactivity following DENV infection. We compared cross-reactive binding of ZIKV-immune sera to the full proteomes of 28 arboviruses. We independently confirmed a purported ZIKV-specific IgG antibody response targeting ZIKV nonstructural protein 2B (NS2B) that was recently reported in ZIKV-infected people and we showed that antibody reactivity in pregnant animals can be detected as late as 127 days post-infection (dpi). However, we also showed these responses wane over time, sometimes rapidly, and in one case the response was elicited following DENV infection in a previously ZIKV-exposed animal. These results suggest that epidemiologic studies assessing seroprevalence of ZIKV immunity using linear epitope-based strategies will remain challenging to interpret due to potential for false positive results.

However, the method we used demonstrates the potential for rapid profiling of proteome-wide antibody responses to a myriad of neglected diseases simultaneously and may be especially useful for distinguishing antibody reactivity among closely related pathogens.

10. “Structural Mechanisms of the Antiviral APOBEC3 Enzymes: DNA, RNA, and HIV1-Vif”
Nadine M. Shaban, Ke Shi, Michael A. Carpenter, Jiayi Wang, Kate V. Lauer, Hideki Aihara, and Reuben S. Harris

APOBEC3s are single-stranded DNA cytosine deaminases involved in restricting the replication of HIV-1 and other viral pathogens. At least four APOBEC3s (APOBEC3F, APOBEC3G, APOBEC3D, and APOBEC3G) are capable of restricting HIV1 by deamination of viral DNA during reverse transcription and/or via a deamination independent mechanism involving RNA. To counter this robust antiviral activity, HIV1 evolved its accessory protein Vif to bind to the APOBEC3s and target them for proteasomal degradation via the E3-ligase complex. In this interplay between host and virus, there are three critical interactions that must occur: APOBEC3 interaction with RNA, APOBEC3 interaction with DNA, and APOBEC3 interaction with Vif. Here, we describe the current understanding of these physical interactions and present our recent resolution of the APOBEC3-DNA structure, APOBEC3-RNA structure, and ongoing work with the APOBEC3-HIV1-Vif-E3-ligase complexes.

11. “The Host Factor EPS8 Enhances Influenza Virus Entry”
Gloria P. Larson, Vy Tran, Shuiqing Yu, Vy Tran, Christina A. Higgins, Danielle Smith, Jens H. Kuhn, and Andrew Mehle

Influenza A virus (IAV), like all viruses, must co-opt host cellular machinery to support successful replication. Several screening methodologies have mapped interactions between host cellular proteins and IAV, identifying numerous host factors with the potential to regulate IAV infection. These methods frequently rely on endpoint phenotypes, manipulation of the host cell, and stable interactions between viral and cellular proteins. To overcome these limitations, we utilized a gene correlation analysis approach. This method correlates IAV infection susceptibility with naturally occurring differences in gene expression among different cell lines. Our screen identified the host factor epidermal growth factor receptor (EGFR) pathway substrate 8 (EPS8) as a high confidence candidate with pro-viral activity. EPS8 is a multifunctional protein involved in signaling pathways, actin remodeling, and endocytic processes. Immunofluorescence assays showed that EPS8 levels were elevated in infected cells but not in uninfected bystander cells, consistent with the results of our screen. We confirmed a pro-viral role for EPS8 by showing that EPS8 overexpression increased viral gene expression and titer. Knockout of EPS8 decreased viral infection and replication. We then probed the role of EPS8 at various steps throughout the viral life cycle. These experiments revealed that EPS8 functions during the process of viral entry,

after virion attachment and before viral gene transcription. Additionally, this process is independent of the route of entry, suggesting EPS8 functions after transit through the endosome to enhance uncoating of IAV virions or import of viral ribonucleoprotein complexes into the nucleus. Using this alternative screening strategy, we identified host factors involved in IAV infection and defined a previously unknown functional impact of EPS8 on IAV replication. Our work elucidates additional molecular mechanisms by which IAV co-opts host cellular machinery to support successful infection, providing insight into potential anti-viral drug targets.

12. “Protein Conjugated Gold Nanoparticles for HIV-1 Entry Inhibition”
Allison Siehr, Bin Xu, Ronald A. Siegel, Wei Shen

Infection of HIV-1 into T-cells is mediated through interactions of the viral glycoprotein gp120 and the CD4 T-cell receptor. One possible approach to treating HIV-1 is to block the function of gp120, inhibiting its interaction with CD4 and ultimately impairing HIV-1 infection. To this end, we are developing a novel antiviral platform and assessing its efficacy to inhibit viral entry of HIV-1. As an antiviral platform, we have developed a gold nanoparticle (GNP) based system that exhibits multivalency and in situ assembly to target gp120 with high binding avidity. Each GNP is modified with two recombinant leucine zipper proteins, denoted as A and B. The proteins contain a cysteine, which is used to covalently attach the proteins to GNPs through gold-thiol chemistry. The protein labelled cysA is fused with an anti-gp120 peptide, which is displayed on the exterior of the modified GNP. The presence of the multiple anti-gp120 contributes to multivalent targeting after which in situ self-assembly is expected to be initiated by the heterodimerization of cysA and Bcys on neighboring GNPs. The modified GNPs are expected to exhibit high affinity binding to gp120 further stabilized by the in situ self-assembly, which serves to block the virus from entering the cell. This system could potentially be used to prevent viral entry into host cells, thus offering an alternative to current HIV-1 therapies. Furthermore, this system can be applied to other viruses by interchanging anti-gp120 peptide sequence with other antiviral peptide sequences.

13. “Development of Humanized Mouse Models for Epstein-Barr Virus (EBV)-Induced Lymphomas
Shannon Kenney (UW)

EBV causes a number of different types of human lymphomas and transforms B cells into long-lived lymphoblastoid cell lines (LCLs) *in vitro*. Although LCL studies have been invaluable for defining some of the transforming functions of various different EBV proteins expressed during the latent forms of viral infection, *in vitro* LCL studies have not identified a role for the lytic form of viral infection in promoting malignancy, and do not take into account the potential effects of the tumor microenvironment or the host immune response. Since EBV cannot infect mouse cells, small animal models have not been available to study how EBV

causes lymphomas *in vivo*. Our lab has developed two different humanized mouse models that we have used to examine the potential roles of lytic EBV infection and T cells in promoting and inhibiting EBV-induced tumors, and to re-examine the requirement for specific EBV latency proteins for the development of EBV-infected lymphomas *in vivo*. The results of these studies suggest that the lytic form of EBV infection promotes EBV-induced malignancies, and reveal that the functions of certain EBV-encoded oncoproteins that are required for transformation *in vitro* can be replaced by helper T cells *in vivo*.

Poster Abstracts

1. “Differential Splicing of ANP32A in Birds Alters its Ability to Stimulate RNA Synthesis by Restricted Influenza Polymerase”
Steven F. Baker, Mitchell P. Ledwith, Andrew Mehle (UW)

Adaptation of viruses to their host can result in specialization and a restricted host range. Species-specific polymorphisms in the influenza virus polymerase restrict its host range during transmission from birds to mammals. ANP32A was recently identified as a cellular co-factor impacting polymerase adaption and activity. Avian influenza polymerases require ANP32A containing an insertion resulting from an exon duplication uniquely encoded in birds. Here we find that natural splice variants surrounding this exon create avian ANP32A proteins with distinct effects on polymerase activity. We demonstrate species-independent direct interactions between all ANP32A variants and the PB2 polymerase subunit. This interaction is enhanced in the presence of viral genomic RNA. In contrast, only avian ANP32A restored ribonucleoprotein complex assembly for a restricted polymerase by enhancing RNA synthesis. Our data suggest that ANP32A splicing variation amongst birds differentially impacts viral replication, polymerase adaption, and the potential of avian hosts to be reservoirs.

2. “Exosomes Secreted by Microglia During Virus-Induced Demyelinating Disease Contribute to Neuroinflammation”
Nhungoc Luong and Julie K. Olson (UMN)

Theiler’s murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) is a mouse model for multiple sclerosis. TMEV infection of susceptible mice leads to a persistent viral infection of microglia in the central nervous system (CNS) which contributes to the development of a chronic progressive demyelinating disease associated with an inflammatory immune response. Exosomes are membrane-bound nanovesicles released from cells that can be taken up by other cells, thus mediating intercellular communication. Exosomes can contain miRNA, mRNA, and proteins that are specific to the cells from which they were released. Previously, we found that microglia infected with TMEV secrete exosomes containing viral RNA/genome that can be taken up by bystander CNS cells. Moreover, these exosomes activated an innate immune response in bystander cells by increasing the expression of type I interferons and pro-inflammatory cytokines. Our present study showed that exosomes secreted by microglia during TMEV-IDD (63-98 days) contained viral RNA and could be taken up by uninfected microglia, astrocytes, and infiltrating macrophages to activate an inflammatory response. Most importantly, exosomes secreted by microglia during persistent TMEV infection when mice have demyelinating disease could be transferred to uninfected mice, and these exosomes induced inflammation in the CNS of uninfected mice. These results suggest that exosomes secreted during virus-induced demyelinating disease may contribute to the viral persistence and

neuroinflammation associated with TMEV-IDD. This research was supported by grants from UMN Academic Health Center (R1638) and NIH (T32DA007097).

3. “Ultrastructural Studies of RSV Assembly by Cellular Cryo-Electron Microscopy”
Jae Yang, Zunlong Ke, Rebecca S. Dillard, Tatiana Chirkova, Christopher C. Stobart, Cheri M. Hampton, Joshua D. Strauss, Martin L. Moore, Larry J. Anderson, Elizabeth R. Wright (UW)

RSV is an enveloped RNA virus that may display both filamentous and spherical morphologies. Virus assembly, budding, and release are critical steps for RSV egress. The surface glycoproteins, in particular F, are important for coordinating with the matrix protein M to drive the formation and elongation of mature RSV particles. Despite great advances in understanding of the RSV budding process, many of the steps and associated macromolecular structures remain unclear. Using combinatory structural and functional approaches, we defined some of these structures and proposed a model that defines several stages of RSV assembly. We used cryo-electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET), and conventional transmission electron microscopy to examine the structures of several RSV strains propagated in non-polarized cell lines and polarized airway epithelial cells. Functional assays including flow cytometry, viral titers, RNA quantification of RSV-infected cells were conducted. Whole-cell cryo-ET of RSV-infected cells combined with sub-volume averaging was used to determine the structures of complexes associated with the major stages of RSV assembly. To preserve the integrity of macromolecular and cellular architecture, native-immunolabeling approaches of cryo-preserved RSV and infected cells was developed. This provided greater clarity into the three-dimensional structures and localization of RSV macromolecules at virus assembly sites. We reported that RSV is filamentous when newly released from both transformed non-polarized cell lines and polarized airway epithelial cells. RSV structure is independent of virus strain and cell type. Imaged RSV particles were infectious. The conclusion is further supported by the result that the majority of RSV F is in pre-fusion conformation at sites of assembly and on filamentous virus particles, while F is in the post-fusion state on spherical particles. Furthermore, the structural analysis resolved interactions retained between F and M. This approach captured the range of steps associated with RSV assembly at high, macromolecular-level resolution. Here, we proposed an RSV assembly model where RSV assembly starts with the accumulation of viral proteins and genomic RNAs at the plasma membrane. During the active assembly state, coordinated viral and host cell components drive the protrusion and elongation of RSV filaments from the membrane. The narrowing of the cell proximal end of the RSV filaments signals the scission and subsequent release of RSV particles. This study is important because it provides direct structural evidence of processes associated with RSV assembly and budding.

4. “TCR Affinity Influences Helper T Cell Differentiation by Regulating Eef1e1 and Gbp2 Expression”
Dmitri I. Kotov, Jason S. Mitchell, Thomas Pengo, Christiane Ruedl, Sing Sing Way, Ryan A. Langlois, Brian T. Fife, Marc K. Jenkins (UMN)

Naive CD4⁺ T lymphocytes differentiate into various helper (Th) cell subsets following TCR binding to microbial peptide:MHCII (p:MHCII) complexes on dendritic cells (DCs). The affinity of the TCR interaction with p:MHCII plays a role in Th differentiation by mechanisms that are not completely understood. We found that low affinity TCRs biased naïve T cells to become T follicular helper (Tfh) cells while high affinity TCRs promoted the formation of Type 1 (Th1) or Type 17 (Th17) cells. TCR affinity could influence Th fate by controlling interactions with non-Tfh-promoting XCR1⁺ or Tfh-promoting SIRPz⁺ DCs, which differ in p:MHCII production, or intrinsically by regulating the expression of genes that influence lineage commitment. We found that TCR affinity did not bias T cell interactions with XCR1⁺ or SIRPz⁺ DCs, but instead regulated expression of IL-2 receptor, Eef1e1, and Gbp2. By driving expression of these genes, among others, strong TCR signaling repressed Tfh formation, while promoting non-Tfh fates.

5. “Characterizing the Putative Antiviral Factor SYNCRIP During Influenza Virus Infection”
Katherine Amato, Vy Tran, Andrew Mehle (UW)

Influenza A virus (IAV) interacts with a variety of cellular host factors during replication and the sum of these interactions determines the outcome of infection. Studying host proteins in the context of infection can yield insight into both viral biology and cellular biology. To this end, we infected a panel of 59 cell lines from the National Cancer Institute with an IAV-GFP reporter virus. We then correlated cellular susceptibility to infection with gene expression to identify putative pro- and anti-viral factors involved in IAV infection. Among our highest-ranking antiviral hits was synaptotagmin binding cytoplasmic RNA interacting protein (SYNCRIP, hnRNP Q). SYNCRIP is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family of proteins and is involved in pre-mRNA processing, splicing, mRNA stability, and translation. In addition to SYNCRIP, several other hnRNPs have known roles during IAV infection or have been implicated in the IAV interactome. SYNCRIP has been previously implicated as a proviral factor for HIV, hepatitis C, and mouse hepatitis virus infections. Here, our screen uncovered SYNCRIP as a putative antiviral host factor for IAV, and mass spectrometry analysis is suggestive of a physical interaction between SYNCRIP and the viral polymerase protein PB2. Furthermore, we confirmed the antiviral role of SYNCRIP during IAV infection by demonstrating a decrease in viral gene expression upon transient overexpression of SYNCRIP. This overall decrease in viral gene expression does not appear to come at the expense of polymerase function, as cells transfected with SYNCRIP demonstrated enhanced polymerase activity compared to controls. These results indicate a network of complex interactions by which SYNCRIP is able to regulate the outcome of IAV infection. .

6. “Efficient Systemic Treatment with Mesothelin-Targeted Oncolytic Adenovirus in Patient-Derived Xenograft (PDX) Model of Pancreatic Cancer”
Mizuho Sato-Dahlman, Yoshiaki Miura, Praveensingh Hajeri, Hideki Yoshida, Kari Jacobsen, Chikako Yanagiba, and Masato Yamamoto (UMN)

Pancreatic cancer is an aggressive malignant disease. Despite extensive efforts, systemic therapies have provided only limited efficacy for patients with this disease. Oncolytic Adenovirus (OAd) is a promising therapeutics, and it is also known for its efficient in vivo gene delivery. However, when adenovirus vectors are injected intravenously into mice, most of the virus goes to the liver and can lead to liver toxicity at high dosage. One of the reason for liver tropism is that hepatocytes express high levels of the primary adenovirus receptor, and non-parenchymal liver cells, such as Kupffer cell and epithelial cell, also capture the viral particle. As a consequence of large sequestration of adenovirus by liver, the tumor transduction rate is low and the in vivo efficacy is limited. Therefore, the improvement of cancer selective transduction and vector distribution to avoid liver sequestration would overcome the obstacles for systemic delivery required for efficient systemic treatment of spread and/or metastatic lesions of pancreatic cancer with OAd. To improve the tumor transduction, we have generated the pancreatic cancer-targeted OAd by high-throughput screening of Ad-fiber library in mesothelin (MSLN) expressing cells. The pancreatic cancer-targeted OAd binds to MSLN protein, which is overexpressed on the surface of pancreatic cancer. MSLN-targeted OAd showed selective and powerful anti-tumor effect against Panc-1 xenograft tumor model in both intratumoral (i.t.) and intravenous (i.v.) injection. Importantly, when we assessed viral distribution after i.v. injection, the liver sequestration of MSLN-targeted OAd was lower than untargeted OAd (Ad5 WT virus) at 48 hrs after injection. By day 7, the viral copy number of MSLN-targeted OAd in the tumor was significantly higher than Ad5 WT virus. These results suggest that systemic injection of the tumor targeted-OAd showed significantly lower liver sequestration and better tumor accumulation. Additionally, we performed multiple time point injection of MSLN-targeted OAd against regrown tumors. Four out of six tumors were controlled with repeated injection. Next, antitumor effect of MSLN-targeted OAd was assessed in patient-derived xenograft (PDX) model. After intravenous administration, only the MSLN-targeted OAd showed significant antitumor effect compared to the untreated group ($p < 0.05$), while the growth of Ad5 WT virus injected group was same as untreated group. In this study, systemic injection of MSLN-targeted OAd showed remarkable anti-tumor effect in both systemic and intratumoral injections at low dose. Our results indicated that tumor targeted-OAd can embody efficient systemic treatment for pancreatic cancer which are mostly found with spread or metastatic lesions.

7. “Human APOBEC3B and HPV E6 and E7 Oncoproteins in Tumorigenesis in Vivo”
Cameron Durfee, Lindsay Larson, Emily Law, Reuben Harris (UMN)

In vitro and in silico data indicate that APOBEC DNA cytosine to uracil deaminases induce a large percentage of mutations in multiple human tumor types. These mutations contribute to observed tumor heterogeneity including metastasis. In cervical cancer, APOBEC3B overexpression is caused by the E6 and E7 oncoproteins of high-risk human papillomaviruses (HPV). We hypothesize that in addition to inducing APOBEC3B overexpression, the HPV E6 and E7 oncoproteins sensitize the human genome to APOBEC3B catalyzed mutagenesis in part by p53 inactivation and in part by additional mechanisms. To test this hypothesis, we have combined a new model for human APOBEC3B expression in mice with an established model E6 and E7 driven tumorigenesis. Standard breeding was used to generate 3 cohorts of 80 animals each, with each cohort consisting of 10 wild-type, 10 E6, 10 E7, 10 E6/E7 animals both with and without APOBEC3B. The first cohort was observational, the second was treated rectally with DMSO, and the third was treated rectally with the chemical carcinogen DMBA. These studies are ongoing but the results to-date will be presented including progression-free survival data.

8. “Human Cytomegalovirus Enters Latency through Macropinocytosis and Endocytosis”
Jeong-Hee Lee, Joseph R. Pasquarella, and Robert F. Kalejta (UW)

To deliver their contents to the sub-cellular compartment where viral genome replication takes place, virions penetrate cellular membranes. After membrane penetration, most virions, in whole or in part, disassemble to activate viral replication or transcription. Complex virions like those of the herpesviruses deliver not only genome containing capsids but also a set of packaged tegument proteins, some of which are critical to establishing the infection. When the herpesvirus human cytomegalovirus (HCMV) enters a terminally-differentiated cell, the tegument protein pp71 migrates to the nucleus and initiates a productive (lytic) infection. When HCMV enters incompletely differentiated myeloid cells, tegument-delivered pp71 remains in the cytoplasm, allowing for the establishment of latency in these cells. Here we show that HCMV enters into the cells in which it establishes latency by macropinocytosis and endocytosis, processes through which extracellular fluids and solids are routinely internalized by cells into vesicles called endosomes. Capsids and a capsid-associated tegument protein are released from maturing endosomes and migrate to the nucleus whereas other tegument proteins, including pp71, remain in the cytoplasm. Inhibition of macropinocytosis or endocytosis impairs latency-associated transcription and precludes viral reactivation. We conclude that HCMV enters latency through the processes of macropinocytosis and endocytosis.

9. “Guinea Pig Cytomegalovirus Infects the Amnion and Induces Pathways Associated with Preterm Birth”
Dira S. Putri, Claudia Fernandez-Alarcon, Juan E. Abrahante, Mark R. Schleiss, and Craig J. Bierle (UMN)

Congenital cytomegalovirus (CMV) infection is significantly associated with preterm birth. In utero CMV infection of the amnion has been associated with adverse pregnancy outcomes, including preterm birth, and human CMV can infect amnion- and chorion-derived cells in vitro. The fetal membranes function as an immunologic and physical barrier during pregnancy. However, infection of the chorioamnion or exposure of the membranes to inflammatory signaling can trigger structural changes that lead to the premature rupture of membranes and preterm birth. To assess the impact of CMV infection on fetal membrane biology and identify virus-induced molecular pathways that could underlie preterm birth, we sought to determine whether guinea pig CMV (GPCMV) infects the amnion. After a mid-gestation viral challenge, GPCMV was detected in the placenta and amnions of pups collected at 21 days post-infection. To develop an in vitro model of fetal membrane infection, amniotic epithelial cells were isolated from late-term guinea pig amnions. GPCMV replicated in both primary and HPV16 E6/E7-transduced amniotic epithelial cells. Amniotic epithelial cells were less susceptible to GPCMV infection than fibroblasts, and GPCMV replication in amniotic epithelial cells was less lytic, was sustained for a longer duration, and resulted in markedly less extracellular virus production. RNA-Seq was used to profile the transcriptomes of GPCMV-infected and uninfected amniotic epithelial cells and fibroblasts. Amniotic epithelial cells expressed significantly higher levels of TLR1 (56.1-fold), TLR2 (9.5-fold), and TLR3 (2.9-fold) than fibroblasts. Infection elicited a robust immune response from amniotic epithelial cells, significantly increasing the expression of several cytokines associated with preterm birth, including CCL5/RANTES (25.1-fold), IL-8 (2.6-fold), and IL-1 (2-fold). These results demonstrated the utility of GPCMV as an experimental model of viral chorioamnionitis and identified candidate immune pathways that regulate CMV infection of the amnion and that may contribute to fetal membrane rupture and preterm birth.

10. “Human Cytomegalovirus UL138 Inhibits STING to Silence Viral Lytic-phase Transcription and Counteract Host Innate Immune Defenses During Latency”
Emily R Albright and Robert F Kalejta (UW)

Latency allows human cytomegalovirus (HCMV) to persist for the lifetime of its host by avoiding immune detection and clearance. The establishment and maintenance of HCMV latency is achieved, in part, through the assembly of heterochromatin on the viral major immediate early promoter (MIEP) resulting in the repression of viral lytic-phase transcription. We have previously shown that the viral latency protein UL138 contributes to the maintenance of this repressive chromatin structure at the MIEP by impairing the recruitment of cellular lysine demethylases via NFκB and CRE/ATF sites in the MIEP. Here, we show that UL138 prevents recruitment of NFκB subunits to both the viral MIEP and to the cellular

IFN² promoter during the establishment of latency, correlating with the repression of both promoters. Inhibition of the cytoplasmic cGAS/STING/TBK1 foreign DNA sensing pathway upstream of NFkB prevented the activation of both IFN² and viral IE transcription following HCMV infection of undifferentiated myeloid cells, phenocopying the expression of UL138. Furthermore, we found that UL138 co-localizes with, binds to, and inhibits STING. Our results identify UL138 as a novel regulator of the host innate immune response during latency and suggest that UL138 prevents the NFkB-mediated activation of viral IE transcription by inactivating the cGAS/STING/TBK1 foreign DNA sensing pathway. These findings illuminate how the activation of innate immunity impairs HCMV latency, and reveal a link between cytoplasmic pathogen sensing and the epigenetic control of viral lytic phase transcription in the nucleus during latency.

11. “Bacteriophage Attack Can Break Metabolic Dependency and Indirectly Affect Non-Host Species”

Lisa Fazzino, Jeremy Anisman, Jeremy Chacón, Rick Heineman, William R. Harcombe (UMN)

Bacteriophage (phage) are understudied members of microbial communities and can impact community composition and dynamics. Phage alter community composition by suppressing dominant bacteria, and increasing competitive pressure between species. Less is known about the effect of phage on obligate mutualisms. We predict that phage attack on an obligate mutualist limits growth of mutualistic community members. This was tested with an engineered community of *Escherichia coli* and *Salmonella enterica* in which species share or compete for metabolites depending on provided media. We tested the impact of *E. coli*-specific phage (T7) or *S. enterica*-specific phage (SP6) in cooperating or competing communities. As expected, phage limited host population sizes until resistance evolved. Interestingly, the effect on the non-host varied by phage. Rather than constraining the growth of an obligate mutualist, killing *E. coli* with phage led to 2-3 fold increases in *S. enterica* population size. Our resource-explicit mathematical models suggest that consumption of nutrients released by lysis caused this. However, increase in non-host was not observed when targeting *S. enterica*. Here, *E. coli* provides carbon to *S. enterica* and receives methionine. The difference in the indirect effects on phage on non-host may be because the specific metabolites exchanged cause differential scavenging benefits. In conclusion, phage attack on a microbial mutualism can break constraints on species ratios and modulate non-host population sizes, though the extent of these impacts may be determined by the metabolites exchanged. Determining how microbial interactions influence the impact of phage is critical for understanding the eco-evolutionary dynamics of microbial communities.

12. “Hypoxia Mimics Target EBV-Positive Gastric Cancer Cells in Lytic Induction Therapy”

Blue-Leaf A Cordes, Richard J Kraus, Andrea Bilger, James C Romero-Masters, Parita A Patel, Paul F Lambert, Shannon C Kenney, and Janet E Mertz (UW)

Latent infection of Epstein-Barr virus (EBV) is associated with cancers of lymphoid and epithelial cell origin, including most nasopharyngeal carcinomas and approximately 10% of gastric carcinomas. We are working to develop lytic-induction therapies for treating patients with EBV-associated tumors by inducing reactivation of EBV into its lytic phase along with providing a nucleoside analog such as ganciclovir, that when activated by the EBV-encoded kinases, will specifically kill the EBV-infected cells. We previously reported that expression of EBVs latent-to-lytic switch gene, BZLF1, can be activated by sequence-specific binding of hypoxia inducible factor-1 (HIF-1) to Zp (Kraus et al., 2017). Here, we show that incubation of the EBV-positive gastric cancer cell lines AGS-Akata, AGS-BD, and SNU-719 with a variety of HIF-stabilizing drugs [i.e., Deferoxamine (DFO), MLN-4924 (Pevonedistat), Dimethylfumarate (DMF), and BAY 85-3934 (Molidutstat)] results in synthesis of lytic EBV proteins. Incubation of the cells with drug for 24 h was sufficient to induce sustained lytic-gene expression, with DFO inducing EBV reactivation in up to 30% of AGS-Akata cells by two days. Cell viability assays indicated that incubation with DFO and Molidustat led to preferential killing of EBV-positive gastric cells compared to their matched EBV-negative control cells. Thus, these drugs (FDA-approved and in phase 3 clinical trials, respectively) may be good candidates for use in lytic-induction therapy. We are beginning to examine the efficacy of DFO to induce EBV reactivation and to inhibit tumor growth in vivo using immunosuppressed NSG mice in which we generate xenografts from EBV-positive vs. EBV-negative gastric carcinoma cell lines. Preliminary data indicate that in EBV-positive xenografts, a higher frequency of Zta-positive cells are located near blood vessels in mice treated with DFO than in control-treated mice, a finding suggesting DFO may induce EBV reactivation in vivo.

13. “Role of Tuft Cells During Influenza A Infection”

Shanley Roach, Jessica Fiege, Ian Stone, and Ryan Langlois (UMN)

Tuft cells are a rare epithelial cell type marked by their unique morphology and apex chemosensory microvilli. These cells can be found in many mucosal tissues, such as the respiratory and intestinal tract, as well as non-mucosal tissues such as the thymus. Clear functions for these cells were not described until several papers identified intestinal tuft cells as the primary epithelial source of IL-25 in the gut. Upon intestinal infection with worms or protozoans, tuft cells release floods of IL-25 and initiate the type 2 immune response to clear infection. A deficiency in tuft cells is marked by an altered immune response and delayed clearance of infection. Although these cells are emerging as important regulators of the immune response and tissue homeostasis in the gut, the role of tuft cells during respiratory infections has yet to be explored. The goal of this project is to explore the role of respiratory

tuft cells in the immune response to and recovery from influenza infection. I also aim to understand how intestinal tuft cells may play a role in repairing the intestinal damage that occurs during respiratory infection. Here we show how respiratory infection with influenza A virus impacts tuft cell numbers in the respiratory and gastrointestinal tract.

14. “The Influence of Keratin 17 on MmuPV1-induced Pathogenesis”
Aayushi Uberoi, Wei Wang, Megan Spurgeon, Xiang-Yang Xue, Vladimir Majerciak, Pierre Coulombe, Zhi-Ming Zheng, Paul F. Lambert (UW)

Mouse papillomavirus (MmuPV1) is a virus that was originally found to cause cutaneous papillomas in nude mice. Our lab has recently reported that this virus also is capable to cause papillomas immunocompetent mice when they are treated with UVB, which correlated with the ability of UVB to induce systemic immunosuppression. RNA seq data of MmuPV1-induced warts in nude mice indicated that stress keratins, such as keratin 17 (K17), are amongst the host genes most highly upregulated in their expression in warts. In immunocompetent mice that developed MmuPV-1 induced papilloma, we found that K17 also was significantly upregulated. Importantly, K17-null mice had early onset of MmuPV1-induced pathogenesis but faster regression: these mice when infected with MmuPV1 developed papillomas at 3 weeks post infection that regressed by 7 weeks, whereas wild type mice developed papillomas around 7 weeks. Our results indicate that stress keratins may contribute to the persistence of MmuPV1-induced papilloma in the skin. The mechanism(s) by which stress keratins contribute to the susceptibility of mice to MmuPV1-induced pathogenesis is now under investigation.

15. “Development of a Pichinde Virus-Based Vaccine Vector for the Treatment of Tuberculosis”
Sophia Vrba, Wanjun Zhu, Vikram Verma, Anna Tischler, and Yuying Liang (UMN)

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, is one of the top ten causes of death worldwide. The current vaccine for TB, the BCG vaccine, is only effective against the most severe forms of childhood disseminated TB and has limited effectiveness in adults and adolescents. Therefore, there needs to be another effective prophylactic and therapeutic method. To address this need, a trisegmented Pichinde Virus-Based Vaccine Vector (rP18tri) was developed for the prevention and/or treatment of TB. Control of TB infection is mediated primarily by cellular immune responses, and the rP18tri-based viral vector can strongly induce both humoral and cellular immunity. The virus vector is composed of three single strands of RNA. The L RNA segment contains the RNA polymerase and the Z protein; S1 contains the nucleoprotein in the negative sense, and the TB antigen, Ag85, in the positive sense; S2 contains the envelope glycoprotein precursor in the positive sense, and the TB antigens EsxA and EsxH in the negative sense. The vaccine vector plasmids were created through traditional cloning techniques and the live viral vaccine was generated after plasmid transfection. Expression of the

three TB antigens, Ag85, EsxA, and EsxH, in the viral vaccine-infected cells was detected by western blot analysis with TB antiserum. A survival assay was performed on immunized mice challenged with an infectious strain of TB to assess protection. Future experiments will include direct flow cytometry (FACS) to characterize the rP18tri-induced TB-specific T cell immune response.

16. “Substitutions in SIV Nef that Selectively Impair Anti-SERINC5 Activity”
Sanath Kumar Janaka, Alexandra V Palumbo, Aidin Tavakoli-Tameh, David T Evans (UW)

Recent studies have revealed that SERINC5, and to a lesser extent SERINC3, are incorporated into lentiviral particles as they bud from infected cells and inhibit the fusion of virions with target cells. The Nef proteins of HIV-1 and SIV enhance viral infectivity by preventing the incorporation of SERINC5 into virions. In addition to counteracting SERINC5, SIV Nef also downmodulates simian tetherin, CD4 and MHC class I (MHC-I) molecules from the surface of infected cells. To assess the role of SERINC5 in lentiviral pathogenesis, we sought to uncouple anti-SERINC5 activity from other Nef functions. From a systematic analysis of alanine substitutions throughout the SIVmac239 Nef protein, we identified two residues in the N-terminal domain and one residue in the C-terminal domain that are specifically required to modulate SERINC5. Although there was significant overlap with sequences required for tetherin antagonism and CD4 downregulation, resistance to SERINC5 was genetically separable from other Nef functions. These observations can now be used to engineer an infectious molecular clone of SIVmac239 that is specifically sensitive to SERINC5 to assess the impact of this factor on lentiviral replication and pathogenesis in infected macaques.

17. “Characterization of Species-specific Molecular Determinants of Innate Immune Suppression in Pathogenic Arenavirus Infection”
Morgan Brisse, Junji Xing, Junjie Shao, Yuying Liang, Hinh Ly (UMN)

The major natural reservoirs for arenaviruses are rodents that can be persistently and often non-symptomatically infected. On the contrary, several of these viruses are known to cause highly pathogenic infections in humans and primates. Inhibition of the type-1 interferon (e.g., IFN β) production seems to be a major determinant of pathogenicity.¹³ Our laboratory has recently discovered that the arenaviral zinc finger-containing (Z) matrix proteins from all known pathogenic arenaviral strains can bind to the cytosolic innate immune receptors RIG-I and MDA5 in human cells to inhibit IFN β production.^{4,5} Additionally, we have found that this inhibitory mechanism of IFN β production may help determine the species-specific pathogenicity, as it only occurs in human and non-human primate cells but not in rodent cells. Here, we report data on our recent efforts to map the potential species-specific regions in the N-terminal CARD 1 and 2 domains of RIG-I (RIG-iN) that are critical for interaction with the Z proteins of multiple pathogenic strains of the arenavirus.

18. “Evidence for a Novel Species-specific Cellular Barrier to HIV-1 Gene Expression”
Sofia Romero, Edward L. Evans III, Nathan M. Sherer (UW)

Human immunodeficiency virus type 1 (HIV-1) is a positive-sense single-stranded RNA virus that causes the acquired immunodeficiency syndrome (AIDS). HIV-1 is unable to produce virions in cells derived from mice and other rodents. These barriers have hindered the development of a small animal model for studying HIV/AIDS. However, because many of these barriers are recessive mouse cells have served as a useful gain-of-function genetic platform for identifying and studying species-specific host-dependency factors (HDFs) required for HIV replication in humans. For instance, mouse Cyclin T1 (mCCNT1) is incompatible with the HIV-1 Tat protein but a single point mutation (Y261C) renders mCCNT1 competent for Tat-mediated viral transcription elongation. Additionally, supplementing mouse cells with human Exportin-1 (hXPO1, also known as CRM1) enhances viral unspliced mRNA trafficking to the cytoplasm regulated by the viral Rev protein. We recently showed that mouse cells can be engineered to support robust HIV-1 virion production through stable expression of HIV-1 compatible versions of CCNT1 and XPO1 (3T3.CX cells)¹. Interestingly, however, 3T3.CX cells exhibited an ~24-hour delay to the onset of viral gene expression when compared to human (HeLa) cells. Herein, we studied the source of this delay using high resolution single-molecule fluorescence in situ hybridization (smFISH) and live cell imaging. Our results show that, similar to human cells, 3T3.CX cells are readily infectible with HIV-1 but, unexpectedly, only a subset of cells (~5%) are capable of efficiently trafficking Rev-dependent viral RNAs from the nucleus to cytoplasm. These results suggest the existence of one or more additional, species-specific factors affecting efficient HIV-1 mRNA nuclear export and regulating an unanticipated, non-stochastic mode of viral gene expression.

19. “An Improved Oncolytic Adenovirus for Radioiodine Therapy and Imaging”
Lisa Koodie (UMN)

The use of oncolytic viruses encoding sodium-iodide symporter (NIS) is an attractive approach to achieve radionuclide imaging of cancer. However, the potential of virus-induced radiotherapy has not been fully explored. Previously, we designed an oncolytic adenovirus (OAd) modified to express high quantities of NIS (Ad5/3-Cox2-NIS-ADP). In original vectors, enhanced oncolysis was mediated by overexpression of adenoviral death protein (ADP). Although these vectors were operative in detection and therapeutic regimens, we were concerned that ADP cytolytic effect may diminish iodine uptake. Here, we have evaluated an identical ADP-deleted virus as a theranostics tool for pancreatic adenocarcinoma (PDAC). SPECT/CT studies assessing ^{99m}TcO₄⁻ accumulation in mice to visualize PDAC Patient Derived Xenografts showed that ADP(-) produced greater ^{99m}TcO₄⁻ uptake that was maintained until day 32, and significantly outlasted ADP(+) and replication-deficient AdCMV-NIS. ADP(+) produced a punctate NIS staining pattern, with little to no cell membrane localization, while ADP(-) produced a distinct NIS distribution as it co-localized with cell membrane bound-cytokeratin-

4. Next, we treated PDAC tumors with virotherapy in combination with ¹³¹I. The therapeutic effect of ADP(-) combined with ¹³¹I outperformed that with ADP(+) or AdCMV-NIS combination therapies. Quantitative analyses of ¹³¹I uptake in tumor tissues showed that ADP(-) retained higher ¹³¹I uptake than ADP(+). These results support our hypothesis that ADP-cytolytic effect impacts virus-induced radionuclide uptake, and that the ADP-deleted OAds are effective tools for NIS-based cancer diagnosis and therapy. We are currently investigating the effect of double-dosed ¹³¹I versus the single dose and are evaluating bio-distribution and toxicity of our vectors in pigs.

20. “Potential Interactions Between HIV-1 and HPV”
Soyeong Park, Denis Lee, Paul Lambert, Nate Sherer (UW)

The prevalence of human papillomavirus (HPV) infection and incidence of HPV-related cancers are increased in human immunodeficiency virus (HIV)-1-infected people. In turn, HPV-infected individuals have a higher risk for HIV-1 infection than HPV-negative population. Although HIV-1 and HPV infect different cells, CD4+ T-cells and epithelial cells respectively, they might interact each other since both viruses are sexually transmitted viruses so they can meet at the same sites and HIV-1 must cross epithelial barrier to infect CD4+ T-cells. Indirectly, epithelial cells can be affected by HIV-1 Tat protein, which is secreted from HIV-1 infected cells and can enter non-target cells. On the other hand, it was suggested that a subset of epithelial cells can be infected with HIV-1 by unconventional mechanism. Thus, we exploited normal immortal keratinocytes(NIKS) as a model system for exploring the potential for HIV-1 and HPV interactions and HIV-1 latency during co-infection or co-culture. First, as a co-infection model, we infected NIKS/NIKS18 with VSV-G pseudotyped HIV-1 and performed assembly assay to see if they can produce virus particles. After 48 hours, we confirmed that NIKS/NIKS18 can make virus even though there was not significant difference in NIK18 compared to NIKS. Regarding to latency, we utilized dual-reporter system which can reveal latency state of HIV-1 by fluorescent proteins and did live cell imaging for 5 days. When NIKS/NIKS18 were infected with dual-reporter pseudotyped-HIV-1, the latency profiles in NIKS and NIKS18 were similar to each other but after 4 days of infection, we observed that NIKS18 were dying faster than NIKS. Since we were using monolayer NIKS/NIKS18, we performed RT-PCR over HPV18 E6 and E7 and the result showed that NIKS18 express very low levels of E6 and E7, explaining why we could not see difference between NIKS and NIK18 in monolayer. Our study revealed that NIKS/NIKS18 can be infected with VSV-G pseudo-HIV-1 and produce HIV-1 virus particles. Even though there was no apparent difference of HIV-1 gene expression and latency between NIKS and NIKS18, NIKS18 showed accelerated cell death upon HIV-1 infection.

21. “The Actin Cortex as a Physical Barrier in Retroviral Assembly”
C Isaac Angert, Morgan E Meissner, Louis M Mansky, Joachim D Mueller (UMN)

Retroviral assembly requires Gag oligomerization at the plasma membrane, which forms an immature Gag lattice that is crucial for particle assembly. We have previously used two-photon fluorescence z-scan measurements to analyze the association of retroviral Gag proteins with the plasma membrane. Here, we report the application of this technique to study the intracellular distribution of HIV-1 Gag in living cells. Using a soluble fluorescent protein marker to identify the cytoplasm, we found that HIV-1 Gag mainly resides in a confined cytoplasmic volume, with significantly reduced Gag concentrations within 100-200 nm of the plasma membrane. Gag depletion near the plasma membrane correlates with the general dimensions of the actin cortex. Latrunculin A, which disrupts actin polymerization, was used to alter the actin cortex. We observed a latrunculin-mediated modulation of Gag depletion, which provides evidence in support of cortical actin selectively depleting Gag access to the plasma membrane. Additionally, latrunculin can transiently increase particle release from cells. We also found that Gag depletion is strongly associated with domains of Gag containing RNA binding motifs. This is consistent with other proteins (i.e., high affinity RNA-binding proteins) where a depletion effect is also observed near the plasma membrane. Collectively, our observations are consistent with the model of large ribonucleoprotein complexes being prevented from readily accessing the plasma membrane due to the dense network of the actin cortex which may act as a physical barrier. These findings have important implications for understanding the details of retroviral assembly and infectious particle formation.

22. “HIV-1 Vif Induces a Prolonged Metaphase Cell Cycle Arrest Characterized by Pronounced Mitotic Spindle Defects and Centrosome Amplification”
Edward L. Evans III, Christopher Bastin, Jordan T. Becker, Laraine M. Zimdars and Nathan M. Sherer (UW)

The human immunodeficiency virus type 1 (HIV-1) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). HIV-1 primarily infects and kills CD4+ T-cells and virus-induced T cell death results, at least in part, due to G2/M cell cycle arrest caused by the viral accessory proteins Vpr and Vif. Why these factors evolved to arrest the cell cycle, and the cell signaling involved, are unknown. In a previous study, we observed that HIV-1 Vif (strain NL4-3) induces cell cycle arrest ~24 hours post-infection, and that this activity is specific to human cell types. Infected human cells maintained an arrested state for up to 40 hours before succumbing to cell death via apoptosis. Here we studied the molecular details underpinning VifNL4-3-mediated cell cycle arrest. Live cell imaging revealed that VifNL4-3 causes cell cycle arrest not in G2 but in metaphase, with arrested cells exhibiting cohesin fatigue and undergoing a remarkable amplification of spindle poles, yielding up to six independent centrosomes per cell. Treating VifNL4-3 arrested cells with the drug reversine, an MPS1 inhibitor that bypasses the AURKB checkpoint, allowed VifNL4-3 arrested cells to proceed past

metaphase to cytokinesis. Taken together, these data reveal that VifNL4-3 causes cell death by dysregulating checkpoint protein turnover at the kinetochore.

23. “Subcellular Localization of APOBEC3 Proteins Regulate Interactions with and Restriction of HIV-1”

Jordan T. Becker, Nathan M. Sherer, Reuben S. Harris (UMN)

HIV-1 mRNAs and RNA genomes must fight to exist within a diverse and complex host cytoplasm. Here, we report studies on how wild-type and mutant HIV-1 RNAs affect the subcellular distribution of RNA-binding proteins including HIV-1 Gag, APOBEC3G (A3G), and APOBEC3H (A3H). A3G is a steady-state component of processing bodies (PB) and can accumulate in stress granules (SGs). A3H, however, has been observed to localize to the nucleus, nucleolus, and cytoplasm. Using cells engineered to stably express fluorescently tagged versions of A3G, A3H, and other cellular RNA-binding proteins, we used live cell imaging to demonstrate that HIV-1 specifically perturbs A3G and A3H subcellular distribution. Specifically, A3G and A3H preferentially associate with and accumulate on HIV-1 gRNAs even when artificially targeted to non-native subcellular locales. Forced localization of A3G to intracellular vesicles (via a heterologous membrane targeting motif) redirects HIV-1 gRNA trafficking to lysosomes with A3G and inhibits HIV-1 virion assembly. Fluorescence recovery after photobleaching (FRAP) experiments shows that HIV-1 gRNA, but not Gag, regulates the diffusion of A3G. Fluorescent A3H localizes to nucleoli and throughout the cytoplasm. The results with A3G are consistent with a model wherein A3G exhibits a clear preference for one or more signals along the HIV-1 genomic RNA. Preliminary results with A3H suggest a similar preference for HIV-1 genomic RNA. RNA immunoprecipitation and sequencing of A3G and A3H studies are ongoing to identify specific sequences and structures uniquely preferred by these two proteins. Finally, time-lapse fluorescent imaging in combination with RNA tethering can be used as a tool for examining RNA-protein interactions in living cells.

24. “Elucidating HIV-1 RNA Interactomes”

Rachel A. Knoener, Jordan T. Becker, Edward L. Evans III, Bayleigh Benner, Mark Scalf, Lloyd M. Smith, Nathan M. Sherer (UW)

Complex networks of transient RNA-protein interactions drive HIV-1 gene expression and replication. The HIV-1 splice variant classes (unspliced, partially spliced, and fully spliced) each have unique characteristics and purpose, and therefore utilize different RNA-protein interactions to achieve balanced expression of mRNA variants and their protein products. Presented is a strategy for the hybridization-capture and isolation of the three classes of HIV splice variants from natively infected cells and the determination of their protein interactomes using mass spectrometry. Comparative analysis revealed over 200 proteins that interact differentially with the various splice-forms in Jurkat cells; a T cell line commonly used for studies of HIV-1 gene expression and latency reversal. Proteins enriched in intron-containing (unspliced and partially-spliced) RNA-interactomes include

many with known functions in the regulation of RNA splicing and stability; and many proteins enriched in the completely spliced HIV RNA interactome had known functions in protein localization and translation. Furthermore, a screen utilizing gene specific siRNA knockdown followed by infection with a 2-color HIV reporter virus revealed the impact of each host factor on the expression of both the unspliced and completely spliced HIV RNA protein products. We confirm roles for over 100 HIV RNA interactors, many not previously implicated as HIV host factors, including splicing factor RBM4, mitochondrial ribosomal protein MRPS9, and DNA-binding protein RBMS1. This strategy, hybridization purification of HIV RNA-protein complexes followed by protein identification by mass spectrometry (HyPR-MS), enables the discovery of host proteins integral to the process of HIV replication.

25. “Analysis of HTLV-1 RNA Packaging and its Interrelationship with RNA Dimerization”
Ruth J. Blower, Heather M. Hanson, Weixin Wu, Joshua Hatterschide, William A Cantara, Karin Musier-Forsyth and Louis M Mansky (UMN)

Human T-cell leukemia virus type 1 (HTLV-1, a deltaretrovirus) is an oncogenic human retrovirus and is related to human immunodeficiency virus type 1 (HIV-1, a lentivirus). In HIV-1, specific interactions between the nucleocapsid (NC) domain of the Gag protein and genomic RNA (gRNA) mediate gRNA dimerization and selective packaging; however, the mechanism for gRNA packaging in HTLV-1, a deltaretrovirus, is unclear. In this study, we conducted gRNA packaging studies in order to investigate whether newly identified HTLV-1 dimer initiation sequence (DIS) can impact the efficiency of HTLV-1 gRNA packaging. Surprisingly, a mutant partially defective in dimer formation in vitro exhibited a significant increase in RNA packaging into HTLV-1-like particles, suggesting that efficient RNA dimerization may not be strictly required for RNA packaging in HTLV-1. These observations highlight distinct differences between the processes of gRNA packaging and dimerization of HTLV-1 relative to that of HIV-1.

26. “An in vitro System for Studying Mouse Papillomavirus”
Simon Blaine-Sauer, Denis Lee, Andrea Bilger, and Paul F. Lambert (UW)

The discovery of the murine papillomavirus (MmuPV1), which infects the common laboratory mouse *Mus musculus*, has allowed researchers to study papillomavirus infections in an animal model. However, studying the MmuPV1 virus in vivo can be both costly and time-consuming. To complement our current in vivo models of papillomavirus infection and neoplastic disease, we have, for the first time, cultured cells from papillomas arising on an immunocompromised mouse naturally infected with MmuPV1. Four separate cell strains were established from keratinocytes that were derived from cutaneous lesions on the tail of a Foxn1^{nu/nu} mouse. Each cell strain has been cultured with a fibroblast feeder layer and in specialized epithelial media containing ROCK inhibitor, a Rho kinase

inhibitor that prolongs the lifespan of primary keratinocytes and preserves their basal-like state. These cell strains have remained viable for over 15 passages, and the viral genome has been maintained at a level of multiple copies per cell. Additionally, preliminary results suggest that these cells may express the viral late gene products E1^{E4} and L1 at low levels even in the basal layer. Current studies are underway to determine whether these MmuPV1-infected mouse keratinocytes can be grown using organotypic raft cultures. These cell strains will provide a valuable platform to study the MmuPV1 lifecycle in vitro, which will be useful for further characterization of MmuPV1 and its relationship with the host. In addition, these cell strains will provide an efficient preliminary screening system for drugs aimed at inhibiting the viral lifecycle prior to in vivo testing.

27. “Perturbing the HIV-1 Programmed Ribosomal Frameshift Site: Translational Regulation Dictates Transcript Fate and Gag-Pol Incorporation”
Bayleigh E. Benner, Jordan T. Becker, Pablo Garcia-Miranda, Samuel E. Butcher, and Nathan M. Sherer (UW)

Perturbing the HIV-1 Programmed Ribosomal Frameshift Site: Translational Regulation Dictates Transcript Fate and Gag-Pol Incorporation Bayleigh E. Benner^{1,2}, Jordan T. Becker^{1,2}, Pablo Garcia-Miranda^{2,3}, Samuel E. Butcher³, and Nathan M. Sherer ^{1,2} ¹The McArdle Laboratory for Cancer Research, ²The Institute for Molecular Virology, and ³The Department of Biochemistry, University of Wisconsin-Madison, WI 53706 The HIV-1 unspliced RNA secondary structure is a key regulator of viral replication utilizing cis-acting features to modulate production of viral proteins. The conserved stem-loop within the gag reading frame dictates the proportions of essential polyproteins Gag and Gag-Pol. The pol gene is translated due to a -1 programmed ribosomal frameshift (PRF) occurring near the end of the gag coding region, producing the Gag-Pol polyprotein approximately 5% of the time. Gag-Pol contains the viral enzymes necessary for production of mature, infectious virions. We have recently shown that perturbing the PRF stem-loops thermodynamic stability alters frameshifting efficiency as much as 3-fold, yielding large reductions to viral infectivity¹. Herein we investigated the mechanism(s) underpinning this loss of infectivity. We report that a subset of HIV-1 PRF mutants exhibit significant decreases to viral RNA (vRNA) genome packaging into virions and, unexpectedly, that completely abolishing frameshifting using a slippery site mutation (-SS) caused marked decreases to unspliced vRNA cytoplasmic abundance despite having little to no effect on Gag translation kinetics. Surprisingly, vRNA stability and infectious virion production was rescued by adding Gag-Pol in trans, thus revealing an unanticipated role for Gag-Pol in stabilizing packageable pools of unspliced vRNA in the cytoplasm. In addition, Gag-Pol incorporation into virus-like particles (VLPs) exhibited a cis preference, where increasing levels of cytoplasmic Gag-Pol could not be incorporated into VLPs above a specific threshold, unless made in cis. Taken together, we show that frameshifting fidelity is crucial not only for maintaining Gag:Gag-Pol ratios in virions, but also for HIV-1 genome stabilization in the

cytoplasm, trafficking, and packaging into new virus particles. These findings suggest an importance for HIV frameshifting beyond regulating protein production. 1. Garcia-Miranda P, Becker JT, Benner BE, Blume A, Sherer NM, Butcher SE. HIV frameshift site RNA stability correlates with frameshift efficiency and decreased viral infectivity. *J Virol*. 2016 May 18.

28. “Development of a New Model for Papillomavirus-Induced Head and Neck Cancer Using Mouse Papillomavirus”
Tao Wei, Andrea Bilger, Paul F. Lambert (UW)

High-risk human papillomavirus (HPVs), such as HPV16, 18 and 33, cause 5% of human cancers, including cervical, anal and an increasing percentage of head and neck squamous cell carcinoma (HNSCC). To study the papillomavirus-associated HNSCC in the context of infection, the recently characterized mouse papillomavirus (MmuPV1) is a promising tool to establish an in vivo infection model for HNSCC. Previous work in our lab has shown that MmuPV1 can cause cutaneous wart in immunodeficient mice, and in immunocompetent mice treated with the UV treatment that induces systematic immunosuppression (Uberoi, et al, 2016, *Plos Pathogen*). Our lab and others also showed that MmuPV1 can infect the mouse female reproductive tract (Cladel, et al., 2017, *Sci Rep*; Uberoi & Spurgeon et al, in preparation). Therefore, we wanted to test whether MmuPV1 can infect the mouse in the oral cavity, and furthermore, whether it causes cancer by itself or with the help of a chemical carcinogen. We initiated our studies by infecting two types of immunodeficient with 10^8 VGE of MmuPV1 (an amount of virus known to cause warts in the skin and neoplastic disease in the female reproductive tract) in two locations: 1) Nod Scid Gamma mice (NSG) mice were infected on their tongue; 2) Fox1nu/nu mice were infected in the epithelium covering the outer rear mandible. Presence of virus in lavage samples taken from the oral cavity was detected by performing MmuPV1 E2-specific PCR. By the end of 4 weeks after infection, 3 out of 4 NSG mice that were orally infected were positive by PCR for E2, and all of these 3 mice developed papilloma-like lesions on their tongues. H&E staining confirmed the existence of a papilloma at the infection site, and the presence of koilocytes in the papilloma, a hallmark of papillomavirus induced lesions. BrdU staining indicates increased numbers of cells undergoing DNA synthesis in the papilloma. Immunofluorescent staining for the viral major capsid protein, L1, indicates that the productive phase of MmuPV1 life cycle was established in the papilloma. Presence of pERK and pS6, two phosphoproteins known to be upregulated in HPV-related tumorigenesis, were increased in the papilloma induced by MmuPV1. Similarly, one of two orally infected nude mice developed two tumors that showed robust expression of the L1 capsid protein. These observations demonstrate that MmuPV1 can infect and cause papillomatosis on the tongue and in the oral cavity of immunodeficient mice. These data raise the potential for establishing an in vivo infection model for HNSCC using MmuPV1.

29. “Influenza Virus Repurposes the Canonical Antiviral Protein IFIT2 as a Pro-Viral Effector to Promote Translation of Viral mRNAs”

Vy Tran, Mitchell P. Ledwith, Thiprampai Thamamongood, Christina A. Higgins, Shashank Tripathi, Max W. Chang, Christopher Benner, Adolfo Garcia-Sastre, Martin Schwemmle, Adrianus C. M. Boon, Michael S. Diamond, Andrew Mehle (UW)

Hosts mount large-scale responses to viral infection in an attempt to block viral replication. Viruses must adapt to replicate in a hostile anti-viral cellular state that is established as part of this host response to pathogens. Interferon stimulation or pathogen challenge robustly induces the interferon induced proteins with tetratricopeptide repeats (IFIT). IFITs are a family of vertebrate proteins that are broadly recognized for their RNA-binding and anti-viral roles during infection, and it has been suggested that they impair translation of viral mRNAs. IFITs have been shown to restrict multiple viruses, including poxviruses, rhabdoviruses, flaviviruses, and coronaviruses. Thus, we were surprised to identify the IFIT family as candidate pro-viral host factors for influenza A virus (IAV) in our genome-wide CRISPR-Cas9 knockout screen. Cells lacking IFIT2 or IFIT3 were selectively enriched over sequential rounds of challenge with influenza virus. We validated the screen by showing that IFIT2-deficient cells support lower levels of IAV replication and exhibit defects in viral gene expression. IFIT2 expression selectively enhanced translation of viral proteins by binding viral mRNAs, but not genomic RNAs. Further experiments revealed that IFIT2 associates with actively translating ribosomes in infected cells where it recruits viral mRNA into the pool of highly translated messages. IFIT2 RNA-binding mutants failed to stimulate gene expression during infection, linking changes in translational efficiency by IFIT2 to its stimulatory role on viral replication. These data demonstrate a positive role for IFIT2 in the post-transcriptional regulation of influenza virus mRNAs, in contrast to its anti-viral role for other viruses. They further reveal how IAV re-purposes a canonically anti-viral protein to enhance its replication. Moreover, it is highly unlikely that the re-purposing of anti-viral genes is specific to influenza virus, but rather our work suggests a new virus:host interface that may be generalizable to other re-purposed anti-viral proteins and viruses.

30. “Polymorphisms in Rhesus Macaque Tetherin are Associated with Differences in Acute Viremia in SIV DNef-Infected Animals”

Sanath Kumar Janaka, Aidin Tavakoli-Tameh, William J. Neidermyer Jr., Ruth Serra-Moreno, James A. Hoxie, Ronald C. Desrosiers, R. Paul Johnson, Jeffrey D. Lifson, Steven M. Wolinsky and David T. Evans (UW)

Tetherin (BST-2 or CD317) is an interferon-inducible transmembrane protein that inhibits virus release from infected cells. To determine the extent of sequence variation and the impact of polymorphisms in rhesus macaque tetherin on SIV infection, tetherin alleles were sequenced from 146 rhesus macaques, including 68 animals infected with wild-type SIVmac239 and 47 animals infected with SIVmac239DNef. Since Nef is the viral gene product of SIV that counteracts restriction by tetherin, these groups afford a comparison of the effects of tetherin

polymorphisms on SIV strains that are, and are not, resistant to tetherin. We identified 15 alleles of rhesus macaque tetherin with dimorphic residues at 9 positions. The relationship between these alleles and plasma viral loads was compared during acute infection, prior to the onset of adaptive immunity. Acute viremia did not differ significantly among the wild-type SIV-infected animals; however, differences in acute viral loads were associated with polymorphisms in tetherin among the animals infected with SIVDnef. In particular, polymorphisms at positions 43 and 111 (P43 and H111) were associated with lower acute phase viral loads for SIVDnef infection. These observations reveal extensive polymorphism in rhesus macaque tetherin, maintained perhaps as a consequence of variability in the selective pressure of diverse viral pathogens, and identify tetherin alleles that may have an inherently greater capacity to restrict SIV replication in the absence of Nef.

31. “The Suitability of the Porcine Model for Assessing Performance of the Species B Adenoviruses”

Matthew Robertson, Lisa Koodie, Malavika Chandrashekar, Kari Jacobsen, George Ruth, Michele Dunning, Praveensingh Hajeri, Richard Bianco, Julia Davydova (UMN)

Engineering oncolytic adenoviruses (Ad) utilizing species B (Ad3, Ad35, Ad11) receptors have greatly improved oncolytic potential of Ad-based therapies. However, lack of a viable animal model has impeded clinical translation of these Ads. Here, we explore pigs as a model to evaluate performance of group B Ads by employing Ad5/Ad3 chimeric vector. We analyzed binding, gene transfer, replication, and cytolysis ability of Ad5 and Ad5/Ad3 in non-human cell-lines (porcine, hamster, murine, canine). Among tested cell-lines, only porcine cells supported Ad5/Ad3 binding. Cell-viability analyses with Ad5/Ad3 revealed cytolysis solely in swine cells, substantiating porcine as the only non-human model to permit Ad5/Ad3 replication. Viral DNA and replication-dependent luciferase expression was observed in swine lungs and spleen suggesting active viral replication. These results indicate that pigs are a valuable model to assess safety and bio-distribution of oncolytic adenoviruses utilizing the Adenovirus type 3 receptors.

32. “Phosphorylation Regulates Influenza Virus Polymerase Function”

Anthony R Dawson, Arindam Mondal, Elyse Freiburger, Joshua J. Coon, and Andrew Mehle (UW)

The influenza ribonucleoprotein complex (RNP) performs both viral gene transcription and genome replication. Consequently, proper RNP formation and function are indispensable for influenza virus replication. The RNP is composed of the heterotrimeric viral RNA-dependent RNA polymerase bound to both ends of the negative sense genomic RNA that is coated by nucleoprotein (NP). During RNP generation, monomeric NP oligomerizes to encapsidate nascent RNA produced by

the viral polymerase. We have previously shown that members of the host protein kinase C (PKC) family phosphorylate NP and regulate its oligomerization, specifically controlling replication and de novo RNP assembly. Our current work reveals that phosphorylation also controls the activity of the viral polymerase. We show that PKC delta (PKCd) phospho-regulates the polymerase independent of its effect on NP function. In experiments that measure polymerase function independent of NP, PKCd expression reduced production of viral mRNAs shifting the balance between replication and transcription. Mass spectrometry of the polymerase identified phosphosites on all 3 polymerase subunits (PB1, PB2, and PA) that are enriched or only present in the presence of active PKCd. We assessed the functional consequences of polymerase phosphorylation by ablating or mimicking phosphorylation at each position. Polymerase activity assays and virus replication experiments with these mutants revealed key phosphosites required for polymerase activity and potential inhibitory phosphorylation events. Primer extension assays confirmed these results and characterized the ability of each mutant polymerase to synthesize transcripts or replication products, discrete functions required at different times during infection. Additionally, we identified suppressor mutations that are refractory to PKCd-driven changes in polymerase function, reinforcing the biological importance of these newly identified phosphosites. These experiments show that host PKCd phospho-regulates the influenza virus polymerase and provide insight into how polymerase activity might be controlled to bias transcription versus replication at discrete times during the infectious cycle.

33. “A Novel Chemotype as Hepatitis B Virus Capsid Assembly Effectors”

Jing Tang, Carlos J. A. Ribeiro, Andrew D. Huber, Jayakanth Kankanala, Jiashu Xie, Stefan G. Sarafianos and Zhengqiang Wang (UMN)

Chronic hepatitis B virus (HBV) infection represents a global health threat. Current FDA-approved direct acting antivirals consist of only one class of drugs, the nucleoside analogues (NAs), which do not cure HBV. Among novel antiviral approaches, targeting HBV core protein (Cp) represents a particularly attractive approach toward inhibition and infection cure of HBV. We present herein a novel chemotype as capsid assembly effectors (CAEs). The original hit with confirmed binding affinity to Cp and low micromolar antiviral activity was identified through high-throughput screening (HTS) of commercial libraries. Subsequent hit optimization through extensive structure-activity relationship (SAR) and structure-property relationship (SPR) studies involved analogue synthesis, biological assays, in vitro absorption, distribution, metabolism and excretion (ADME), and animal pharmacokinetics (PK). These medicinal chemistry efforts led to the identification of multiple analogues strongly binding to Cp and potently inhibiting HBV replication in nanomolar range without cytotoxicity. Two of our analogues, ZW-1066 (EC₅₀ = 0.11 uM, F = 25%, CC₅₀ >100 uM) and ZW-1042 (EC₅₀ = 0.31 uM, F = 46%, CC₅₀ >100 uM), displayed overall lead profiles superior to reported CAEs used as controls in our studies. Molecular modeling and resistance profiling provided valuable insights into their binding mode and

mechanism of action.

34. “Type 2 EBV Induced Highly Lytic B-cell Lymphomas in a Cord Blood-Humanized Mouse Model”

James C Romero-Masters, Shane M Huebner, Makoto Ohashi, Elizabeth Barlow, Eric Johannsen, Shannon C Kenney (UW)

EBV strains are classified into major types known as type 1 or type 2 which is determined by their EBNA2/EBNA3s latent gene sequences. Type 2 EBV infection is most commonly found in Africa (where approximately 25% of the population is infected) and occurs with or without concomitant type 1 infection. Type 2 EBV transforms EBV-infected B cells in vitro into lymphoblastoid cell lines (LCLs) much less efficiently than type 1 EBV; this difference is due to a single amino acid change in EBNA2 that results in higher level LMP1 expression with type 1 EBV infection (Tzellos et al., J. Virol., 2014). Interestingly, type 2 EBV was recently reported to infect T cells much more efficiently in vitro than type 1 EBV (Coleman et al., J. Virol., 2015). Here, we have compared the in vivo phenotypes of two type 1 EBV strains (Akata and Mutu) versus two type 2 EBV strains (AG876 and BL5) in a cord blood-humanized (CBH) mouse model, as well as in in vitro generated LCLs containing each virus strain. Both type 1 and 2 EBV strains induced activated diffuse large B-cell lymphomas (DLBCLs) with a similar high efficiency in CBH mice. All tumors had type III viral latency, but tumors infected with type 2 AG876 EBV expressed less LMP1. We found very few EBV-infected T cells in either type 1 or type 2 EBV-infected CBH mice, although uninfected T cells infiltrated both tumor types extensively. Interestingly, type 2 AG876 EBV-infected lymphomas were highly lytic compared to either type 1 Akata EBV-infected lymphomas and expressed high levels of immediate-early (BZLF1) and late (Gp350 and p18) lytic proteins. Similarly, AG876- and BL5-infected LCLs had highly lytic infection in comparison Akata- and Mutu infected LCLs. These results indicate that type 2 EBV efficiently induces B-cell lymphomas in the CBH mouse model and suggest that type 2 EBV (which contain the Zp-V3 variant of the BZLF1 promoter) may promote especially high levels of lytic infection.

