'Wisc-e-sota'

2nd Joint UMN-UW Virology Training Grant Symposium

Friday, October 10th, 2014

UW-La Crosse

Cartwright Center

A 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

2nd Annual *'Wisc-e-sota'* Virology Symposium
Friday, October 10th, 2014
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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	Ann Palmenberg (UW) **"EMCV leader protein complexes with RanGTPase, Crm1 transportin and Erk1/2 kinases to direct nuclear pore phosphorylation and NP trafficking inhibition"**
2	10:00	Barbara R Tschida, Timothy P Kuka, Pauline J Jackson, Vincent W Keng, and David A Largaespada (UMN) **"Differing oncogenic effects of hepatitis B virus gene X mutant variants in liver cancer"**
3	10:15	Coral K. Wille, Shidong Ma, Dhannanjay M. Nawandar, and Shannon C. Kenney (UW) **"5-hydroxymethylation of the EBV genome inhibits BZLF1 (Z), but enhances BRLF1 (R), mediated viral reactivation"**
	10:30-10:45	Morning coffee break and poster set up
		Convenor: Rob Pugh
4	10:45	Sally Robinson, Diem Ngo, Anne Hoybook, Michael Murtaugh (UMN) **"Mechanisms of age-dependent resistance to PRRSV infection"**
5	11:00	Ryan T. Behrens, Mounavya Aligeti, Ginger M. Pocock, Johannes Schindelin, Christian Dietz, Kevin W. Eliceiri, Chad M. Swanson, Michael H. Malim, Paul Ahlquist, Nathan M. Sherer (UW) **"Cooperativity among Rev-associated nuclear export signals regulates HIV-1 gene expression and is a determinant of virus species tropism"**
6	11:15	Megan Bracken, Michael Hoffman (UW-Lax) **"Viral protein requirements for efficient human parainfluenza virus 3 virus-like particle formation"**

7	11:30	Scott J. Schachtele, Manohar B. Mutnal, Shuxian Hu, Wen S. Sheng, and James R. Lokensgard (UMN) **"**Sustained reactive gliosis following regulatory T cell depletion during acute viral encephalitis"
	Noon-2:45	**Luncheon & Poster Session – *Featuring 30+ posters***
		Convenor: Andrew Bennett
8	2:45	Bailey, AL Lauck, M Weiler, A Sibley, SD Dinis, JM Bergman, Z Nelson, CW Correll, M Gleicher, M Hyeroba, D Tumukunde, A Weny, G Chapman, C Kuhn, JH Hughes, AL Friedrich, TC Goldberg, TL O'Connor, David H (UW) **"Understanding GBV-C-mediated protection from AIDS"**
9	3:00	John P. Eichorst, Joachim D. Mueller and Louis M. Mansky (UMN) **"Viewing the biogenesis of HTLV-1 particles in living cells"**
10	3:15	Megan E. Spurgeon, Jingwei Cheng, James A. DeCaprio, and Paul F. Lambert (UW) **"Skin tumorigenesis in transgenic mice expressing the Merkel cell polyomavirus T antigens"**
	3:30-3:45	Afternoon coffee break and poster take down
		Convenor: Lizzie Steinert
11	3:45	Brett D. Anderson and Reuben S. Harris (UMN) **"HIV-1 Vif Couples Two Independent Mechanisms for Promoting Viral Infectivity by Hijacking the Cellular Transcription Factor CBFβ"**
12	4:00	Adityarup Chakravorty, Bill Sugden (UW) **"Netropsin Inhibits the Growth of EBV-positive Burkitt's Lymphoma Cells"**
13	4:15	D. Park, J. Lalli, L. Sedlackova, and Stephen Rice (UMN) **"Letting go": how does herpes simplex virus get out of cells (and why is ICP27 involved)?"**
	4:45	Closing Remarks
	5:00	**Symposium Reception – Holiday Inn Hotel La Crosse**

Poster Presentations

Poster abstract numbers in the left column

1. **"Analysis of authentic HTLV-1 particles using cryo-electron tomography"**
   Sheng Cao, José O. Maldonado, Iwen F. Grigsby, Louis M. Mansky and Wei Zhang

2. **"Pathogen fee-for-service sequencing at the WNPRC"**
   Dane Gellerup & Shelby O'Connor

3. **"Env Substitutions Can Protect Vif-null HIV-1 from APOBEC3G Deamination"**
   Terumasa Ikeda, John S Albin, Dan Mundt, Menelaos Symeonides, Markus Thali and Reuben S Harris

4. **"Role of Membrane-Shaping Host Reticulons in HIV-1 Replication"**
   Justin Massey, James Bruce and Paul Ahlquist

5. **"Elucidating the Interaction between HIV-1 Vif with human APOBEC3F"**
   John S. Albin , Elizabeth M. Luengas, Allison M. Land, Christopher Richards, Brett D. Anderson, Nadine M. Shaban,Ozlem Demir, John R. Holten, John S. Anderson, Daniel A. Harki , Rommie E. Amaro and Reuben S. Harris

6. **"Multimerization, Functions, and Mechanisms of the Purified Bromovirus RNA Helicase Domain"**
   Robert A. Pugh and Paul Ahlquist,

7. **"Porcine Epidemic Diarrhea Virus: Immunity Following Feedback"**
   CMT Dvorak, S Stone, A Rovira, I Levis, S Brown, M Turner, S Hough, T Snider, and MP Murtaugh

8. **"Hypoxia-induced lytic reactivation of EBV requires both HIF-1± and activated p53."**
   Richard J. Kraus, Xianming Yu, Saranya Sathiamoorthi, Shannon C. Kenney, and Janet E. Mertz

9. **"Deep Sequencing Analysis of PRRSV Genetic Variation Among Cell Types"**
   Xiong Wang, Michael P. Murtaugh

10. **"Evidence for HIV-1 Genomic RNA Elements Regulating the Efficiency of HIV-1 Virus Particle Assembly"**
    Jordan T. Becker & Nathan M. Sherer

11. **"Striking Contribution of G-to-A Hypermutants to Viral Mutagenesis in HIV Types 1 & 2"**
    Jonathan Rawson, Sean Landman, Cavan Reilly, and Louis Mansky

12. **"Dissecting HIV-1 s post-transcriptional stages using an imaging based approach"**
    Ginger M Pocock, Jordan T. Becker, Sirisha R Dommaraju, Paul Ahlquist, Nathan M Sherer

13. **"Comparative analysis of retrovirus virus-like particles using cryo-TEM"**
    Jessica Martin, Sheng Cao, Jose Maldonado, Wei Zhang, Louis Mansky

14. **"Production of West Nile Virus replicon particles in insect cells using packaged replicons to deliver recombinant RNA"**
    Brendan T. Boylan, Lindsey A. Moser, Kristen A. Bernard

15. **"A re-evaluation of the distribution and recirculation of memory CD8 T cells"**
    Elizabeth Steinert, Jason M. Schenkel, David Masopust

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

17. **"Mechanisms of age-dependent resistance to PRRSV infection"**
Sally Robinson, Diem Ngo, Anne Hoybook, Michael Murtaugh

18. **"Infection and pathogenesis of the recently discovered Murine Papillomavirus (MmuPV-1) in vivo"**
Aayushi Uberoi, Satoshi Yoshida and Paul F. Lambert

19. **"pH dependent kinetics and ssDNA binding of HIV-1 restriction factor APOBEC3G-ctd using time resolved NMR"**
William C. Solomon, Stefan Harjes, Ming Li, Reuben S. Harris and Hiroshi Matsuo

20. **"Examining the Role of Sirt1 in HPV-positive Head and Neck Squamous Cell Carcinoma"**
Patrick Nyman, Paul Lambert, Henry Pitot

21. **"HPV E6 triggers up-regulation of the antiviral DNA cytosine deaminase APOBEC3B"**
Valdimara C. Vieira, Brandon Leonard, Nuri A. Temiz, Elizabeth A. White, Peter M. Howley, Laurel Lorenz, Denis Lee, Paul Lambert, Marcelo A Soares and Reuben S. Harris

22. **"HIV-1 Env s cytoplasmic tail domain regulates multiple aspects of viral cell-to-cell transmission at the virological synapse"**
Jaye C. Gardiner, Eric J. Mauer, and Nathan M. Sherer

23. **"Cytokine-Mediated Proliferation and Differentiation of Porcine B Cells into Ig Secreting Plasma Cells"**
Michael C Rahe and Michael P Murtaugh

24. **"CD8 T Cell Based HIV Vaccines Is Targeting Conserved Epitopes the Answer?"**
Shelby O'Connor, Dane Gellerup, and Alexis Balgeman

25. **"Endocytosis of the Viral Envelope Glycoprotein Protects Human and Simian Immunodeficiency Virus Infected Cells from Antibody-Dependent Cell-Mediated Cytotoxicity"**
Benjamin von Bredow, Juan F Arias, Lisa N Heyer, David T Evans

26. **"When (neutralizing) antibodies fail: inducing cross-reactive immunity to influenza"**
Thomas Friedrich

Abstracts – Oral Presentations

1. "EMCV Leader protein complexes with RanGTPase, Crm1 transportin and Erk1/2 kinases to direct nuclear pore phosphorylation and NP trafficking inhibition"
Jessica J. Ciomperlik, Valjean Bacot-Davis and Ann C. Palmenberg

Encephalomyocarditis virus (EMCV) Leader protein (L) directs host kinases to hyper-phosphorylate various Phe-Gly- containing nucleoporin proteins (Nups), inhibiting active host nucleocytoplasmic trafficking. Necessary to this functionality, L binds RanGTPase localizing the complex to the periphery of the nuclear pores (NP). Here, we demonstrate that L alone, or in complex with Ran, additionally binds karyopherin Exportin1 (Crm1), a broad range exportin responsible for the nuclear export of several kinases, including those in the MAPK pathways. The L:Crm1 interaction is very high affinity, able to withstand 500 mM NaCl. The dual phosphorylation of L by CK2 and Syk kinases1 is not required for L:Ran binding, but L:Crm1 (or L:Ran:Crm1) interactions are markedly enhanced by prior phosphorylation of L. These same L phosphorylation events are required for subsequent L-directed Nup phosphorylation1, presumably by increasing Crm1 affinity for the complex. A 1:1:1 stoichimetric complex of L:Ran:Crm1 can be isolated from cells or cell extracts, suggesting that L lies structurally at the Crm1:Ran interface, binding both simultaneously. Previous work implicated MAPK activation as part of the L-directed Nup phosphorylation cascade2. Using siRNAs, we now demonstrate that MAPK kinases ERK1/2 are directly responsible for phosphorylating the Nups. Although activated ERK1/2 translocates into nuclei especially when Crm1 is in deficit, these kinases do not usually phosphorylate Nups. Yet recombinant GST-L pulls ERK1/2 and additional MAPKs from HeLa whole cell lysates. This dictates a model where phosphorylated L:Ran:Crm1 complexes localize within the nuclear pores, interact with activated ERK1/2, trapping it for Nup hyper-phosphorylation. The consequence is cessation of active nucleocytoplasmic trafficking, rendering the cell defenseless to viral infection.

2. "Differing oncogenic effects of Hepatitis B virus gene X mutant variants in liver cancer"
Barbara R Tschida, Timothy P Kuka, Pauline J Jackson, Vincent W Keng, and David A Largaespada

Hepatocellular carcinoma (HCC), or liver cancer, is the 2nd leading cancer-related cause of death worldwide. It has extremely limited treatment options and a dismal prognosis, with an average 5 year survival rate of approximately 15%. It is sex biased, affecting more men than women. The mechanisms driving HCC are not completely understood, and insight into HCC development and maintenance is needed to improve prevention and treatment. 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 oncogenesis in a model of somatic HBx overexpression in the mouse liver. 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 may be more oncogenic than wildtype HBx. We tested their oncogenic effects directly in vivo using the Sleeping Beauty (SB) transposon system to stably integrate expression vectors in hepatocytes in a selective mouse model. We injected cohorts of mice with SB transposon expression plasmids for point mutated (HBxM), truncated (HBxTr), or wildtype (HBxWT) HBx. All cohorts were co-injected with shTP53 to predispose to tumor formation. Male mice injected with HBxWT developed tumors at 100% penetrance at approximately 170 days post-injection. Male mice injected with HBxM had a higher average tumor burden than male mice injected with HBxWT (7.6 and 3.1 tumors per mouse, respectively; $p = 0.03$). No increase in tumor burden or penetrance was seen with HBxTr in either sex, or with either HBx variant in females. There was, however, a non-significant trend toward increased tumor penetrance in female mice injected with HBxM compared to HBxWT (57% and 17%, respectively;; $p = 0.13$), and a significant increase in liver size (6.2 and 5.6 percent of body weight,respectively, $p = 0.02$). In sum, we have developed new mouse models of liver cancer driven by two variants of HBxassociated with increased HCC incidence in humans, and we found the point mutant variant to have increased oncogenicpotential

compared to wildtype HBx. Experiments are underway to determine the mechanism underlying the oncogenic roles ofHBx variants by expressing them in human hepatocyte cell lines and examining cellular gene expression changes,transformation, and alterations in HBx protein stability.

3. "5--hydroxymethylation of the EBV genome inhibits BZLF1 (Z), but enhances BRLF1 (R), mediated viral reactivation"

Coral K. Wille, Shidong Ma, Dhannanjay M. Nawandar, and Shannon C. Kenney

Viral genome CpG methylation differentially affects the ability of the two EBV Immediate--early proteins, Z and R, toinduce lytic reactivation, but whether 5--hydroxymethylcytosine (5hmC) modification affects Z or R transcriptional function remains unknown. 5--methylcytosine (5mC) is converted to 5hmC by the TET family of enzymes. To determine whether 5hmC modification of CpG--containing Z and R binding motifs affects Z or R DNA binding, we performed electromobility shift assays. As expected, Z bound efficiently to methylated, but not unmethylated, forms of CpG--containing Z--response elements. However, 5hmC modification inhibited Z binding. Furthermore, a catalytically active form of TET2, which decreased the ratio of methylated versus hyroxymethylated R promoter in latently infected 293 cells, decreased the amount of in vivo Z binding to this promoter in ChIP assays. TET2 expression also inhibited Z activation of methylated (but not unmethylated) lytic EBV promoters in reporter gene assays, and decreased Z--mediated viral reactivation in EBV--infected 293 cells (where the viral genome is heavily methylated). In contrast, R binding was not affected by methylation or 5hmC modification, and TET2 increased the ability of R to activate methylated lytic viral promoters. These results suggest that 5hmC modification of the EBV genome differentially affects the ability of Z versus R to induce lytic reactivation. Importantly, IHC studies in normal tonsil tissue revealed that global 5hmC modification is very low in undifferentiated basal epithelial cells, but greatly increases in partially differentiated cells. Thus, 5hmC modification of the viral genome during epithelial cell differentiation may promote R--mediated lytic EBV reactivation. In addition, since we find that TET2 expression and global 5mC modification is strikingly different in Burkitt lines with type I versus type III latency, variation in the amount of viral genome 5hmC versus 5mC modification may also regulate EBV lytic reactivation in B--cell environments.

4. "Mechanisms of age-dependent resistance to PRRSV infection"

Sally Robinson, Diem Ngo, Anne Hoybook, Michael Murtaugh

Porcine reproductive and respiratory syndrome virus (PRRSV) causes a severe, economically devastating disease inMinnesota, and swine herds worldwide. PRRSV infection has very different outcomes in young and adult pigs. Whereas young pigs suffer serious respiratory disease, secondary infections, morbidity and mortality, adult pigs often show inapparent or mild infections. We have observed that PRRSV grows much more vigorously in young pigs compared to adult animals; a difference that is evident in susceptibility of macrophages, the key permissive cell, to infection. Young macrophages support higher levels of virus growth than do macrophages from older animals. There is no difference in expression of CD163 and CD169, proteins expressed on the surface of macrophages that are required for or associated with the infection process. Therefore, the difference in viral growth in macrophages appears to be an intrinsic, innate characteristic of macrophages that is dependent on animal age. Discovery and characterization of viral restriction factors, proteins that suppress (restrict) viral growth in permissive cells, has provided novel insights toward prevention and treatment of infection, such as in HIV infection of humans. Age related restriction of PRRSV growth in porcine alveolar macrophages (PAMs), unrelated to cellular expression of receptor molecules for the virus, suggests the possibility of age--dependent expression of viral restriction factors. We hypothesize that PAM expression of anti--PRRSV restriction factors constitutes cellular mechanisms of resistance to viral infection, and that age-- dependent expression accounts for the observed diminution of PRRSV growth in adult pigs. To this end we have examined PAMs from young and old pigs for susceptibility to PRRSV infection in vitro. Transcriptomes from these infected and uninfected young and old macrophage will be examined by high--throughput sequencing and bioinformatics to identify differences in age--dependent restriction factors that are expressed constitutively or are induced following viral infection.

The differential response to infection between PAMs of susceptible young pigs, and more resistant adult pigs is expected to be a powerful approach to identify changes in gene expression or macrophage polarization that will enhance future studies determining molecular mechanisms of resistance.

5. "Cooperativity Among Rev--Associated Nuclear Export Signals Regulates HIV--1 Gene Expression and is a Determinant of Virus Species Tropism"

Ryan T. Behrens, Mounavya Aligeti, Ginger M. Pocock, Johannes Schindelin, Christian Dietz, Kevin W. Eliceiri, Chad M. Swanson, Michael H. Malim, Paul Ahlquist, Nathan M. Sherer

Murine cells exhibit a profound block to HIV-1 virion production recently mapped to a species-specific structural attribute of the murine version of the CRM1 (mCRM1) nuclear export receptor, and rescued by expression of human CRM1 (hCRM1). In human cells, the HIV-1 Rev protein recruits hCRM1 to intron-retaining viral mRNAs encoding the Rev response element (RRE), thereby facilitating viral late gene expression. Here we exploited murine 3T3 fibroblasts as a gain-of-function system to study hCRM1 s species--specific role in regulating Rev s effector functions. We show that Rev is rapidly exported from the nucleus by mCRM1 despite only relatively weak contributions to HIV-1 s post-transcriptional stages. Indeed, Rev preferentially accumulates in the cytoplasm of murine cells with or without hCRM1 expression, by contrast to human HeLa cells where Rev often exhibits striking en masse transitions between the nuclear and cytoplasmic compartments. Efforts to bias Rev s trafficking into or out of the nucleus revealed that Rev encoding a second CRM1 binding domain (Rev-2xNES), or Rev-dependent viral gag-pol mRNAs bearing tandem RREs (GP-2xRRE), rescue virus particle production in murine cells even in the absence of hCRM1. Combined, these results suggest a model wherein Rev-associated nuclear export signals cooperate to regulate the number or quality of CRM1 s interactions with viral Rev/RRE ribonucleoprotein complexes in the nucleus. This mechanism regulates CRM1-dependent viral gene expression and is a determinant of virus species tropism.

6. "Viral protein requirements for efficient human parainfluenza virus 3 virus-like particle formation"

Megan Bracken, Michael Hoffman

Paramyxoviruses exit infected cells after specific viral proteins assemble at the cell membrane and bud from the host cell as infectious virions. To investigate assembly and budding requirements for human parainfluenza virus 3 (HPIV-3), its matrix (M) protein, a protein present between the envelope and RNA core of the virus particle, was expressed in 293T cells. The HPIV--3 M protein was sufficient for inducing release of M--containing, enveloped, virus--like particles (VLPs) from cells, indicating that M protein is critical for virus particle budding. Next, to understand the roles of additional HPIV-3 proteins in assembly and budding, viral envelope proteins hemagglutinin-neuraminidase (HN) and fusion (F), as well as the RNA-binding nucleoprotein (N), and the phosphoprotein (P) were expressed individually in cells. These proteins were unable to trigger their own release from cells. However, when M protein was co-expressed with N, F, or HN, VLPs that contained M and N, M and F, and M and HN, respectively, were released from cells. The P protein was not released from cells when co-expressed with M, but did release when co-expressed with M and N, indicating that the N protein recruits the P protein during virus particle assembly. The release efficiency of M protein was not enhanced by co-expression of any other viral protein. When M, N, and HN were co-expressed together, release of all three proteins as a VLP occurred. Analysis by electron microscopy verified that the VLPs had expected morphologies, such as containing spikes when the envelope proteins were expressed with M, and were similar in size to HPIV-3 virions. These findings show that the M protein plays a coordinating role in HPIV-3 viral particle assembly and release, and suggest that M protein interacts, independently, with the N, F and HN proteins.

7. "Sustained reactive gliosis following regulatory T cell depletion during acute viral encephalitis"
Scott J. Schachtele, Manohar B. Mutnal, Shuxian Hu, Wen S. Sheng, and James R. Lokensgard

Long-term, persistent inflammation of the central nervous system is commonly seen with neurodegenerative disease, stroke, and viral brain infection. Using a murine model of viral encephalitis (murine cytomegalovirus, MCMV) we have previously shown that the post-encephalitic brain is maintained in an inflammatory state consisting of prolonged glial cell (microglia and astrocyte) activation, retention of brain-infiltrating MCMV-specific effector-memory CD8+ T-cells, and long-term persistence of antibody--producing cells of the B-lineage. In the present study we report that this lingering neuroinflammation occurs concomitantly with accumulation and retention of immunosuppressive regulatory T-cells (Tregs) within the brain. However, the extent to which Tregs help control prolonged neuroimmune activation following viral encephalitis is unknown. Here, we used Foxp3 promoter--diphtheria toxin receptor (Foxp3-DTR) transgenic mice which, upon administration of low--dose diphtheria toxin (DTx), results in the specific depletion of Tregs. We found that Treg depletion during acute viral brain infection (0-7 d p.i.), using scheduled injections of DTx, resulted in exacerbation of encephalitis (i.e., CD4+ and CD8+ T-cell brain infiltration and retention) and sustained activation of resident glial cells (i.e., elevated major histocompatibility complex (MHC) class II, as well as programmed death ligand-1 (PD-L1) expression on microglia, and increased glial fibrillary acidic protein (GFAP) expression on astrocytes) when compared to undepleted animals. This sustained proinflammatory environment was associated with increased neuropathogenesis and reduced cognitive performance in spacial learning and memory tasks (i.e., Barnes Maze) by convalescent animals. Taken together, these data demonstrate that sustained glial cell activation, unremitting post-encephalitic neuroinflammation, and its associated long--term neurological sequelae are modulated by the immunoregulatory properties of Tregs during acute viral encephalitis.

8. "Whole genome sequencing of SIV-infected macaques identifies candidate loci that may contribute to host control of virus replication"
Adam J. Ericsen, Gabriel J. Starrett, Justin M. Greene, Michael Lauck, Muthuswamy Raveendran, David Rio Deiros, Mariel S. Mohns, Nicolas Vince, Brian T. Cain, Ngoc H. Pham, Jason T. Weinfurter, Adam L. Bailey, Melisa L. Budde, Roger W. Wiseman, Richard Gibbs, Donna Muzny, Thomas C. Friedrich, Jeffrey Rogers and David H. O'Connor

A small percentage of human immunodeficiency virus (HIV)-infected people and simian immunodeficiency virus (SIV)-infected macaques control virus replication without antiretroviral treatment. The major determinant of this control is host expression of certain major histocompatibility complex (MHC) alleles. However, this association is incompletely penetrant, suggesting that additional loci modify the MHC s protective effect. Here, to identify candidate control-modifying loci, we sequenced the genomes of 12 SIV-infected Mauritian cynomolgus macaques that experienced divergent viral load set points despite sharing the protective M1 MHC haplotype. Our genome-wide analysis of haplotype-level variation identified seven candidate control-modifying loci on chromosomes 2, 3, 7, 8, 9, 10, and 14. The highest variant density marks the candidate on chromosome 7, which was the only control-modifying locus to comprise genes with known immunological function. Upon closer inspection, we found an allele for one of these genes, granzyme B, to be enriched in M1(+) controllers. Given its established role as a cytotoxic effector molecule that participates in CD8-mediated killing of virus-infected cells, we tested the role of variation within gzmb in modifying SIV control by prospectively challenging M1(+) granzyme B-defined macaques. Our study establishes a framework for using whole genome sequencing to identify haplotypes that may contribute to complex clinical phenotypes. Further investigation into the immunogenetics underlying spontaneous HIV control may contribute to the rational design of a vaccine that prevents acquired immune deficiency syndrome.

9. "Viewing the biogenesis of HTLV-1 particles in living cells"
John P. Eichorst, Joachim D. Mueller and Louis M. Mansky

The assembly of human T-cell leukemia virus type 1 (HTLV-1) and the human immunodeficiency virus (HIV-1) particles at the plasma membrane is driven by the retroviral Gag protein. Our previous observations have indicated that the oligomeric state of Gag that trafficks to the plasma membrane is different between HTLV-1 and HIV-1 Gag, despite the similarities between the two proteins of these closely related human retroviruses. In our current studies, we sought to better understand how these observations may impact the biogenesis of virus particles. Gag puncta are formed over the course of a few minutes. Yet the dynamics for how the punctum recruits additional Gag molecules is poorly understood. There are two main pools of Gag available: cytoplasmic Gag and non-punctate, membrane-bound Gag. Here, we have conducted experiments to determine if the puncta mainly recruit cytoplasmic or membrane-bound HTLV-1 Gag. The relatively long duration of the assembly process (>= 5 minutes) allows us to use photoconversion of membrane-bound proteins to identify the recruitment pathway. Our studies to date indicate that Gag protein at the plasma membrane plays an important role in the biogenesis of mature Gag puncta.

10. "Skin tumorigenesis in transgenic mice expressing the Merkel cell polyomavirus T antigens"
Megan E. Spurgeon, Jingwei Cheng, James A. DeCaprio, and Paul F. Lambert

Merkel cell polyomavirus (MCPyV) is a recently discovered human polyomavirus that is currently the focus of intense study due to its association with Merkel cell carcinoma (MCC), a rare but highly aggressive neuroendocrine cancer of the skin. MCPyV is a ubiquitous virus in the general population, yet the viral genome is specifically detected in over 80% of MCC tumors. Integration of the viral genome, continued expression of MCPyV large and small tumor antigens (T antigens), and tumor-- specific truncations in large T antigen that render the virus replication-defective are all hallmarks of viral oncogenesis and proposed mechanisms of MCPyV-induced tumor initiation. In order to study MCPyV-associated disease and the potential role of the virus in MCC carcinogenesis, we developed a transgenic mouse model in which MCC-derived T antigen sequences were directed in their expression to epithelial cells of the skin, including stratified squamous epithelial cells as well as Merkel cells. Expression of the MCPyV small and truncated, large T antigens in these mice was documented by western analysis and associated with multiple acute skin phenotypes including hyperplasia, hyperkeratosis, and acanthosis. Mild alopecia and progressive whisker shortening and/or loss were also observed over time. Approximately 45% of transgenic mice on the FVB/N genetic background spontaneously developed cutaneous papillomas within several months of birth. These overt phenotypes were associated with immunohistological evidence of increased cellular proliferation, unscheduled DNA synthesis, induction of E2F-responsive genes consistent with pRb inactivation, and disruption of normal differentiation within stratified epithelia. In sum, these observations provide clear evidence that MCPyV T antigens are tumorigenic in vivo, consistent with an etiological role of MCPyV in human cancer.

11. "HIV-1 Vif Couples Two Independent Mechanisms for Promoting Viral Infectivity by Hijacking the Cellular Transcription Factor CBF²"
Brett D. Anderson and Reuben S. Harris

Several members of the APOBEC3 family of DNA cytosine deaminases can potently restrict HIV-1 replication by catalyzing extensive cytosine deamination in the viral cDNA during reverse transcription. HIV-1 encodes the small protein Vif, which commandeers a cellular ubiquitin ligase to target the APOBEC3 proteins for polyubiquitination and proteasomal degradation. Recently, we identified the cellular transcription factor CBF² as an obligate Vif binding partner and essential component of this ubiquitin ligase(1). We hypothesized that the viral hijacking of CBF² to promote APOBEC3 degradation may concurrently promote viral replication by altering the expression of cellular genes normally regulated by CBF². These studies identify a set of antiviral genes regulated by CBF², and provide an elegant solution to the question of why HIV-1 evolved to hijack CBF² as opposed to any other cellular protein to promote APOBEC3 neutralization and viral infectivity. Selected Reference: 1. Jager et al. Vif

hijacks CBF-² to degrade APOBEC3G and promote HIV-1 infection. Nature 481, 376-379 (2011)

12. "Netropsin Inhibits the Growth of EBV-positive Burkitt's Lymphoma Cells"
Adityarup Chakravorty & Bill Sugden

INTRODUCTION Epstein Barr Virus (EBV) is a Herpesvirus causally linked to multiple cancers, including Burkitt's lymphoma, the most common childhood cancer in sub-Saharan Africa. EBV provides survival factors to its associated tumors; tumor cells that lose EBV die by apoptosis. Identifying compounds that force the loss of viral genomes from tumor cells could yield potential therapeutic leads. In tumor cells EBV DNA is maintained as circular, extrachromosomal plasmids. Only one viral protein, EBNA1, is required for the maintenance of EBV genomes. Netropsin is a naturally-derived small molecule that binds to the minor groove of AT-rich DNA. EBNA1 is hypothesized to tether EBV genomes to cellular DNA through its N-terminal AT-hook DNA-binding domain. We hypothesized that netropsin would inhibit this tethering activity of EBNA1, leading to the loss of EBV genomes from cells, and the death of those cells dependent on the virus.

MATERIALS & METHODS We cultured different cell lines harboring EBV or EBV-derived plasmids in the presence and absence of netropsin, and measured the rate of loss of plasmids in them. The growth rates of Wp-restricted tumor cell lines that depend on EBV were also measured in the presence and absence of Netropsin. We replaced the N-terminal AT-hook DNA-binding domain of EBNA1 with the histone, H1. This fusion protein is able to maintain EBV-derived plasmids in cells, but lacks an AT-hook DNA-binding domain. We tested whether the rate of loss of EBV-derived plasmids maintained in cells expressing the H1-EBNA1 fusion protein differed in the presence and absence of netropsin.

RESULTS & DISCUSSION Netropsin is able to force the loss of EBV DNA and EBV-derived plasmids from different tumor cell lines. This forced loss occurred without any deleterious effects on the cell for multiple cell lines. In tumor cell lines that depend on EBV, the forced loss led to significant decreases in the rate of cell proliferation. Netropsin acts specifically to force the loss of EBV genomes from Primary Effusion Lymphoma cells, while not affecting the genomes of the closely related Kaposi Sarcoma-associated Herpes Virus in the same cells. Netropsin also did not increase the rate of loss of EBV-derivative plasmids maintained in cells expressing the H1-EBNA1 fusion protein.

CONCLUSIONS Netropsin is able to inhibit the growth of Burkitt's lymphoma cell lines by specifically forcing the loss of EBV genomes from these cells. These data serve as proof-of-concept for small molecule screens to identify other compounds that inhibit binding of EBNA1 s AT-hooks to DNA, which can be used therapeutically for EBV-induced cancers.

13. "Letting go": how does herpes simplex virus get out of cells (and why is ICP27 involved)?"
D. Park, J. Lalli, L. Sedlackova, and S. Rice

Numerous studies have focused on ICP27, an immediate-early (IE) protein of herpes simplex virus type 1 (HSV-1) with diverse roles in viral gene regulation. However, its homolog in HSV-2, termed ICP27t2, has been little studied. Here, we have used two different approaches to functionally compare ICP27t2 and ICP27. In transfection-based assays, ICP27t2 closely resembled ICP27 in its capacity to enhance HSV-1 late mRNA expression and suppress the splicing of a viral intron. To study ICP27t2 in the context of viral infection, we engineered K2F1, an HSV-1 mutant that encodes ICP27t2 in place of ICP27. In Vero cells, K2F1 replicated with wild-type (WT) kinetics and yields, and expressed viral genes normally. Thus, we conclude that ICP27t2 and ICP27 are functionally very similar, and that ICP27t2 can mediate all of the ICP27 activities that are required for HSV-1 replication in cell culture. Surprisingly, however, we found that K2F1 forms plaques that have a different morphology than WT HSV-1 plaques. Investigation of this trait showed that it results from decreased release of K2F1 progeny virions into the media. Two previously-isolated HSV-1 ICP27 mutants show a similar release defect. Together, our results reveal that ICP27 plays a previously unappreciated role in virion release. The mechanism of HSV-1 release and the possible functions of ICP27 in release will be discussed.

Abstracts – Poster Presentations

1 "Analysis of authentic HTLV-1 particles using cryo-electron tomography"
Sheng Cao, José O. Maldonado, Iwen F. Grigsby, Louis M. Mansky and Wei Zhang

Human T cell leukemia virus type 1 (HTLV-1) was the first human cancer-causing retrovirus discovered. The morphology of cell-associated virus particles has been studied with limited resolution using thin--section transmission electron microscopy. Using cryo-electron tomography, we studied the HTLV-1 particles purified from chronically infected MT-2 cells. HTLV-1 virions were polymorphic, roughly spherical particles that varied in size. The discernible capsid cores in HTLV-1 were typically poorly defined polyhedral structures that also contained regions of smooth surface. The average density inside the capsid core appeared similar to that in the compartment between the capsid and the viral membrane. The majority of the particles lacked a defined capsid core and had evenly distributed electron densities occupying the interior of the particle. This property likely affects the overall infectivity in the cell-free HTLV-1 virion samples.

2 "Pathogen fee-for-service sequencing at the WNPRC"
Dane Gellerup & Shelby O'Connor

Understanding viral evolution in response to host immune responses is of critical importance for numerous infectious disease researchers. Deep sequencing viral genomes can provide valuable insight into this relationship. We have the experience and the tools to deep sequence populations of RNA viruses using a variety of different approaches. We use Illumina MiSeq technology to deep sequence virus populations. We offer full-service sequencing of the full coding region of an SIV RNA genome. For viruses with a known reference, we isolate vRNA and generate four overlapping ~2.5 kb cDNA amplicons spanning the entire coding sequence of the viral genome. For virus inoclua with unknown sequences, we isolate viral RNA from plasma and generate viral cDNA using random hexamers and the SuperScript Double-Stranded cDNA synthesis kit (Invitrogen). Purified cDNA is fragmented and tagged using the Nextera XT in vitro transposition kit (Illumina). Libraries are pooled and sequenced on the Illumina MiSeq. Over 30 uniquely tagged genomes can be multiplexed on a single run of the instrument, generating approximately one million reads spanning each genome, which allows for >1000x coverage at each nucleotide. We offer additional sequencing alternatives that afford greater flexibility to clients. A client can generate their own viral RNA or cDNA amplicons and send them to us for processing. This reduces cost, gives the client more control over their sample preparation, and gives us the flexibility to sequence non-SIV pathogens. Once sequencing is complete, we provide FASTQ sequences and, if desired, a BAM alignment of the reads mapped against a reference. This gives the client a data set to explore sequence variation in their chosen virus population. With our service, we can help clients answer questions about sequence diversity in their pathogen of interest, which ultimately gives the investigator greater information about their study.

3. "Env Substitutions Can Protect Vif-null HIV-1 from APOBEC3G Deamination"
Terumasa Ikeda, John S Albin, Dan Mundt, Menelaos Symeonides, Markus Thali and Reuben S Harris

Several APOBEC3 proteins restrict the replication of Vif-deficient HIV-1 by deaminating nascent cDNA cytosines to uracils. HIV-1 encodes Vif to counteract the antiviral activity of these enzymes through a proteasome-mediated degradation pathway. The APOBEC3-Vif axis is therefore an attractive target for antiviral drug development. However, this strategy could be undermined if HIV-1 can develop an alternative mechanism to circumvent restriction. To explore this possibility, we performed a series of cell culture selection studies to determine whether HIV-1 with an irreparable 230 bp deletion in vif could develop the capacity to replicate in the presence of restrictive levels of APOBEC3G. Vif-null viruses were selected in step--wise increments to resist increasing amounts of APOBEC3G. All viruses that emerged from this stepwise selection procedure maintained the original 230 bp vif deletion and acquired

large numbers of mutations in various viral open reading frames. Interestingly, all viruses had one or more amino acid substitutions in Env. Molecular clones containing only the original vif deletion and these changes in Env were protected from APOBEC3G dependent restriction and hypermutation in T cell spreading infections but not in 293T-based single cycle experiments despite high levels of APOBEC3G packaging in both systems. The studies demonstrate a novel Vif-independent mechanism that HIV-1 can use to escape restriction by APOBEC3G.

4. "Role of Membrane-Shaping Host Reticulons in HIV-1 Replication"
Justin Massey, James Bruce and Paul Ahlquist

HIV-1 is a retrovirus that must direct synthesis, nuclear import and chromosomal integration of viral cDNA to successfully replicate. While host factors in the endoplasmic reticulum (ER), such as the SET complex of nucleases, are important for HIV-1 replication, the role of the ER in HIV-1 trafficking remains undefined. Reticulon 3 (RTN3) is an ER-resident, membrane--shaping protein and a member of the reticulon homology domain (RHD) family that is crucial for ER morphogenesis, stabilization of positive membrane curvature, and replication of certain positive strand RNA viruses. A recent siRNA screen reported that knocking down RTN3 inhibited an early but undetermined stage of HIV-1 infection by two-fold (Koenig et al, Cell 2008). We found that RTN3 knockdown inhibited early stages of HIV-1 replication at least four-fold, significantly more than previously reported. Additionally, we defined the phase of viral replication that is inhibited by knocking down RTN3. RTN3 knockdown inhibited HIV-1 replication after reverse transcription, as levels of viral cDNA were the same in RTN3 knockdown cells and cells treated with control siRNAs. However, we observed decreased levels of nuclear products of HIV-1 cDNA, including two LTR (long terminal repeat) circles and chromosomally integrated viral cDNA as measured by Alu-PCR. Therefore, in RTN3 knockdown cells, HIV-1 reverse transcription proceeds with normal efficiency, but entry of the resulting cDNA products into the nucleus is inhibited. In keeping with this, RTN3 knockdown had no effect on the transcription or translation of a previously integrated HIV-1 provirus. We hypothesize that RTN3 may affect trafficking or nuclear entry of the viral pre-integration complex or host factors required for these processes. Understanding how HIV-1 cDNA travels to the nucleus should facilitate developing new antivirals that prevent the establishment of latent and productive infections.

5. "Elucidating the Interaction between HIV-1 Vif with human APOBEC3F"
John S. Albin , Elizabeth M. Luengas, Allison M. Land, Christopher Richards, Brett D. Anderson, Nadine M. Shaban,Ozlem Demir, John R. Holten, John S. Anderson, Daniel A. Harki , Rommie E. Amaro and Reuben S. Harris

Lentiviruses are a subset of retroviruses that produce illnesses characterized by a delay in the onset of symptoms following infection. Lentiviruses are responsible for causing a variety of diseases in mammals such as immunodeficiencies, blood cancers, and neuropathies. Mammals have evolved a variety of mechanisms to combat lentivirus infections. The APOBEC3 (A3) family of DNA cytosine deaminases function by directly attacking viral nucleic acid replication intermediates and converting normal DNA cytosine bases into mutagenic uracils. Every mammal has multiple A3 proteins that combine to suppress infection by lentiviruses. However, lentiviruses fight back by producing Vif which directly binds to CBF² to recruit E3 ubiquitin ligase complex that mediates proteasomal degradation of A3 protein1 3. High-resolution structures of HIV-1 Vif3 and several human A3 enzymes4 8 are now available, but detailed information on the pathogen-host protein interaction surfaces have been elusive. Here, we use a combination of genetic, biochemical, and computational approaches to develop and test a model for the direct interaction between Vif and A3F. First, Vif-resistant A3F-E324K was used to select compensatory adaptations in Vif in long-term infection experiments. A single amino acid change restored Vif function and served as an anchor for docking studies. A combination of published and new site directed mutation data were used to distinguish between multiple interaction models and deduce the most likely binding interface. These surfaces are attractive targets for drug development with the long--term goal of identifying small molecules that disrupt the interaction with Vif and suppress HIV-1 infection through A3-mediated restriction.

6. "Multimerization, Functions, and Mechanisms of the Purified Bromovirus RNA Helicase Domain"
Robert A. Pugh and Paul Ahlquist,

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 5' to 3', superfamily 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 identified a soluble truncation of the 1a replication protein that contains the helicase domain. This truncation has been purified to homogeneity and examined for helicase activity. We found that it preferentially interacts with itself to form oligomeric structures, binds RNA substrates and is stimulated to hydrolyze ATP by ssRNA. Its unwinding activity appears to be tightly regulated by an accessory protein or domain. Further results from our ongoing studies of these 1a functions and the complex relationship between the oligomeric structures and activities of this crucial RNA replication domain will be reported.

7. "Porcine Epidemic Diarrhea Virus: Immunity Following Feedback"
CMT Dvorak, S Stone, A Rovira, I Levis, S Brown, M Turner, S Hough, T Snider, and MP Murtaugh

Introduction: Porcine epidemic diarrhea virus (PEDV), an enteric coronavirus related to transmissible gastroenteritis virus (TGEV), appeared suddenly in the Unites States in April, 2013. Epidemic sow herd outbreaks, characterized by severe diarrhea, vomiting, and high mortality in nursing pigs for several weeks, continue to spread the disease. PEDV appeared in Canada in February, 2014, and multiple outbreaks have already been reported despite extensive efforts to prevent and control the disease. In the absence of vaccines for PEDV in the US, producers have used feedback of infected intestinal tissues to induce immunity. However, there is little information on (1) the efficacy of various feedback protocols on induction of immunity, including duration of the serological response and mucosal antibody production or (2) the effect of re-exposure of a herd by feedback on immunity and re-infection. We performed two experiments to (1) determine the effect of feedback on antibody responses in sows and piglets and (2) determine the effect of re-exposure by feedback on sows and naïve gilts.

Materials and Methods: To examine the efficacy of prevention protocols, feedback materials were titered for PEDV by qRT-PCR and either fed to pregnant sows once with a second administration about 3 weeks later (protocol 1) or fed in multiple doses over a one week period (protocol 2). Samples were obtained at feedback, 3-5 weeks post-feedback, and 9 weeks post-feedback. Piglet samples were obtained at weaning. Infection was assessed by viral qRT-PCR and immune response by anti-capsid ELISA. The effect of re-exposure to PEDV on a herd was examined by performing a whole herd feedback followed by herd closure and then 4-5 months later introducing gilts and performing feedback on the herd again (except farrowing sows). Thirty sows and 10 gilts were examined for PEDV-specific antibodies in serum by ELISA and IFA on the day of feedback (day 0), day 7, and day 35 post-feedback. On day 0, 2, 7, and 35 fecal samples were obtained and viral presence was monitored by PEDV PCR.

Results: Feedback efficacy was observed on farms using both feedback protocols as well as re-exposure of the herd. Uniform seroconversion was observed at 3-5 weeks, but by 9 weeks antibody levels had returned to baseline in the majority of animals examined. Piglets had no detectable serum anti-capsid antibodies at weaning in the majority of animals, and anti-capsid antibodies were not detected in sow milk or feces. However, we have found antibodies to envelope spike protein in milk, indicating transfer of maternal immunity.

Conclusions: Feedback of infected gut material is an effective method for inducing anti-PEDV immunity in adult sows, even though the capsid ELISA response appears to be short-lived. ELISA analysis shows transfer of IgG maternal immunity to piglets in colostrum, and IgA anti-spike antibodies in milk. Further testing of sow and piglet feces is needed to more fully understand immunity in the gut to PEDV. In a re-exposed herd 5 months after an outbreak,, few previously exposed sows shed virus and, by day 35, the majority were antibody positive by ELISA and all were antibody positive by IFA. Duration of immunity thus is ≥5 months, whereas newly introduced naïve gilts shed virus in feces. Feedback, therefore, is effective in boosting herd-level protection after introduction of naïve gilts, without inducing disease in previously exposed animals or re-contaminating the environment in the gestation barn or farrowing rooms.

8. "Hypoxia-induced lytic reactivation of EBV requires both HIF-1± and activated p53."
Richard J. Kraus, Xianming Yu, Saranya Sathiamoorthi, Shannon C. Kenney, and Janet E. Mertz

Jiang et al. previously reported that hypoxic growth conditions can lead to induction of EBV lytic reactivation (J Clin Virol 37:98, 2006). We show here that hypoxia does so in some EBV(+) cell lines by inducing accumulation of HIF-1± which directly binds to a hypoxia-responsive element (HRE) located within the BZLF1 gene promoter, Zp. Incubation of gastric carcinoma AGS-Akata or EBV(+) Burkitt lymphoma (BL) Sal cells with CoCl2, desferrioxamine (DFO) or L-mimosine, iron-competing or chelating mimics of hypoxia, promoted accumulation of HIF-1± and enhanced expression of EBV s lytic antigens; ChIP assays showed that HIF-1± bound Zp within the context of these whole EBV genomes. ShRNA knockdown of HIF-1± in Sal cells significantly reduced DFO-mediated EBV lytic reactivation. Importantly, expression of HIF--1± efficiently induced EBV lytic-gene expression in 293T cells harboring WT virus, but not cells infected with variants containing 3--bp substitution mutations within the Zp HRE. While HIF-1± directly activated Zp via its HRE, it failed to activate transcription from the BRLF1 promoter, Rp, in either reporter assays or Zp HRE mutant EBV genomes. Thus, HIF-1± specifically targets Zp. Interestingly, DFO failed to induce reactivation in BL MutuI cells which contain a mutant variant of p53; shRNA knockdown of p53 in Sal cells also inhibited DFO-induced reactivation. Along with stabilizing HIF-1±, DFO treatment also signaled enhanced accumulation and phosphorylation of p53 at ser15. Nutlin-3, an mdm2 inhibitor, strongly synergized with DFO, but not TPA, in inducing EBV reactivation in Sal and AGS-Akata cells, but not in MutuI cells. Inhibition of the protein kinase ataxia--telangiectasia mutated (ATM) blocked both p53 phosphorylation at ser15 and DFO-induced EBV reactivation. Thus, we conclude that hypoxia--induced EBV lytic reactivation requires both ATM -mediated phosphorylation of p53 and HIF-1± sequence--specific binding to Zp.

9. "Deep Sequencing Analysis of PRRSV Genetic Variation Among Cell Types"
Xiong Wang, Michael P. Murtaugh*

Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus with an extremely high mutation rate estimated at ~10-2/site/year. Mutations can arise from viral RNA polymerase infidelity, genomic recombination, and host cell mutator activity. However, the frequency of nucleotide variations across individual sites in the viral genome, which might help address the contribution of various mutational mechanisms, has not been investigated in permissive cells or host animals. Since biological and antigenic variation arising from these mutations may contribute to disease severity, incomplete effectiveness of vaccination, and prolonged infection, we examined this question using ultra--deep sequencing. Strain variation has been examined extensively through consensus ORF5 sequencing analysis, however, nucleotide sequence variation across the entire genome of individual viral genomes in a viral population has not been evaluated. We sequenced three independent virulent PRRSV strains grown in two different permissive cell types at an average redundancy between 6,000- and 50,000-fold. Fifty bp, paired-end reads were mapping to the corresponding reference genome and single nucleotide polymorphisms were detected across the genome. Our preliminary results show that the highest mutation frequencies were detected in nonstructural protein 2 coding region (nsp2), nsp3, and nsp11. Overall nucleotide substitution patterns were random, but at frequencies higher than 1%, A to G and G

to A substitutions were over-represented, suggesting the potential editing activities of a cytoplasmic form of the apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3 (APOBEC3) family. PRRSV whole genome SNP analysis showed that the mutational spectrum was dependent on both virus strain and permissive cell type, either porcine macrophages or MA-104, a simian epitheliod cell line. Overall, host cellular anti-viral mechanisms appear to have a limited effect on the PRRSV mutation rate, suggesting that antigen-specific adaptive immunological responses may play a dominant role in driving PRRSV mutation.

10. "Evidence for HIV-1 Genomic RNA Elements Regulating the Efficiency of HIV-1 Virus Particle Assembly"

Jordan T. Becker & Nathan M. Sherer

Human immunodeficiency virus type 1 (HIV-1) virion assembly is a stepwise process driven by the structural polyproteins Gag and Gag-Pol that multimerize at the plasma membrane to form enveloped, immature capsids. Assembly is a cooperative process with Gag-Gag interactions promoted both through the binding of Gag s N-terminal Matrix (MA) domain to plasma membrane phospholipids, and also Gag s C-terminal Nucleocapsid (NC) domain binding to both viral and cellular derived RNA molecules in the cytoplasm. NC s core RNA--inding function is to ensure encapsidation of two copies of the viral positive--strand genomic RNA (gRNA). gRNAs also serve as the viral mRNAs encoding Gag and Gag-Pol, and thus Gag translation and genome packaging may be integrated processes. However, in the absence of package-able gRNAs Gag is still able to drive formation of non--infectious virus-like particles through promiscuous interactions between NC and cellular mRNAs and some highly structured non-coding RNAs. Thus, how HIV-1 coordinates and guarantees the specific packaging of gRNA molecules despite the abundance of other cellular and viral RNAs remains a puzzle. We have hypothesized that crosstalk between gRNAs and Gag s MA domain may govern at least part of the mechanism because gRNA nucleocytoplasmic transport pathways can markedly impact the efficiency of Gag s MA-dependent targeting plasma membrane assembly sites in diverse cell systems (e.g., murine cells; Sherer et al. J. Virol, 83(17):8525-35, 2009). However, it has been difficult to distinguish between contributions of Gag expression kinetics vs. the presence of one or more gRNA-intrinsic assembly signals. Here, we established a tractable cell based system to compare gRNA/Gag interactions in cis and in trans using a combination of live cell imaging and functional assembly assays. We show that full-length HIV-1 gRNAs mutated to no longer express Gag/Gag-Pol can markedly enhance HIV-1 particle assembly when provided in trans, so that gRNAs do likely encode one or more elements capable of directly stimulating efficient virion production. Second, we observe a loss in gRNA-provided assembly enhancement in the presence of assembly-competent Gag variants lacking all or portions of the native Gag MA domain, so that this process appears to be MA-dependent. Importantly, ongoing studies also indicate that gRNA-MA interactions play a role in regulating the integrity of gRNA encapsidation. Taken together, these results emphasize the importance of functional crosstalk between MA and gRNAs in regulating both efficient virion assembly and the specificity of gRNA encapsidation.

11. "Striking Contribution of G-to-A Hypermutants to Viral Mutagenesis in HIV Types 1 & 2"

Jonathan Rawson, Sean Landman, Cavan Reilly, and Louis Mansky

Human immunodeficiency virus type 1 (HIV-1) mutates at an extraordinary rate, promoting rapid evolution of drug resistance, immune system escape, and invasion of multiple compartments within infected individuals. Here, we have harnessed the immense power of Illumina high-throughput sequencing to characterize the mutagenesis of HIV-1 and human immunodeficiency virus type 2 (HIV-2) in one round of replication. We obtained approximately 2.4 million paired reads passing all filters, containing ~230 million paired bases and ~90,000 paired mutations. We found that the total mutation frequencies of HIV-1 & 2 were 5.9 and 4.6 × 10-4 mutations per base pair per replication cycle respectively, a difference that was not statistically significant. Intriguingly, approximately two-thirds of all mutations observed were found to be G-to-A transition mutations. The high levels of G-to-A transition mutations were largely due to the presence of G-to-A hypermutants, observed in approximately 1 of 160 read pairs for HIV-1 and 1 of 190 read pairs for HIV-2. The identified

hypermutated sequences contained between 2 and 18 G-to-A mutations per read pair (medians of 4 [HIV-1] and 3 [HIV-2]). About 99% of the observed G-to-A transition mutations occurred at GA or GG dinucleotides, implicating the activity of one or more APOBEC3 family members. Remarkably, G-to-A hypermutants were responsible for about half of all observed mutations in both HIV-1 & 2. Taken together, these observations imply G-to-A hypermutation may significantly contribute to viral mutagenesis in vivo for both HIV-1 & 2.

12. "Dissecting HIV-1 s post-transcriptional stages using an imaging based approach"
Ginger M Pocock, Jordan T. Becker, Sirisha R Dommaraju, Paul Ahlquist, Nathan M Sherer

The late stages of the HIV-1 life cycle involve a series of integrated post-transcriptional regulatory events mediated by interactions between key viral and cellular factors. These stages are initiated at nuclear export of unspliced and partially spliced viral mRNAs, including full-length genomic RNAs (gRNAs) that encode the Gag and Gag-Pol polyproteins that form the viral capsid. gRNA nuclear export is regulated by the viral Rev protein that multimerizes on the Rev response element (RRE), a cis-acting structural RNA element found in intron--containing viral transcripts, and recruits the cellular chromosomal region maintenance 1 (CRM1) nuclear export receptor through its C-terminal leucine-rich nuclear export signal (NES). It is not fully understood why HIV-1 gRNAs (and some other retroviruses) use the CRM1 pathway, in contrast to other retroviruses that lack Rev equivalents and utilize the conventional NXF1/NXT-regulated mRNA nuclear export machinery. However, Rev provides the virus with a mechanism for pre-programming viral ribonucleoprotein (vRNP) complexes in the nucleus in ways that influence downstream cytoplasmic events including mRNA translation, Gag/gRNA trafficking, genome packaging and the efficiency of virus particle assembly. We are applying a systematic comparative imaging approach to study the cell biology linking gRNA trafficking to the efficiency of infectious virion production. We aim to directly visualize the dynamic interplay occurring among Rev, gRNA, CRM1 and Gag molecules in single living cells using a combination of time-lapse imaging, fluorescence recovery after photobleaching (FRAP) and fluorescence resonance energy transfer (FRET). In this system, we are exploiting defined cellular blocks to virion production in order to interrogate trafficking bottlenecks relevant to instances of viral manipulation of host transport pathways. These studies have revealed dynamic regional kinetics, including orchestrated, en masse redistributions of labelled Rev, CRM1 and gRNA species between the nuclear and cytoplasmic compartments. We are currently exploring the hypothesis that CRM1 serves as a mechanism to regulate staged, transient bursts of late stage viral gene expression that provide a mechanism to circumvent aspects of the intrinsic cellular immune defense.

13. "Comparative analysis of retrovirus virus-like particles using cryo-TEM"
Jessica Martin, Sheng Cao, Jose Maldonado, Wei Zhang, Louis Mansky

The retroviral Gag protein is the primary structural protein responsible for virus particle formation and budding. The Gag protein alone is sufficient for production of virus-like particles (VLPs). VLPs from several retroviral genera have been studied via microscopy in the past, but no comprehensive study had analyzed retroviral VLPs together in parallel with the same methodologies. Here, in this study, we evaluated VLPs generated from the Gag proteins of six different genera alpharetrovirus, betaretrovirus, deltaretrovirus, gammaretrovirus, foamy virus, and lentivirus (i.e., Rous sarcoma virus, Mason-Pfizer monkey virus, bovine leukemia virus, murine leukemia virus, human foamy virus, and human immunodeficiency virus type 2) using cryo-TEM. This analysis revealed that within each genera there are particle size distributions. Furthermore, the average sizes and morphology of VLPs varies, spanning from particle diameter of 82.9 nm to 151.2 nm. The findings of this study reveal that the known differences retrovirus life cycle and Gag trafficking patterns among retroviral genera correspond with changes in particle morphology. This observation implies that differences in the virus assembly pathway influence particle morphology.

14. "Production of West Nile Virus replicon particles in insect cells using packaged replicons to deliver recombinant RNA"

Brendan T. Boylan, Lindsey A. Moser, Kristen A. Bernard

Packaged replicon particles (RPs) play essential roles in vaccine development and as a tool to investigate the first round of infection. The replicon genome lacks most or all of the viral structural genes and is unable to package viral progeny and undergo second rounds of infection. RPs are produced by supplying the structural proteins in trans using expression vectors or packaging cell lines. Packaging techniques often use mammalian cells; however, insect cell-derived RPs better mimic natural infection for arboviruses. Differences in the glycosylation of West Nile virus (WNV) envelope protein for mosquito and mammalian cell-derived virus can affect cell tropism and the host response in animal models. Our goal is to produce WNV RPs from mosquito cells, which to our knowledge have not been used to package RPs. Two methods were compared using a WNV replicon genome that contains a reporter gene in place of the structural genes. For the packaging vector, an alphavirus replicon genome containing genes for the WNV structural proteins was used. In the first method, cells were electroporated with in vitro transcribed WNV replicon RNA, incubated for 24-48 hours, and then electroporated with in vitro transcribed packaging vector RNA. Although this method produced high titers of WNV RPs in BHK-21 mammalian cells, no RPs were produced in C6/36 mosquito cells. In the second method, cells were infected with WNV RPs, which were produced in BHK-21 mammalian cells, and 24-48 hours later, these cells were electroporated with in vitro transcribed packaging vector RNA. This second method resulted in production of RPs from mammalian and mosquito cells. We are now optimizing the technique to produce high titer WNV RPs in mosquito cells. This will allow us to mimic arboviral infections in vivo and to examine any differences in early virus-host interactions between mammalian and insect cell-derived virus particles.

15. "A re-evaluation of the distribution and recirculation of memory CD8 T cells"

Elizabeth Steinert, Jason M. Schenkel, David Masopust

Non-lymphoid memory CD8 T cells contribute to protection from viral re--infection. 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 and quantity of antigen specific memory CD8 T cells after LCMV infection in mice utilizing 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. We found that cell isolation efficiency varied among tissues and was particularly inefficient in the mucosae. Further, isolations from whole organs are compartmentally and phenotypically biased, altering our perception of the populations present in non-lymphoid tissues. Thus, histological enumeration methods are necessary to appreciate the true distribution of the memory CD8 T cell population. Applying this approach to parabiotic mice whose blood supply was conjoined five months after LCMV infection, we quantified resident and recirculating memory CD8 T cells within numerous organs. Recirculation varied considerably between different organs, and among different tissue compartments within a given organ. Together these results provide a systematic quantification of the distribution and compartmentalization of memory CD8 T cell subsets and highlight the relative numerical abundance of resident memory CD8 T cells.

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

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

Epstein-Barr virus (EBV) usurps B lymphocyte growth and survival signaling pathways in order to establish life-long latent infection in humans. Remarkably, four EBV nuclear antigens (EBNAs) regulate cell gene expression by targeting RBPJ, a DNA binding protein in the Notch signaling pathway. The mechanism by which EBNA2, EBNA3A, EBNA3B, and EBNA3C regulate different genes via RBPJ remains unclear. Our results demonstrate that EBNA3A, EBNA3B and EBNA3C bind to distinct genomic locations. Of the 5520 sites bound by EBNA3 proteins, only 1518 are bound by more than one.

Surprisingly, only 30-40% of EBNA3A, EBNA3B, or EBNA3C bound sites co--localize with RBPJ. In an effort to determine which factors in addition to RBPJ determine binding specificity, we identified sequences enriched at EBNA3A, EBNA3B and EBNA3C bound sites. Additionally, using published ENCODE ChIP-Seq data we found that EBNA3 bound sites were enriched for RUNX3, EBF1, and PAX5. Using LCLs conditional for EBNA3A or EBNA3C activity we demonstrate that our ChIP-Seq binding data predicts the genome locations at which EBNA2 binding is regulated by each EBNA3. Finally, we examined changes in the H3K27me3 mark that is believed to mediate EBNA3 repression of cell genes. Surprisingly, we observed no correlation between changes in this mark upon EBNA3A or EBNA3C inactivation with EBNA3A or EBNA3C binding. This suggests that EBNA3 proteins may exert their transcriptional effects through indirect mechanisms. Our study represents the first genome wide characterization of EBNA3A, EBNA3B and EBNA3C binding in LCLs. Moreover, our results suggest a mechanism by which EBNA3 proteins regulate distinct, but partially overlapping, sets of cell genes and provide a basis for understanding their role in lymphomagenesis.

17. "Mechanisms of age-dependent resistance to PRRSV infection"

Sally Robinson, Diem Ngo, Anne Hoybook, Michael Murtaugh

Porcine reproductive and respiratory syndrome virus (PRRSV) causes a severe, economically devastating disease in Minnesota, and swine herds worldwide. PRRSV infection has very different outcomes in young and adult pigs. Whereas young pigs suffer serious respiratory disease, secondary infections, morbidity and mortality, adult pigs often show inapparent or mild infections. We have observed that PRRSV grows much more vigorously in young pigs compared to adult animals; a difference that is evident in susceptibility of macrophages, the key permissive cell, to infection. Young macrophages support higher levels of virus growth than do macrophages from older animals. There is no difference in expression of CD163 and CD169, proteins expressed on the surface of macrophages that are required for or associated with the infection process. Therefore, the difference in viral growth in macrophages appears to be an intrinsic, innate characteristic of macrophages that is dependent on animal age. Discovery and characterization of viral restriction factors, proteins that suppress (restrict) viral growth in permissive cells, has provided novel insights toward prevention and treatment of infection, such as in HIV infection of humans. Age related restriction of PRRSV growth in porcine alveolar macrophages (PAMs), unrelated to cellular expression of receptor molecules for the virus, suggests the possibility of age-dependent expression of viral restriction factors. We hypothesize that PAM expression of anti-PRRSV restriction factors constitutes cellular mechanisms of resistance to viral infection, and that age-dependent expression accounts for the observed diminution of PRRSV growth in adult pigs. To this end we have examined PAMs from young and old pigs for susceptibility to PRRSV infection in vitro. Transcriptomes from these infected and uninfected young and old macrophage will be examined by high-throughput sequencing and bioinformatics to identify differences in age-dependent restriction factors that are expressed constitutively or are induced following viral infection. The differential response to infection between PAMs of susceptible young pigs, and more resistant adult pigs is expected to be a powerful approach to identify changes in gene expression or macrophage polarization that will enhance future studies determining molecular mechanisms of resistance.

18. "Infection and pathogenesis of the recently discovered Murine Papillomavirus (MmuPV-1) in vivo"

Aayushi Uberoi, Satoshi Yoshida and Paul F. Lambert

The recent discovery of the cutaneotropic murine papillomavirus (MmuPV1) provides us, for the first time, a unique opportunity to define the role of papillomavirus (PV) genes in PV-associated pathogenesis and life cycle in vivo, in the context of a genetically manipulatable host organism. A naturally occurring PV infection in the laboratory mice can provide a major new means for investigating the molecular pathogenesis of cutaneous papillomaviruses, which are relatively less understood. We have developed an infection model to study MmuPV1 in vivo by using MmuPV1 quasivirions developed in 293FT cells to infect mice following scarification. We have found that MmuPV1 causes overt pathogenesis seen in the form of papillomatosis around the muzzle, tail and ear regions in the

FoxN1nu/nu immune deficient strain of mice. Upon serial passage of papillomas we can see papillomas as early as week 2 after infection. Monitoring of wart growth profiles indicate that genetic background can impact size of papilloma formation. Preliminary analysis of cytokeratin and histochemical markers in the papilloma indicates a delay in terminal differentiation. Using different immune deficient strains of mice, we found that complete T-cell deficiency is required for MmuPV1 induced papillomatosis. While MmuPV1 failed to induce papillomatosis in immune competent strains of mice, we now have preliminary evidence that indicates that ultraviolet radiation assists MmuPV1 in causing wart formation and squamous cell carcinoma in such mice.

19. "pH dependent kinetics and ssDNA binding of HIV-1 restriction factor APOBEC3G-ctd using time resolved NMR"

William C. Solomon, Stefan Harjes, Ming Li, Reuben S. Harris and Hiroshi Matsuo

APOBEC3G has an important role in human defense against retroviral pathogens including HIV-1. We used time resolved NMR to determine kinetic parameters of A3Gctd s deamination reactions within a 5´-CCC hot spot sequence. A3Gctd exhibited 45-fold preference for 5´-CCC substrate over 5´-CCU substrate, which explains why A3G displays almost no processivity within a 5'-CCC motif containing two potential 5-CC targets. A3Gctd as well as full--length A3G showed peak deamination velocities at pH 5.5. We found that H216 is responsible for this pH dependence, suggesting that protonation of H216 could play a key role in substrate binding. Protonation of H216 appears important for HIV-1 restriction activity as well, since substitutions of H216 resulted in lower restriction in vivo.

20. "Examining the Role of Sirt1 in HPV-positive Head and Neck Squamous Cell Carcinoma"

Patrick Nyman, Paul Lambert, Henry Pitot

Human Papillomavirus (HPV) is a non-enveloped double-strand DNA virus that infects the stratified squamous epithelia of humans. HPV is known to be the primary cause of cervical cancer as well as a subset of head and neck cancers, with the viral proteins E5, E6 and E7 being the driving oncoproteins. The longevity protein Sirt1, a protein and histone deacetylase, has been implicated as both a tumor promoter and tumor suppressor depending on the tissue context. Sirt1 was previously found to be upregulated by E7 in HPV-expressing cells, and in cervical cancer cell lines, loss of Sirt1 expression led to apoptosis. We hypothesized that Sirt1 would act as a tumor promoter in E7-mediated head and neck cancer, and that loss of Sirt1 would inhibit tumor formation. We utilized an HPV16 E7 transgenic mouse (K14E7) on a background with wild type or inactivated Sirt1 gene, and monitored these cohorts for head and neck cancer upon treatment with the oral carcinogen 4NQO. We found that loss of Sirt1 did not decrease tumor incidence in the context of E7. Furthermore, loss of Sirt1 increased tumor incidence and higher tumor grade in the absence of E7. Sirt1-deficient mice also exhibited more hyperplastic tissue compared to those with a functional Sirt1 gene. E7-expressing mice deficient in Sirt1 had a higher tumor multiplicity than those sufficient with Sirt1, but this difference was not statistically significant. Further examination is needed to determine if loss of Sirt1 had a bona fide impact on tumor incidence in the context of E7-mediated tumorigenesis. Our findings show that Sirt1 acts as a tumor suppressor in the head and neck.

21. "HPV E6 triggers up-regulation of the antiviral DNA cytosine deaminase APOBEC3B"

Valdimara C. Vieira, Brandon Leonard, Nuri A. Temiz, Elizabeth A. White, Peter M. Howley, Laurel Lorenz, Denis Lee, Paul Lambert, Marcelo A Soares and Reuben S. Harris

The APOBEC3 proteins are a family of DNA cytosine deaminases that function broadly in innate immunity by restricting viral, transposon and foreign DNA1. Recently, the cytosine-to-uracil activity of APOBEC3B has been implicated as a source of mutagenesis in multiple human cancers2,3. Interestingly, cervical and head/neck cancers, both known to be HPV- associated, are among the tumors displaying the highest APOBEC3B expression and mutational signature3. We therefore hypothesized that HPV infection contributes to the reported up-regulation of APOBEC3B. Here, we show that APOBEC3B is the only APOBEC3 family member significantly up-regulated upon transduction

of N/Tert-1 human keratinocytes with a retrovirus encoding E6 from several high-risk HPV types (p=0.016). No change in expression was detected for cells transduced with low risk or non-cancer HPV E6. To further test our hypothesis, we transfected normal immortal keratinocytes with either the full-length clone of the wildtype HPV18 genome or with an HPV18 genome containing a stop codon in the E6 ORF. This comparison indicated that at least half of the increase caused by HPV18 was dependent on the E6 ORF. Finally, mining of TCGA head and neck cancer samples with reported HPV status showed a trend toward HPV up-regulation in virus-positive tumors (p=0.15). Taken together, these results suggest a model in which HPV E6 induces APOBEC3B up-regulation, which could explain a large proportion of observed mutations in both cervical and head/neck cancers.

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22. "HIV-1 Env s cytoplasmic tail domain regulates multiple aspects of viral cell-to-cell transmission at the virological synapse"

Jaye C. Gardiner, Eric J. Mauer, and Nathan M. Sherer

Retroviral Envelope (Env) glycoproteins act as fusion machines that mediate the merger of virion and cellular membranes during viral entry. In the context of interactions between a virally infected and uninfected cell, Env and receptor can also facilitate the formation of a tight cell-cell adhesion zone known as a virological synapse (VS). The efficiency of viral transmission can be markedly enhanced at the VS relative to cell-free infection, and may also provide for viral spread despite the presence of antiviral factors. How different retroviruses form a VS, undergo cell-cell transmission and ensure VS turnover is only partially characterized. Here, we describe a genetically tractable visual approach for studying VS formation and turnover for the lentivirus human immunodeficiency virus type 1 (HIV-1). First, we demonstrate that recruitment of the HIV-1 Gag structural protein to the VS requires crosstalk between Gag s N-terminal Matrix (MA) domain and a bipartite signaling determinant found within Env s cytoplasmic tail domain (CTD). Second, we use long-term (>48 h) live cell imaging to confirm that Env-receptor binding regulates the duration and nature of interaction between infected and uninfected target T cells. Moreover, the Env CTD plays a key role in regulating VS morphology and, ultimately, in preventing syncytia formation. Interestingly, preliminary data suggests that the MA domain also contributes to the stability and duration of VS-regulated cell-cell interactions, even in the presence of wild-type Env glycoproteins. Taken together, our results are consistent with a model wherein communication between the Env CTD and Gag s MA domain coordinates virion assembly to the VS, provides for cell-to-cell transmission of virions while simultaneously preventing cell-cell fusion, and strengthens linkages between infected and uninfected cells for prolonged periods of time thereby promoting the efficient spread of infection.

23. "Cytokine-Mediated Proliferation and Differentiation of Porcine B Cells into Ig Secreting Plasma Cells"

Michael C Rahe and Michael P Murtaugh

The US swine industry has witnessed the recent introduction and emergence of two novel coronaviruses, porcine epidemic diarrhea virus (PEDV) and swine delta coronavirus (SDCoV), while also continuing to battle with porcine reproductive and respiratory syndrome virus (PRRSV) and swine influenza virus (SIV). How the swine immune system develops an antibody response to these viruses has not been thoroughly studied. Improved understanding of this basic response to infection should lead to enhanced development of vaccines and immunotherapeutics to assist in the fight against viral infection. The B cell is the driving force behind the humoral immune response to viral infection, as the terminal differentiation of B cells into immunoglobulin secreting plasma cells is crucial for the

neutralization, opsonization, and complement fixation of many viral pathogens. Enriched CD21+ porcine B cells were activated in vitro with CD40L and then stimulated with various cytokines for up to 7 days in order to screen cytokines for the capacity to cause cellular proliferation and differentiation. IL-21 was found to be a potent inducer of proliferation on activated CD21+ B cells, when compared to unstimulated cells and other purported stimulatory cytokines. Additionally, stimulation with IL-21 resulted in the differentiation of B cells into immunoglobulin secreting plasma cells. Cells that were stimulated with IL-4, APRIL, and IL-21 differentiated into plasma cells and secreted a significant amount of IgA when compared to cytokines that have been reported to drive an IgA antibody response. These findings identify many essential components of the basic humoral immune response to viral challenge while also setting the stage for future research into how this response may be targeted in order to protect against viral infection.

24. "CD8 T Cell Based HIV Vaccines Is Targeting Conserved Epitopes the Answer?"
Shelby O'Connor, Dane Gellerup, and Alexis Balgeman

Immunodominant CD8 T cell responses emerge in the first few weeks after HIV/SIV infection, suppress virus replication, and select for escape variants. Although T cell based HIV vaccines have not successfully provided sterilizing immunity, vaccine-elicited CD8 T cells may contribute to the control of replication of breakthrough viruses. Designing a vaccine to elicit these potent CD8 T cells in all vaccinated individuals, however, is a daunting challenge. Conceptually, a vaccine that elicits CD8 T cells targeting invariant epitopes that do not accumulate escape mutations may be valuable, but the efficacy of these T cells is unknown. We employ a model of SIVmac239 nef-infected MHC-identical Mauritian cynomolgus macaques to test the hypothesis that CD8 T cells targeting epitopes that do not accumulate escape mutations are unable to detect and destroy virally infected cells. Accordingly, we expect that CD8 T cells targeting epitopes that accumulate variants are more effective at controlling virus replication. To test this hypothesis, we are using the sequences of virus populations and the corresponding host immune responses to create variants of live attenuated SIVmac239 nef designed to elicit CD8 T cells targeting epitopes that do and do not accumulate variants. We will determine whether acute CD8 T cells targeting invariant epitopes can emerge during acute infection and control virus replication.

25. "Endocytosis of the Viral Envelope Glycoprotein Protects Human and Simian Immunodeficiency Virus Infected Cells from Antibody-Dependent Cell-Mediated Cytotoxicity"
Benjamin von Bredow, Juan F Arias, Lisa N Heyer, David T Evans

Lentiviral envelope glycoproteins, including those of the human and simian immunodeficiency viruses, have particularly long cytoplasmic domains. Although the function of this domain is not fully understood, it is known to contain sequences important for regulating the trafficking of the viral envelope glycoprotein (Env) in HIV-1- and SIV-infected cells. Perhaps the best characterized of these is a highly conserved membrane--proximal Yxx¦ motif in the gp41 cytoplasmic tail required for AP-2-dependent endocytosis. Amino acid substitutions in this motif increase Env expression on the surface of infected cells and Env incorporation into virions. Using an assay designed to measure the killing of virus--infected cells by antibody--dependent cell--mediated cytotoxicity (ADCC), we show that substitutions in the membrane--proximal endocytosis motif of gp41 cause an increase in susceptibility to ADCC that correlates with elevated levels of Env on the surface of HIV-1- and SIV--infected cells. In the case of HIV-1, this effect is additive with a deletion in vpu recently shown to increase the susceptibility of HIV-1-infected cells to ADCC as a result of tetherin-mediated accumulation of virons on the cell surface. These results reveal a previously unappreciated role for the gp41 cytoplasmic domain in protecting HIV-1- and SIV-infected cells from ADCC by regulating the amount of Env present on the cell surface prior to virus assembly.

26. "When (neutralizing) antibodies fail: inducing cross-reactive immunity to influenza"
Thomas Friedrich

Background : Current influenza vaccines primarily aim to induce neutralizing antibodies (NAbs). These vaccines are highly effective against well-matched strains, but do not offer broad protection against emerging strains. A vaccine that elicits both T cell and B cell responses might induce more broadly protective immunity. Modified vaccinia Ankara (MVA) is a safe and well-characterized vector for inducing both antibody and cellular immunity; in this study, we evaluated the immunogenicity and protective efficacy of MVA vaccines expressing hemagglutinin (HA) and/or nucleoprotein (NP) in a translational macaque model. Methods We tested 4 MVA constructs encoding either HA from an H1N1 virus; the H1 HA with NP from an H5N1 virus; HA and NP from an H5N1 virus; or H5N1 NP alone. Vaccines were given to cynomolgus macaques (Macaca fascicularis) intradermally in 2 doses of 10^8 pfu 4 weeks apart. 8 weeks later, animals were challenged with 10^7 pfu of the pandemic isolate A/California/04/2009 (H1N1pdm). During the vaccination and challenge phases, we monitored neutralizing and HA- binding antibody responses; virus-specific CD4+ and CD8+ T cells;; and antibody-dependent natural killer (NK) cell activation.
Results : MVA-based vaccines encoding HA induced potent serum antibody responses against homologous H1 or H5 HAs, but did not stimulate strong T cell responses prior to challenge. However, animals vaccinated with MVA encoding influenza antigens made strong virus-specific CD4+ and CD8+ T cell responses within the first 7 days of H1N1pdm infection, while animals vaccinated with MVA encoding irrelevant antigens did not. There was little or no H1N1pdm replication in animals that received vaccines encoding H1 (homologous) HA, while in contrast, the vaccine encoding only NP from an H5N1 virus provided no protection. Surprisingly, there was a trend toward reduced shedding of H1N1pdm in animals vaccinated with MVA encoding HA and NP from an H5N1 isolate. This reduced shedding was associated with cross-reactive antibodies capable of binding H1 HA protein and eliciting antibody--dependent cellular cytotoxicity (ADCC) effector functions from NK cells. Conclusions Our results, together with previous studies in our lab, suggest that ADCC may play a previously unrecognized role in heterosubtypic immunity to influenza. Vaccines optimized to induce cross-reactive ADCC antibodies may provide an important measure of protection against emerging antigenic variants, including emerging pandemic viruses, when NAbs are ineffective.