'Wisc-e-sota'

5th Joint UMN-UW Virology Training Grant Symposium

October 11th, 2017 7-10pm

Opening reception & keynote lecture

October 12th, 2017 8:00am-4:00pm

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

5th Annual Wisc-e-sota Virology Symposium

Wednesday-Thursday, October 11-12, 2017
Radisson Hotel
La Crosse, Wisconsin

Oral Presentations

Talk abstract numbers in left column

	October 11, 2017 (Wednesday)	
	6:30-7:30pm	Registration
	7:00-10:00pm	Opening reception
	7:40-7:45pm	Welcome and opening remarks
	7:45-8:45pm	Opening talk – James Neaton (UMN)
	October 12, 2017 (Thursday)	
		Convenor: Reza Djavadian (UW)
	7:30-8:00am	Check-in and poster set-up
	8:00am	Breakfast
1	9:00am	Robert Geraghty (UMN)
2	9:30am	Patrick Nyman (UW)
3	9:45am	Morgan Meissner (UMN)
	10:00-10:30am	Morning break
		Convenor: Liang Guo (UMN)
4	10:30am	Aidin Tavakoli Tameh (UW)
5	10:45am	Dmitri Kotov (UMN)
6	11:00am	James Bruce (UW)

	11:30-2:00pm	Lunch, poster session, and group picture
		Convenor: Isaac Angert (UMN)
7	2:00pm	Yoshi Kawaoka (UW)
8	2:30pm	Christopher Richards (UMN)
9	2:45pm	Aurelia Faure (UW)
		Convenor: Myeong Kyun Shin (UW)
10	3:00pm	Ti Luong (UMN)
11	3:15pm	Tony Dawson (UW)
12	3:30pm	Ryan Langlois (UMN)
	4:00pm	Closing Remarks

Poster Presentations

1. "HSV-1 Replication is Inhibited by the Antiviral DNA Cytosine Deaminase"
   Adam Cheng, Thomas Frost, Stephen Rice, Reuben Harris (UMN)

2. "Gene Correlation Analysis Identifies Functional Pro- and Anti-Viral Factors for Influenza Virus Infection"
   Gloria P. Larson, Vy Tran, Shuiqìng Yú, Yíngyún Caì, Danielle Smith, Jens H. Kuhn, and Andrew Mehle (UW)

3. "HIV-1 Adaptation Studies Reveal a Novel Vif-Independent Mechanism for Evading Lethal Restriction by APOBEC3G"
   Terumasa Ikeda, Menelaso Symeonides, John S. Albin, Ming Li, Markus Thali, and Reuben Harris (UMN)

4. "Polymorphisms in Rhesus Macaque Tetherin are Associate with Difference in Peak Viremia During Acute Infection with SIV"
   Sanath Kumar Janaka, Aidin Tavakoli-Tameh, William J. Neidermyer Jr., Ruth Serra-Moreno, Bin Jia, James A. Hoxie, Ronald C. Desrosiers, Paul Johnson, Jeffrey D. Lifson, Steven M.Wolinsky, and David T. Evans (UW)

5. "APOBEC3B Lysine Residues are Dispensable for DNA Cytosine Deamination, HIV-1 Restriction, and Nuclear Localization"
   Amy M. Molan, Health M. Hanson, Cynthia M. Chweya, Brett D. Anderson, Gabriel J. Starrett, Christopher M. Richards, Reuben S. Harris (UMN)

6. "UVB Mediated Immunosuppression is Necessary for Papillomavirus Mediated Pathogenesis"
   Aayushi Uberoi, Megan Spurgeon, Tao Wei, Jeremiah Ye, Ed Glover, Ian Frazer, Chris Bradfield, Paul Lambert (UW)

7. "Lethal Mutagenesis of HIV-1 Induced by 5-aza-2-deoxycytidine in Human Primary CD4+T Cells"
   Emily Julik, Morgan E. Meissner, Megan E. Roth, Marzena Baran, Jerry Daniel, Daryl M. Gohl, Kenneth B. Beckman, Joshua A. Baller, Steven E. Patterson, Louis M. Mansky (UMN)

8. "Bacterial Viral Infection Influences Metabolic Interactions of Microbial Community Members"
   Lisa Fazzino, William Harcombe (UMN)

9. "Perturbations to the HIV-1 Programmed Ribosomal Frameshift Site Dictates Translational Regulation and Viral Genomic RNA Transcript Fate"
   Bayleigh E. Benner, Jordan T. Becker, Pablo Garcia-Miranda, Samuel E. Butcher, and Nathan M. Sherer (UW)

10. "Single Nucleotide Editing Using Chimeric APOBEC-Cas9 Complexes"
Amber St. Martin, Daniel Salamango, and Reuben S. Harris (UMN)

11. "Latent Epstein-Barr Virus Infection in a Keratinocyte Model Impairs Differentiation"
Mark Eichelberg, Rene Welch, Ahmed Ali, Makoto Ohashi, Joseph Guidry, Shannon Kenney, Sunduz Keles, Rona Scott, and Eric Johannsen (UW)

12. "Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes"
K. Shi, M.A. Carpenter, S. Banerjee, N.M. Shaban, K. Kurahashi, D.J. Salamango, J.L. McCann, G.J. Starrett, J.V. Duffy, Ö Demir, R.E. Amaro, D.A. Harki, H. Aihara, and R.S. Harris (UMN)

13. "Epstein Barr Virus Transcription Factors Rta and Zta Play Distinctly Different Roles in Promoting EBV Replication in Epithelial Cells"
Ahmed Ali, Mark Eichelberg, Makoto Ohashi, Reza Djavadian, Shannon Kenney, and Eric Johannsen (UW)

14. "Translation Control of HIV-1"
Kathleen Boris-Lawrie (UMN)

15. "HIV, Ratio Normalization and Winning the Game of T Cells"
Rob Striker, Dawit Wolday, Mitchell Kirsh, Ajay Sethi, Joe McBride, and Irene Ong (UW)

16. "The Hepatitis C Viral Protein NS5A Stabilized Growth-Regulatory Human Transcripts"
Liang Guo, Suresh D. Sharma, Jose Debes, Daniel Beisang, Bernd Rattenbacher, Irina A. Vlasova-St. Louis, Darin L. Wiesner, Craig E. Cameron, and Paul R. Bohjanen (UMN)

17. "Quantitative Imaging of Rhinovirus Infection Spread with Single-Cell Resolution"
Huicheng Shi, Bahar Inankur, and John Yin (UW)

18. "Regulation of EBV Lytic Reactivation by p53 and p63 in Epithelial Cells"
Nick Van Sciver, Dhananjay Nawandar, Denis Lee, Kathellen Makielski, Paul Lambert, and Shannon C. Kenney (UW)

19. "Characterizing Guinea Pig Cytomegalovirus Pathogenesis in the Amnion"
Dira S. Putri, Craig J. Bierle (UMN)

20. "Deep Sequencing Barcoded Zika Virus from Macaques Using a Small Amplicon Approach"
Katie Zarbock, Paola Silveira, Team ZEST, Greg Ebel, David O'Connor, and Shelby O'Connor (UW)

21. "Cryo-EM Structure of Porcine Delta Coronavirus Spike Protein in the Pre-Fusion State"
Jian Shang, Yuan Zheng, Yang Yang, Chang Liu, Qibin Geng, Wanbo Tai, Lanying Du, Yusen Zhou, Wei Zhang, Fang Li (UMN)

22. "CXCR5-Transduced Primary Rhesus Macaque PBMCs Accumulate in B Cell Follicles in a Novel Ex Vivo B Cell Follicle Migration Assay"
Gwantwa Mwakalundwa, P Haran, S Li, M Pampusch, HM Abdeelal, EG Rakasz, E Connick, PJ Skinner (UMN)

23. "EBNA3C-Deleted Epstein-Barr Virus Causes Lymphomas in a Humanized Mouse Model Despite Being Severely Deficient for B Cell Transformation In Vitro"
James Romero-Masters, Reza Djavadian, Makoto Ohashi, Shidong Ma, Andrea Bilger, Eric Johannsen, and Shannon Kenney (UW)

24. "Novel Features of CRM1-Dependent Retroviral mRNA Nuclear Export Revealed Using Live Cell Imaging"
Ryan T. Behrens, Christina Higgins, and Nathan M. Sherer (UW)

25. "Analysis of Proviral and Particle Structure from Distinct Chronically Infected Cell Lines Demonstrates the Polymorphic Nature of HTLV-1 Particle Cores"
Luiza Mendonça, Morgan E. Meissner, Wei Zhang, Louis Mansky (UMN)

26. "Role of IQGAP1 in HPV-Positive and HPV-Negative Head and Neck Carcinogenesis"
Tao Wei, Suyong Choi, Darya Buehler, Richard Anderson, and Paul Lambert (UW)

27. "SIV Vif and Human APOBEC3B Interactions Resemble Those Between HIV-1 Vif and Human APOBEC3G"
Jiayi Wang, Nadine M. Shaban, Allison M. Land, William L. Brown, Reuben S. Harris (UMN)

28. "Species-Specific Regulation of HIV-1 Gene Expression and Vif-Induced Cell Cycle Arrest"
Edward L. Evans III, Jordan T. Becker, Stephanie S. Fricke, Kishan M. Patel, and Nathan M. Sherer (UW)

29. "Visualizing HTLV-1 Gag Puncta Biogenesis by Super-Resolution Microscopy"
John Eichorst, John Kohler, Joachim Mueller, and Louis Mansky (UMN)

30. "Comprehensive Analysis of the Kinetics of Epstein-Barr Virus Lytic Gene Expression"
Reza Djavadian, Mitch Hayes, and Eric Johannsen (UW)

31. "Identification of Cancer Cell Lines with a Clear APOBEC Mutation Signature"
Matthew C. Jarvis, Diako Ebrahimi, Nuri A. Temiz, and Reuben S. Harris (UMN)

32. "Cryo-Electron Tomography Reveals Novel Features of a Viral RNA Replication Compartment"
Kenneth J. Ertel, Desirée Benefield, Daniel Castaño-Diez, Janice G. Pennington, Mark Horswill, Johan A. den Boon, Marisa S. Otegui, and Paul Ahlquist (UW)

33. "Studies of Non-Punctate, Membrane-Bound HTLV-1 Gag by Fluorescence Fluctuation Spectroscopy and Fluorescence Lifetime Imaging Microscopy"
Isaac Angert, John Eichorst, John Kohler, Jessica Martin, Wei Zhang, Louis M Mansky, and Joachim D Mueller (UMN)

34. "Whole-Transcriptome Sequencing to Identify Immune Gene Variants"
Amelia K. Haj, Julie A. Karl, Roger W. Wiseman, and David H. O'Connor (UW)

35. "Human Papillomavirus Promotes Epstein-Barr Virus Lytic Reactivation in Immortalized Oral Keratinocytes"
Kathleen Makielski, Denis Lee, Laurel Lorenz, Dhananjay Nawandar, Ya-Fang Chiu, Shannon Kenney, and Paul Lambert (UW)

Abstracts – Oral Presentations

"Two Viruses (HIV and Ebola), Two Research Networks (INSIGHT and PREVAIL), Too Busy!"
James Neaton

Two major international collaborations involving the University of Minnesota, the International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) and the Partnership for Research on Ebola Virus in Liberia (PREVAIL), have resulted in the design and conduct of several major clinical trials. In this talk, we will describe the origin of each of the research groups, challenges we faced in the design and conduct of clinical trials, some of the trial results, and what future research is being planned.

1. "Inhibition of HCMV pUL89 Endonuclease Activity and Virus Replication"
Yan Wang, Jayakanth Kankanala, Jing Tang, Zhengqiang Wang and Robert J. Geraghty

Human cytomegalovirus infection in individuals lacking a fully functioning immune system, such as newborns and transplant patients, can result in severe and debilitating consequences. Approved anti-human cytomegalovirus drugs mainly target the viral polymerase and resistance to those drugs has appeared. Therefore, new drugs from novel targets are needed for use instead of, or in combination with, current polymerase inhibitors. Human cytomegalovirus terminase complex cleaves the concatemeric genomic viral DNA into unit lengths during genome packaging and particle assembly. Terminase complex ATPase and endonuclease activity is provided by the viral protein pUL89. pUL89 is an attractive drug target because its activities are required for infectious virus production. A domain located in the C-terminus of pUL89 has an RNase H/integrase-like fold and endonuclease activity that can be inhibited by compounds featuring a chelating triad motif. We have identified a variety of small molecule chelating scaffolds that inhibit pUL89 endonuclease activity in vitro and demonstrate characteristics of pUL89 inhibitors in cell culture. Taken together, the results substantiate our pharmacophore hypothesis and validate our ligand-based approach toward identifying novel inhibitors of pUL89 endonuclease.

2. "Examining the Role of the Notch Signaling Pathway and HPV in Head and Neck Squamous Cell Carinoma"
Patrick Nyman, Darya Buehler and Paul Lambert

Human Papillomavirus (HPV) is a small DNA virus that infects stratified squamous epithelia, and it is well known to be the primary cause of cervical cancer, as well as a subset of Head and Neck Squamous Cell Carcinomas (HNSCC). One pathway recently implicated in HNSCC is the Notch signaling pathway. The Notch signaling pathway is an important pathway in cell differentiation decisions with differential downstream targets and effects depending on tissue type and developmental stage, and the receptor protein Notch1 has been found to be one of the most highly mutated genes in HNSCC. We have therefore hypothesized that the Notch signaling

pathway would act as a tumor suppressor in HPV-positive and HPV-negative HNSCC. Here, we have utilized a mouse strain with inactivated Notch signaling via expression of a dominant negative form of MAML1 (DNMAML1), a required transcriptional coactivator of Notch signaling. We utilized this gene in conjunction with either HPV16 E6/E7 transgenes or a mutated p53 to simulate HPV-positive and HPV-negative cancer drivers, respectively, and monitored the groups for head and neck cancer upon long-term treatment with the carcinogen 4NQO. Mice with inactivated Notch signaling exhibited a greater disease severity and tumor multiplicity both with HPV16 E6/E7 and with mutant p53. We also found that DNMAML1-expressing mice exhibited an increased presence of nuclear ^{2}-catenin, a marker for increased tumorigenic potential. Finally, despite having similar rates of tumor incidence, HPV16 E6/E7 mice with lost Notch signaling developed a higher number of high-grade carcinomas than p53R172H mice with lost Notch signaling. These results indicate that the Notch signaling pathway can serve in a tumor suppressing function in both HPV-positive and HPV-negative HNSCC and that there may be an addtional synergistic effect between HPV and lost Notch signaling in developing high-grade carcinomas.

3. "Molecular Mechanisms of HIV-2 Mutagenesis During Replication"
Morgan Meissner, Emily Julik, and Louis Mansky

More than 36 million individuals are infected with HIV worldwide. Nearly 95% of these individuals are infected with HIV type 1 (HIV-1), which has a high rate of viral mutation that helps drive immune evasion, disease progression, and rapid emergence of drug resistance. HIV type 2 (HIV-2) accounts for fewer than 2 million infections overall, remains primarily restricted to West Africa, and exhibits a significantly attenuated disease phenotype compared to HIV-1, characterized by lower rates of transmissibility and a slower progression to AIDS. HIV-2 has recently been found to have a significantly lower rate of mutation compared with HIV-1, which may be related to the differences in viral disease progression and persistence. Although the main driver of HIV mutagenesis is the low fidelity of the virally encoded reverse transcriptase, host factors such as the APOBEC3 (A3) family of cytosine-deaminases have been found to contribute to viral mutation as well. Here we report that HIV-1 and HIV-2 are restricted by different subsets of APOBEC3 proteins in vitro. Infection of the CEM-GFP T-cell line with HIV-2 does not elicit a robust change in A3 expression as has been seen for HIV-1. Using a single-cycle infectivity assay for viruses produced in the presence of the seven different A3 proteins, we demonstrate that while A3D, -F, -G and -H restrict HIV-1 infection in vitro, only A3F, -G, and H (and to a lesser extent A3C) are able to restrict HIV-2 infection while increasing mutant frequency. This is consistent with previously reported differences in the frequency and rate of G-to-A mutations between the two viruses, and with differences in their packaging into budded virions in the absence of Vif proteins. Sequencing of proviral sequences will be done to determine if restriction by the specific A3 proteins correlates with increased rates of G-to-A mutation and hypermutation, and accumulation of viral RNA products will be analyzed to determine if reverse transcription is inhibited. Preliminary results of this work suggest that HIV-2 experiences less mutagenesis from A3 proteins compared with HIV-1, consistent with previous results. Future studies will expand upon this work in an attempt to correlate mutation

frequency with infectivity for the viruses using nucleoside analogs and small molecule compounds to provide insights into the contrasting phenotypes observed between the viruses.

4. "Mutations in Nef that Selectively Disrupt Tetherin Antagonism Impair SIV Replication during Acute Infection of Rhesus Macaques"
Aidin Tavakoli-Tameh, Sanath Kumar Janaka, Lauren Callahan, Ksenia Bashkueva, Katie Zarbock, Shelby O'Connor, Kristin Crosno, Saverio Capuano, Ruth Serra-Moreno, Hajime Uno, Jeffrey D. Lifson, David. T. Evans

Nef promotes the release of SIV from infected cells by counteracting restriction of non-human primate tetherin (BST-2 or CD317). Nef also downmodulates cell-surface CD4 and MHC class I (MHC I) molecules and enhances infectivity by preventing incorporation of SERINC3/5 into virions. We previously demonstrated that tetherin antagonism by Nef is genetically separable from CD4- and MHC I-downmodulation and infectivity enhancement. Here we show that selective disruption of tetherin antagonism impairs virus replication during acute SIV infection of rhesus macaques. Separate groups of four rhesus macaques were infected with either wild-type SIVmac239 or an SIVmac239 mutant (SIVmac239-NefAAA) with a combination of three amino acid substitutions in the flexible loop region of Nef that impair tetherin antagonism, but not CD4- or MHC I-downmodulation or infectivity enhancement. Viral RNA loads in plasma were significantly lower during acute infection (weeks 1-4 post-infection) for animals infected with SIVmac239-NefAAA compared to animals infected with wild-type SIVmac239 ($p = 0.0044$, mixed-effects model). Sequence analysis of the virus population in plasma confirmed that the substitutions in Nef were retained during acute infection; however, changes were observed by week 24 post-infection that either restored the wild-type residue or introduced alternative residues at these positions, consistent with selective pressure on Nef to regain anti-tetherin activity. These observations provide the most direct evidence to date that the ability to counteract restriction by tetherin is important for lentiviral replication in primates.

5. "TCR Affinity Influences Helper T Cell Differentiation by Biasing Dendritic Cell Interactions"
Dmitri I. Kotov, Thomas Pengo, Jason S. Mitchell, Jessica A. Kotov, Christiane Ruedl, Sing Sing Way, Ryan A. Langlois, Brian T. Fife, Marc K. Jenkins

CD4+ T lymphocytes utilize their T cell receptor to detect microbial proteins presented by dendritic cells causing T cells to differentiate into various subsets. However the mechanism by which T cell receptor affinity biases T cell differentiation is unknown. We demonstrate that low affinity T cell receptors biased T cells into B cell helpers while high affinity T cell receptors skewed towards non-B cell helper fates, suggesting that the default fate is a B cell helper. This T cell receptor-driven bias in differentiation was identified following infection with the bacteria Listeria monocytogenes as well as the PR8 strain of influenza A virus. To examine T cell-dendritic cell interactions, we created a software for high-throughput, multicolor, quantitative imaging called Chrysalis. With Chrysalis, we show that high affinity T cells maintain prolonged interactions with XCR1+ dendritic cells biasing T cells into macrophage helpers, while lower affinity T cells preferentially interacted with SIRPz+ dendritic cells and became B cell helpers.

Thus, T cell receptor affinity influences T cell-dendritic cell interactions thereby dictating T cell differentiation during bacterial and viral infections.

6. "ZBTB2 Represses HIV-1 Gene Expression in a ZASC1- and ATR-Dependent Manner"
James Bruce, Megan Bracken, Ginger Pocock and Paul Ahlquist

Previously, we reported that cellular transcription factor ZASC1 facilitates DNA-dependent / RNA-independent recruitment of TAT/P-TEFb to the HIV-1 promoter and is a critical factor in regulating HIV-1 transcriptional elongation (PLoS Path e1003712). Subsequently, we identified transcription factor ZBTB2 as a strong ZASC1 interactor and showed that ZBTB2 represses the HIV-1 promoter, dependent on functional ZASC1 binding sites in the HIV-1 promoter and the ZBTB2 POZ domain, which we showed to recruit HDAC1 and HDAC4. Accordingly, siRNA knockdown or CRISPR/CAS9 knockout of ZBTB2 in T cell lines enhanced expression from WT HIV-1, but not from proviruses lacking ZASC1 binding sites. ChIP of infected primary T-cell extracts revealed that ZBTB2 is recruited to the WT HIV-1 LTR but not to an LTR lacking ZASC1 binding sites. Thus, ZASC1 recruits ZBTB2 and represses the HIV-1 promoter. HIV-1 VPR activates the viral LTR by inducing the ATR kinase DNA damage response. Since a consensus ATR phosphorylation site is located in ZBTB2s ZASC1 binding region, we investigated if ZBTB2 function was regulated by VPR and the DNA damage response. Co-expressing ZASC1 dramatically relocalized ZBTB2 from the cytoplasm to the nucleus. Mutations abolishing ZASC1/ZBTB2 interaction prevented ZBTB2 nuclear relocalization and ZBTB2-mediated repression of HIV-1 transcription. Mutating the putative ATR phosphorylation site in ZBTB2 increased its nuclear localization and repressive activity. Conversely, expressing wt VPR, but not VPR mutants deficient in ATR activation, increased ZBTB2 cytoplasmic localization. Significantly, stimulating the ATR pathway with DNA damaging agents reproduced VPRs effects on ZBTB2 localization. Moreover, the effects of DNA damaging agents and VPR on ZBTB2 localization could be blocked by ATR kinase inhibitors or overexpressing WIP1 phosphatase, which removes ATR phosphorylations. These data support a model in which ATR-phosphorylated ZBTB2 is preferentially cytoplasmic, while de-phosphorylated ZBTB2 is nuclear and actively represses HIV-1 transcription in a ZASC1- and ATR-dependent manner.

7. "Working With Deadly Viruses: Battling Ebola and Influenza"
Yoshi Kawaoka

Every year, influenza epidemics occur, causing increased morbidity and mortality, particularly in vulnerable populations, such as the very young and very old. In addition, worldwide epidemics, such has the 1918 pandemic, occasionally occur. Consequently, influenza has an enormous impact on the global economy. By contrast, Ebola virus has only been recognized since 1976, and, until recently, outbreaks of this virus had caused relatively few deaths because they occurred in rural, isolated areas. However, the recent outbreak in West Africa occurred over a large, densely populated urban area and changed our understanding of what constitutes an Ebola virus outbreak. I will discuss our recent research on these viruses.

8. Differential Expression of APOBEC3H Splice Variants and a Potential HIV-1 Protease Defense Mechanism

Christopher Richards, Diako Ebrahimi, Michael Carpenter, Jiayi Wang, Brett Anderson, Nadine Shaban, Adam Cheng, Terumasa Ikeda, and Reuben S. Harris

APOBEC3 enzymes provide innate immune protection from a variety of pathogens including the AIDS virus HIV-1. APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H combine to potently restrict viral reverse transcription by deamination-dependent and -independent mechanisms. In humans, APOBEC3H is the most diverse member of the APOBEC3 family, as it is encoded by at least seven different haplotypes (which are comprised of different combinations of 5 SNPs) and four different isoforms (SV154, SV182, SV183, SV200). Using RNA-Seq from the 1000 Genomes Database, we have found that HapI is prevalent outside of Africa, while HapII is dominant in Africa and that these two different haplotypes have significantly different alternative splicing of APOBEC3H mRNA, which manifests as higher expression of SV200 in HapII indivuals compared to significantly lower expression in HapI individuals. Unexpectedly, while characterizing the A3H splice variants in HIV-1 infectivity assays, we observed that HIV-1 protease was specifically interacting with the SV200 form of A3H. These results suggest that HIV-1 may have adapted to use protease to counteract/modulate A3H SV200.

9. "How Does Epstein-Barr Virus Likely Contribute to Primary Effusion Lymphomas?"

Aurelia Faure, Bill Sugden

Primary Effusion Lymphoma (PEL) is the only human cancer known to be associated with two tumor viruses. Kaposi's sarcoma-associated herpesvirus (KSHV) is present in 100% of PELs and 90% of them are co-infected with Epstein-Barr virus (EBV). We want to understand the likely co-dependence of PELs on these two viruses. KSHV encodes anti-apoptotic genes essential for the survival of PEL cells. We have therefore asked what EBV genes provide selective advantages to PEL cells. Introduction of vectors expressing various EBV genes into BC-1 cells showed that the BART miRNAs were sufficient to lead to the loss of EBV from these cells. We conclude that EBVs BART miRNAs support BC-1 cells and likely other PELs too. We hypothesize that EBV, likely in part via the BART miRNAs, provides proliferative signals initially to B cells that evolve into PELs when co-infected with KSHV because KSHV has failed to support long-term proliferation of B cells in vitro. To test this hypothesis, we have infected peripheral B cells with KSHV BAC16, which encodes a GFP marker (Brulois et al. J. Virol. 2012), plus or minus EBV. The number of cells expressing GFP was measured by flow cytometry from day 4 through 42 following infection with KSHV. Peripheral primary B cells could not be detectably infected with KSHV whereas co-infection with EBV increased the efficiency of infection up to 3%. We have found that EBV infection has two opposing effects on KSHV infection: early events of EBV infection increase the efficiency of infection with KSHV while the efficiency of infection of EBV-infected cells with KSHV decreases over time. KSHV infects the largest fraction of primary B cells when it enters cells prior to EBV infection. In vitro activation of B cells with IL-4 and CD40 ligand partially mimics EBV infection by allowing detectable infection of B cells with KSHV. These experiments reveal that EBV infection is essential for KSHV to infect peripheral B cells

detectably and that the requirements for this co-infection are complex.

10. "Exomsomes Secreted From Virus-Infected Microglia Can Activate an Inflammatory Response in the Central Nervous System"
Nhungoc (Ti) Luong and Julie K. Olson

Exosomes are membrane-bound nanovesicles released from cells that can be taken up by other cells, thus mediating intercellular communication. Exosomes contain miRNA, mRNA, and proteins that are specific to the cells from which they were released. Theilers murine encephalomyelitis virus (TMEV) induced demyelinating disease 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. We have previously shown that TMEV infection of microglia activates an innate immune response with expression of type I interferons, cytokines, chemokines, and effector molecules associated with development and progression of demyelinating disease. Next, we wanted to determine whether microglia infected with TMEV secrete exosomes and whether these exosomes could influence uninfected CNS resident cells, such as microglia, astrocytes, and neurons. The exosomes secreted from TMEV-infected microglia contain viral RNA that could be transferred to bystander uninfected CNS cells. The exosomes secreted from virus- infected microglia activated the innate immune response in bystander uninfected microglia, astrocytes, and neurons by increasing the expression of type I interferons and pro-inflammatory cytokines. The viral RNA was sensed by innate immune receptors in the recipient cells and activated an innate immune response in the uninfected bystander cells. Most interestingly, exosomes secreted from microglia in the brain of TMEV- infected mice could be transferred to uninfected mice and activate an inflammatory immune response in the brain of uninfected mice. These results show that exosomes secreted by TMEV-infected microglia can activate the immune response in bystander uninfected CNS cells which may contribute to the inflammatory response associated with persistent virus infection and demyelinating disease. This research was supported by grants from UMN Comparative Medicine (R21742) and NIH (T32DA007097).

11. "Phospho-Regulation of the Influenza Virus Replication Machinery"
Anthony R. Dawson, Arindam Mondal, Gregory K Potts, Elyse C Freiberger, Steven F Baker, Lindsey A Moser, Kristen A Bernard, Joshua J Coon, Andrew Mehle

Influenza virus expresses transcripts early in infection and transitions towards genome replication at later time points. This process requires de novo assembly of the viral replication machinery, large ribonucleoprotein complexes (RNPs) composed of the viral polymerase, genomic RNA and oligomeric nucleoprotein (NP). Despite the central role of RNPs during infection, the factors dictating where and when they assemble are poorly understood. Here we demonstrate that human protein kinase C (PKC) family members regulate RNP assembly. Activated PKC´ interacts with the polymerase subunit PB2 and phospho-regulates NP oligomerization and RNP assembly during infection. Consistent with its role in regulating RNP

assembly, knockout of PKC´ impairs virus infection by selectively disrupting genome replication. However, primary transcription from pre-formed RNPs deposited by infecting particles is unaffected. Thus, influenza virus exploits host PKCs to regulate RNP assembly, a step required for the transition from primary transcription to genome replication during the infectious cycle.

12. "Cellular Resistance and Susceptibility to Influenza Virus Infections"
Louis Sjaastad, Elizabeth Fay, Jessica Fiege, Barbara Waring, Marissa Macchietto, Steven Shen, and Ryan Langlois

Influenza A virus (IAV) infects a broad range of cell types within the respiratory tract. The cells initially targeted by the virus in a naïve host, founder cells, are the site of primary replication and virus spread. Founder cells are difficult to detect using replication competent IAV as the virus spreads too quickly to isolate the cells that were infected first. To overcome this obstacle we utilize a fluorescence expressing single cycle IAV to characterize founder cells in the mouse lung. Using this tool we determined that several district cell types in different anatomical locations in the airway are infected during the first round of replication. Using the intensity of the fluorophore as a surrogate for virus polymerase activity we also discovered two distinct populations of cells with high and low of virus. These data suggest that some cells within the lung are highly permissive to virus replication while others are able to blunt replication. To determine the degree of protection infected founder cells can confer to potential secondary infected cells we used sequential single cycle IAVs that express distinct fluorophores to label primary and secondary infected cells. As expected there was a significant reduction in the overall number of secondary infected cells, likely due to the antiviral environment established by the first round of replication. Surprisingly this reduction was only in observed in the cells with low levels of polymerase activity. Cells with high levels of polymerase activity were not reduced. Together these data demonstrate that there are cells within the lung that are highly permissive to virus replication. We postulate that these cells are the virus factories that amplify virus even in the face of potent IFN responses.

Poster Abstracts

1. "HSV-1 Replication is Inhibited by the Antiviral DNA Cytosine Deaminase APOBEC3G"
Adam Cheng, Thomas Frost, Stephen Rice, Reuben Harris

APOBEC3 (A3) proteins belong to a family of DNA cytosine deaminase enzymes that function in innate immunity and cancer pathogenesis. Of the seven human A3 proteins, APOBEC3G (A3G) is a well-studied restriction factor that inhibits the replication of retroviruses like HIV and HTLV-1 by catalyzing the conversion of cytosines to uracils in viral cDNA replication intermediates, which results in viral DNA degradation or hypermutation. Only one study to-date has addressed the effect of A3s on HSV-1 infectivity and mutagenesis. Interestingly, only APOBEC3C (A3C), but not A3G was found to restrict HSV-1. To further investigate the biological relevance of A3s on HSV-1 replication, we generated a panel of stable A3-expressing 293T cells, infected cells with HSV-1 KOS1.1, and monitored virus replication by plaque assays. We found that A3G induces a log-fold decrease in HSV-1 replication at a low MOIs and a dampened, but consistent reduction in replication at high MOIs. Additionally, HSV-1 does not appear to have the capacity to degrade A3s, which is in contrast to the HIV-1 Vif-mediated degradation of A3s via a ubiquitin-proteasome pathway. Further work is required to elucidate the mechanism HSV-1 restriction, but out initial studies suggest that A3G is a novel biologically relevant innate immune molecule that functions in inhibiting HSV-1 replication.

2. "Gene Correlation Analysis Identified Functional Pro- and Anti-Viral Factors for Influenza Infection"
Gloria P. Larson, Vy Tran, Shuiqìng Yú, Yíngyún Caì, Danielle Smith, Jens H. Kuhn, and Andrew Mehle

Viruses both utilize and are antagonized by host cellular factors, pathways, and post-translational modifications. Several screening methodologies have mapped host-cell interactions with influenza A viruses (IAV). These screens identified numerous host factors that regulate IAV infection, but 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 that correlates IAV infection susceptibility with naturally occurring differences in gene expression among different cell lines. This approach identified several host factors that strongly enhance or suppress IAV replication. We investigated top candidates, including the potential enhancer epidermal growth factor receptor (EGFR) pathway substrate 8 (EPS8) and suppressor poly-ADP-ribose polymerase 8 (PARP8). Our data suggested EPS8 is upregulated upon infection, and overexpressing EPS8 increases viral gene expression and viral titer, validating its role as a pro-viral host factor. Probing steps throughout the viral life cycle suggested EPS8 functions post-attachment during viral entry. EPS8 is involved in actin remodeling and endocytosis processes, and mutant analyses suggested these functions are important for viral entry enhancement. The other host factor, PARP8, is a member of the poly-ADP-ribose polymerase (PARP) family of enzymes. PARPs facilitate the post-translational modification ADP-ribosylation, which has been shown to be upregulated in

response to diverse viral infections. We showed PARP8 inhibits IAV polymerase activity in reporter assays, reduces viral gene expression during infection, and decreases overall viral replication. Our data further demonstrate that PARP8 and ADP-ribosylation may directly target the viral polymerase to repress IAV replication, supporting our screen results. By exploring an alternative screen approach gene correlation analysis we identified previously unknown viral host factors with opposing effects on viral infection outcome. These results expand our knowledge of how IAV co-opts host cellular machinery to support successful infection and potential anti-viral drug targets.

3. "HIV-1 Adaptation Studies Reveal a Novel Vif-Independent Mechanism for Evading Lethal Restriction by APOBEC3G"
Terumasa Ikeda, Menelaos Symeonides, John S. Albin, Ming Li, Markus Thali, and Reuben S Harris

HIV-1 replication requires Vif-mediated neutralization of APOBEC3 antiviral enzymes. Viruses lacking Vif succumb to deamination-dependent and -independent restriction processes. Here, HIV-1 adaptation studies are leveraged to ask whether viruses with an irreparable vif deletion could develop resistance to restrictive levels of APOBEC3G. Several resistant viruses were recovered with multiple amino acid substitutions in Env, and these changes alone are sufficient to protect Vif-null viruses from APOBEC3G-dependent restriction in T cell lines. Env adaptations cause decreased fusogenicity, which allows for higher levels of Gag-Pol packaging. Increased concentrations of packaged Pol in turn correlate with faster virus DNA replication and reduced APOBEC3G-mediated hypermutation of viral replication intermediates. Taken together, these studies reveal a novel Env- and Pol-dependent mechanism that HIV-1 can use to escape restriction. This mechanism may provide transmitting viruses with an additional layer of protection, and may also be relevant to other viruses that lack obvious Vif-like defense mechanisms.

4. "Polymorphisms in Rhesus Macaque Tetherin are Associated with Differences in Peak Viremia during Acute Infection with SIV"
Sanath Kumar Janaka, Aidin Tavakoli-Tameh, William J. Neidermyer Jr., Ruth Serra-Moreno, Bin Jia, James A. Hoxie, Ronald C. Desrosiers, Paul Johnson, Jeffrey D. Lifson, Steven M. Wolinsky, and David T. Evans

Tetherin (BST-2 or CD317) is an interferon-inducible 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, full-length tetherin cDNA clones were sequenced from 139 rhesus macaques, including 69 animals infected with wild-type SIVmac239 and 47 animals infected with SIVmac239 nef. 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 14 alleles of rhesus macaque tetherin with dimorphic residues at 9 positions throughout the protein. The relationship between these alleles and peak plasma viral loads was compared

during acute infection, prior to the onset of adaptive immunity. Peak viremia did not differ significantly among the wild-type SIV-infected animals; however, differences in peak viral loads were associated with polymorphisms in tetherin among the animals infected with SIV nef (Kruskal-Wallis test, $p<0.05$). In particular, polymorphisms at positions 14, 43 and 111 (D14, P43 and H111) were associated with significantly lower peak viral loads for SIV nef infection (Kruskal-Wallis test, $p<0.05$). 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.

5. "APOBEC3B Lysine Residues are Dispensable for DNA Cytosine Deamination, HIV-1 Restriction, and Nuclear Localization"
Amy M. Molan, Heather M. Hanson, Cynthia M. Chweya, Brett D. Anderson, Gabriel J. Starrett, Christopher M. Richards, Reuben S. Harris

The APOBEC3 family of DNA-cytosine deaminases are well characterized as innate defense mechanisms against viral infection and are perhaps best known for their restriction of HIV-1. The APOBEC3s restrict HIV-1 by mutating proviral ssDNA cytosines to uracils disrupting the viral message. APOBEC3s have also been implicated in restriction of other viruses, as well as transposable elements, and represent a critical part of the human innate immune system. As these defenders are themselves mutagens, what is to prevent them from turning against the cells they evolved to protect? Recent work has shown that for APOBEC3B (A3B), the regulatory mechanisms can fail and hypermutation of the human genome results. There is a multitude of evidence that A3B is regulated through its expression level, nuclear localization, cell type-dependent restriction of HIV-1. Many proteins are subjected to post-translational modifications (PTMs) as part of their regulatory mechanisms. Of the over 400 known PTMs, many occur at lysine residues including acetylation, ubiquitination, and methylation to name a few. To investigate whether or not lysine residues are part ofA3Bs post-translational regulatory mechanism we constructed a lysine-less (K-free) A3B where all 10 of the lysine residues were mutated to arginine. The impact of these mutations on protein catalytic activity, viral restriction, SIV-Vif mediated degradation, and localization was assessed. Surprisingly we found that A3B lysine residues are dispensable for all measurable activities except sensitivity to SIV Vif-mediated degradation.

6. "UVB Mediated Immunosuppression is Necessary for Papillomavirus Mediated Pathogenesis"
Aayushi Uberoi, Megan Spurgeon, Tao Wei, Jeremiah Ye, Ed Glover, Ian Frazer, Chris Bradfield, and Paul Lambert

Papillomaviruses (PVs) are species tropic double-stranded DNA viruses that have been implicated in causing several forms of cancer in humans and other animals. PVs can infect mucosal or cutaneous epithelium and their life cycle is dependent upon the epithelial differentiation program thus limiting our ability to monitor and study the progression of the

disease in their natural hosts in depth. Our lab and others have extensively studied mucosotropic high-risk Human papillomaviruses (HPVs) implicated in anogenital and head and neck cancers. However, the biology and progression of skin diseases associated with their cutaneous PVs is relatively less well understood. Epidemiological studies have implicated that ultraviolet radiation (UVR) from sunlight drive papillomavirus-induced disease in healthy as well as immunocompromized humans. In order to study this phenomenon, we have developed a papillomavirus-based in vivo infection model using the recently discovered murine papillomavirus (MmuPV1), which is the first ever papillomavirus to naturally infect the laboratory strain of mice. While MmuPV1 failed to induce skin papillomatosis in most immune competent strains of mice, we discovered that UVR makes such mice highly susceptible to MmuPV1- induced skin disease, including formation of warts and squamous cell carcinoma (Uberoi et al., PLoS Pathogens, 2016). We also found a correlation between UVR's ability to make mice susceptible to infection and its capacity to cause systemic immunosuppression (ibid). We have now made efforts to test whether UVR's ability to cause systemic immunosuppression is necessary to make mice susceptible to MmuPV1-induced skin disease. Several factors mediate the capacity of UVR to cause systemic immunosuppression in mice, including: a) the activation of the aryl hydrocarbon receptor (AHR), a transcription factor that modulates several arms of the immune system and b) the elicitation of the immune-suppressive cytokine, IL10. We first confirmed that UVR induces activation of AHR and elicitation of IL10 upon UV-irradiation. We then determined that UVR-induced susceptibility to MmuPV1-induced skin disease was abolished in both AHR-null and IL10-null mice, as was the ability of UVR to cause systemic immunosuppression. These results demonstrate that UVR's ability to cause systemic immunosuppression is necessary to make mice susceptible to MmuPV1-induced skin disease. These studies also shed further light into the role of UVR in skin disease caused by cutaneous HPVs.

7. "Lethal mutagenesis of HIV-1 Induced by 5-aza-2-deoxycytidine in Human Primary CD4+ T Cells"

Emily Julik, Morgan E. Meissner, Megan E. Roth, Marzena Baran, Jerry Daniel, Daryl M. Gohl, Kenneth B. Beckman, Joshua A. Baller, Steven E. Patterson, and Louis M. Mansky

HIV-1 replicates with an intrinsically high mutation rate due to the error-prone nature of its reverse transcriptase enzyme. The experimental antiviral strategy of lethal mutagenesis, which has not yet entered clinical use, exploits and increases this high mutation rate to the point of catastrophic error and viral elimination due to excessive accumulation of deleterious mutations induced by small molecule mutagens. Previously, our group demonstrated that a divalerate prodrug form of the deoxcytidine analog 5-aza-2-deoxycytidine was able to eliminate HIV-1 infectivity in U373-MAGI cells. Here, we extend these findings into a more physiologically relevant cell type, human primary CD4+ T cells isolated from whole blood. As in cell lines, non-cytotoxic doses of 5-aza-2-deoxycytidine are competent to eliminate viral infectivity in these primary cells. The decline in viral infectivity is accompanied by an increase in the viral mutation rate and, in particular, elevation of G-to-C transversion levels, consistent with the proposed mechanism of action of this drug. We are further evaluating the antiviral specificity of 5-aza-2-

deoxycytidine using a single cell next generation sequencing approach wherein single primary CD4+ T cells infected with an HIV-1 vector and treated with 5-aza-2-deoxycytidine at its 50% effective concentration are isolated on an integrated fluidic circuit for whole genome amplification and sequence-based genotyping of host and proviral DNA. We expect to observe increased mutational load, skewed toward G-to-C transversions, in proviral but not host DNA in cells treated with 5-aza-2-deoxycytidine compared to untreated cells, demonstrating that this drug is specifically a viral mutagen without off-target mutagenic effects for patient DNA. These results will promote the clinical translation of antiviral lethal mutagens, which has so far proved elusive.

8. "Bacterial Viral Infection Influences Metabolic Interactions of Microbial Community Members"
Lisa Fazzino and William Harcombe

Bacteriophage (phage) are an understudied member of natural microbial communities. Phage infect specific bacterial species, which can evolve phage-resistance. Infection is influenced by the host cell's physiological state, which is affected by metabolic interactions between host cells and other members of the microbial community. Furthermore, competition with other bacterial community members influence a host cells ability to evolve phage resistance. Our synthetic bipartite microbial community of Escherichia coli and Salmonella enterica is genetically engineered to cooperate by sharing metabolic byproducts, or compete for carbon sources, depending on provided metabolites. By growing this co-culture system cooperatively or competitively in the presence or absence of an E. coli-specific phage (T7), we can determine how phage infection affects community members in different metabolic contexts by tracking species-specific population dynamics and evolution of phage resistance. Results indicate that cooperation favors the evolution of T7-resistant E. coli, while competition suppresses the evolution of T7-resistant E. coli. This is also predicted by our resource-explicit ODE mathematical model. However, mathematical models do not predict the observed increase in S. enterica yield when grown in the cooperative community with phage compared to without phage. This shows that phage attack can break metabolic interactions of microbial community members. We hypothesize that S. enterica is either consuming burst E. coli cellular contents, or burst E. coli lysate contains extracellularly active enzymes that break down supplied lactose into monosaccharide components that S. enterica can metabolize. Determining the effect that phage has on microbial communities will allow us to more accurately model community-phage interactions, and will provide an ecological framework to understand phage infection and evolution of phage resistance within a microbial community context.

9. "Perturbations to the HIV-1 Programmed Ribosomal Frameshift Site Dictates Translational Regulation and Viral Genomic RNA Transcript Fate"
Bayleigh E. Benner, Jordan T. Becker, Pablo Garcia-Miranda, Samuel E. Butcher, and Nathan M. Sherer

The HIV-1 genomic RNA (gRNA) secondary structure is a key regulator of viral replication

utilizing cis-acting features to modulate production of viral proteins. A conserved stem-loop within the gag reading frame dictates the proportions of essential polyproteins Gag and Gag-Pol. The pol gene is translated via a -1 programmed ribosomal frameshift (PRF) region within the gag gene, producing the Gag-Pol polyprotein approximately 5% of the time. Gag-Pol contains the viral enzymes necessary for production of mature virions. Previously we have shown that perturbing frameshifting efficiency 2-3 fold greatly reduces viral infectivity. Here we investigate the mechanism underpinning this loss of infectivity, and how altered frameshifting affects mRNA trafficking and stability, subsequently impacting gRNA packaging. Utilizing RT-PCR on our panel of PRF mutants with altered frameshifting efficiency, we provide evidence suggesting that mutants which frameshift greater or less than 5% exhibit a significant decrease in packaging of viral gRNA into virions. We further demonstrate using fluorescence in situ hybridization (FISH) and live-cell imaging, that inhibiting frameshifting reduces the cytoplasmic viral gRNA pool, resulting in less gRNA available for packaging. We hypothesize that inhibiting HIV-1 frameshifting results in decreased viral gRNA stability through cellular mRNA degradation mechanisms. Lastly, by complementing our panel of frameshift site mutants with wild-type virus provided in trans, we see a partial rescue to infectivity in mutants with abolished frameshifting but not mutants with increased frameshifting. These data suggest that Gag-Pol may help stabilize the unspliced viral gRNA, and rescue it from mRNA degradation. Thus, frameshifting fidelity is critical not only for regulating Gag:Gag-Pol ratios but, unexpectedly for HIV-1 gRNA stabilization, packaging and infectivity.

10. “Single Nucleotide Editing Using Chimeric APOBEC-Cas9 Complexes”
Amber St. Martin, Daniel Salamango, and Reuben S. Harris

Base editing is an exciting new application for genome engineering technology. C-to-T mutations in genomic DNA have been achieved using ribonucleoprotein complexes comprised of rat APOBEC1 single-stranded DNA deaminase, Cas9 nickase (Cas9n), uracil DNA glycosylase inhibitor (UGI), and guide (g)RNA. Here, we report the first real-time lentiviral-based system for quantification of base editing in living human cells as well as next-generation editing constructs that achieve higher editing frequencies. Mutation of an APOBEC-preferred trinucleotide, 5-TCA-to-TTA, restores mCherry fluorescence in a reporter marked by eGFP, and editing frequencies are quantified through ratios of mCherry-positive to eGFP-positive cells. Using this system as both an episomal and a chromosomal editing reporter, we show that human APOBEC3A and APOBEC3B base editing constructs are more efficient than a rat APOBEC1 construct. We also demonstrate an enrichment of editing events at a heterologous chromosomal locus in reporter-activated, mCherrypositive cells. The combination of a rapid, fluorescence-based base editing reporter system and more efficient, structurally defined DNA editing enzymes expands the versatility of this powerful new technology.

11. "Latent Epstein-Barr Virus Infection in a Keratinocyte Model Impairs Differentiation"
Mark Eichelberg, Rene Welch, Ahmed Ali, Makoto Ohashi, Joseph Guidry, Shannon Kenney, Sunduz Keles, Rona Scott, and Eric Johannsen

Epstein-Barr virus (EBV) is a human herpesvirus associated with the development of specific forms of lymphoma, nasopharyngeal carcinoma, and gastric carcinomas. Although carcinomas account for almost 90% of EBV associated cancers, progress had been limited due to lack of a physiologic in vitro model of EBV epithelial infection. Recently, EBV infection of normal oral keratinocytes (NOKs) has emerged as a model that recapitulates aspects of EBV infection in vivo, such as differentiation-associated viral replication. Using NOK and NOK-EBV cells we characterized gene expression changes by RNA-seq due to EBV infection and differentiation. Although latent EBV infection in undifferentiated cells resulted in more subtle changes in gene expression, NOK-EBV gene changes during differentiation were markedly different than those observed in NOK. The down-regulation cellular growth and metabolism pathways associated with differentiation were attenuated in NOK-EBV compared to NOK. To assess which EBV gene products were responsible for these effects, we infected NOK cells with EBV mutants defective for microRNA expression, replication, and EBER expression. In addition, we have grown NOKs, NOK-EBV, and NOK-EBVmutants in organotypic raft cultures and are characterizing them for differences in differentiation, viral replication, and BrdU incorporation. We expect to define the mechanism by which EBV latent infection alters keratinocyte differentiation and provide a basis for understanding the role of EBV in epithelial cancers.

12. "Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes"
K. Shi, M.A. Carpenter, S. Banerjee, N.M. Shaban, K. Kurahashi, D.J. Salamango, J.L. McCann, G.J. Starrett, J.V. Duffy, Ö. Demir, R.E. Amaro, D.A. Harki, H. Aihara, R.S. Harris

APOBEC-catalyzed cytosine-to-uracil deamination of single-stranded DNA (ssDNA) has beneficial functions in immunity and detrimental effects in cancer. APOBEC enzymes have intrinsic dinucleotide specificities that impart hallmark mutation signatures in mutated viral DNA and in cancer. Although numerous structures have been solved, mechanisms for global ssDNA recognition and local target-sequence selection remain unclear. Here we report crystal structures of human APOBEC3A and a chimera of human APOBEC3B and APOBEC3A bound to ssDNA at 3.1-Å and 1.7-Å resolution, respectively. These structures reveal a U-shaped DNA conformation, with the specificity-conferring 1 thymine flipped out and the target cytosine inserted deep into the zinc-coordinating active site pocket. The 1 thymine base fits into a groove between flexible loops and makes direct hydrogen bonds with the protein, accounting for the strong 52-TC preference. These findings explain both conserved and unique properties among APOBEC family members, and they provide a basis for the rational design of inhibitors to impede the evolvability of viruses and tumors. Shi, Carpenter et al. NSMB 2017

13. "Epstein Barr Virus Transcription Factors Rta and Zta Play Distinctly Different Roles in Promoting EBV Replication in Epithelial Cells"
Ahmed Ali, Mark Eichelberg, Makoto Ohashi, Reza Djavadian, Shannon Kenney, and Eric Johannsen

Epstein Barr Virus (EBV) is a human herpesvirus associated with lymphomas and specific epithelial malignancies. EBV replication is controlled by the viral transcription factors Zta and Rta. Although both Zta and Rta are required for the lytic cascade, dissecting the specific role played by Rta or Zta is challenging because each activates the other expression. To circumvent this, we generated an EBV mutant deleted for both Rta and Zta in the Akata strain BACmid (designated Akata-dRdZ). Stable latent infection of Akata-dRdZ was established in telomerase-immortalized normal oral keratinocytes (NOKs). The resultant NOK-Akata-dRdZ cells were transfected with Rta, Zta, or Rta + Zta and EBV lytic gene expression examined by RNA-seq at 48 hours post transfection. Our data revealed that the majority of lytic transcripts were induced by Rta, but surprisingly, Zta activated only the two transcripts (LF3 and BHLF1) driven by the OriLyt promoters. This suggests that, at least in this epithelial infection model, EBV replication may be much more similar to that of KSHV that previously appreciated. In KSHV, the Rta ortholog (ORF50) drives expression of all lytic promoters whereas the Zta ortholog (K8) serves only as an OriLyt binding protein for DNA replication. We are currently investigating whether this limited role for Zta is specific to epithelial cells or holds true in B cells, but was previously unappreciated (e.g., obscured by Zta induction of Rta expression). In addition, we are investigating the mechanisms by which Zta synergizes with Rta to activate lytic promoters in NOK-Akata-dRdZ that it is unable to activate when expressed alone. Our results have important implications for lytic induction therapy as a means of killing EBV positive carcinomas.

14. "Translation control of HIV-1"
Kathleen Boris-Lawrie

In-solution, HIV 5UTR forms two interchangeable long-range nt-pairings, one sequesters the gag start codon promoting dimerization while the other sequesters the dimer initiation signal preventing dimerization. While the effect of these nt-pairings on dimerization and packaging has been documented their effect on authentic HIV translation in cellulo has remained elusive until now. HIVNL4-3 5UTR substitutions were designed to individually stabilize the dimer-prone or monomer-prone conformations, validated in-solution, and introduced to molecular clones. The effect of 5UTR conformation on ribosome loading to HIV unspliced RNA and rate of Gag polypeptide synthesis was quantified in cellulo. Monomer- and dimer-prone 5UTRs displayed equivalent, basal rate of translation. Gain-of- function substitution U103, in conjunction with previously defined nt-pairings that reorient AUG to flexible nt-pairing, significantly activated the translation rate, positing basal translation rate is under positive selection. The observed translation up-regulation is attributable to nt-pairings at the junction of R and U5 upstream of previously characterized HIV riboswitch and demonstrates the basal translation rate of authentic HIV RNA is regulated independently of monomer:dimer

equilibrium of the 5UTR.

15. "HIV, Ratio Normalization and Winning the Game of T Cells"
Rob Striker, Dawit Wolday, Mitchell Kirsh, Ajay Sethi, Joe Mcbride and Irene Ong

Currently HIV therapy can drop the viral load to undetectable and allow a depleted CD4 response to recover. Therapy does less to reverse the stimulation of CD8s. While therapy dramatically reduces mortality, it does not completely reverse the negative health consequences of HIV. For example, patient living with HIV still have higher rates of cancer and cardiovascular disease than age matched controls. Recent data suggests that this excess morbidity is largely concentrated in patients with CD4/8 ratios below 0.4, but it is unclear if normalization of the ratio is possible with therapy, and if so how long it takes. Furthermore immune defects coexist when the ratio doesnt rise above 1.0. To begin to address these and other questions we have constructed long term follow-up of three distinct cohorts of people living with HIV. One, from Ethiopia, is one of the few cohorts to contain more than a decade of follow-up in women living with HIV. A second cohort is from our Madison VA, and a third is from the Wisconsin Department of Corrections. Normalization is variable in all three cohorts and does not occur universally in these cohorts despite a decade of therapy or more. In the Ethiopian cohort which is only 35% women though, 8% of females reach a CD4/CD8 ratio of 1.5, while only 4% of men reach 1.5. The length of therapy increases the difference between men and women. Factors associated with lack of ratio recovery will be discussed and as well as cohort specific average ratio rises. Ratio normalization maybe a more useful measurement of immune recovery than the more traditional CD4 count in the modern antiviral therapy era.

16. "The Hepatitis C Viral Protein NS5A Stabilizes Growth-Regulatory Human Transcripts
Liang Guo, Suresh D. Sharma, Jose Debes, Daniel Beisang, Bernd Rattenbacher, Irina A. Vlasova-St. Louis, Darin L. Wiesner, Craig E. Cameron, and Paul R. Bohjanen

Numerous mammalian proto-oncogene and other growth-regulatory transcripts are upregulated in malignancy due to abnormal mRNA stabilization. In hepatoma cells expressing a hepatitis C virus subgenomic replicon, we found that the viral NS5A, a protein known to bind to viral RNA, also bound specifically to human cellular transcripts that encode regulators of cell growth and apoptosis, and this binding correlated with transcript stabilization. An important subset of human NS5A-target transcripts contained GU-rich elements, sequences known to destabilize mRNA. We found that NS5A bound to GU-rich elements in vitro and in cells. Mutation of the NS5A zinc finger abrogated its GU-rich element-binding and mRNA stabilizing activities. Overall, we identified a molecular mechanism whereby HCV manipulates host gene expression by stabilizing host transcripts in a manner that would promote growth and prevent death of virus-infected cells, allowing the virus to establish chronic infection and lead to the development of hepatocellular carcinoma.

17. "Quantitative Imaging of Rhinovirus Infection Spread with Single-Cell Resolution"
Huicheng Shi, Bahar Inankur, and John Yin

Due to the complexity of viral and host cellular processes involved, it is generally not known how the kinetics of viral gene expression within individual cells relates to the overall dynamics of infection spread through a population of susceptible host cells. To address this challenge, HeLa cells were infected with human rhinovirus (HRV) engineered to express green fluorescent protein (GFP) as a byproduct of viral gene expression. Fluorescent signal was tracked over several rounds of infection and kinetic parameters were estimated from reporter expression at single-cell resolution. Speed-up of detected gene expression between the initial and subsequent infected cells provides preliminary evidence for a mechanism of non-lytic infectious particle release. Shorter durations of reporter signal across four infection cycles correlate with cell aging. This work shows how single-cell imaging can reveal subtle changes in the dynamics of virus-cell interaction during infection spread.

18. "Regulation of EBV Lytic Reactivation by p53 and p63 in Epithelial Cells"
Nick Van Sciver, Dhananjay Nawandar, Denis Lee, Kathleen Makielski, Paul Lambert, and Shannon C. Kenney

Epstein-Barr Virus (EBV) infects 95% of the adult population worldwide and is associated with a number of malignancies such as Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma (NPC), and 10% of gastric cancers. p53 is a tumor suppressor and is mutated in approximately 10% of all NPCs. Whether a p53 mutation confers any alteration to the EBV life cycle in epithelial cells is not clear. Our lab previously showed that EBV lytic reactivation in rafted EBV-infected oral keratinocytes is limited to the differentiated epithelial layers. In order to investigate whether p53 regulates EBV lytic reactivation during differentiation of normal keratinocytes, we have created (in collaboration with the Lambert lab) EBV-infected isogenic normal oral keratinocyte lines (NOKs) that have either wild-type p53, no p53, or a mutant p53 (K120E). Our preliminary results comparing cells with wild-type versus mutant p53 reveal a marked increase in EBV lytic reactivation and cellular differentiation in the p53 mutant cells (in comparison to wildtype cells) during organotypic raft culture. We are currently examining how complete knockout of p53 affects epithelial cell differentiation and lytic EBV reactivation. In addition, we are determining if a related p53 family member, Np63, which is known to regulate epithelial differentiation and proliferation, also affects EBV reactivation in normal keratinocytes. Our preliminary results suggest that loss of Np63 expression using either siRNAs or shRNAs in EBV latently infected epithelial cell lines induces lytic EBV reactivation. Additionally, when Np63 is over-expressed in the a constitutively lytic EBV-infected gastric carcinoma cell line (which normally expresses no Np63), AGS- Akata we find that lytic EBV expression is reduced. Together these results suggest that both p53 and Np63 regulate lytic EBV reactivation during differentiation of normal oral keratinocyte cells.

19. “Characterizing Guinea Pig Cytomegalovirus Pathogenesis in the Amnion”
Dira S. Putri and Craig J. Bierle

Human cytomegalovirus (CMV) is most significant infectious cause of congenital disease and preventable sensorineural birth defects. Congenital CMV infection occurs in up to 2% of pregnancies in the United States, and an estimated 8,000 children are disabled by the virus each year. Recent studies have observed that persistent human CMV infection of the amnion is associated with premature birth, intrauterine growth restriction, and congenital CMV disease. As the innermost layer of the fetal membranes, the amnion is the final barrier separating the fetus from the outside world. A single layer of amniotic epithelial cells protects against viral and bacterial pathogens by secreting cytokines and other factors to modulate the innate and adaptive immune responses. How human CMV establishes infection in the amnion and how persistent viral infection impacts the membranes normal function are poorly understood. We sought to determine whether guinea pig CMV, a well-established animal model of in utero CMV transmission, also infects the amnion during natural infection. After a mid-gestation viral challenge, guinea pig CMV was detected in the placenta and amnions of pups collected at 21 days post-infection. Guinea pig amniotic epithelial (CPAE) cells were isolated from late-term amnions and immortalized with HPV16 E6/E7. Both primary and immortalized CPAE cells supported GPCMV replication. Compared to guinea pig CMV infections in lung fibroblasts, CMV replication in CPAE cells is less lytic, sustained for a longer duration, and results in markedly less extracellular virus production. Additionally, experiments have revealed that CPAE cells secrete trans-acting antiviral factors of unknown identity. Together, these results indicated that the guinea pig is an appropriate animal model for studying viral infection of the amnion in vivo and in vitro.

20. “Deep Sequencing Barcoded Zika Virus from Macaques Using a Small Amplicon Approach”
Katie Zarbock, Paola Silveira, Team ZEST, Greg Ebel, David O’Connor, and Shelby O'Connor

Sequencing Zika virus (ZIKV) has historically been challenging because viral titers are low and transient. It is difficult to isolate sufficient virus template numbers to successfully amplify viral cDNA for deep sequencing. We adapted a process by Quick et. al. to sequence the ZIKV genome. Amplicons are tagged and sequenced on an Illumina MiSeq instrument. We used this technique to sequence a unique Zika-barcode stock, and its comparable wild type clone. In nonpregnant animals, barcode diversity mirrors the stock at days 3 and 5 post infection. In one pregnant animal, there is a dramatic reduction in barcode diversity between days 7 and 8 that corresponds to decreased viremia. Future studies are needed to determine the mechanism leading to this bottleneck. A second version of the Zika barcode virus has also been generated and is more diverse than version 1. We will infect nonpregnant animals with the Zika barcode version 2 stock soon.

21. “Cryo-EM Structure of Porcine Delta Coronavirus Spike Protein in the Pre-Fusion State”
Jian Shang, Yuan Zheng, Yang Yang, Chang Liu, Qibin Geng, Wanbo Tai, Lanying Du, Yusen Zhou, Wei Zhang, and Fang Li

Oronavirus spike proteins from different genera are divergent, although they all mediate viral entry into cells by binding to host receptors and fusing viral and cell membranes. Here we determined the cryo-EM structure of porcine delta coronavirus (PdCoV) spike protein at 3.3-angstrom resolution. It contains three receptor-binding S1 subunits and a trimeric membrane-fusion S2 stalk. S1 contains two domains, N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD). PdCoV S1-NTD has the same galectin fold as alpha- and beta-coronavirus S1-NTDs, and recognizes sugar as its potential receptor. PdCoV S1-CTD has the same structural fold as alpha-coronavirus S1-CTDs, which differs from the structure of beta-coronavirus S1-CTDs; it binds to an unidentified receptor on host cell surfaces. PdCoV S2 is locked in the pre-fusion conformation by structural restraint of S1 from a different subunit. PdCoV spike possesses several structural features that may facilitate immune evasion by the virus, such as its compact structure, concealed receptor-binding sites, and shielded critical epitopes. Overall, this study reveals that delta-coronavirus spikes are structurally and evolutionally more closely related to alpha-coronavirus spikes than to beta-coronavirus spikes; it also has important implications for the receptor recognition, membrane fusion, and immune evasion by delta coronaviruses as well as coronaviruses in general.

22. “CXCR5-Transduced Primary Rhesus Macaque PBMCs Accumulate in B Cell Follicles in a Novel Ex Vivo B Cell Follicle Migration Assay”
Gwantwa Mwakalundwa, Haran P, Li S, Pampusch M, Abdeelal HM, Rakasz EG, Connick E, and Skinner PJ

Background: CD8 T cells are crucial for controlling both human and simian immunodeficiency virus (HIV/SIV) infections. Low levels of virus-specific CD8 T cells within B cell follicles permit ongoing replication of HIV and SIV. The B cell homing molecule, CXCR5, is required for homing to B cell follicles. We hypothesize that engineering virus-specific CD8 T cells to express CXCR5 will enable them to home to B cell follicles and suppress viral replication. To begin to test this hypothesis we developed a novel ex vivo B cell migration assay. Method: We first determined the optimal time that lymph node tissue slabs could be incubated and maintain follicular morphology. Next we evaluated whether CD8 T cells engineered to express CXCR5, accumulated within B cell follicles. We transduced CD8 T cells from SIV infected rhesus macaques, with a control vector or a CXCR5 transducing vector, labeled with a live cell dye CTV, layered these cells onto 300um thick fresh tissue slabs and incubated for six hours. Sections were then fixed and stained via IHC with anti-CD20 to stain B cell follicles. Sections were imaged using a confocal microscope and CTV labeled cells were quantified inside and outside of B cell follicle. Results: We determined that follicular morphology was maintained after 4 and 6 hours of incubation, but was lost after overnight incubation. Preliminary migration results showed a fivefold increase in the follicular to extra follicular ratio (F: EF) of CTV labeled cells in the CXCR5 transducing vector samples compared to control samples. Conclusion: We successfully created

an ex vivo B cell migration assay. Preliminary results using this assay suggest that CXCR5 transduction induces T cell migration into B cell follicles.

23. "EBNA3C-Deleted Epstein Barr Virus Causes Lymphomas in a Humanized Mouse Model Despite Being Severely Deficient for B Cell Transformation in Vitro"
James Romero-Masters, Reza Djavadian, Makoto Ohashi, Shidong Ma, Andrea Bilger, Eric Johannsen, and Shannon Kenney

EBNA3C is an EBV-encoded latent protein essential for B cell transformation in vitro. A critical function of EBNA3C during EBV-mediated transformation in vitro is down-regulation of the tumor suppressor gene products, p14ARF, p16INK4a, and the pro-apoptotic protein, BIM. Here we have used a cord-blood humanized mouse model to examine the phenotype of an EBNA3C-deleted (EBNA3C) EBV mutant in vivo. We find that EBNA3C induces lymphomas in approximately 40% of animals, while wildtype (WT) virus induces lymphomas in virtually all animals. EBNA3C-induced lymphomas have a delayed onset (60-90 days) compared to WT tumors (30-40 days), but once present are as large as the WT tumors and highly invasive. Both WT- and EBNA3C-infected lymphomas are CD20-positive activated diffuse large B cell lymphomas. In comparison to WT tumors, EBNA3C tumors have increased numbers of lytically infected cells. EBNA3C tumors also have greatly enhanced expression of p16. However, EBNA3C tumors express high levels of Cyclin E, c-Myc, and BCL2, which may allow cells to bypass p16-mediated cell cycle inhibition and apoptosis. Unexpectedly, we also find that EBNA3C tumors have greatly increased T cell infiltration in comparison to WT tumors. RNAseq and qPCR analysis of WT- and EBNA3C-infected lymphomas reveals a significantly increased expression of the T cell cytokines, CCL5 and CCL20, in the EBNA3C tumors; EBNA3C tumors also have increased expression of interferon alpha and multiple interferon-responsive genes response. Together, these results reveal that EBNA3C expression is not absolutely essential for EBV-induced lymphomagenesis in cord-blood humanized mice, and suggest that previously unappreciated roles of EBNA3C include inhibiting the T cell response to EBV-infected B cells, as well as the interferon response.

24. "Novel Features of CRM1-Dependent Retroviral mRNA Nuclear Export Revealed Using Live Cell Imaging"
Ryan T. Behrens, Christina Higgins, and Nathan M. Sherer

Primate lentiviruses including HIV-1, HIV-2, and SIV and also deltaretroviruses such as HTLV-1 have necessarily adapted to overcome profound cell- and/or species-specific barriers to the nuclear export of essential late-stage, intron-retaining viral mRNAs. These barriers critically influence virus cell tropism and are key determinants of host immune detection and pathogenic outcomes. Both lentiviruses and deltaretroviruses overcome these barriers using Rev or Rex proteins, respectively, that route unspliced or incompletely spliced viral mRNAs out of the nucleus using the highly specialized cellular CRM1/RanGTP nuclear export machinery. Rev/Rex-equivalent proteins from diverse retroviral pathogens (e.g., HIV-1, HIV-2, SIV, and HTLV) are unified in that they all link viral RNAs to CRM1. However, whether Rev/Rex-encoded

activities have evolved to circumvent identical selective pressures or, instead, govern multiple unique modes of RNA transport and/or other features of regulated gene expression is unknown. Live-cell imaging represents a powerful new strategy for direct characterization of these pathways. Here, for the first time, we present comparative imaging of Rev/Rex equivalent proteins and RNA trafficking dynamics for HIV-1, SIV, HIV-2, HTLV, and 6 additional retroviruses (BIV, EIAV, FIV, Visna, HTLV-2 and BLV) over >30 hours of continuous imaging in single living cells. We show that the HIV-1 Rev protein exhibits striking, highly unique subcellular transport dynamics and demonstrate that Rev regulates a novel CRM1-dependent, burst-likeRNA export behavior, activating rapid, late-stage upregulation of gene expression just prior to the onset of virus particle production. Our comparative experiments reveal both distinct features of Rev/Rex-regulated export mechanisms and, in some instances, remarkable interoperability of viral elements relevant to natural co-infections (e.g., for HIV/HTLV or BIV/BLV) and, potentially, cross-species transmission. Moreover, we discuss address the potential utility of next-generation, low-toxicity CRM1 inhibitors as broad-spectrum inhibitors of these viral pathogens, and how we have exploited species-specific features of CRM1 to expose additional vulnerabilities.

25. "Analysis of Proviral and Particle Structure from Distinct Chronically Infected Cell Lines Demonstrates the Polymorphic Nature of HTLV-1 Particle Cores"
Luiza Mendonça, Morgan E Meissner, Wei Zhang, Louis Mansky

Human T-cell leukemia virus type 1 (HTLV-1) was the first described human retrovirus. It is the etiological agent of adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1 particle structure is still poorly understood, and previous studies have analyzed viruses produced by the MT-2 cell line, a transformed lymphocytic cell chronically infected with HTLV-1, which harbors truncated proviruses and expresses truncated Gag proteins. In this study, we demonstrate that the chronically infected SP cell line harbors a relatively low number of proviruses, making it a promising experimental system for the study of HTLV-1 particle structure. We identified the genomic sites of integration and characterized the genetic structure of the gag region in each provirus. We determined that despite encoding for a truncated Gag, only full length protein is incorporated into budding viral particles. Moreover, cryo-EM analysis of the purified particles produced by SP cells revealed that particles can be divided into three classes regarding the core structure: viruses with complete cores, viruses with incomplete cores, and particles with no cores but with homogeneously distributed electron density. Observed cores were loosely polygonal in shape, and virus particles averaged 115 nm in diameter. Studies of retroviral particle core morphology have demonstrated a correlation between capsid core stability and relative infectivity of the virus. In this study, we demonstrate that HTLV-1 particles produced from distinct chronically infected cell lines are polymorphic in nature, with many particles exhibiting a lack of organized particle density associated with a complete core structure. Ensuring that this phenotype is a true characteristic of HTLV-1 particles is the first step in correlating the diversity in particle structures observed for this virus with the low levels of transmissibility associated with HTLV-1 infection. These data corroborate the results seen for particles produced from MT-2 cells and

further indicate that HTLV-1 particles exhibit highly polymorphic cores, which may contribute to the remarkably low infectivity that is characteristic of the virus.

26. "Role of IQGAP1 in HPV-Positive and HPV-Negative Head and Neck Carcinogenesis"
Tao Wei, Suyong Choi, Darya Buehler, Richard Anderson and Paul Lambert

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). PI3K/Akt signaling, along with its downstream effector mTOR, is one of the most frequently altered pathways in both HPV-positive and HPV-negative HNSCC with PIK3CA, the gene encoding the catalytic subunit of PI3K, being the most frequently altered gene in the HPV-positive HNSCC (~56%). These alterations lead to an increased activity of this pathway. Recently, IQ motif-containing GTPase activation protein 1 (IQGAP1), a 190-kDa protein that contains multiple protein-interaction domains, has been reported to scaffold the PI3K/Akt pathway. This gene is increased in its expression in human HNSCCs, and we found that interfering with the ability of IQGAP1 to scaffold PI3K inhibits the growth of human HNSCC cell lines in vitro. Additionally we found that IQGAP1 associates with HPV16 E6 & E7, two viral oncoproteins implicated in carcinogenesis, and that these interactions correlate with an upregulation of the PI3K/AKT/mTOR signaling in cell culture. These data have led us to test the hypothesis that IQGAP1 contributes to head and neck carcinogenesis. To test this hypothesis we made use of a well-validated mouse model for HNSCC, in which a synthetic oral carcinogen, 4-nitroquinoline 1-oxide (4NQO), is used to drive tumorigenesis, and genetically engineered mice that are deficient in the expression of IQGAP1 (IQGAP1-/- mice). Utilizing the latter, we first verified that IQGAP1 is necessary for efficient PI3K signaling upon EGF-stimulation in vivo. We then pursued HNSCC studies. After treating mice with 4NQO in their drinking water for 16 weeks followed by 5 weeks on normal drinking water, Iqgap1+/+ mice showed significantly higher cancer multiplicity as well as significantly higher numbers of high grade cancers than Iqgap1-/- mice. These results support the above stated hypothesis that IQGAP1 contributes to head and neck carcinogenesis. Future studies focus on whether IQGAP1 is necessary specifically for HPV-positive HNSCC, and utilize our HPV-transgenic mouse model for HNSCC. Experimental mice generated by crossing Iqgap1-/- mice and K14-E6/E7 will be put under 4NQO treatment to determine whether loss of IQGAP1 affects head and neck carcinogenesis in the context of HPV.

27. "SIV Vif and Human APOBEC3B Interactions Resemble Those Between HIV-1 Vif and Human APOBEC3G"
Jiayi Wang, Nadine M. Shaban, Allison M. Land, William L. Brown, and Reuben S. Harris

Several members of the APOBEC3 family of DNA cytosine deaminases can potently inhibit HIV-1 replication by catalyzing extensive cytosine deamination in viral cDNA and impeding reverse transcription. HIV-1 counteracts restriction with the virally encoded Vif protein, which targets the APOBEC3 proteins to a cellular ubiquitin ligase for proteasomal degradation. HIV-1 Vif is highly optimized for degrading the human APOBEC3 repertoire and, in general, lentiviral Vif

proteins specifically target the restricting APOBEC3 enzymes of each relevant host species. However, SIVmac239 Vif elicits curiously wide range of APOBEC3 degradation capabilities that includes several human APOBEC3s and even human APOBEC3B, a non-HIV-1 restricting APOBEC3 enzyme. To better understand the molecular determinants of the interaction between human APOBEC3B and SIVmac239 Vif, we analyzed an extensive series of mutants. We found that SIVmac239 Vif interacts with the N-terminal domain of human APOBEC3B and, interestingly, this occurs within a structural region homologous to the HIV-1 Vif interaction surface of human APOBEC3G. An alanine scan of SIVmac239 Vif revealed several residues required for human APOBEC3B degradation activity. These residues overlapped with HIV-1 Vif surface residues that interact with human APOBEC3G, and are distinct from those that engage APOBEC3F or APOBEC3H. Overall, these studies indicate that the molecular determinants of the human APOBEC3B-SIVmac239 Vif interaction strongly resemble those of human APOBEC3G-HIV-1 Vif interaction, and may be a remnant of an ancestral Vif activity used for cross-species transmission.

28. "Species-Specific Regulation of HIV-1 Gene Expression and Vif-Induced Cell Cycle Arrest"
Edward L. Evans III, Jordan T. Becker, Stephanie S. Fricke, Kishan M. Patel, and Nathan M. Sherer

Cells derived from mice and other rodents exhibit profound blocks to HIV-1 virion production reflecting species-specific incompatibilities between viral Tat and Rev proteins and the essential host factors Cyclin T1 (CCNT1) and Exportin-1 (XPO1, also known as CRM1), respectively. Here we asked if CCNT1 and XPO1 represent the final barriers to HIVs post-integration stages in mouse cells engineered to constitutively express HIV-compatible versions of CCNT1 and XPO1 (3T3.CX cells). 3T3.CX cells were infected with single round (Env-/Vpr-/Nef-) HIV-1/NL4-3, and viral gene expression and virus particle production were monitored for up to two weeks post-infection. 3T3.CX cells yielded >40-fold increases to HIV-1 virus particle output relative to parental 3T3 cells, but exhibited an ~24 h lag to the onset of early and late viral gene expression compared to permissive human cells (HeLa). Unexpectedly, long-term (> 6 day) monitoring of infection revealed 3T3.CX cells to be remarkably tolerant of HIV-1-associated cytopathic effects relative to human cells, an effect we mapped to the capacity of the viral accessory protein Vif to induce G2/M cell cycle arrest in human cells but not in non-human cell types. Vif retained the capacity to degrade known Cullin 5-targeted substrates including APOBEC3G and a B56 subunit of the PP2A holoenzyme in both mouse and human cells, thus indicating that Vifs role in the cell cycle can be uncoupled mechanistically from its other defined roles in protein turnover. Combined, our results reinforce that CCNT1 and XPO1 are the predominant blocks to HIV-1s late stages in mouse cells and expose additional novel cell- or species-specific HIV-host interactions influencing viral RNA metabolism and cell cycle control.

29. “Visualizing HTLV-1 Gag Puncta Biogenesis by Super-Resolution Microscopy”
John Eichorst, John Kohler, Joachim Mueller, and Louis Mansky

Human T-cell leukemia virus type-1 (HTLV-1) particle assembly occurs at the plasma membrane and is driven by Gag oligomerization. However, the precise mechanism(s) that initiates Gag puncta biogenesis is not well established. Here, we sought to establish quantitative imaging approaches to analyze this process at single molecule resolution by using super-resolution microscopy. To do this, HTLV-1 Gag was labeled with the fluorescent protein mEos3.2 and monitored in cells using the super-resolution approach of photoactivation localization microscopy (PALM). In PALM, the lateral (xy) spatial resolution of the image output is improved to nearly 40 nm by locating the position of each single fluorescently-labeled molecule, independent of any other in the cell. This results in images that contain intricate details about the distribution and organization of HTLV-1 Gag protein at the plasma membrane, which are not readily visible using confocal or epi-fluorescence imaging. Nonetheless, PALM requires detailed corrections and controls in order to translate the data into absolute densities and concentrations. Here, we describe the ongoing studies to improve the amount of quantitative information available PALM to study HTLV-1 particle assembly. Using multiple control methods, including measurements of fast fluorescence fluctuations, the effects of the non-ideal photophysics of the fluorescent proteins utilized in PALM on the subsequent quantitative analysis will be discussed. Furthermore, with the improved spatial resolution of PALM, we are conducting studies to determine whether non-punctate HTLV-1 Gag represents an assembly intermediate. In parallel, ongoing experiments seek to determine if the spatial organization, using paired correlation methods, of non-punctate HTLV-1 Gag at the plasma membrane are requisite for virus particle biogenesis. These experimental strategies are extending the information content available from PALM measurements and aid in providing new insights into HTLV-1 particle assembly.

30. “Comprehensive Analysis of the Kinetics of Epstein-Barr Virus Lytic Gene Expression”
Reza Djavadian, Mitch Hayes, and Eric Johannsen

We and others previously demonstrated that 6 Epstein-Barr virus (EBV) genes that have orthologs in beta- and gamma-, but not alpha-herpesviruses mediate late gene transcription in a viral lytic DNA replication-dependent manner. We proposed a model in which these beta-gamma genes encode a viral pre-initiation complex (vPIC) that mediates transcription from nascent viral DNA. Subsequently it was reported that some late gene transcripts do not require vPIC. To rigorously define which transcription start sites (TSS) are dependent on viral lytic DNA replication or vPIC, we performed Cap Analysis of Gene Expression (CAGE)-SEQ on cells infected with wildtype EBV or EBV mutants defective for DNA replication, vPIC function, or lacking an origin of lytic replication. Using conservative criteria, we defined 18 true-late and 32 early TSS as well as 12 TSS that are active at low levels early and are upregulated in a vPIC and lytic DNA replication-dependent manner. Our results represent the most complete characterization of lytic gene expression kinetics reported to date and suggest that most, but

not all EBV late genes are vPIC dependent.

31. "Identification of Cancer Cell Lines with a Clear APOBEC Mutation Signature"
Matthew C. Jarvis, Diako Ebrahimi, Nuri A. Temiz, and Reuben S. Harris

Mutations fuel tumor evolution and contribute to multiple processes including immune evasion, metastasis, and therapy resistance. Multiple endogenous and exogenous sources of DNA damage contribute to the overall mutation burden in cancer, with distinct and overlapping combinations contributing to each cancer type. Each mutation source results in a characteristic signature, which can be deduced from tumor genomic DNA sequences. Genomic DNA cytosine deamination by cellular APOBEC enzymes is the most abundant newly appreciated source of mutation in many cancers, including certain cancer types with a viral association. HPV-positive cancers, such as head/neck and cervical cancers have been linked with mechanisms leading to enriched APOBEC mutagenic signature, defined as C-to- T and C-to- G mutations occurring in 5-TCW motifs. Resolving the complex mutational profiles in these cancer types is crucial to understanding pathogen-related APOBEC mutagenesis in these cell systems. Here we identify cancer cell lines with a strong APOBEC mutation signature by analyzing the base substitution mutation spectra from over 1000 cancer cell lines through the COSMIC database. These cell lines may provide model systems for fundamental APOBEC research and for advancing clinical strategies to target tumor evolvability in HPV-positive cancers.

32. "Cryo-Electron Tomography Reveals Novel Features of a Viral RNA Replication Compartment"
Kenneth J. Ertel, Desirée Benefield, Daniel Castaño-Diez, Janice G. Pennington, Mark Horswill, Johan A. den Boon, Marisa S. Otegui, and Paul Ahlquist

Positive-strand RNA viruses are the largest genetic class of viruses and include many established and emerging human pathogens such as Zika virus. These viruses invariably replicate their RNA genomes on rearranged intracellular membranes, frequently inside virus-induced membrane invaginations. The organization of viral proteins and RNAs in these compartments remains largely unknown. Here, we report striking new results from the first cryo-EM tomography structural analyses of a positive-strand RNA virus RNA replication compartment. Using the advanced flock house nodavirus model system, we have visualized many previously unrecognized features of membrane-associated viral RNA replication complexes. The images reveal that the FHV-induced spherular invaginations of outer mitochondrial membranes are densely packed with coiled template RNAs and that a dramatic ringed structure crowns the necked aperture of each complex to the cytoplasm. Subtomogram averaging of these crowns reveals a detailed twelve-fold symmetrical structure with concentric flanking protrusions and a central electron density. Immunogold labeling shows that the crowns contain protein A, the major multifunctional viral RNA replication protein. Many crowns are associated with long extruding cytoplasmic fibrils, likely to be exported progeny RNA. We show that the volume distributions of the replication compartments are closely correlated with the size and complexity of the viral RNA templates being replicated. Taken together, these

structural findings and the complementary genetic and biochemical results provide important new mechanistic insights and imply dynamic new models for the structure, assembly and function of positive-strand RNA virus RNA replication complexes and, by extension, important leads to design new antiviral treatment approaches.

33. "Studies of Non-Punctate, Membrane-Bound HTLV-1 Gag by Fluorescence Fluctuation Spectroscopy and Fluorescence Lifetime Imaging Microscopy"
Isaac Angert, John Eichorst, John Kolher, Jessica Martin, Wei Zhang, Louis M. Mansky, and Joachim D Mueller

Human T-cell leukemia virus type 1 (HTLV-1) particle assembly occurs at the plasma membrane and is driven by the Gag protein. HTLV-1 particle biogenesis is poorly understood. Previous studies support a model in which non-punctate, plasma membrane (PM)-bound Gag acts as a reservoir for HTLV-1 Gag puncta biogenesis. In order to gain further insight into the role of non-punctate PM-bound Gag in HTLV-1 particle assembly, fluorescence fluctuation spectroscopy (FFS) has been utilized to characterize the density of non-punctate Gag at the plasma membrane of living cells. The density of non-punctate, PM-bound Gag was found to vary with cytoplasmic Gag concentration in a manner consistent with a Langmuir-isotherm binding model. To further investigate non-punctate Gag at the PM, we are currently employing fluorescence lifetime imaging microscopy (FLIM) measurements of fluorescence resonance energy transfer (FRET) between red and green labeled fluorescent Gag proteins co-expressed in cells. The short length-scale sensitivity provided by FLIM/FRET measurements of non-punctate Gag at the PM provides complementary data to that of ongoing FFS and super-resolution microscopy studies. An update of these studies will be presented. The characterization of non-punctate, membrane-bound HTLV Gag will provide important clues for the biogenesis of HTLV-1 Gag puncta, which is crucial for driving virus particle production. Such processes are vital for infectious HTLV-1 spread, and a detailed understanding should aid in the discovery of novel targets for antiretroviral intervention.

34. "Whole-Transcriptome Sequencing to Identify Immune Gene Variants"
Amelia K. Haj, Julie A. Karl, Roger W. Wiseman, and David H. O'Connor

The rhesus MHC class I allele Mamu-B*08 is associated with control of viral replication, but is likely only one of many genetic modifiers of susceptibility to HIV. Other immune genes, including killer cell immunoglobulin-like receptor and Fc gamma receptor genes, are also likely to be important in determining the rate of HIV disease progression. Much of the current work in macaque genomics employs whole-exome or whole-genome sequencing; however, these approaches rely on flawed reference genomes and annotations for variant calling, and perform especially poorly in complex immune genes. Long-read RNA sequencing allows for identification of full-length transcript isoforms and phased SNPs from multiple immune gene families simultaneously, without a reference genome. Here we describe whole-transcriptome sequencing of peripheral blood mononuclear cells from one rhesus and one cynomolgus macaque using Pacific Biosciences long-read RNA sequencing (Iso-Seq) technology performed

on a PacBio Sequel machine. We obtained over 800,000 consensus reads per animal, with an average read length of approximately 3000 bases. Using data produced from the PacBio Iso-Seq analysis pipeline (version 4.0.0), we identified reads aligning to expected MHC class I alleles as well as to specific KIR alleles and Fc gamma receptor isoform sequences. We anticipate that this technology will be useful in characterizing cell type-specific expression of immune gene isoforms in macaques during the course of SIV infection.

35. "Human Papillomavirus Promotes Epstein-Barr Virus Lytic Reactivation in Immortalized Oral Keratinocytes"
Kathleen Makielski, Denis Lee, Laurel Lorenz, Dhananjay Nawandar, Ya-Fang Chiu, Shannon Kenney, and Paul Lambert

Epstein-Barr virus (EBV) and human papillomaviruses (HPVs) are human tumor viruses that cause head and neck cancers. Both viruses infect and replicate in upper aerodigestive tract epithelia. Some studies have detected co-infection in both oropharyngeal and nasopharyngeal cancers raising the possibility that these viruses could potentially affect each other's life cycles and/or oncogenic potential. Our lab has established an in vitro model system using organotypic raft cultures to test the effects of EBV and HPV on each other in stratified squamous oral epithelial cells. We found that the presence of HPV promotes EBV lytic reactivation in suprabasal layers of the oral epithelium. Specifically, HPV promotes EBV genome amplification and expression of an EBV immediate-early protein, Z, both of which are hallmarks of EBV lytic reactivation. HPV's ability to promote EBV lytic reactivation is driven by HPV oncoprotein E7, and more specifically, its ability to bind pocket proteins. In addition, HPV promotes infectious EBV production in squamous oral epithelium. Studying the effects of HPV on EBV lytic reactivation and virus production could elucidate mechanisms by which co-infection might contribute to viral infection and head and neck carcinogenesis.