‘*Wisc*-*e*-*sota*’

1st Joint UMN-UW Virology Training Grant Symposium

Friday, September 20th, 2013

UW-La Crosse

Cartwright Center

9 am - 4 pm

The inaugural collaborative symposium of the
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

***'Wisc-e-sota'* Virology Symposium**
Friday, September 20th, 2013
9:00 am - 4:00 pm
UW-La Crosse
Cartwright Center

ORAL PRESENTATIONS

Talk abstract numbers in left column

	9:00-9:30	Registration
	9:20-9:30	Welcome & Opening Remarks
		Convenor: Casey Solomon
1	9:30	Rob Striker (UW) **"Optimizing Hepatitis C treatment where it is needed most"**
2	10:00	Jonathan Rawson, Richard Heineman, Lauren Beach, Jessica Martin, Erica Schnettler, Michael Dapp, Steven Patterson, and Louis Mansky (UMN) **"5,6-Dihydro-5-aza-2-deoxycytidine potentiates the anti-HIV-1 activity of ribonucleotide reductase inhibitors"**
3	10:15	Adam Bailey (UW) **"Discovery and characterization of novel simian hemorrhagic fever viruses in a wild primate population"**
	10:30-10:45	Morning coffee break and poster set up
		Convenor: Megan Spurgeon
4	10:45	Elizabeth Steinert, Jason M. Schenkel, David Masopust (UMN) **"Cell isolations massively underestimate and misrepresent the non-lymphoid memory CD8 T cell compartment after viral infection"**
5	11:00	Louise Moncla (UW) **"A novel nonhuman primate model for influenza transmission"**
6	11:15	Reuben Harris (UMN) **" HIV-1 Restriction by a Swarm of APOBEC3 DNA Deaminases"**
	11:45-2:30	**Luncheon & Poster Session – *Featuring 30+ posters***

Convenor: Chad Kuny

7	2:30	Shelley Grimes (UMN) **"The Role of pRNA in the DNA Packaging Motor of Bacteriophage ø29"**
8	3:00	Nathaniel Byers, Rianna Vandergaast and Paul Friesen (UW) **"Baculovirus Inhibitor-of-Apoptosis (IAP) Stabilizes Host IAP to Prevent Apoptosis"**

Convenor: Bobbi Tschida

9	3:15	Joshua Boyer (UMN) **"Impaired antiviral response associated with human disease variant of Ptpn22"**
10	3:30	Eric Johannsen (UW) **"The EBNA3 family of Epstein-Barr virus nuclear proteins targets the USP46 deubiquitination complex to regulate cell growth"**
	4:00	Closing Remarks

POSTER PRESENTATIONS

Poster abstract numbers in left column

1 Adam Swick, Jay Warrick, Ashley Baltes, and John Yin (UW)
"Visualizing vesicular stomatitis virus (VSV) infection spread and resulting host immune responses in vitro with a dual color fluorescent reporter system"

2 William C. Solomon (UMN)
"Finding determinants of ssDNA specificity in HIV-1 restriction factor APOBEC3G"

3 Chad V. Kuny and Robert F. Kalejta (UW)
"Deoxyribonucleotide Availability is Not the Source of the UL97 Deletion Virus DNA Replication Defect"

4 Anurag Rathore, Michael A Carpenter, Terumasa Ikeda, Ming Li, Emily K. Law, William L. Brown, Reuben S. Harris (UMN)
"A single amino acid substitution in human APOBEC3G alters the dinucleotide substrate specificity"

5 Anthony Hanson, Masaki Imai, Gabriele Neumann and Yoshihiro Kawaoka (UW)
"Identification of Stabilizing Mutations in an H5 HA Influenza Protein"

6 Judd F. Hultquist and Reuben S. Harris (UMN)
"The Cofactors and Targets of Primate and Nonprimate Lentiviral Vif Proteins"

7 Jincan Zhang and Aurelie Rakotondrafara (UW)
"Functional analysis of an atypical plant viral IRES"

8 Brett Anderson, Markus-Frederik Bohn , Shivender Shandilya, John, S. Albin, Takahide Kono, Rebecca M. McDougle, Michael A. Carpenter, Anurag Rathore, Leah Evans, Ahkillah N. Davis, JingYing Zhang, Yongjian Lu, Mohan Somasundaran, Hiroshi Matsuo, Reuben S. Harris, Celia A. Schiffer (UMN)
"Crystal structure of the Vif-binding and catalytically active domain of the DNA cytosine deaminase APOBEC3F"

9 Coral K. Wille, Dhananjay M. Nawandar, and Shannon C. Kenney (UW)
"EBV genome hydroxymethylation differentially affects Z- versus R-mediated lytic gene expression"

10 Lauren B. Beach, Jessica van Oploo, Michael J. Dapp, Ben D. Duckworth, Steven E. Patterson, and Louis M. Mansky (UMN)
"Investigating the Molecular Anti-HIV-1 Mechanism of Clofarabine"

11 Jessica A. Reusch, Dhananjay M. Nawandar, Shannon C. Kenney, and Janet E. Mertz (UW)
"BLIMP1, master regulator of plasma cell differentiation, induces EBV lytic replication by activating transcription from the Z and R promoters"

12 Michael A. Carpenter, Ming Li, Anurag Rathore, Lela Lackey, Emily K. Law, Allison M. Land, Brandon Leonard, Shivender M. D. Shandilya, Markus-Frederik Bohn, Celia A. Schiffer, William L. Brown and Reuben S. Harris (UMN)
"The foreign DNA restriction enzyme APOBEC3A can deaminate both normal and 5-methylcytosine"

13 Megan E. Spurgeon and Paul F. Lambert (UW)
"Development of a transgenic mouse model to study Merkel Cell Polyomavirus T antigen expression in the stratified epithelia"

14 Marisa Barbknecht, Sol Sepsenwol, Eric Leis, Maren Tuttle-Lao, Mark Gaikowski, Susan E. Kelly, Nick J. Knowles, Becky Lasee, Michael A. Hoffman (UW-Lax)
"Characterization of a new picornavirus isolated from bluegills"

15 Andrea Timm and John Yin (UW)
"Kinetics of virus-host interactions in single-cells using a high-throughput assay"

16 Juliet Crabtree, Vikram Verma, Yaya Wang, Yuying Liang, Erik Peterson (UMN)
"PTPN22 Promotes Protective Responses to Influenza A Immunization"

17 Mounavya Aligeti, Ryan T Behrens, Nathan M Sherer (UW)
"Species-Specific Regulation Of HIV-1 Rev Activity"

18 Jose O. Maldonado, Iwen F. Grigsby, Sheng Cao, Joachim Mueller, Wei Zhang, and Louis M. Mansky (UMN)
"Analysis of HTLV-1 Particle Morphology and Gag Stoichiometry"

19 Reza Djavadian (UW)
"Epstein-Barr Virus (EBV) Late Gene Regulation"

20 Peter R. Wilker, Jorge M. Dinis, Gabriel Starrett, Masaki Imai, Masato Hatta, Chase W. Nelson, David H. OConnor, Austin L. Hughes, Gabriele Neumann, Yoshihiro Kawaoka and Thomas C. Friedrich (UW-Lax)
"Selection on hemagglutinin imposes a genetic bottleneck during transmission of reassortant H5N1 influenza viruses"

21 Adam J. Ericsen, Gabriel J. Starrett, Justin M. Greene, Michael Lauck, Brian T. Cain, Ngoc H. Pham, David Rio Deiros, Muthuswamy Raveendran, Roger W. Wiseman, Jeffrey Rogers, Richard Gibbs, Donna Muzny, Thomas C. Friedrich, and David H. O' Connor (UW)
"Granzyme B is a Genetic Determinant of AIDS Virus Control"

22 Barbara R Tschida, Vincent W Keng, Timothy P Kuka, and David A Largaespada (UMN)
"Determining the role of Hepatitis B virus gene X mutant variants in hepatocellular carcinoma"

23 Jay W Warrick, Adam D Swick, Ashley Baltes, John Yin (UW)
"Single-cell spatiotemporal kinetics of innate immune activation and viral protein expression in monolayer cultures

24 Allison M. Land, Reuben S. Harris (UMN)
"Conversion of Rhesus Macaque APOBEC3F from HIV-1 Vif-Resistant to Vif-Sensitive"

25 Robert A. Pugh and Paul Ahlquist (UW)
"Purification, Multimerization, RNA Binding, and ATPase Activity of Bromovirus RNA Helicase Domain"

26 Eric W. Refsland, Elizabeth M. Luengas, Judd F. Hultquist, Leah C. Evans, Rachel Prosser, Keith Henry, and Reuben S. Harris (UMN)
"Stable APOBEC3H haplotypes confer resistance to HIV infection"

27 Eric W. Refsland, Judd F. Hultquist, and Reuben S. Harris (UMN)
"Gene targeting and knockdown in the T cell Line CEM2n demonstrate a role for APOBEC3D, APOBEC3F, and APOBEC3G in HIV-1 restriction and hypermutation"

28 Melba Marie Tejera, M. Suresh (UW)
"Forkhead Transcription Factor FOXO1 Regulates the Function and Survival of Memory CD8 T Cells"

29 Sheng Cao and Wei Zhang (UMN)
"Characterization of an Early-Stage Membrane Fusion Intermediate of Sindbis Virus using Cryo-electron Microscopy"

30 Alexandra Torres, GC Blitzer, MA Smith, EA Armstrong, PM Harari, PF Lambert, RJ Kimple (UW)
"Epidermal growth factor inhibition of HPV positive head and neck cancer cells and primary tumorgrafts results in significant growth inhibition mediated by apoptosis"

31 Wenbo Zhou, Feng Chen, Yuri Klyachkin, Yuk Y. Sham, Robert J. Geraghty (UMN)
"Mutations in the amino terminus of herpes simplex virus type 1 gL can reduce cell-cell fusion without affecting gH/gL trafficking"

32 Anqi Wang, Bo Zhao, Tram Ta, Rene Welch, Elliott Kieff, Sunduz Keles and Eric Johannsen (UW)
"Genome-wide analysis of EBNA3 binding in EBV transformed cells"

Abstracts-Oral Presentations

1 "Optimizing Hepatitis C treatment where it is needed most"

Rob Striker, Amanda Carlson, Micheal Lucey, Tim Hess and David Burnett (UW)

The United States incarcerates ~1% of its citizens, 20% more than Russia the next closest nation in the world, but most of these incarcerations are for much less than a decade. This mass incarceration is largely driven by the war on illicit drugs and therefore 12-41% of the imprisoned population is infected with Hepatitis C Virus (HCV). Release from prison may result in reinfection. Guidelines recommend arbitrary durations of therapy from 24-72 weeks depending upon viral response measured at 1 month. Disease from HCV is relatively slow and HCV therapy is both expensive, until recently not widely effective, and generally not available to inmates who frequently have short sentences relative to the duration of therapy. We reviewed the mortality in general and from HCV specifically over a 12-year period of time in the WI Department of Corrections (annual census of ~22,000), and found liver disease to be a common cause of death. Furthermore successful therapy for HCV protected against death. Therapy in prison was no less effective than a contemporary cohort outside of prison. Finally we present preliminary work inside and outside the prison that suggests response within 1 week of initiation therapy can guide treatment decisions leading to shorter, less expensive therapy available to individuals with short sentences.

2 "5,6-Dihydro-5-aza-2-deoxycytidine potentiates the anti-HIV-1 activity of ribonucleotide reductase inhibitors"

Jonathan Rawson, Richard Heineman, Lauren Beach, Jessica Martin, Erica Schnettler, Michael Dapp, Steven Patterson, and Louis Mansky (UMN)

The nucleoside analog 5,6-dihydro-5-aza-2-deoxycytidine (KP-1212) has been proposed as a first-in-class lethal mutagen of human immunodeficiency virus type-1 (HIV-1). While initial studies in cell culture appeared quite promising, Phase II clinical trials of a prodrug form (KP-1461) revealed that monotherapy could not significantly reduce plasma viral loads in HIV-1-infected individuals. Here, we tested whether the antiretroviral activity of KP-1212 could be enhanced in combination with inhibitors of ribonucleotide reductase (RNRIs). While we observed that KP-1212 had a minimal effect on HIV-1 infectivity and mutant frequency in a single-round replication assay, KP-1212 potentiated the activity of the RNRIs gemcitabine and resveratrol, leading to both significant reductions in HIV-1 infectivity without cellular toxicity and corresponding increases in the viral mutant frequency. DNA sequencing analysis revealed that the combination of KP-1212 with resveratrol (a phytostilbene and phytoalexin with known RNRI activity) led to a marked induction of G-to-C transversion mutations, an uncommon mutation type rarely observed during HIV-1 replication. Taken together, these observations represent the first demonstration of a mild anti-HIV-1 mutagen potentiating the antiretroviral activity of RNRIs. These observations could enhance the potential for the clinical translation of KP-1212 in treating HIV-1 infection.

3 " Discovery and characterization of novel simian hemorrhagic fever viruses in a wild primate population"

Adam Bailey (UW)

RNA viruses have a remarkable capacity for evolution and adaptation. As such, these viruses rank among the most globally-important emerging infectious diseases. Identifying natural reservoirs of RNA viruses and characterizing these viruses in their pre-emergent state is considered a top priority for predicting and preventing the emergence of new infectious diseases. We recently discovered two highly-divergent viruses related to simian hemorrhagic fever virus (SHFV) infecting wild red colobus monkeys in Kibale National Park, Uganda. In depth characterization of these infections showed that these viruses are prevalent in the red colobus population, sustain high levels of viremia in infected monkeys, and exhibit an extraordinary amount of genetic diversity, both among and within hosts. Analysis of within-host viral diversity revealed distinct signatures of natural selection with implications for immune evasion and persistence. Taken together, these findings show that these viruses share several important properties with some of the most rapidly evolving, emergent RNA viruses.

4 "Cell isolations massively underestimate and misrepresent the non-lymphoid memory CD8 T cell compartment after viral infection"

Elizabeth Steinert, Jason M. Schenkel, David Masopust (UMN)

Non-lymphoid memory CD8 T cells contribute to protection from re-infection as they are localized to frontline tissues where infections occur. Interrogation of this population is often highly dependent upon cell isolation and ex vivo analysis; and interpretation is predicated on presumptions of near complete extraction. We evaluated the distribution of LCMV Armstrong infection-specific memory CD8 T cells in mice via the complementary approaches of cell isolation and flow cytometry vs. in situ immunohistochemistry. Memory cells were established in every non-lymphoid tissue tested, including trachea, bladder, gall bladder, stomach, and heart, revealing the remarkable diaspora of cellular immune responses following viral infection. Surprisingly, we found that enzymatic processing isolates as few as 2% of memory CD8 T cells truly present in non-lymphoid organs, and representation of harvested cells was biased. In contrast, isolations from lymphoid tissue were more efficient. This underestimated population likely plays a key role in subsequent interactions with the invading virus. Understanding the quantity of pathogen specific memory CD8 T cells available to survey a given tissue after primary infection is the first step to estimating the true potential contribution this population can make to secondary immune responses at non-lymphoid sites.

5 "A novel nonhuman primate model for influenza transmission"

Louise Moncla (UW)

Studies of influenza transmission are necessary to predict the pandemic potential of emerging influenza viruses. Currently, both ferrets and guinea pigs are used in such studies, but these species are distantly related to humans. Nonhuman primates (NHP) share a close phylogenetic relationship with humans and may provide an enhanced means to model the virological and immunological events in influenza virus transmission. Here, for the first time, it was demonstrated that a human influenza virus isolate can productively infect and be transmitted between common marmosets (Callithrix jacchus), a New World monkey species. We inoculated four marmosets with the 2009 pandemic virus A/California/07/2009 (H1N1pdm) and housed

each together with a naïve cage mate. We collected bronchoalveolar lavage and nasal wash samples from all animals at regular intervals for three weeks post-inoculation to track virus replication and sequence evolution. The unadapted 2009 H1N1pdm virus replicated to high titers in all four index animals by 1 day post-infection. Infected animals seroconverted and presented human-like symptoms including sneezing, nasal discharge, labored breathing, and lung damage. Transmission occurred in one cohabitating pair. Deep sequencing detected relatively few genetic changes in H1N1pdm viruses replicating in any infected animal. Together our data suggest that human H1N1pdm viruses require little adaptation to replicate and cause disease in marmosets, and that these viruses can be transmitted between animals. Marmosets may therefore be a viable model for studying influenza virus transmission.

6 "HIV-1 Restriction by a Swarm of APOBEC3 DNA Deaminases"
Reuben Harris (UMN)

The past decade has witnessed the unveiling of an extraordinary mechanism of innate immunity in which several cellular APOBEC3 proteins enter assembling viral particles and destroy the integrity of the viral nucleic acid by deaminating viral cDNA cytosines to uracils. This mechanism explains the phenomenon of G-to-A hypermutation, which is evident in viral sequences from patients. However, HIV-1 avoids lethal mutagenesis through its accessory protein Vif, which nucleates the formation of an APOBEC3 degradation complex. Human cells have the capacity to express up to seven APOBEC3 family members. We have been working to test the hypothesis that only a subset of these family members is relevant to HIV-1 pathogenesis. Knockout experiments have demonstrated roles for APOBEC3G in restriction and mutation in a 5GG context, and roles for APOBEC3D and APOBEC3F in restriction and mutation in a 5GA context. Individual APOBEC3 protein over-expression experiments were concordant, with evidence for the same three proteins as well as indications that APOBEC3H may also contribute to virus restriction and mutation in a 5GA context. Primary cell experiments have supported this idea as stable alleles of APOBEC3H appear to provide a natural barrier to infection by some HIV-1 genotypes. These data combine to indicate that swarm of four APOBEC3 proteins has capabilities for HIV-1 restriction and mutagenesis, with implications for drug resistance, immune escape and virus evolution.

7 "The Role of pRNA in the DNA Packaging Motor of Bacteriophage ø29"
Shelley Grimes (UMN)

During assembly, double-stranded DNA bacteriophages package their genomes into the viral head, compacting the DNA to near-crystalline density. This process is driven by powerful ATP-dependent molecular motors that assemble on the portal vertex. In ø29, the motor is comprised of three ring-shaped complexes: the dodecameric head-tail connector, a pentameric ring of prohead RNA (pRNA), and the ring ATPase gp16. The ø29 motor has been shown to be highly coordinated. Packaging involves a two-step cycle in which at least four ATPases each bind ATP during a static dwell phase, followed by a burst where 10bp of DNA is translocated into the head. This mechanism implies a high degree of communication within the motor. We have employed an integrated approach to probe the structure/function role of these three components and how they interact and communicate to form the functional DNA packaging motor. Here we present our current model of pRNA structure and function. The functional domains of pRNA have been characterized and demonstrated to perform crucial roles in motor assembly and function. In particular, the centrally located position of pRNA in the motor suggests a potential role as mediator of communication. Fitting the known structures of the connector, pRNA, and

gp16 into cryoEM maps of the motor will generate a nearly complete pseudo-atomic model of a DNA packaging motor that will provide insight into the spatial relationships amongst the various motor components, identify functional targets for mutagenesis, and provide a structural framework for evaluating potential mechanisms for force transduction.

8 "Baculovirus Inhibitor-of-Apoptosis (IAP) Stabilizes Host IAP to Prevent Apoptosis"
Nathaniel Byers, Rianna Vandergaast and Paul Friesen (UW)

Apoptosis is a host defense that can be detrimental to virus multiplication. Thus, many viruses encode genes that subvert apoptosis. Baculoviruses encode homologues of the host inhibitor-of-apoptosis (IAP) proteins. Host IAPs form dimers that block apoptosis by neutralizing caspases, pro-death proteases. After an apoptotic stimulus, host IAP is degraded, releasing its inhibition on the caspases. Despite its relatedness to host IAP, Op-IAP3, the prototypic baculovirus IAP, has no direct caspase inhibitory activity. Thus, viral IAP works through a noncanonical mechanism. We observed that viral IAP (vIAP) prohibited the loss of endogenous host IAP after virus infection. Therefore, we hypothesized that vIAP functions by binding and stabilizing host IAP so that host IAP continues inhibiting caspases. We report here that vIAP immunoprecipitated with host IAP, demonstrating a physical interaction that may be essential for vIAP function. Viral IAP, which is about five times more stable than host IAP, significantly increased the half-life of host IAP. Viral IAP that was engineered to have a short half-life, but retained physical interaction with host IAP, did not increase host IAP stability, suggesting that interaction between the host and viral IAPs is not sufficient for vIAPs anti-apoptotic function. Rather, vIAP requires its long half-life to confer stability to host IAP. The destabilized vIAP failed to protect cells from virus-induced apoptosis, correlating vIAP stability with anti-apoptotic function. Consistent with the hypothesis that vIAP functions through host IAP, vIAP failed to block apoptosis when host IAP was depleted by RNAi. Collectively, our findings support the hypothesis that viral IAP functions by stabilizing host IAP through direct interaction, illuminating a novel viral mechanism to prevent apoptosis.

9 "Impaired antiviral response associated with human disease variant of Ptpn22"
Joshua Boyer (UMN)

PTPN22 is a potent risk allele for diverse human autoimmune diseases. We recently documented a key role for Ptpn22 in promoting interferogenic Toll-like receptor signaling. As type 1 IFN are critical mediators of antiviral host defense, we investigated a possible role for Ptpn22 in anti-viral immunity. Protective immune responses against lymphochoriomeningitis virus (LCMV) require pattern recognition receptors including TLRs. We found that after LCMV-Armstrong exposure, Ptpn22/mice displayed reduced infection-induced serum IFN± and IFN² levels and blunted activation of splenic CD8±+ DC and plasmacytoid DC (pDC). Moreover, Ptpn22/ mice exhibited impaired expansion of LCMV-specific CD8+ T cells. We utilized mice made transgenic for the human disease-associated gene variant (PTPN22W) to ask about the role of LypW in antiviral responses. We found that PTPN22W-expressing mice also exhibit reduced type 1 IFN production and impaired CD8 T cell expansion in response to LCMV. Taken together, these data indicate that Ptpn22 is required for efficient innate immune activation and cytotoxic T lymphocyte proliferation, and that the human disease associated variant is a reduced-function allele in anti-viral responses.

10 "The EBNA3 family of Epstein-Barr virus nuclear proteins targets the USP46 deubiquitination complex to regulate cell growth"

Eric Johannsen, Makoto Ohashi, Amy M Holthaus, Michael A Calderwood, Bryan Krastins (UW)

The Epstein-Barr virus (EBV) nuclear proteins EBNA3A, EBNA3B, and EBNA3C interact with the cell DNA binding protein RBPJ and regulate partially overlapping subsets of cell and viral genes. To elucidate the other proteins targeted by the EBNA3 proteins, we generated lymphoblastoid cell lines (LCLs) expressing flag-HA tagged EBNA3A, EBNA3B, or EBNA3C and used tandem affinity purification to isolate each EBNA3 complex. Mass-spectrometry revealed that each EBNA3 complex forms a distinct complex with RBPJ, that does not include any other EBV encoded proteins. The EBNA3A and EBNA3B complexes also contained the deubiquitinase (DUB) complex consisting of USP46, WDR48, and WDR20 and EBNA3C complexes contained WDR48. By direct immunoprecipitation an EBNA3C association with the USP46 complex was evident, but to a lesser degree than observed for EBNA3A and EBNA3B. Mapping revealed that WDR48 binds to EBNA3 domains that are important for LCL growth, suggesting a role for the USP46 complex in EBNA3 growth transformation effects. We speculate that EBNA3 proteins may regulate cell gene expression by recruiting the USP46 complex to promote the ubiquitin removal from chromatin bound proteins near RBPJ sites. Attempts to identify the specific USP46 substrates are ongoing.

Abstracts- Poster Presentations

1 "Visualizing vesicular stomatitis virus (VSV) infection spread and resulting host immune responses in vitro with a dual color fluorescent reporter system"

Adam Swick, Jay Warrick, Ashley Baltes, and John Yin (UW)

The spread of a viral infection is a highly complex process, one that integrates functions across massively disparate scales, from the intracellular to the intercontinental. In this work we focused on the spread of an infection at the level of cell to cell propagation using an in vitro tissue culture model of spread. Using a model RNA virus, the prototypical rhabdovirus vesicular stomatitis virus (VSV) we investigated its ability to spread in the face of the host innate immune response. In order to study the spatial and temporal patterns of its spread we developed a dual color fluorescent protein reporter system that used recombinant strains of VSV that express RFP as indicator of viral replication, and stable transgenic human cell lines that produce GFP as a measure of host cell innate immune activity. We validated that the introduction of the reporter genes did not perturb normal growth or health of the cells or virus and that the reporter signal was correlated with the associated biological function. Using the dual reporter system we performed time lapse live-cell fluorescent microscopy, which when combined with new methods of image processing and analysis, enabled the tracking the dynamic activity of viral and antiviral processes in tens of thousands of individual cells. We found that effective propagation of VSV infections was dependent on its ability to efficiently suppress the host innate immune response. The VSV mutant strain M51R, which lacks the ability to inhibit the export of host cell mRNA, was a robust activator of IFIT2, which can be classified as both a viral stress inducible gene (VSIG) and an interferon stimulated gene (ISG). While activation of this host antiviral response gene had little effect on a single infection cycle, over a multi-round spreading infection, its activity was correlated with inhibition of infection propagation. In M51R infections, IFIT2 activation beyond the infection front, likely through paracrine signaling, was indicative of a primed antiviral state in uninfected cells which led to the containment of the infection. Conversely, WT VSV which was able to largely suppress IFIT2 activity, spread efficiently throughout the cell monolayer. These results both better elucidate the mechanism of IFIT2 function in antiviral defense and more broadly highlight the critical need for viruses to suppress innate immunity in order to propagate efficiently.

2 "Finding determinants of ssDNA specificity in HIV-1 restriction factor APOBEC3G"

William C. Solomon (UMN)

APOBEC3 (A3) proteins are involved in multiple cellular processes ranging from inhibition of LINE-1 retrotransposons to innate immune response to viral DNA. A3 proteins are single strand DNA (ssDNA) cytidine deaminases that convert deoxycytidine residues in ssDNA substrates to deoxyuridine (dU). The incorporation of these non-native dU residues into the template DNA strand results multiple deoxyguanosine (dG) to deoxythymidine (dT) transversion mutations following transcription. These dG to dT transversion mutations can result in the introduction of changes in the coding sequence or premature stop codons into the open reading frames of target DNA promoting nonsense mediated decay. This can be beneficial as is the case in APOBEC3G (A3G) mediated hypermutation and restriction of HIV-1 viral pathogenesis. However, sub-lethal levels of mutation can result in enhancement of HIV-1 escape potential and, in the recently published case of APOBEC3B (A3B), can actually promote oncogenesis. Since A3 proteins act primarily through this deamination dependent pathway, an understanding of the

mechanism of ssDNA targeting and substrate selection is crucial to understanding A3 proteinseffects on HIV-1 restriction and oncogenesis.

3 **"Deoxyribonucleotide Availability is Not the Source of the UL97 Deletion Virus DNA Replication Defect"**

Chad V. Kuny and Robert F. Kalejta (UW)

Human Cytomegalovirus (HCMV), a member of the Herpesviridae family, requires considerable cellular resources to replicate its large, double-stranded DNA genome. To facilitate this process, HCMV manipulates the cell cycle, forcing the cell into a pseudo S-phasewhere the cellular DNA replication resources are available to the virus. HCMV uses the UL97 protein kinase, a virally-encoded cyclin-dependent kinase (v-Cdk), to manipulate the cell cycle, phosphorylating the Retinoblastoma tumor suppressor protein (Rb) that allows for production of the genes that are necessary for cellular genome replication, including Nucleotide Biosynthetic Enzymes (NBEs). In the absence of UL97 and the corresponding Rb phosphorylation, these genes are not expressed, and HCMV exhibits a DNA synthesis defect. Since HCMV encodes a number of its own DNA replication enzymes, but is notably lacking NBEs, we have hypothesized that deoxyribonucleotide availability is a root cause of the UL97 deletion mutant (UL97) DNA synthesis defect. To test this hypothesis, we have examined viral DNA replication in the presence of an exogenous supply of deoxyribonucleosides, which can be transported into cells and converted to deoxyribonucleotides in the absence of S-phase gene expression, and found that this treatment partially rescues DNA replication of HCMV UL97. However, this treatment also allows WT HCMV to replicate DNA more efficiently, therefore deoxyribonucleotide availability generally represents a limiting factor for HCMV DNA replication, and v-Cdk UL97 must play other roles in genome replication.

4 **"A single amino acid substitution in human APOBEC3G alters the dinucleotide substrate specificity"**

Anurag Rathore, Michael A Carpenter, Terumasa Ikeda, Ming Li, Emily K. Law, William L. Brown, Reuben S. Harris (UMN)

APOBEC3G (A3G) is innate immune single stranded DNA cytosine deaminases with biological functions in retrovirus and retrotransposons restriction. To determine whether these functions might relate to local substrate preferences, we analyzed a series of chimeras in which putative DNA binding loop regions of A3G were replaced with the corresponding loops from A3A. Loop 1 replacement attenuated A3G activity while Loop 3 replacement enhanced A3G catalytic activity without altering its intrinsic 5-CC dinucleotide substrate preference. Loop 7 replacement caused A3G to become A3A-like and strongly prefer 5-TC substrates. Simultaneous loop 3 and loop 7 replacement resulted in a hyperactive A3G variant that also preferred 5-TC dinucleotides. A series of single amino acid swaps revealed D317 as the major determinant of dinucleotide substrate specificity. All of the catalytically active A3G variants, regardless of dinucleotide preference, retained HIV-1 restriction activity. These data support a model in which the loop 7 region of A3G, and likely most other DNA deaminases serves to govern the selection of local dinucleotide substrates for deamination but may not be part of the regulatory mechanisms that these enzymes use to engage relevant biological substrates such as retroviral cDNA.

5 "Identification of Stabilizing Mutations in an H5 HA Influenza Protein"
Anthony Hanson, Masaki Imai, Gabriele Neumann and Yoshihiro Kawaoka (UW)

There have been isolated cases of human infection with highly pathogenic H5N1 influenza viruses but these viruses are still unable to efficiently transmit between humans. However, multiple groups have recently observed respiratory droplet transmission of H5 viruses in a ferret model. The transmitted viruses possessed mutations in the hemagglutinin (HA) receptor binding domain that resulted in a change of preference from avian to human-type receptors. Additional mutations were also present in HA that conferred increased protein stability. After cleavage of the HA0 precursor protein into the HA1 and HA2 subunits, HA undergoes a switch to its fusogenic form when exposed to acidic conditions or heat. To identify additional stabilizing mutations that affect the pH at which HA-mediated fusion occurs, we generated a virus library possessing random mutations in the extracellular domain of an H5 HA altered to bind human-type receptors. The library was subjected to three rounds of heat inactivation and subsequent amplification. From this, we isolated individual mutants that displayed a significant increase in heat stability, a decrease in their fusion pH relative to the wild-type HA, and maintained their human-type receptor binding preference. Next, we plan to test these variants in a ferret model for respiratory droplet transmission. It is critical to further characterize and understand what molecular features are essential to confer HA stability and transmissibility to H5N1 viruses in order to best monitor field isolates and prepare for the emergence of a new pandemic.

6 "The Cofactors and Targets of Primate and Nonprimate Lentiviral Vif Proteins"
Judd F. Hultquist and Reuben S. Harris (UMN)

Human Immunodeficiency Virus type 1 (HIV-1) and related lentiviruses require the accessory protein Vif to neutralize members of the APOBEC3 family of retrovirus restriction factors and render their hosts cells permissive for replication. Vif neutralizes the APOBEC3 proteins by recruitment of an E3 ubiquitin ligase complex that polyubiquitinates the APOBEC3 proteins and targets them for proteasomal degradation. Despite this conserved function and general mechanism of action, Vif exhibits almost no primary sequence conservation between lentiviral subgroups and may be playing additional alternate roles that ultimately contribute to the distinct pathogenesis of each virus. We used an affinity purification-mass spectrometry approach coupled with targeted functional studies to probe the interactome of each lentiviral Vif protein and identify pertinent cofactors and targets. For example, we and others recently demonstrated that HIV-1 Vif requires the transcription factor CBFbeta to assemble the E3 ligase complex necessary to degrade the restrictive repertoire of human APOBEC3 proteins (1, 2, 3). CBFbeta is also required for Simian Immunodeficiency Virus (SIV) Vif function (1, 3), but is dispensable for FIV (Feline), BIV (Bovine), and MVV (Ovine) Vif function (unpublished observations). The recruitment of CBFbeta by HIV-1 Vif has recently been reported to alter gene expression at CBFbeta responsive loci (4) and may contribute to unique aspects of primate versus nonprimate lentiviral pathogenesis. A number of differences between lentiviral subgroups in the composition of the APOBEC3 degrading Vif E3 ligase complexes, as well as the capacity of these proteins to form other independent complexes, strongly suggest that the lentiviral Vif proteins play multiple essential and likely distinct roles in viral infection and pathogenesis. References 1. Jäger*, S., D.Y. Kim*, J.F. Hultquist*, K. Shindo, R.S. LaRue, E. Kwon, M. Li, B.D. Anderson, L. Yen, D. Stanley, C. Mahon, J. Kane, K. Franks-Skiba, P. Cimermancic, A. Burlingame, A. Sali, C. Craik, R.S. Harris#, J.D. Gross# & N.J. Krogan# (2011) Vif hijacks CBFbeta to degrade APOBEC3G and promote HIV-1 infection. Nature 481:371-5. 2. Zhang, W., J. Du, S.L. Evans, Y. Yu & X.F. Yu (2011) T-cell differentiation factor CBFbeta regulates HIV-1 Vif-mediated evasion of host restriction. Nature 481:376-9. 3. Hultquist, J.F., M.

Binka, R.S. LaRue, V. Simon, R.S. Harris (2012) Vif proteins of human and simian immunodeficiency viruses require cellular CBFbeta to degrade APOBEC3 restriction factors. Journal of Virology 86:2874-77. 4. Kim DY, E. Kwon, P.D. Hartley, D.C. Crosby, S. Mann, N.J. Krogan, J.D. Gross (2013) CBFbeta stabilizes HIV Vif to counteract APOBEC3 at the expense of RUNX1 target gene expression. Molecular Cell 49:632-44

7 "Functional analysis of an atypical plant viral IRES"

Jincan Zhang and Aurelie Rakotondrafara (UW)

Internal ribosome entry site (IRES) are RNA sequences that eliminate the need for the cap structure to recruit the ribosomes in a 5-end independent manner. Since their discovery in animal picornaviruses as long and highly compacted structures, different classes of IRESes have been reported including in plant viral 5leaders. Great divergences are observed between animal and plant viral IRESes, where the latter are notable for their smaller size, structure simplicity and where function appears in some cases independent of specific RNA structure. Triticum Mosaic Virus (TriMV) is a new member of the RNA picornavirus super-group, which infects plants. Like other members, its polyadenylated genomic RNA naturally lacks a 5cap structure. In this study, we analyzed the function of the TriMV atypically 739 nt long 5untranslated region (UTR), which is uncharacteristic of any known plant-hosted picornaviruses. Here we showed that the TriMV 5leader directs cap-independent translation of a heterologous monocistronic mRNA in vitro. In an in vitro competition assay, the TriMV 5UTR sequence strongly inhibits cap-dependent translation in trans by sequestering the initiation factor eIF4F. Using a bicistronic reporter mRNA, we revealed that TriMV 5leader can initiate translation internally, and with 100 fold higher efficiency than prototype plant viral IRESes. For full activity it requires the entire 739nt 5UTR. TriMV IRES may be a good plant model system to study human pathogenic viruses.

8 "Crystal structure of the Vif-binding and catalytically active domain of the DNA cytosine deaminase APOBEC3F"

Brett Anderson, Markus-Frederik Bohn , Shivender Shandilya, John, S. Albin, Takahide Kono, Rebecca M. McDougle, Michael A. Carpenter, Anurag Rathore, Leah Evans, Ahkillah N. Davis, JingYing Zhang, Yongjian Lu, Mohan Somasundaran, Hiroshi Matsuo, Reuben S. Harris, Celia A. Schiffer (UMN)

Human APOBEC3F is an anti-retroviral single-stranded DNA cytosine deaminase, susceptible to degradation by the HIV-1 protein Vif. In this study the crystal structure of the HIV Vif binding, catalytically active, C-terminal domain of APOBEC3F (A3F- CTD) was determined. The A3F- CTD shares structural motifs with portions of APOBEC3G-CTD, APOBEC3C and APOBEC2. Residues identified to be critical for Vif-dependent degradation of APOBEC3F all fit within a predominantly negatively charged contiguous region on the surface of A3F-CTD. Specific sequence motifs, previously shown to play a role in Vif susceptibility and virion encapsidation, are conserved across APOBEC3s and between APOBEC3s and HIV-1 Vif. In this structure these motifs pack against each other at intermolecular interfaces providing potential insights both into APOBEC3 oligomerization and Vif interactions.

9 "EBV genome hydroxymethylation differentially affects Z- versus R-mediated lytic gene expression"

Coral K. Wille, Dhananjay M. Nawandar, and Shannon C. Kenney (UW)

We recently showed that viral genome methylation differentially affects the ability of the two EBV immediate-early proteins, BZLF1 and BRLF1, to reactivate EBV: BZLF1 preferentially activates methylated viral promoters while the reverse is true for BRLF1. In this study, we have examined the effect of 5-hydroxymethylcytosine (5hmC) modification of the EBV genome on the ability of BZLF1 versus BRLF1 to activate lytic gene promoters. 5hmC is produced by oxidizing methylcytosine, and this modification is mediated by the TET family of proteins. To examine the effect of 5hmC modification on BZLF1 and BRLF1 binding to EBV promoters, we performed electromobility shift assays using unmethylated, methylated, and 5hmC-modified BZLR1 and BRLF1 binding sites. BZLF1 bound much more efficiently to the methylated, versus 5hmC-modified, form of CpG-containing BZLF1-response elements in the BRRF1 and BRLF1 promoters. In contrast, BRLF1 binding to unmethylated, methylated, and 5hmC-modified binding motifs was similar. In reporter gene assays, co-transfection with a TET2 expression vector inhibited BZLF1 activation of methylated lytic EBV promoters, but increased the ability of BRLF1 to activate methylated EBV promoters. Over-expression of TET2 also inhibited the ability of BZLF1 to reactivate lytic gene expression in a latently infected cell line containing a highly methylated EBV genome. These results suggest that 5hmC modification of the EBV genome differentially affects the ability of BZLF1 versus BRLF1 to induce lytic reactivation. Furthermore, our preliminary data suggest that the EBV genome undergoes 5hmC modification in some cell lines. Since 5hmC modification has been recently shown to be increased during epithelial cell differentiation, but is often largely absent in undifferentiated cells and cancer cells, 5hmC modification of the EBV genome may play a role in determining the latency state of the viral genome in certain cellular environments.

10 "Investigating the Molecular Anti-HIV-1 Mechanism of Clofarabine"

Lauren B. Beach, Jessica van Oploo, Michael J. Dapp, Ben D. Duckworth, Steven E. Patterson, and Louis M. Mansky (UMN)

The deoxyadenosine analog 2-Chloro-9-(2-deoxy-2-fluoro-²-D-ara¬binofuranosyl)-9H-purin-6-amine, or clofarabine, is FDA approved to treat refractory pediatric acute lymphoblastic leukemia. The main anti-cancer mechanism of action for clofarabine includes the inhibition of ribonucleotide reductase (RNR) and non-canonical DNA chain termination. In addition, clofarabine may be incorporated into cellular RNA and cause RNA chain termination as well as inhibit mRNA polyadenylation. In this study, we determined that clofarabine has anti-HIV-1 activity. The selectivity index of clofarabine in these experiments was determined to be 1591 in MagiU373 cells, 33 in CEM-GFP cells, and 36 in 293T cells. We also found that clofarabine-treated cells at the EC50 concentration led to a 2.2 fold increase in mutant frequency. Proviral DNA sequencing revealed significant differences in the mutation spectra between clofarabine and no drug controls. In particular, clofarabine-treatment was associated with significantly higher G-to-A transition mutations. Analysis of dNTP pools revealed that clofarabine significantly depleted dATP and dGTP nucleotide pools. To date, our data demonstrates that clofarabine has potent anti-HIV-1 activity, and results in an increase in HIV-1 mutational loads. Ongoing studies are further investigating the antiretroviral mechanism of action of clofarabine.

11 Jessica A. Reusch, Dhananjay M. Nawandar, Shannon C. Kenney, and Janet E. Mertz (UW)
"BLIMP1, master regulator of plasma cell differentiation, induces EBV lytic replication by activating transcription from the Z and R promoters"

Epstein-Barr virus (EBV) persists as a life-long latent infection in host memory B cells, while plasma cell differentiation in humans is associated with the lytic form of EBV infection. Additionally, in normal nasopharyngeal tissue, lytic EBV infection has only been detected in the more differentiated layers. Therefore, cellular transcription factors involved in mediating cellular differentiation may contribute to EBV reactivation. Here, we have investigated the ability of the master regulator of plasma cell differentiation, BLIMP1, to induce EBV lytic reactivation. We show that BLIMP1 expression is sufficient to induce viral reactivation in latently infected nasopharyngeal carcinoma CNE-2 cells, gastric carcinoma AGS-Akata cells, and undifferentiated normal oral keratinocyte (NOK) cells. Interestingly, the ability of BLIMP1 expression to stimulate EBV lytic reactivation appears to be cell-type-dependent in B cells. For example, the Burkitt lymphoma cell lines, Sal I and Kem III, strongly respond to BLIMP1 expression, whereas Mutu I and Kem I cell lines do not. Since viral reactivation is mediated by transcriptional activation of the two EBV immediate-early genes, BZLF1 (Z) and BRLF1 (R), we investigated the effect of BLIMP1 on transcription from the Z and R promoters, Zp and Rp, respectively. Using reporter assays in transiently transfected EBV-negative cell lines, we demonstrate that BLIMP1 independently activates both Zp and Rp. To our knowledge, Zp and Rp are the first promoters shown to be activated by BLIMP1, which normally functions as a transcriptional repressor. Furthermore, we have mapped activation by BLIMP1 of Rp to a 6-bp region located at -665 upstream from the transcription initiation site and activation of Zp to within -83 base pairs upstream of the transcription initiation site. To begin to elucidate the mechanisms behind these activations, we have performed ChIP studies that show BLIMP1 associates with both Rp and Zp in the context of EBV-infected cells despite not directly binding to these promoters in EMSAs. These data indicate that BLIMP1 may associate with these promoters as a component of transcription complexes. Taken together, we conclude that BLIMP1 plays a direct, important role in inducing EBV lytic replication during cellular differentiation in a cell-type-dependent manner.

12 **"The foreign DNA restriction enzyme APOBEC3A can deaminate both normal and 5-methylcytosine"**
Michael A. Carpenter, Ming Li, Anurag Rathore, Lela Lackey, Emily K. Law, Allison M. Land, Brandon Leonard, Shivender M. D. Shandilya, Markus-Frederik Bohn, Celia A. Schiffer, William L. Brown and Reuben S. Harris (UMN)

Multiple studies have indicated that the TET oxidases and, more controversially, the AID/APOBEC deaminases have the capacity to convert genomic DNA 5-methylcytosine (MeC) into altered nucleobases that provoke excision repair and culminate in the replacement of the original MeC with a normal cytosine (C). We show that human APOBEC3A (A3A) efficiently deaminates both MeC to thymine (T) and normal C to uracil (U) in single-stranded DNA substrates. In comparison, the related enzyme APOBEC3G (A3G) has undetectable MeC-to-T activity and 10-fold less C-to-U activity. Upon 100-fold induction of endogenous A3A by interferon, the MeC status of bulk chromosomal DNA is unaltered whereas both MeC and C nucleobases in transfected plasmid DNA substrates are highly susceptible to editing. Knockdown experiments show that endogenous A3A is the source of both of these cellular DNA deaminase activities. This is the first evidence for non-chromosomal DNA MeC-to-T editing in human cells. These biochemical and cellular data combine to suggest a model in which the expanded substrate versatility of A3A may be an evolutionary adaptation that occurred to fortify its innate immune function in foreign DNA clearance

by myeloid lineage cell types. This research has been supported by the NIGMS F32 GM095219 and by NIDCR T32 DE07288. Selected Reference M. A. Carpenter et al. (2012). Methyl- and Normal-Cytosine deamination by the Foreign DNA Restriction Enzyme APOBEC3A. J. Biol. Chem. 287, 34801-34808.

13 "Development of a transgenic mouse model to study Merkel Cell Polyomavirus T antigen expression in the stratified epithelia"

Megan E. Spurgeon and Paul F. Lambert (UW)

The newly discovered Merkel cell polyomavirus (MCPyV or MCV) ubiquitously infects humans during early childhood, although the route of transmission has not been determined. The virus can be detected on surfaces and in skin swabs, and emerging data suggests a potential tropism for the skin. MCPyV was first isolated from a Merkel cell carcinoma (MCC), and multiple studies now confirm clonal integration of the viral genome in over 80% of MCCs. The portion of the viral genome integrated in MCCs preserves expression of both small and large tumor antigens (or T antigens). Interestingly, all tumor-derived large T antigen sequences observed to date contain mutations that yield a truncated protein unable to promote viral DNA replication but that can still bind the tumor suppressor pRb. However, whether MCPyV is the primary etiological agent of this rare, neuroendocrine malignancy or whether MCPyV is associated with other malignancies remains to be determined. To further elucidate the role of MCPyV in neoplastic progression in the context of the stratified squamous epithelium, we are generating a transgenic mouse model of MCPyV-induced carcinogenesis. In this model, expression of the viral T antigens from the early region of the MCPyV genome is directed to the stratified squamous epithelia using the keratin 14 promoter. We have identified a positive founder mouse, and preliminary data suggests that expression of the MCPyV T antigen proteins induces phenotypes within the stratified squamous epithelia associated with neoplastic progression. An introduction to MCPyV, along with an overview of our transgene design and preliminary data, will be discussed.

14 "Characterization of a new picornavirus isolated from bluegills"

Marisa Barbknecht, Sol Sepsenwol, Eric Leis, Maren Tuttle-Lao, Mark Gaikowski, Susan E. Kelly, Nick J. Knowles, Becky Lasee, Michael A. Hoffman (UW-Lax)

The freshwater fish Lepomis macrochirus (bluegill) is common to North American waters and important both ecologically and as a sport fish. In 2001 an unknown virus was isolated from bluegills following a bluegill fish kill. This virus was identified as a picornavirus [termed bluegill picornavirus (BGPV)] and a diagnostic RT-PCR was developed. A survey of bluegills in Wisconsin waters showed the presence of BGPV in 5 of 17 waters sampled, suggesting the virus is widespread in bluegill populations. Experimental infections of bluegills confirmed that BGPV can cause morbidity and mortality in bluegills. Molecular characterization of BGPV revealed several distinct genome characteristics, the most unique of which is the presence of a short poly(C) tract in the 32 UTR. Additionally, the genome encodes a polyprotein lacking a leader peptide and a VP0 maturation cleavage site, and is predicted to encode two distinct 2A proteins. Sequences comparison showed that the virus is most closely related to a phylogenetic cluster of picornaviruses which includes the genera Aquamavirus, Avihepatovirus and Parechovirus. However it is distinct enough, for example sharing only about 38% sequence identity to the parechoviruses in the 3D region, that it may represent a new genus in the Picornaviridae.

15 "Kinetics of virus-host interactions in single-cells using a high-throughput assay"
Andrea Timm and John Yin (UW)

Virus populations are composed of a highly heterogeneous mix of individual particles. This is due to the high error rates in genome replication rates that are highest in RNA viruses such as vesicular stomatitis virus (VSV). These heterogeneous populations, known as quasi-species, allow virus populations to evolve to meet the challenges of adaptive immunity and anti-viral drugs. Similarly, individual cells in a population are also highly variable in many ways including cell-cycle stage, available resources (i.e. amino acids, nucleotides, ribosomes), and ability to respond to pathogens. All of these cellular characteristics can affect the dynamics of the virus-host interaction that ultimately dictates if the infection becomes established. To study these complex systems, we have developed techniques to analyze virus infections on a single-cell level. We are able to study distributions in behavior, rather than the averaged behavior that population level measures provide. In order to study isolated single-cell infections, we have developed a massively-parallel platform, which combines microfluidics, automated live-cell imaging capabilities, and computational and image processing tools and also utilizes fluorescent reporter viruses and cells. The experimental device is constructed with PDMS, and is composed of 10 separated sections, distributed in a 2x5 array, which covers approximately the same area as a standard glass slide. Each section is composed of thousands of microwells that physically isolate single-infected cells. The device is sealed and placed in an environmentally controlled chamber on a fluorescent microscope for time-lapse imaging. Here we use a dual-color reporter system developed previously in our lab to demonstrate applications of our new high-throughput, single-cell infection platform. This reporter system combines a VSV-strain encoding a gene for RFP and a PC3 cell that expresses GFP off of a cloned IFIT2 promoter. Both of these fluorescent proteins appear to accurately report on the state of either the virus infection or the level IFIT2 expression within a given cell. Using this system, we have been able to characterize a few general phenotypes that fit our single-cell populations, and we have been able to find interesting correlations between several kinetic parameters extracted from the reporter protein production curves. We were also able to show that when the virus reporter was detected long before the IFIT2 reporter, IFIT2 reporter production was severely limited. Likewise, when we detected the IFIT2 reporter long before the virus reporter, the cell appeared to be able to mostly shut down virus reporter production. Using this new system, we are able to investigate the competition between viruses and their hosts, under multiple conditions.

16 "PTPN22 Promotes Protective Responses to Influenza A Immunization"
Juliet Crabtree, Vikram Verma, Yaya Wang, Yuying Liang, Erik Peterson (UMN)

A variant allele of PTPN22 is a potent susceptibility factor for autoimmune diseases, including Rheumatoid Arthritis and Type I Diabetes. Ptpn22 is required in myeloid cells for selective promotion of Toll-Like Receptor signaling leading to Type I IFN production. Since Type I IFN are essential for anti-viral immunity and for efficient responses to viral immunization, we investigated the role of PTPN22 in response to vaccination. We tested the ability of Ptpn22 knockout mice to mount a protective immune response to vaccination against Influenza A virus. We found that Ptpn22 is required for efficient generation of both influenza-specific cytotoxic T lymphocyte and neutralizing humoral responses after vaccination with inactivated influenza. Further, upon challenge with live influenza, vaccinated Ptpn22 KO animals exhibit increased mortality, suggesting that defects in antibody and CTL responses associated with Ptpn22 deficiency result in decreased protection engendered by inactivated influenza vaccination. These findings indicate a requirement for Ptpn22 in protective immune responses to influenza vaccine. Moreover, they

suggest that further studies of vaccine responsiveness should be mounted in human carriers of the disease-associated PTPN22 variant, since such carriers exhibit diminished Toll-Like Receptor-induced Type I IFN production by myeloid cells.

17 "Species-Specific Regulation Of HIV-1 Rev Activity"

Mounavya Aligeti, Ryan T Behrens, Nathan M Sherer (UW)

Murine cells exhibit severe blocks to the post-transcriptional stages of the HIV-1 life cycle. We (and others) recently identified a polymorphism in the murine version of the CRM1 nuclear export receptor (mCRM1) that limits the nuclear export of HIV-1 partially spliced and unspliced viral mRNAs. This process is regulated by the viral Rev auxiliary protein, that recruits CRM1 to the cis-acting Rev response element (RRE) encoded by these viral mRNAs. We mapped the species-specific residues in CRM1 that govern this activity to a surface patch of amino acids 19-30 Å from the CRM1 nuclear export signal (NES) binding cleft that engages Revs leucine-rich NES. The cellular function of this patch domain and how it regulates HIV-1 Rev-dependent RNA transport are yet to be defined. Here, we used a comparative visual and viral genetic approach to further define the mechanism underlying mCRM1/Rev incompatibility. Surprisingly, we found that the CRM1-dependent nuclear export of Rev is fully intact in murine cells even in the absence of human CRM1 (hCRM1), so that viral RNA trafficking is uncoupled from Rev trafficking under these conditions. We rescued HIV-1 virus-like particle (VLP) production in murine cells (in the absence of hCRM1) by recoding or duplicating Revs native NES sequence, suggesting that the defect represents a sub-optimal configuration of NES sequences in the Rev/RRE ribonucleoprotein transport complex. Finally, we show that in addition to muroid rodent cells, the provision of hCRM1 markedly improves HIV-1 particle production in avian cell lines but not in cells derived from other non-primate mammalian species such as cat and rabbit. Low levels of CRM1 activity in the context of Rev correlate with previous observations of HIV-1 pre-mRNA oversplicing occurring in both murine and avian cells, and are likely to represent important determinants of retroviral host range.

18 "Analysis of HTLV-1 Particle Morphology and Gag Stoichiometry"

Jose O. Maldonado, Iwen F. Grigsby, Sheng Cao, Joachim Mueller, Wei Zhang, and Louis M. Mansky (UMN)

The Gag polyprotein is the main structural protein of retroviruses and is essential for the assembly and release of virus particles. In the absence of other retroviral proteins, Gag is sufficient for the production and release of viral-like particles (VLPs), which are structurally similar to immature infectious virions. Even with more tractable retroviral systems, the details for how Gag oligomerizes, trafficks to the plasma membrane, and drives the release of immature virus particles is poorly understood, particularly for the deltaretroviruses. We are currently focused on two areas of study: 1) analyze the morphology of the immature lattice formed in particles by the Gag polyprotein, and 2) study the determinants of Gag copy number per virus particles. Using cryogenic transmission electron microscopy (cryo-TEM), we have been analyzing the morphology of HTLV-1 VLPs. We have been investigating the average Gag copy number of HTLV-1 VLPs by scanning transmission electron microscopy (STEM). These fundamental studies of HTLV-1 assembly will lead to detailed information about these processes that will be useful for a better understanding of how these viruses replicate in cells. Such information may inform new therapeutic strategies.

19 "Epstein-Barr Virus (EBV) Late Gene Regulation"
Reza Djavadian (UW)

Like all herpesviruses, Epstein-Barr Virus (EBV) replication occurs through an ordered cascade of gene expression: First, immediate early genes are expressed, which upregulate early genes followed by replication of the viral DNA. Finally EBV late genes are expressed, which primarily encode structural proteins of the EBV virion. These proteins have been shown to elicit a robust immune response by the host. Although late gene expression is tightly controlled by EBV, the mechanisms regulating this process largely remain unknown. EBV late gene promoters frequently contain a modified TATA box in which a T is found in the forth position (TATT). Recently, EBV BcRF1 has been shown to encode a TATA Binding Protein (TBP) homolog, which preferentially recognizes the TATT sequence and is essential for late gene expression. EBV BcRF1 is one of seven genes present only in ²- and ³-herpesviruses and absent in ±-herpesviruses. Interestingly, five of these genes, including the BcRF1 homolog, have the same phenotype when deleted in a murine ³-herpesvirus model: intact DNA replication with markedly impaired late gene expression. We hypothesize that these seven proteins constitute a complex required for late gene expression and present preliminary data defining a second mechanism, in addition to TATT motif binding, by which this complex may be recruited to late gene promoters.

20 "Selection on hemagglutinin imposes a genetic bottleneck during transmission of reassortant H5N1 influenza viruses"
Peter R. Wilker, Jorge M. Dinis, Gabriel Starrett, Masaki Imai, Masato Hatta, Chase W. Nelson, David H. OConnor, Austin L. Hughes, Gabriele Neumann, Yoshihiro Kawaoka and Thomas C. Friedrich (UW-Lax)

The emergence of human-transmissible H5N1 avian influenza viruses poses a major pandemic threat. H5N1 viruses are thought to be highly genetically diverse both among and within hosts, but the effects of this diversity on viral replication and transmission are poorly understood. Here, we evaluate the impact of within-host viral genetic diversity on avian influenza host adaptation and transmission dynamics using samples collected during previously described experiments evaluating H5N1 virus transmission in ferrets. We apply deep sequencing to measure sequence variation in the hemagglutinin (HA), neuraminidase (NA), and matrix (M) gene segments during infection of inoculated ferrets and in matched contact ferrets subsequently infected via respiratory droplet transmission. We show that although within-host genetic diversity in the HA segment increased during replication in inoculated ferrets, HA diversity was dramatically reduced upon respiratory droplet transmission, where infection was established by only 1-2 distinct HA segments from a diverse source virus population in transmitting animals. Moreover, minor HA variants present in as little as 5.9% of viruses within the source animal became dominant in ferrets infected via respiratory droplets. This work establishes that respiratory droplet-mediated transmission of reassortant H5 influenza viruses imposes a significant genetic bottleneck.

21 "Granzyme B is a Genetic Determinant of AIDS Virus Control"
Adam J. Ericsen, Gabriel J. Starrett, Justin M. Greene, Michael Lauck, Brian T. Cain, Ngoc H. Pham, David Rio Deiros, Muthuswamy Raveendran, Roger W. Wiseman, Jeffrey Rogers, Richard Gibbs, Donna Muzny, Thomas C. Friedrich, and David H. O' Connor (UW)

A small percentage of HIV-infected people and SIV-infected macaques control chronic-phase viral replication in the absence of antiretroviral treatment. Control is strongly associated with the presence of certain protective major histocompatibility complex (MHC) class I alleles, implicating

CD8+ T cells and natural killer cells as key mediators of viral containment. Not all individuals possessing protective MHC class I alleles, however, successfully suppress viral replication. Here we sequenced the whole genomes of 12 SIV-infected Mauritian cynomolgus macaques (6 controllers and 6 progressors) that share the protective M1 MHC haplotype, and defined alleles for immune-related genes implicated in cytotoxic T cell and natural killer cell function and looked for associations with chronic-phase viral loads. We found that inheritance of a single granzyme B allele was strongly associated with chronic-phase control of SIV (P=0.0001), and that animals possessing this control-associated granzyme B allele exhibited higher expression of granzyme B in CD3-CD8+ natural killer cells. Identification of the specific mechanism responsible for SIV control among individuals with beneficial combinations of MHC and granzyme B alleles will clarify the types of cellular immune responses that should be elicited by prophylactic and therapeutic vaccination.

22 "Determining the role of Hepatitis B virus gene X mutant variants in hepatocellular carcinoma"

Barbara R Tschida, Vincent W Keng, Timothy P Kuka, and David A Largaespada (UMN)

Hepatocellular carcinoma (HCC), or liver cancer, is the 3rd leading cause of cancer-related death worldwide. It has extremely limited treatment options and a dismal prognosis. Greater understanding of pathogenesis is needed to improve prevention and treatment, but the mechanisms driving HCC are not yet understood. Hepatitis B virus (HBV), which infects 350 million people worldwide, is a major risk factor for HCC, accounting for up to 80% of primary liver cancers cases. We have shown the HBV viral regulatory protein HBx contributes to HBV-associated liver oncogenesis in a mouse model. HBx activates transcription from both viral and cellular promoters to facilitate viral replication. Mutant variants of HBx including a 2 nucleotide point mutant and a C-terminal truncated mutant are associated with increased HCC incidence, and are likely more oncogenic than wildtype HBx. We have synthesized expression plasmids for the wildtype, point mutated, and truncated HBx variants. To test the oncogenic roles of HBx mutant variants, we will express them in hepatocytes in vitro and examine cellular gene expression changes, transformation, alterations in HBx protein stability, and interaction between HBx and PRMT1 compared to wildtype. We are also testing their oncogenic effects directly vivo using the Sleeping Beauty (SB) transposon system to stably integrate expression vectors in hepatocytes in a selective mouse model. This model uses mice deficient for the fumarylacetoacetate hydrolase (Fah) enzyme which die of liver failure unless maintained on the protective drug NTBC. Transposon vectors co-expressing HBx variants and Fah are delivered to the livers of Fah-/- mice by hydrodynamic tail vein injection and integrated by SB transposase. As Fah-/- hepatocytes die, the liver is repopulated by cells stably expressing the delivered transgenes, producing a transgenic liver in 6-8 weeks. The inflammation, hepatocyte death, and liver regeneration caused by the hydrodynamic injection and drug withdrawal mimics the liver inflammation and cycles of hepatocyte death and regeneration associated with chronic viral infection, making this system an excellent tool to model the effects of viral gene expression in vivo. We have injected cohorts of mice with point mutated, truncated, or wildtype HBx along with shTP53 to predispose to tumor formation. These mice are currently aging. We will compare tumor penetrance and latency caused the mutant variants to wildtype, and then examine expression of genes found to be differentially regulated by the mutant HBx variants in vitro. This will allow us to examine the oncogenic effect of HBx variants and their mechanisms of tumor induction.

23 "Single-cell spatiotemporal kinetics of innate immune activation and viral protein expression in monolayer cultures

Jay W Warrick, Adam D Swick, Ashley Baltes, John Yin (UW)

Epidemiological studies are typically focused at the organismal level, gathering many organism-environment-phenotype data points to identify potentially important factors that influence a disease or process. A similar approach is now being used to study infections in vitro where the natural spatial inhomogeneity and intrinsic cell-to-cell variability within the culture is quantified using fluorescent microscopy and image analysis to gather analogous cell-microenvironment-phenotype data. Currently, this type of data is acquired at a specific time-point to identify previously unknown correlations. Here, we gather this type of data over time for prostate cancer cells (PC-3) infected with vesicular stomatitis virus to specifically examine the dynamics of viral protein expression and innate immune response (IFIT2 and IFN-activation). In this way, we can examine how the dynamics of infection change depending on the context of the host response. The spatiotemporal resolution of this technique is extremely valuable given that diffusion and direct cell-cell transfer of virus and cytokines mediate these processes. The technique also allows us to mine the data for different contextual motifs or common patterns that naturally arise. Such information will allow us to begin answering questions like, When and how intensely does innate immunity need to be activated to prevent propagation of an infection.

24 "Conversion of Rhesus Macaque APOBEC3F from HIV-1 Vif-Resistant to Vif-Sensitive"

Allison M. Land, Reuben S. Harris (UMN)

Four related human DNA cytosine deaminases restrict HIV-1 replication in T cells: APOBEC3D, 3F, 3G and 3H. To combat these innate immune molecules, HIV-1 encodes the protein Vif, which binds these restrictive APOBEC3 proteins and targets them for proteasomal degradation, allowing HIV-1 replication to occur. The crystal structure of the C-terminal domain of APOBEC3F has recently been solved, but the structure of Vif remains a mystery. To delineate the APOBEC3F/Vif interaction surface, we used a genetic approach, comparing the amino acid sequences of human APOBEC3F with rhesus macaque APOBEC3F. Although the proteins are phylogenetically related and share 88% identity, rhesus APOBEC3F is not targeted for degradation by HIV-1 Vif. Our previous studies demonstrated that a single amino acid change at position 324 in human APOBEC3F from a glutamate to a lysine (the corresponding residue in the rhesus protein) was sufficient to render human APOBEC3F resistant to HIV-1 Vif, but the reciprocal substitution failed to make rhesus APOBEC3F fully susceptible to HIV-1 Vif (1). We hypothesize that multiple reciprocal changes will be necessary to render rhesus APOBEC3F fully susceptible to HIV-1 Vif. We generated a series of human/rhesus APOBEC3F chimeric proteins along with single amino acid residue changes and tested them for their sensitivity to HIV-1 Vif and restriction of HIV-1 in a single-cycle infectivity assay. We are therefore progressing towards identifying the regions and residues that define the APOBEC3F/Vif interaction surface and mapping them to the current APOBEC3F structural model. This interaction between Vif and APOBEC3 proteins is of considerable interest as a potential therapeutic target; preventing Vif-mediated degradation of the APOBEC3 deaminases could allow our natural innate immune proteins to successfully repress HIV-1 replication. 1. Albin, J.S. et al. J Biol Chem 2010. 285: 40785-40792.

25 "Purification, Multimerization, RNA Binding, and ATPase Activity of Bromovirus RNA Helicase Domain"
Robert A. Pugh and Paul Ahlquist (UW)

RNA replication by brome mosaic virus (BMV) occurs in ~70 nm spherular endoplasmic reticulum (ER) membrane invaginations induced by the multifunctional viral replication protein, 1a. 1a contains an N-proximal methyltransferase domain involved in capping viral RNA, and a putative Superfamily (SF) 1 helicase at its C-terminus. Both 1a domains are conserved throughout the entire alphavirus-like superfamily of positive-strand RNA viruses, and have parallels in other virus families. Extensive mutational analysis shows that the BMV 1a helicase domain has independent, essential functions in forming the membrane-bounded RNA replication compartments, recruiting viral RNA templates and the viral RNA-dependent RNA polymerase, and continuing synthesis of all forms of viral RNA, including (+) and (-) strand genomic RNAs and subgenomic mRNA. However, many critical aspects of the precise interactions and mechanisms by which the 1a helicase domain carries out these processes have yet to be determined. Here we have purified a fragment containing the helicase domain of the replication protein, found that it preferentially interacts with itself to form oligomeric structures, binds RNA substrates and is stimulated to hydrolyze ATP by ssRNA.

26 "Stable APOBEC3H haplotypes confer resistance to HIV infection"
Eric W. Refsland, Elizabeth M. Luengas, Judd F. Hultquist, Leah C. Evans, Rachel Prosser, Keith Henry, and Reuben S. Harris (UMN)

HIV pathogenesis requires the virus to evade or neutralize components of its hosts adaptive and innate immune systems. For example, the HIV accessory protein Vif targets the innate APOBEC3 family of DNA cytosine deaminases for degradation. Without Vif, the virus is unable to overcome the lethal mutations inflicted by four APOBEC3s: APOBEC3D, F, G, and H. Interestingly, A3H is the most variable family member with at least 7 distinct haplotypes currently present in the human population. Over-expression studies have shown that a subset of these variants express stably (haplotypes II, V and VII) while others do not (haplotypes I, III, IV, VI). Stable haplotypes II and V are common in individuals of African descent, whereas unstable haplotype I is commonly found in individuals of European descent. A remaining question is whether or not the previously reported APOBEC3H stabilities extend to individuals and whether people encoding stable APOBEC3H haplotypes are more resistant to an HIV infection. We hypothesize that stable APOBEC3H protein expression provides a natural barrier to HIV infection. Preliminary studies of volunteers recruited at the HIV Clinic at Hennepin County Medical Center have identified 8 persons of African descent with the stable variants and 8 more individuals (4 Caucasian and 4 African descent) with unstable variants of APOBEC3H. To test our hypothesis, we have performed a series of ex vivo studies with primary CD4+ T lymphocytes isolated from this subset of individuals to assess whether or not the APOBEC3H haplotype and stability confer differential susceptibility to an HIV infection.

27 "Gene targeting and knockdown in the T cell Line CEM2n demonstrate a role for APOBEC3D, APOBEC3F, and APOBEC3G in HIV-1 restriction and hypermutation"
Eric W. Refsland, Judd F. Hultquist, and Reuben S. Harris (UMN)

The AIDS virus HIV-1 interacts with many host factors during replication and pathogenesis. These cellular proteins are generally classified as either virus dependence factors or restriction factors. The latter class is unique because it dominantly blocks virus replication and forces the virus to maintain a counterdefense strategy. The DNA cytosine deaminase APOBEC3G is a prototypical

restriction factor, which converts retroviral cDNA cytosines to uracils during reverse transcription. The resulting genomic plus-strand guanine-to-adenine (G-to-A) mutations can render viruses non-infectious and contribute to drug resistance. Here we report the isolation of a near-diploid T cell line CEM2n, which is amendable to gene targeting as demonstrated by systematic APOBEC3 gene deletion studies. Parental CEM2n expresses six APOBEC3 genes, is nonpermissive for Vif-deficient HIV-1 replication, and causes G-to-A hypermutations in GG and GA dinucleotide motifs. APOBEC3G-null derivatives become semi-permissive for Vif-deficient HIV-1 replication and lose the capacity to inflict GG-to-AG hypermutations. APOBEC3F-null clones also become semi-permissive, but the hypermutation patterns remain unaltered. Systematic shRNA-mediated depletion of other endogenous APOBEC3s in an APOBEC3F-null background demonstrates an unanticipated overlapping involvement of APOBEC3D and APOBEC3F in HIV restriction and GA-to-AA hypermutation. These data demonstrate that endogenous levels of at least three human APOBEC3 proteins combine to restrict Vif-deficient HIV-1 and cause the associated dinucleotide hypermutation biases, which are also evident in patient-derived viral sequences. CEM2n may be useful for studying a multitude of host factors relevant to HIV-1, other T lymphotrophic viruses such as HTLV-1, and T cell leukemias and lymphomas.

28 "Forkhead Transcription Factor FOXO1 Regulates the Function and Survival of Memory CD8 T Cells"

Melba Marie Tejera, M. Suresh (UW)

CD8 T cells are important in defense against viral, and intracellular infections. Therefore, vaccines against these agents need to engender potent CD8 T cell memory. However, the molecular mechanisms that govern the establishment and maintenance of CD8 T cell memory are not well understood. There is accumulating evidence that the transcription factor FOXO1 plays crucial roles in regulating T cell homeostasis, but its role in CD8 T cell memory remains unknown. To investigate the role of FOXO1 in establishment and maintenance of CD8 T cell memory we infected mice that are conditionally deficient for FOXO1 in T cells (KO) and littermate wild type (WT) mice with lymphocytic choriomeningitis virus (LCMV). We found that FOXO1 in is not required for CD8 T cells to clonally expand and differentiate into effector cells. Additionally, the numbers of LCMV-specific memory CD8 T cells were stably maintained in KO mice at levels comparable to those in WT mice. Strikingly however, LCMV-specific memory CD8 T cells in KO mice display phenotypic attributes associated with senescence and exhibit progressive erosion in their functional ability. Consequently, FOXO1-deficient memory CD8 T cells showed poor recall responses and impaired protective immunity to re-infection. Next, we compared the responses of FOXO1-deficient CD8 T cells and WT CD8 T cells in a competitive environment of bone marrow chimeric mice. In bone marrow chimeras, WT CD8 T cells differentiated into effector and memory CD8 T cells. In striking contrast, FOXO1-deficient CD8 T cells differentiated into effectors, but failed to survive and form memory CD8 T cells in the same chimeric mice. Thus, using competitive and non-competitive conditions, we have dissected the T cell-intrinsic pivotal roles of FOXO1 in supporting survival versus maintenance of functional and protective CD8 T cell memory. These findings have implications in the development of strategies to modulate FOXO1 activity and augment vaccine-induced CD8 T cell immunity.

29 "Characterization of an Early-Stage Membrane Fusion Intermediate of Sindbis Virus using Cryo-electron Microscopy"
Sheng Cao and Wei Zhang (UMN)

The sequential steps in the alphavirus-membrane fusion pathway have been postulated based on the pre- and post-fusion crystal structures of the viral fusion protein E1 in conjunction with biochemical studies. However, the molecular structures of the hypothesized fusion intermediates have remained obscure due to difficulties inherent in the dynamic nature of the process. We developed an experimental system that uses liposomes as the target membrane to capture Sindbis virus, a prototypical alphavirus, in its membrane-binding form at pH 6.4. Cryo-electron micrograph analyses and three-dimensional reconstructions showed that the virus retains its overall icosahedral structure at this mildly acidic pH, except in the membrane-binding region, where monomeric E1 associates with the target membrane, and the E2 glycoprotein retains its original trimeric organization. The structure of the liposome-bound Sindbis virus suggests that low pH triggers dissociation of the E1-E2 heterodimer, which precedes dissociation of the E2 trimer. Moreover, the remaining E2 trimers may hinder E1 homotrimerization and could be a potential target for antiviral drugs.

30 "Epidermal growth factor inhibition of HPV positive head and neck cancer cells and primary tumorgrafts results in significant growth inhibition mediated by apoptosis"
Alexandra Torres, GC Blitzer, MA Smith, EA Armstrong, PM Harari, PF Lambert, RJ Kimple (UW)

Epidermal growth factor inhibition of HPV positive head and neck cancer cells and primary tumorgrafts results in significant growth inhibition mediated by apoptosis. Authors: AD Torres, GC Blitzer, MA Smith, EA Armstrong, PM Harari, PF Lambert, RJ Kimple Purpose: Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor (EGFR), is approved for use in the treatment of head and neck cancer (HNC). One recent study (SPECTRUM) suggested that patient with recurrent or metastatic HPV+ HNC do not benefit from treatment with EGFR targeted therapy. However, most studies of EGFR inhibition have been performed with HPV-negative cells and clinical trials have been performed without regard to HPV status. We performed this study to determine whether HPV+ HNC cells and patient-derived tumorgrafts respond to cetuximab and to examine the mechanisms through which cetuximab affects HPV+ HNC. Methods: Four HPV+ cell lines (UD-SCC2, UM-SCC47, UPCI-SCC90, 93-VU-147T) were assessed for EGFR expression by western blot. Sensitivity to cetuximab was tested by assessing cell density 2, 4, 6, and 8 days after cetuximab treatment and by assessing colony formation 2 weeks after plating. Apoptosis was measured by caspase activation, flow cytometry for Annexin V and propidium iodide staining, and immunoblot. Cell cycle was assessed by immunoblot for cyclin D1, cyclin B1, and p27Kip-1 and confirmed by flow cytometry for propidium iodide stained cells. Subcutaneous flank xenografts and tumorgrafts were performed in Hsd:athymic Nude-Foxn1nu female mice treated with intraperitoneal cetuximab (0.2mg/mouse) delivered twice weekly for 2 weeks. Time to tumor quadrupling from baseline was assessed by Kaplan-Meier method. Results: Significant variation in EGFR expression was seen in HPV+ cells. Cetuximab treatment resulted in significant delay in cell proliferation ($p<0.005$ for all lines) and decrease in colony formation ($p<0.04$ for all lines). In these cell lines, cetuximab caused an increase in apoptosis as measured by caspase activity, Bax activation, and Annexin V labeling. In addition, as previously seen in HPV- cell lines, cetuximab increased p27Kip-1 and decreased cyclin B1 and cyclin D1 coinciding with in a G1 cell cycle arrest. Using both a cell line xenograft model and a direct-from-patient tumorgraft model of HPV+ HNCs, cetuximab resulted in significant tumor growth delay (median time to tumor quadrupling: 15 vs. 24 days, $p=0.02$; and 42 vs. 89 days, $p=0.0001$, respectively). Conclusions: Epidermal growth

factor receptor inhibition by cetuximab appears to be effective in slowing proliferation and inducing apoptosis in HPV+ HNC. The proposed mechanism of action appears to be similar to that shown over a decade ago in HPV- HNC. These results suggest that cetuximab may play a role in the management of patients with HPV+ HNC.

31 "Mutations in the amino terminus of herpes simplex virus type 1 gL can reduce cell-cell fusion without affecting gH/gL trafficking"

Wenbo Zhou, Feng Chen, Yuri Klyachkin, Yuk Y. Sham, Robert J. Geraghty (UMN)

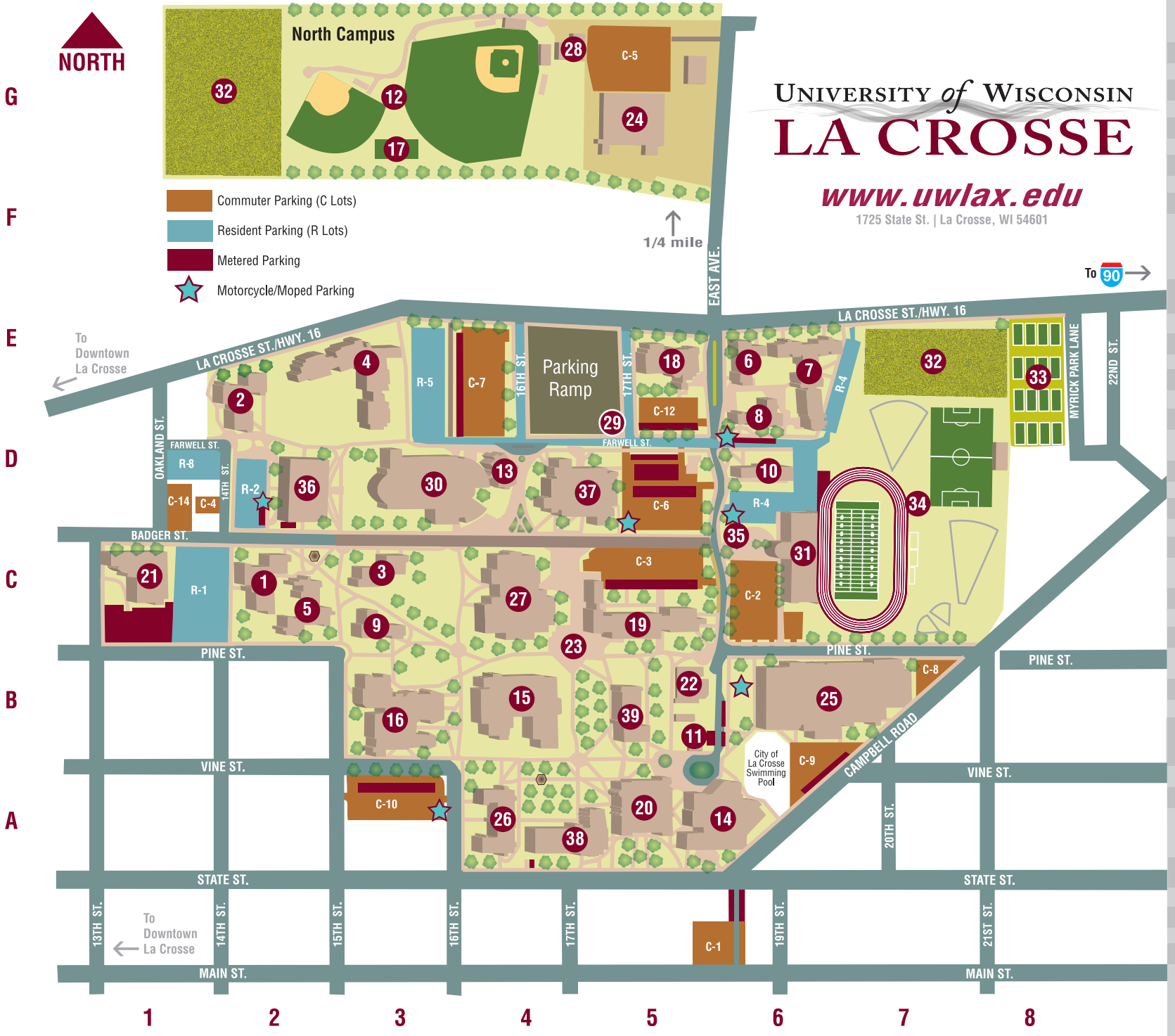
Herpes simplex virus (HSV) requires four envelope glycoproteins to conduct membrane fusion during virus entry and spread. Two of those required glycoproteins, H (gH) and L (gL), form a functional heterodimer. Clear roles for gL in the fusion process are to promote gH trafficking out of the endoplasmic reticulum. It is unclear, however, if gL plays any other roles in HSV-glycoprotein-induced membrane fusion. In this study, we generated amino-terminal deletions and point mutations covering gL residues 24-43 to dissect the role of that region in the gL-dependent actions required for membrane fusion. The most interesting mutants promoted gH cell-surface expression levels equivalent to that seen with wild-type gL but were defective in their ability to promote membrane fusion. For example, a gL mutant missing residues 27-31 displayed a 60% reduction in cell-cell fusion when expressed with the other HSV-1 fusion glycoproteins and no defect in promoting gH cell-surface expression. In addition, two single amino acid substitution mutants, tyrosine to glutamic acid at residue 26 and valine to aspartic acid at residue 27, showed a >40% reduction in cell fusion but no defect in gH cell-surface expression. The amino terminus of gL plays an important but as yet undetermined role in HSV-1 membrane fusion.

32 "Genome-wide analysis of EBNA3 binding in EBV transformed cells"

Anqi Wang, Bo Zhao, Tram Ta, Rene Welch, Elliott Kieff, Sunduz Keles and Eric Johannsen (UW)

Epstein Barr virus (EBV) is a DNA tumor virus that infects over 90% of the population by adulthood and it is causally associated with several human malignancies. Epstein-Barr virus (EBV) is able to drive the transformation of B-cells, resulting in the generation of lymphoblastoid cell lines (LCLs) in vitro. EBV nuclear proteins EBNA2, EBNA3A and EBNA3C are necessary for transformation and maintain LCL growth, whereas EBNA3B is dispensable. All four of the EBNA proteins regulate cell gene expression in LCLs by binding to the RBPJ transcription factor. EBNA2 is a strong transactivator, whereas the EBNA3 proteins up-regulate some genes and down-regulate others. In the study, we mapped the genome-wide binding of each EBNA3 protein, EBNA2 and RBPJ in LCLs using ChIP-seq. We demonstrated that despite the large number of site bound by each EBNA3 protein throughout human genome and a substantial overlap among them, each EBNA3 also targeted to a unique subset of genes. In addition, there are significant number of EBNA3s bound sites that are not bound by RBPJ. We also demonstrated that EBNA3 binding correlated weakly with gene regulation, but was stronger for down-regulated genes than for up-regulated genes. Most importantly, we discovered that EBNA3C regulated genes show reduced H3K27 trimethylation to a greater extent than EBNA3 bound genes in LCLs. Because changes in H3K27me3 at the p16INK4a tumor suppressor locus have been previously shown to mediate EBNA3A and EBNA3C effects on LCL growth, our result suggest that these changes may be indirect. These studies should not only provide a basis for understanding the differential binding of each EBNA3 protein, but also lend insight into whether EBNA3 proteins regulate genes through direct and indirect mechanisms or delimitate the epigenetic changes associated with EBNA3 bound sites in LCLs.

NOTES



RESIDENCE HALLS

- **1 Angell Hall — C2
- *2 Coate Hall — D2
- **3 Drake Hall — C3
- *4 Eagle Hall — E3
- 5 Hutchison Hall — C2
- 6 Laux Hall — E6
- *7 Reuter Hall — E6
- *8 Sanford Hall — E6
- *9 Wentz Hall — C3
- *10 White Hall — D6

OTHER FACILITIES

- *11 Archaeology Center and Laboratories — B5
- 12 Baseball/Softball Fields — G3
- **13 Campus Child Center — D4
- *14 Cartwright Center — A5
- *15 Centennial Hall — B4
- *16 Center for the Arts — B3
- 17 Challenge and Ropes Course — F3
- *18 Cleary Alumni & Friends Center (Admissions Office) — E5
- *19 Cowley Hall of Science — C5
- *20 Graff Main Hall — A5
- *21 Health Science Center — C1
- 22 Heating Plant — B5
- 23 Hoeschler Tower — B4
- *24 Maintenance Building/ Campus Stores — G5
- *25 Mitchell Hall — B6
- *26 Morris Hall — A4
- *27 Murphy Library — C4
- *28 North Campus Field and Equipment Building — G4
- * 29 Police Services — D5
- *30 Recreational Eagle Center — D3
- *31 Roger Harring Stadium at Veterans Memorial Field Sports Complex — C6
- 32 Student Recreation Fields — G1, E7
- 33 Tennis Courts — D8
- 34 Veterans Memorial Field Sports Complex (football, practice, soccer, track and field) — D7
- 35 Veterans Memorial Monument — C6
- *36 Whitney Center — D2
- *37 Wimberly Hall — D4
- *38 Wing Technology Center — A4
- *39 Wittich Hall — B5

*Handicapped accessible
**Limited accessibilty