'Wisc-e-sota'

3rd Joint UMN-UW Virology Training Grant Symposium



September 24th, 2015 7-10pm

Opening reception & Keynote at Piggy's Restaurant

September 25th, 2015 9am-4:30pm

UW-La Crosse, Cartwright Center

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

3rd Annual *'Wisc-e-sota'* Virology Symposium
Thursday-Friday, September 24-25th, 2015
UW-La Crosse
Cartwright Center

ORAL PRESENTATIONS
Talk abstract numbers in left column

Thursday, September 24, 2015

6:30 – 7:00 pm	Registration Piggy's 501 Front Street S, La Crosse, WI 54601 (608) 784-4877
7:00 – 10:00pm	Opening Reception at Piggy's
7:40 – 7:45pm	Welcome & Opening Remarks
7:45 – 8:25pm	Opening Talk David Largaespada, American Cancer Society Professor, UMN (UMN & UW, McArdle Lab alumnus) "Using Transposons to Understand Cancer"

Friday, September 25, 2015

	8:30am	Continental breakfast and registration
		Convenor: Erik Eriksen
1	9:00am	Mark Schleiss (UMN) **"Cytomegalovirus: Pathogenesis, Prevention and Vaccination"**
2	9:30am	Reza Djavadian and Eric Johannsen (UW) **"Genetic analysis implicates an Epstein-Barr virus (EBV)-encoded pre-initiation complex as a critical mediator of late gene expression"**
3	9:45am	Terumasa Ikeda, John S. Albin, Menelaos Symeonides, Ming Li, Dan P. Mundt, George M. Shaw, Beatrice H. Hahn, Markus Thali and Reuben S Harris (UMN) **"Env Substitutions Enhance Gag-Pol Packaging and Protect from APOBEC3G Restriction"**
	10:00 – 10:30am	Morning coffee break and poster set-up
		Convenor: Christopher Richards
4	10:30am	Aayushi Uberoi and Paul Lambert (UW) **"Examining the Role of the Recently Discovered Murine Papillomavirus (MmuPV-1) in Skin Tumorigenesis"**
5	10:45am	Jessica Martin, Sheng Cao, Jose Maldonado, Wei Zhang and Lou Mansky (UMN) **"Distinct morphologies observed among retrovirus-like particles as determined by cryo-electron microscopy"**

6	11:00am	Jamie L. Schafer, Moritz Ries, Natasha Guha, Michelle Connole, Arnaud D. Colantonio, Emmanuel J. Wiertz, Nancy A. Wilson, Amitinder Kaur and David T. Evans (UW) **"Suppression of a Natural Killer Cell Response by Simian Immunodeficiency Virus"**
	11:30 – 2:30pm	Lunch & Poster Session
		Convenor: DJ Nawandar
7	2:30pm	Michael Murtaugh, Cheryl Dvorak, Diem Ngo and Suzanne Stone (UMN) **"Porcine circovirus 2 life history, and why it matters"**
8	3:00pm	Lindsey Moser, Pei-Yin Lim, Linda M. Styer, Laura D. Kramer and Kristen Bernard (UW) **"Influence of location, dose, and timing of mosquito saliva on enhancement of West Nile virus replication"**
9	3:15pm	Jessica Fiege and Ryan Langlois (UMN) **"Influenza A Virus tropism in hematopoietic and non-hematopoietic cells in the lung"**
		Convenor: Isaac Angert
10	3:30pm	Matthew Sutton, Alexis Balgeman, Dane Gellerup, Amy Ellis, Andrew Van Pay, Max Harris, Ericka Becker, and Shelby O'Connor (UW) **"Evaluating Immunity Elicited by CD8 T Cell Responses Targeting Invariant Epitopes"**
11	3:45pm	Prasad, S, Hu, S, Sheng, WS and Lokensgard JR. (UMN) **"Tregs modulate proliferation of T-cells and microglia following viral brain infection"**
12	4:00pm	Ginger M. Pocock, Jordan T. Becker, Paul Ahlquist and Nathan M. Sherer (UW) **"Export elements couple retroviral mRNA nuclear egress to distinct cytoplasmic trafficking regimes"**
	4:30pm	Closing remarks

Poster Presentations

Poster abstract numbers in the left column

1. "**The role of Hepatitis B virus gene X mutant variants in liver cancer"**
   Barbara R Tschida, Timothy P Kuka, Carlos A Acosta, Lindsey A Lee, Pauline J Jackson, Vincent W Keng, and David A Largaespada

2. **"Genetic and computational approaches elucidate the binding interface between human APOBEC3F and HIV-1 Vif"**
   C. Richards

3. **"Fluorescence Lifetime Imaging for the Study of Gag Assembly"**
   Isaac Angert, Jessica Martin, Joachim Mueller and Louis Mansky

4. **"Determination of human T-cell leukemia virus type 1 proviral load and genome structure in chronically infected T-cell lines"**
   Morgan Meissner, LeAnn Oseth, Jessica Martin, Luiza Mendonca, Wei Zhang, and Louis Mansky

5. **"Degradation of the Cancer Genomic DNA Deaminase APOBEC3B by SIV Vif"**
   Allison M. Land, Jiayi Wang, Emily K. Law, Ryan Aberle, Andrea Kirmaier, Annabel Krupp, Welkin E. Johnson, and Reuben S. Harris

6. **"Targeted mutagenesis of cytomegalovirus mediated by CRISPR/Cas9: an alternative to BAC recombineering"**
   Craig J. Bierle and Mark R. Schleiss

7. **"Microbial translocation during early acute AIDS virus infection"**
   Adam J. Ericsen, Michael Lauck, Mariel S. Mohns, Sarah R. DiNapoli, James P. Mutschler, Justin M. Greene, Jason T. Weinfurter, Gabrielle Lehrer-Brey, Kristin A. Crosno, Eric J. Peterson, Roger W. Wiseman, Benjamin J. Burwitz, Jonah B. Sacha, Thomas C. Friedrich, Jason M. Brenchley, & David H. O'Connor

8. **"Evaluating the Role of MCPyV Large T Antigen-pRb Interactions in Promoting In Vivo Phenotypes"**
   Megan E. Spurgeon and Paul F. Lambert

9. **"Promotion of EBV lytic reactivation in oral keratinocytes"**
   Kathleen Makielski, Dhananjay Nawandar, Denis Lee, Laurel Lorenz, Bill Sugden, Shannon Kenney and Paul Lambert

10. **"Differentiation-dependent KLF4 expression promotes lytic Epstein-Barr Virus infection in epithelial cells"**
    Dhananjay M. Nawandar, Anqi Wang, Kathleen Makielski, Denis Lee, Shidong Ma, Elizabeth Barlow, Jessica Reusch, Coral K. Wille, Deborah Greenspan, John S. Greenspan, Janet E. Mertz, Eric C. Johannsen, Paul F. Lambert, and Shannon C. Kenney

11. **"Location, location, location: HIV-1 genomic RNA trafficking controls virus particle production"**
    Jordan Becker and Nathan Sherer

12. **"Progress on Developing Small Chemical Inhibitors of the APOBEC3 Family of DNA Cytosine Deaminases"**
    Ming Li, Margaret E. Olson, Daniel A. Harki, and Reuben S. Harris

13. **“Transcriptional Regulation of APOBEC3 Antiviral Immunity Through the CBF²/RUNX Axis”**
Brett D. Anderson and Reuben S. Harris

14. **“The Complex Relationship Between Oncoviruses and APOBEC Mutagenesis in Cancer”**
Gabriel J. Starrett, James A. DeCaprio and Reuben Harris

15. **“Is Endogenous APOBEC3H a Transmission Barrier against HIV-1?”**
Jiayi Wang, Allison M. Land, Brian J. Hoium, Eric W. Refsland, Elizabeth M. Luengas, Romel D. Mackelprang, William L. Brown, Michael Emerman, Jairam Lingappa and Reuben S. Harris

16. **“Whole transcriptome analysis of young and adult host porcine macrophages infected by PRRSV”**
Xiong Wang, Sally R. Robinson, Diem K. Ngo and Michael P. Murtaugh

17. **“PCV2 NEUTRALIZING ANTIBODY LEVELS IN COLOSTRUM FROM FARMS WITH HIGH OR LOW LEVELS OF VIREMIA”**
CMT Dvorak, BJ Payne, JL Seate, and MP Murtaugh

18. **“Hypoxia-induced Lytic Reactivation of EBV Requires Both HIF-1± and Activated Wild-type p53”**
Richard J. Kraus, Xianming Yu, Saranya Sathiamoorthi, Shidong Ma, Shannon C. Kenney, and Janet E. Mertz

19. **“1.99 angstrom zinc-free APOBEC3F catalytic domain crystal structure”**
Nadine M. Shaban, Ke Shi, Ming Li, Hideki Aihara, Reuben S. Harris

20. **“Discovery, characterization, and ecology of a Novel Hepatitis A-like virus in wild Olive Baboons (Papio anubis), Uganda”**
Andrew J. Bennett, Samuel D. Sibley, Michael Lauck, Geoffrey Weny, David Hyeroba, David H. OConnor, Tony L. Goldberg

21. **“Viewing the biogenensis of HTLV-1 and HIV-1 particles in living cells”**
John Eichorst, Joachim Mueller and Louis Mansky

22. **“Factors driving porcine B cell proliferation and differentiation dynamics”**
Michael C Rahe and Michael P Murtaugh

Abstract – Opening Talk

"Using Transposons to Understand Cancer"
David Largaespada

Understanding the complexity of genetic and epigenetic alterations in human cancer remains a daunting challenge. Using data from The Human Cancer Genome Atlas (TCGA) it was suggested recently that human tumors fall generally into one of two categories. "M" class tumors are dominated by recurrent somatic mutations (mostly single nucleotide variants), and a second class of tumors, the "C" class, are dominated by recurrent gene copy number alteration and *TP53* mutation (Ciriello et al., *Nature Genet.*, 2013). Defining drivers associated with C class tumors will remain a challenge without strong functional studies to sort through the hundreds of candidates that are altered at the gene copy number level. We have developed an approach to define strong candidate cancer genes in mouse models using unbiased, forward genetic screens based on transposon insertional mutagenesis using the *Sleeping Beauty* transposon system. We hypothesize that data from these screens will help to identify genetic drivers of human cancer that are altered at the gene copy number or epigenetic levels. This screening method was inspired by the study of slow transforming retroviruses, which can cause cancer by insertional mutagenesis. The study of viruses has played a major role in the history of cancer research. Like viruses, transposons exist in equilibrium with their hosts over time. I will briefly discuss viruses and cancer, transposon biology, and results from our *Sleeping Beauty* transposon-based cancer screens in mice, with particular attention to the identification of cancer genes altered at the epigenetic or copy number level.

Abstracts – Oral Presentations

1. "Cytomegalovirus: Pathogenesis, Prevention and Vaccination"
Mark R. Schleiss

In this overview of the epidemiology, molecular biology and prevention of cytomegalovirus infections, I will discuss current concepts regarding immune evasion, viral pathogenesis, correlates of protective immunity, and state-of-the-art review of vaccines in preclinical development and clinical trials. Emphasis will be placed on the value of animal models in the study of cytomegalovirus vaccines.

2. "Genetic analysis implicates an Epstein-Barr virus (EBV)-encoded pre-initiation complex as a critical mediator of late gene expression"
Reza Djavadian and Eric Johannsen

Epstein-Barr virus replication is accomplished by an intricate cascade of gene expression that integrates DNA replication and structural protein synthesis. Most genes encoding structural proteins exhibit true late kinetics their expression is strictly dependent on DNA replication. Recently, the EBV BcRF1 gene was reported to encode a TATA binding protein homolog, which preferentially recognizes the TATT sequence found in true late gene promoters. BcRF1 is one of 7 EBV genes with homologs found only in other beta and gamma-herpesviruses, but absent in alpha-herpesviruses. Using an EBV BAC-mid, we disrupted these beta-gamma genes and found 6 of these genes, including BcRF1, exhibited the identical phenotype: intact viral DNA replication with loss of late gene expression. These same 6 genes have been found by other investigators to be essential for activation of TATT promoters in reporter assays. Our results demonstrate the relevance of these findings for late gene expression in the context of whole virus.

3. "Env Substitutions Enhance Gag-Pol Packaging and Protect from APOBEC3G Restriction"
Terumasa Ikeda, John S. Albin, Menelaos Symeonides, Ming Li, Dan P. Mundt, George M. Shaw, Beatrice H. Hahn, Markus Thali and Reuben S Harris

HIV-1 replication and pathogenesis require Vif-mediated degradation of several APOBEC3 antiviral DNA cytosine deaminases. Without Vif viruses succumb to a combination of deamination-dependent and independent restriction mechanisms. Here, we used a series of HIV-1 adaptation studies to ask whether viruses with an irreparable vif deletion could develop resistance to restrictive levels of APOBEC3G. Interestingly, several resistant viruses had multiple amino acid substitutions in Env and these changes protected Vif-null viruses from APOBEC3G-dependent restriction in T cells despite restrictive levels of APOBEC3G packaging. These Env adaptations led to higher levels of reverse transcriptase in viral particles, which in turn correlated with faster virus DNA replication and reduced APOBEC3G-mediated hypermutation of viral replication intermediates. Taken together, these studies demonstrate a novel Env- and RT-dependent mechanism that HIV-1 can use to escape restriction by APOBEC3G.

4. "Examining the Role of the Recently Discovered Murine Papillomavirus (MmuPV-1) in Skin Tumorigenesis"
Aayushi Uberoi and Paul Lambert

The recent discovery of the 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 presumably due to deregulation of Rb. 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 evidence that indicates that ultraviolet radiation assists MmuPV1 in causing wart formation and squamous cell carcinoma in such mice. Our studies suggest that ultraviolet radiation appears to have a systemic effect on the biology of the host making it susceptible to MmuPV induced cancers. This is a novel finding and shows that MmuPV1 infection model can be used to study infection and pathogenesis of high-risk cutaneous papillomaviruses.

5. "Distinct morphologies observed among retrovirus-like particles as determined by cryo-electron microscopy"
Jessica Martin, Sheng Cao, Jose Maldonado, Wei Zhang and Lou Mansky

The retroviral Gag protein is the main structural protein responsible for virus particle assembly and release. The Gag protein alone is generally sufficient for production of virus-like particles (VLPs). We have previously noted differences in morphologies among retrovirus-like particles. However, there has previously been no comprehensive study done to compare in parallel the morphologies among retrovirus genera using Gag-only VLPs. Here in this study, we have produced VLPs of representatives from all seven of the retroviral genera i.e., alpharetrovirus, betaretrovirus, deltaretrovirus, epsilonretrovirus, gammaretrovirus, lentivirus, and spumaretrovirus in parallel and compared by cryoelectron microscopy (cryoEM). We produced VLPs by transiently expressing in HEK293T cells the Gag and Envelope (Env) proteins of Rous sarcoma virus (RSV, alpha), Mason-

Pfizer monkey virus (MPMV, beta), bovine leukemia virus (BLV, delta), walleye dermal sarcoma virus (WDSV, epsilon), murine leukemia virus (MuLV, gamma), human immunodeficiency virus type 2 (HIV-2, lenti), and human foamy virus (HFV, spuma). VLPs were purified using sucrose density gradients, concentrated, and analyzed by cryoEM. Common among all VLPs studied was a distribution of VLP size, which spanned an overall range of 82 to 151 nm. Some of the differences in VLP morphology included a distinctly large Env-like protein of 16 nm +/- 2nm seen coating HFV-like particles. Other retroviral-like particles had no visible Env-like proteins on their surfaces. A distinct and characteristic immature Gag lattice was observed with HIV-2, MLV and BLV, whereas there was a lack of a distinct Gag lattice observed with RSV and HFV. Intriguingly, the size distribution of HIV-2-like particles was quite narrow compared to that of the other retroviral genera, with more than 60% falling within the range of 140-159 nm, and were quite uniform in their morphology. These observations were in stark contrast with the observations made with other retroviral-like particles, where no greater than 35% of the particles for a given retrovirus fell within 10 nm of the mean. Taken together, our observations indicate that while VLPs from the various retroviral genera have common features, distinct differences were noted for some which may provide important clues for understanding unique aspects of virus particle assembly and virus structure.

6. "Suppression of a Natural Killer Cell Response by Simian Immunodeficiency Virus"
Jamie L. Schafer, Moritz Ries, Natasha Guha, Michelle Connole, Arnaud D. Colantonio, Emmanuel J. Wiertz, Nancy A. Wilson, Amitinder Kaur and David T. Evans

Natural killer (NK) cell responses in primates are regulated in part through interactions between two highly polymorphic molecules, the killer-cell immunoglobulin-like receptors (KIRs) on NK cells and their major histocompatibility complex (MHC) class I ligands on target cells. We previously reported that the binding of a common MHC class I molecule in the rhesus macaque, Mamu-A1*002, to the inhibitory receptor Mamu-KIR3DL05 is stabilized by certain simian immunodeficiency virus (SIV) peptides, but not by others. We have now investigated the functional implications of these interactions by testing SIV peptides bound by Mamu-A1*002 for the ability to modulate Mamu-KIR3DL05+ NK cell responses. Twenty-eight of 75 SIV peptides bound by Mamu-A1*002 suppressed the cytolytic activity of primary Mamu-KIR3DL05+ NK cells, including three immunodominant CD8+ T cell epitopes previously shown to stabilize Mamu-A1*002 tetramer binding to Mamu-KIR3DL05. Substitutions at C-terminal positions changed inhibitory peptides into disinhibitory peptides, and vice versa, without altering binding to Mamu-A1*002. The functional effects of these peptide variants on NK cell responses also corresponded to their effects on Mamu-A1*002 tetramer binding to Mamu-KIR3DL05. In assays with mixtures of inhibitory and disinhibitory peptides, low concentrations of inhibitory peptides dominated to suppress NK cell responses. Consistent with the inhibition of Mamu-KIR3DL05+ NK cells by viral epitopes presented by Mamu-A1*002, SIV replication was significantly higher in Mamu-A1*002+ CD4+ lymphocytes co-cultured with Mamu-KIR3DL05+ NK cells than with Mamu-KIR3DL05- NK cells. These results demonstrate that viral peptides can differentially affect NK cell responses by modulating MHC class I interactions with inhibitory KIRs, and provide a mechanism by which immunodeficiency viruses may evade NK cell responses.

7. "Porcine circovirus 2 life history, and why it matters"
Michael Murtaugh, Cheryl Dvorak, Diem Ngo and Suzanne Stone

Porcine circovirus 2 (PCV2) was recognized as a disease-causing agent in pigs in the 1990's, but no relationship could be discerned between infection and disease based on viral genetics or viral loads. Disease pathogenesis is broadly defined since, even today, clinical signs vary widely and herds suffering outbreaks contain a mixture of affected and unaffected individuals in which there are no apparent differences in viral characteristics or viral loads. Our lab waded into this morass about 10 years ago, when PCV2-associated disease had not been reported in the U.S., to participate in a national survey of swine health to determine PCV2 infection incidence and

characteristics. Surprisingly, infection was extremely widespread, suggesting that the depth of the virus-host interaction was not fully appreciated. Using molecular, immunological and cell biological approaches, we determined that PCV2 establishes a vertically transmitted, persistent, life-long infection in the face of high titers of neutralizing antibodies. While natural infection elicits an ineffective immune response, vaccination with recombinant capsid protein completely prevents disease without curing viral infection. In 8 to 9 years following universal piglet vaccination, viremia levels have decreased dramatically, yet disease reappears rapidly in cases of lapsed vaccination. The PCV2 genome encodes a capsid and a polymerase, with two additional proteins possibly expressed. Thus, it provides an experimental model for complete immunological characterization of a virus-host interaction with ample opportunities for discovery since due to its unusual propensity for nonclinical persistence in the face of a robust immune response.

8. "Influence of location, dose, and timing of mosquito saliva on enhancement of West Nile virus replication"
Lindsey Moser, Pei-Yin Lim, Linda M. Styer, Laura D. Kramer and Kristen Bernard

The arbovirus West Nile virus (WNV) emerged in New York in 1999 and quickly spread throughout the United States. Transmission is maintained in an enzootic cycle in which the virus is spread to susceptible hosts via the bite of infected mosquitoes. Arthropod-derived components within the viral inoculum are increasingly acknowledged to play a role in infection of subsequent hosts. Our laboratory has previously shown that Culex tarsalis mosquito saliva and salivary gland extract (SGE) enhance replication of West Nile virus in a mouse model of infection. Here, we report further characterization of this observation by examining the effective dose, proximity, and timing of saliva or SGE administration. We show that a single mosquito can enhance viral replication. Further investigation demonstrated that as little as 10 ng of SGE increases WNV replication. These data suggest that the active salivary factor is potent. We also investigated whether the interaction of saliva and virus is necessary for increased replication by examining the effects of spatial and temporal separation of salivary treatment and virus infection. We show that enhancement of viral replication requires co-localized injection of SGE and virus; however, enhanced viral titers are observed when SGE is administered up to 24 hours prior to or 12 hours after viral infection. These data suggest that the active factor or its effects are stable and localized. Our laboratory is currently conducting studies to identify the active factor(s) and the mechanisms by which WNV replication is enhanced. These studies could lead to the identification of novel prophylactic or treatment options useful in limiting the spread and disease of WNV and other mosquito-borne viruses.

9. "Influenza A Virus tropism in hematopoietic and non-hematopoietic cells in the lung"
Jessica Fiege and Ryan Langlois

Previously, Influenza A virus (IAV) infected cells have been difficult to track long term as infection results in death of infected cells either through the lytic phase of the virus or the innate and adaptive arms of the immune system. Therefore, we developed an IAV expressing cre recombinase (IAV-cre) to indelibly label infected cells from reporter mice. This made the surprising finding that infected lung epithelial cells can survive the acute infection phase. My initial experiments have identified enrichment of infected cells in both podoplanin and CC10 expressing lung epithelial cells. Additionally, both macrophages and dendritic cells were infected during acute IAV infection, but these infected cells were not detected after clearance of the virus. Both infection by IAV and the resulting immune response can eliminate infected cells, so identifying the specific cells infected can allow us to better understand the mechanisms of lung damage after IAV infection. Future experiments will restrict virus replication to specific cell types, or selectively deplete immune cell populations to elucidate which immune cells are responsible for killing specific virus-infected cell populations and identify sources of both virus and immune related pathology. These findings will aid in our understanding of IAV tropism and the relationship to lung pathology.

10. "Evaluating Immunity Elicited by CD8 T Cell Responses Targeting Invariant Epitopes"
Matthew Sutton, Alexis Balgeman, Dane Gellerup, Amy Ellis, Andrew Van Pay, Max Harris, Ericka Becker, and Shelby O'Connor

Contending with the enormous sequence diversity of HIV is a major challenge for designing a preventative or therapeutic HIV vaccine designed to elicit effective CD8 T cells. These cells can control virus replication, but they also select for T cell escape variants. One rational approach to address this sequence diversity is to elicit CD8 T cells that target the most conserved viral regions. Notably, previous data from our group suggests that T cells targeting epitopes that accumulate variants are necessary for virus control. In this current study, we wanted to test the hypothesis that CD8 T cells specific for invariant epitopes that do not accumulate escape variants are unable to efficiently detect and destroy virally-infected cells, even when such CD8 T cells can produce antiviral cytokines in vitro. We are testing this hypothesis with Mauritian cynomolgus macaques (MCMs) infected with SIVmac239nef and variant SIVnef sequences with CD8 T cell epitopes ablated. We are using MCMs who are homozygous for the M3 MHC haplotype, as animals with this MHC genotype typically do not control SIVmac239 replication. By deep sequencing, we characterized the diversity of twelve M3-restricted CD8 T cell epitopes that are present in SIVmac239nef. We also infected four M3 homozygous MCMs with SIVmac239nef and performed IFN^{3}-ELISPOT assays with PBMC and peptides corresponding to the 12 epitopes and their variants. We are using this information to create SIVnef viruses with ablation of specific T cell epitopes that we will test in vitro and in vivo. We identified a variant in each of the 8 SIV epitopes known to rapidly accumulate mutations within M3 homozygous MCMs that avoid immune detection by IFN^{3}-ELISPOT. These variants were engineered into SIVmac239nef to create an SIVnef-8x virus that retains only the invariant epitope sequences. Currently, we are growing the SIVnef-8x virus and testing this virus in vitro and in vivo replicative to ensure that the ablations made do not affect viral fitness, and to assess control in M3 homozygous and MHC mismatched animals. We have shown that variants within epitopes known to rapidly accumulate mutations can be identified, and that these variants are not detected by IFN^{3}-ELISPOT assays with PBMC from SIVmac239nef infected M3 homozygous MCMs. These findings have enabled the creation of a virus that we expect to direct the immune response towards those conserved regions that are able to resist immune escape. With this virus we will specifically assess the value of CD8 T cell responses directed towards invariant sequences. Establishing the value, or lack thereof, of these epitopes in a vaccine is both timely and highly significant.

11. "Tregs modulate proliferation of T-cells and microglia following viral brain infection"
Prasad, S, Hu, S, Sheng, WS and Lokensgard JR.

Regulatory T-cells (Tregs) are well known to play crucial roles in suppression of immune responses during infection and autoimmunity. Accumulation and retention of Treg cells has been reported within post viral-encephalitic brains. However, the extent to which these Tregs modulate neuroinflammation is yet to be elucidated. Here, we used Foxp3-DTR-GFP knock-in transgenic mice, which upon administration of low dose of diphtheria toxin (DTx) results in specific deletion of Tregs. We investigated the proliferation status of various immune cell subtypes within inflamed central nervous system (CNS) tissue. We observed that depletion of Tregs resulted in increased proliferation frequencies of CD8+ and CD4+ T-cells, as well as brain-resident microglial cells during the acute phase of viral infection (i.e., 7 d post-infection, dpi). In contrast, immune cell proliferation rate was controlled in untreated animals by 14 dpi when compared to Dtx-treated mice. Previous studies by us and others have demonstrated that Treg numbers within the brain rebound by 20 dpi following Dtx treatment to higher numbers than in untreated animals. Despite this rebound, microglia and CD4+ T-cells proliferated at a higher rate when compared to that of Treg-sufficient mice, thus maintaining sustained neuroinflammation. Furthermore, at 30 dpi we found the

majority of CD8+T cells were CD127hi KLRG1- indicating that the cells were long lived memory precursor cells. These memory cells showed marked elevation of CD103 expression (a marker of tissue resident-memory T-cells, Trm), from DTx-treated animals. In contrast, a small percentage of CD4+ T-cells expressed CD103, which was also found at negligible levels in cervical lymph nodes. In summary, our findings demonstrate that Tregs limit neuroinflammatory responses to viral brain infection by controlling proliferation of immune cell types within the brain and may direct a larger proportion of brain-infiltrating CD8+ T-cells to be maintained as Trm cells.

12. "Export elements couple retroviral mRNA nuclear egress to distinct cytoplasmic trafficking regimes"

Ginger M. Pocock, Jordan T. Becker, Paul Ahlquist and Nathan M. Sherer

Retroviruses encode cis-acting RNA elements that override cellular blocks to the nuclear export and cytoplasmic utilization of intron-containing viral mRNAs. HIVs Rev response element (RRE) recruits the CRM1 nuclear export receptor through the activity of the viral Rev protein, while retroviruses that lack Rev-like proteins encode constitutive transport elements (CTEs) that directly recruit components of the NXF1/NXT mRNA nuclear export machinery. Why retroviruses preferentially exploit one or the other pathway is unknown. Here, we employed live cell imaging to directly monitor the integrated stages of retroviral genomic RNA (gRNA) trafficking, translation, and virus particle assembly in real time and in single living cells. We show that HIV-1s RRE governs pulsiform Rev- and CRM1-dependent transitions of gRNAs from the nucleus to the cytoplasm, upstream of viral late gene expression and virus particle assembly that occurs at the plasma membrane. In striking contrast, gRNAs bearing the CTE from Mason-Pfizer monkey virus rapidly target the centrosome that forms the pericentriolar core of the microtubule-organizing center (MTOC) prior to capsid formation that occurs in the cytoplasm for this virus. Interestingly, CTE-bearing transcripts were targeted to centrosomes both during interphase also during metaphase, with transcripts enriched even to the poles of the mitotic spindle coincident with nuclear membrane and subsequently partitioned to the cytoplasm of daughter cells during cytokinesis. These results demonstrate that viral mRNA nuclear history can be coupled to distinct cytoplasmic transport regimes relevant to particular virus replication strategies. Moreover, NXF1 tethering was sufficient to direct non-viral mRNAs to the MTOC even in the absence of a viral RNA element, revealing a novel cellular pathway by which NXF1 and microtubules can cooperate to spatially restrict mRNAs to the centrosome at multiple stages of the cell cycle.

Abstracts – Poster Presentations

1. "The role of Hepatitis B virus gene X mutant variants in liver cancer"

Barbara R Tschida, Timothy P Kuka, Carlos A Acosta, Lindsey A Lee, Pauline J Jackson, Vincent W Keng, and David A Largaespada

Hepatocellular carcinoma (HCC), or liver cancer, is the 2nd leading cause of death from cancer worldwide. Understanding the mechanisms of HCC development and maintenance is needed to develop effective prevention and treatment strategies. 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. The HBV viral regulatory protein, HBx, can activate transcription from both viral and cellular promoters to facilitate viral replication, and is believed to contribute to HBV-associated HCC. We have shown HBx contributes to HBV-associated oncogenesis in a mouse model of somatic HBx expression in the liver. HBV expressing HBx variants including a 2 nucleotide point mutant and a C-terminal truncation are associated with increased HCC risk compared to wildtype HBV. To determine if these variants are more oncogenic than wildtype HBx, we tested them in vivo using the selective fumarylacetoacetate hydrolase-deficient (Fah-/-) mouse model. Fah-/- mice have a tyrosine metabolism defect and die from liver failure unless maintained on a protective drug, Nitisinone. We use hydrodynamic injection into the tail vein to deliver plasmids carrying both a gene of interest and an Fah rescue cDNA on a Sleeping Beauty (SB) transposon to hepatocytes of Fah-/- mice with SB knocked into the Rosa26 locus for ubiquitous expression. Nitisinone is withdrawn from the drinking water, and only the cells in which the transposon has been integrated and is stably expressed survive to regenerate the liver as the Fah-/- hepatocytes die. This allows for the rapid generation of mice with livers expressing the transgene in nearly every hepatocyte. We used this system to generate mice expressing point mutated (HBxM), truncated (HBxTr), or wildtype (HBxWT) HBx along with shTP53 to predispose to tumor formation. Mice injected with HBxM had a higher average tumor burden than mice injected with HBxWT. No increase in tumor burden or penetrance was seen with HBxTr. In sum, we have developed new mouse models of liver cancer driven by two variants of HBx associated with increased HCC incidence in humans, and we found the point mutant variant to have increased oncogenic potential compared to wildtype HBx. Experiments are underway to determine the mechanism underlying the oncogenic roles of HBx variants by expressing them in human hepatocyte cell lines and examining cellular gene expression changes, transformation, and changes in HBx protein stability.

2. "Genetic and computational approaches elucidate the binding interface between human APOBEC3F and HIV-1 Vif"

C. Richards

APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H combine to potently restrict viral reverse transcription by deamination-dependent and -independent mechanisms. However, the HIV-1 accessory protein Vif counteracts restriction by nucleating the formation of an E3 ubiquitin ligase complex to mediate their degradation. Despite advances in both APOBEC3 and Vif structural biology, a molecular understanding of this direct host-pathogen interaction has been elusive. Here,
virus adaptation and computational studies are used to derive a model for the APOBEC3F-Vif interaction that best explains available genetic and biochemical data. A single amino acid derivative of human APOBEC3F (E324K) as well as rhesus macaque APOBEC3F, which naturally has K324, both selected for the emergence of a negative charge at position 71 in HIV-1 Vif (i.e., G71D). The functionality of this compensatory electrostatic change was validated in virus infectivity studies and then used as a constraint to dock the two proteins and produce a model for optimization by molecular dynamics simulations. Interestingly, 1 microsecond simulations revealed a long-lasting electrostatic interaction between APOBEC3F E289 and HIV-1 Vif R15, which was similarly validated by charge swapping experiments. These gain-of-function results fortify data demonstrating the importance of these and other residues in prior loss-of-function studies. Together, we propose the first structural model for

the APOBEC3F-Vif interaction that rationalizes prior observations and provides a foundation for further investigations including structure-guided antiviral drug discovery to leverage the full restriction potential of the APOBEC3 enzymes.

3. "Fluorescence Lifetime Imaging for the Study of Gag Assembly"
Isaac Angert, Jessica Martin, Joachim Mueller and Louis Mansky

Fluorescence lifetime imaging microscopy (FLIM) can give unique insight into molecular interactions through observation of fluorescence resonant energy transfer (FRET) between fluorescently tagged protein constructs. We use the FLIM/FRET technique to study Gag assembly in cells transiently expressing fluorescently labeled Gag constructs. The assembly steps between expression of Gag in the cytoplasm and budding of a virus-like particle are of specific interest because they remain poorly understood. We show preliminary application the FLIM/FRET technique to study these 'intermediate' assembly steps and discuss several challenges that have been encountered. We propose extensions of the FLIM/FRET method to overcome these challenges and facilitate FRET measurements in spatially heterogeneous systems.

4. "Determination of human T-cell leukemia virus type 1 proviral load and genome structure in chronically infected T-cell lines"
Morgan Meissner, LeAnn Oseth, Jessica Martin, Luiza Mendonca, Wei Zhang, and Louis Mansky

Human T-cell leukemia virus type-1 (HTLV-1) infects about 15 million people worldwide and results in an incurable leukemia in about 5% of individuals infected. Progress in the study of the mechanisms of viral replication and particle structure have been limited given the difficulty in growing the virus in cell culture. Previous work utilizing the chronically infected MT-2 cells demonstrated that viral particles are highly polymorphic, particularly in regards to capsid core structure. MT-2 cells contain multiple proviruses, and their genomic structure varies including truncated gag gene sequences. Thus, this provirus diversity could contribute to the diversity of particle structures observed. The goal of this project is to identify an HTLV-1 T-cell line harboring proviruses with intact gag sequences which can be used for the study of viral particle morphology. Using fluorescent in-situ hybridization (FISH), I have determined that the chronically infected SP cell line contains only four proviruses, in comparison to other chronically infected lines (ATL-T, ATL-2, and C91PL), which have a wide range of proviral copy numbers (10-12, 15-16, and 19+, respectively). The integration sites for the proviruses in the SP cell line are currently being determined via splinkerette PCR analysis, which will allow for sequence analysis of the full-length proviral genomes. The SP cell line holds promise for virus assembly studies as well as other aspects of the HTLV-1 life cycle.

5. "Degradation of the Cancer Genomic DNA Deaminase APOBEC3B by SIV Vif"
Allison M. Land, Jiayi Wang, Emily K. Law, Ryan Aberle, Andrea Kirmaier, Annabel Krupp, Welkin E. Johnson, and Reuben S. Harris

APOBEC3B is a newly identified source of mutation in many cancers, including breast, head/neck, lung, bladder, cervical, and ovarian. APOBEC3B is a member of the APOBEC3 family of enzymes that deaminate DNA cytosine to produce the pro-mutagenic lesion, uracil. Several APOBEC3 family members function to restrict virus replication. For instance, APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H combine to restrict HIV-1 in human lymphocytes. HIV-1 counteracts these APOBEC3s with the viral protein Vif, which targets the relevant APOBEC3s for proteasomal degradation. While APOBEC3B does not restrict HIV-1 and is not targeted by HIV-1 Vif, we asked whether related lentiviral Vif proteins could degrade APOBEC3B. Interestingly, several SIV Vif proteins are capable of promoting APOBEC3B degradation, with SIVmac239 Vif proving the most potent. This likely occurs through the canonical polyubiquitination mechanism as APOBEC3B protein levels are restored by MG132 treatment and by altering a conserved E3 ligasebinding motif. We further show that SIVmac239 Vif can prevent APOBEC3B mediated geno/cytotoxicity and degrade endogenous APOBEC3B in

several cancer cell lines. Our data indicate that the APOBEC3B degradation potential of SIV Vif is an effective tool for neutralizing the cancer genomic DNA deaminase APOBEC3B. Further optimization of this natural APOBEC3 antagonist may benefit cancer therapy.

6. "Targeted mutagenesis of cytomegalovirus mediated by CRISPR/Cas9: an alternative to BAC recombineering"

Craig J. Bierle and Mark R. Schleiss

The cytomegaloviruses are among the most genetically complex mammalian viruses, with viral genomes that often exceed 230 kbp. Manipulation of cytomegalovirus genomes is largely done in infectious bacterial artificial chromosomes (BACs), which necessitates the maintenance of the viral genome in E. coli. Using this approach, reconstitution of virus can only occur after transient transfection of fibroblasts. We have developed an alternative strategy that utilizes CRISPR/Cas9-mediated genome editing to introduce targeted mutations directly into the viral genome. In this proof-of-concept study, transfection and drug selection was used to restrict lytic replication of guinea pig cytomegalovirus to cells that express Cas9 and virus-specific guide RNA. The result was highly efficient editing of the viral genome and disruption of nonessential viral genes. Compared to BAC recombineering, this methodology avoids selective pressures that are associated with propagation of the viral genome in bacteria, minimizes the number of passages necessary to develop transgenic viruses, and may facilitate the genetic manipulation of clinical isolates or other low passage cytomegalovirus strains.

7. "Microbial translocation during early acute AIDS virus infection"

Adam J. Ericsen, Michael Lauck, Mariel S. Mohns, Sarah R. DiNapoli, James P. Mutschler, Justin M. Greene, Jason T. Weinfurter, Gabrielle Lehrer-Brey, Kristin A. Crosno, Eric J. Peterson, Roger W. Wiseman, Benjamin J. Burwitz, Jonah B. Sacha, Thomas C. Friedrich, Jason M. Brenchley, & David H. OConnor

Migration of microbial products from the gastrointestinal tract into the blood is observed in people chronically infected with HIV, and in macaques chronically infected with pathogenic simian immunodeficiency virus (SIV). In both humans and macaques, this microbial translocation is accompanied by systemic immune activation, which is associated with the pace of progression to AIDS. However, there is uncertainty as to how early the phenomenon occurs following virus transmission, and whether it occurs as a cause or consequence of disease progression. Here, we used the SIV-infected macaque model to examine the acute-phase kinetics of microbial translocation in order to better understand its potential to affect disease progression. Within the first week of infection, prior to the acute-phase peak of viremia, there was a massive influx of bacterial products into the blood. Increased inflammation and CD4+CCR5+ T cell target generation in the periphery accompanied this translocation, and plasma levels of soluble CD14 (sCD14) at multiple time-points correlated with chronic-phase set-point levels of virus replication. Altogether, our results identify early acute microbial translocation as one of the earliest pathological phenomena to occur during immunodeficiency virus infection.

8. "Evaluating the Role of MCPyV Large T Antigen-pRb Interactions in Promoting In Vivo Phenotypes"

Megan E. Spurgeon and Paul F. Lambert

Evidence continues to grow that supports a causal link between a recently discovered human polyomavirus, Merkel cell polyomavirus (MCPyV), and a rare human skin cancer called Merkel cell carcinoma (MCC). Some of the most compelling evidence for this causal relationship is integration of the viral genome and subsequent maintained expression of the MCPyV small and large tumor antigens (or T antigens) in nearly all virus-positive MCCs. Interestingly, the MCPyV large T antigens found in MCCs contain tumor-specific mutations that truncate the protein in a way that renders the virus unable to replicate yet preserves the pRb-binding motif and transforming capacity. We recently reported the development and characterization of a transgenic mouse model of MCPyV T antigen expression in which tumor-derived small T and truncated large T antigens are expressed under the control of a keratin

14 (K14) promoter. These transgenic mice developed several overt phenotypes that were confined to anatomical sites containing stratified epithelium. Several molecular phenotypes also accompanied MCPyV T antigen expression in murine epithelia including hyperplasia, increased proliferation, and disrupted differentiation. We sought to determine which, if any, of these acute in vivo phenotypes require association between the truncated MCPyV large T antigen and the cellular protein pRb. Transgenic mice were generated in which K14-driven MCPyV T antigens were expressed in the context of a homozygous RbLXCXE knock-in allele that prevents T antigen-pRb interactions through the LXCXE motif. The results of these experiments, together with their potential implications on MCPyV-associated transformation and the relationship between the cellular environment and MCPyV T antigens, will be discussed.

9. "Promotion of EBV lytic reactivation in oral keratinocytes"

Kathleen Makielski, Dhananjay Nawandar, Denis Lee, Laurel Lorenz, Bill Sugden, Shannon Kenney and Paul Lambert

Epstein-Barr virus (EBV) and human papillomaviruses (HPVs) are human tumor viruses that cause head and neck cancers. Both viruses infect and replicate in upper aerodigestive tract epithelia. Some studies have detected co-infection in both oropharyngeal and nasopharyngeal cancers raising the possibility that these viruses could potentially affect each other's life cycles and/or oncogenic potential. Our lab has established an in vitro model system using organotypic raft cultures to test the effects of EBV and HPV on each other in stratified squamous oral epithelial cells. We found that the presence of HPV promotes EBV lytic reactivation. Specifically, HPV promotes EBV genome amplification and expression of an EBV immediate-early protein, Z. Studying the effects of HPV on EBV lytic reactivation could be a first step to determining if co-infection contributes to viral infection and head and neck carcinogenesis.

10. "Differentiation-dependent KLF4 expression promotes lytic Epstein-Barr Virus infection in epithelial cells"

Dhananjay M. Nawandar, Anqi Wang, Kathleen Makielski, Denis Lee, Shidong Ma, Elizabeth Barlow, Jessica Reusch, Coral K. Wille, Deborah Greenspan, John S. Greenspan, Janet E. Mertz, Eric C. Johannsen, Paul F. Lambert, and Shannon C. Kenney

Epstein-Barr virus (EBV) is a human herpesvirus associated with B-cell and epithelial cell malignancies. EBV lytically infects normal differentiated oral epithelial cells, where it causes a tongue lesion known as oral hairy leukoplakia (OHL) in immunosuppressed patients. However, the cellular mechanism(s) that enable EBV to establish exclusively lytic infection in normal differentiated oral epithelial cells are not currently understood. Here we show that a cellular transcription factor known to promote epithelial cell differentiation, KLF4, induces differentiation-dependent lytic EBV infection by binding to and activating the two EBV immediate-early gene (BZLF1 and BRLF1) promoters. We demonstrate that latently EBV-infected, telomerase-immortalized normal oral keratinocyte (NOKs) cells undergo lytic viral reactivation confined to the more differentiated cell layers in organotypic raft culture. Furthermore, we show that endogenous KLF4 expression is required for efficient lytic viral reactivation in response to phorbol ester and sodium butyrate treatment in several different EBV-infected epithelial cell lines, and that the combination of KLF4 and another differentiation-dependent cellular transcription factor, BLIMP1, is highly synergistic for inducing lytic EBV infection. We confirm that both KLF4 and BLIMP1 are expressed in differentiated, but not undifferentiated, epithelial cells in normal tongue tissue, and show that KLF4 and BLIMP1 are both expressed in a patient-derived OHL lesion. In contrast, KLF4 protein is not detectably expressed in B cells, where EBV normally enters latent infection, although KLF4 over-expression is sufficient to induce lytic EBV reactivation in Burkitt lymphoma cells. Thus, KLF4, together with BLIMP1, plays a critical role in mediating lytic EBV reactivation in epithelial cells.

11. "**Location, location, location: HIV-1 genomic RNA trafficking controls virus particle production"**
Jordan Becker and Nathan Sherer

The HIV-1 Gag protein is sufficient to drive the assembly of virus-like particles (VLPs) at the plasma membrane (PM) of cells, even in the absence of all other viral factors. However, during infection HIV-1 must coordinate assembly with precise packaging of two copies of its viral genomic RNA (gRNA), despite a surrounding excess of cellular and viral RNAs. Thus, we hypothesized that, at least to an extent, HIV-1 gRNAs play an active role in regulating the site or efficiency of virus particle assembly. Here, we combined visual and functional assays to study whether gRNA abundance or subcellular localization impacts the site or efficiency of virus particle production. Remarkably, increasing the cytoplasmic abundance of either HIV-1 coding or non-coding gRNAs had little or no effect on virus particle assembly. However, perturbing the cytoplasmic localization of HIV-1 gRNAs using a novel RNA tethering strategy resulted in a profound block to virus particle production. Moreover, we made the surprising finding that mistargeting gRNAs away from the plasma membrane (to subcellular membranes or the cytoskeleton) caused Gag to also accumulate at these sites. Thus, we have demonstrated that gRNAs do indeed encode some active or regulatory functions relevant to Gags trafficking in the cell and virion production. Future studies will assess ways by which altering gRNA subcellular trafficking can be used as a viable anti-HIV therapeutic strategy.

12. "Progress on Developing Small Chemical Inhibitors of the APOBEC3 Family of DNA Cytosine Deaminases"
Ming Li, Margaret E. Olson, Daniel A. Harki, and Reuben S. Harris

APOBECs are a family of nine active DNA cytosine deaminases with a variety of biological roles in human cells, including retrovirus and retrotransposon restriction, foreign DNA restriction, and antibody gene diversification (1). To develop chemical probes for these enzymes, we optimized a fluorescence-based single-strand DNA deaminase assay and used it to screen over 500,000 compounds for enzyme inhibition. Parallel screens were done using APOBEC3A/APOBEC3B and APOBEC3G to help distinguish specific and broad-spectrum chemical inhibitors. These screens have yielded over 2,300 primary hits with various specificities and chemical scaffolds. Biochemical and structural studies demonstrate several distinct mechanisms of inhibition (e.g., 2, 3). Screening data and progress on scaffold validation will be summarized.

13. "Transcriptional Regulation of APOBEC3 Antiviral Immunity Through the CBF²/RUNX Axis"
Brett D. Anderson and Reuben S. Harris

Several members of the APOBEC3 family of DNA cytosine deaminases have the capacity to potently restrict HIV-1 replication by inducing extensive G-to-A mutations in the viral cDNA during reverse transcription. HIV-1 counteracts this antiviral defense by encoding the small protein Vif, which recruits 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). CBF² normally forms a heterodimer with members of the RUNX family of DNA binding transcription factors to regulate expression of numerous genes involved in hematopoietic development and immune function. A recent structural study on the Vif-CBF² complex revealed that Vif engages a surface on CBF² that overlaps significantly with the known RUNX binding surface (2). Therefore, we hypothesized that the viral hijacking of CBF² to promote APOBEC3 degradation may concurrently promote viral replication by precluding the assembly of functional CBF²/RUNX heterodimers and thus altering the expression of cellular genes. Surprisingly, we found that CBF² functions in complex with the RUNX proteins as a critical positive regulator of the APOBEC3 genes themselves in CD4+ T cells, and that genetic knockdown and knockout of CBF² is sufficient to render T cells permissive to vif-deficient HIV-1 replication due to a loss of APOBEC3 gene expression(3). Based on these results, we propose a two-pronged model whereby HIV-1 Vif

counteracts the APOBEC3 antiviral defense by directly promoting APOBEC3 polyubiquitination while simultaneously suppressing APOBEC3 gene transcription by sequestering CBF^2 from RUNX-associated transcription complexes

14. "The Complex Relationship Between Oncoviruses and APOBEC Mutagenesis in Cancer"
Gabriel J. Starrett, James A. DeCaprio and Reuben Harris

As many as one in five cancers has a viral origin. For instance, nearly 100% of cervical cancers and 26% of head and neck squamous cell carcinomas (HNSCC) are due to infections by high-risk human papillomavirus (HPV). Interestingly, these cancers show upregulation of the antiviral DNA cytosine deaminase, APOBEC3B, which causes an increased proportion of cytosine mutations in 5-TC motifs in the tumor genome. We recently showed that high-risk HPV induces the specific upregulation of APOBEC3B. This raises the question - is the mutator phenotype caused by APOBEC3B upregulation required for virus-induced transformation? To begin to address this question, we asked if APOBEC3B upregulation is a general property of DNA tumor viruses or an intrinsic characteristic of HPV. We therefore investigated whether virus-associated Merkel cell carcinoma (MCC) is similarly driven by APOBEC3B-mediated mutation. Merkel cell carcinoma (MCC) is an aggressive form of skin cancer that originates in somatosensory cells. In nearly 80% of MCC cases, Merkel cell polyomavirus (MCV), a small dsDNA virus, is detectable in the tumor. We analyzed mutation spectra and APOBEC3 expression profile in both virus-associated and non-virus-associated MCC through the use of high-throughput DNA and RNA sequencing of patient primary tumors and matched normals. Surprisingly, whole genome sequence analysis revealed that tumors with viral transcripts detectable in the matched RNA sequencing data had low numbers of somatic mutations with no obvious APOBEC3B signature; whereas, those with no detectable virus had large proportions of UV-associated mutations. There was also no discernable change in APOBEC3 mRNA expression in either classification of tumor. These results suggest that MCV has evolved a mechanism to drive tumorigenesis independent of upregulating APOBEC3B and this mechanism is unique to virus-associated MCC. Our data also suggest that MCV has a novel mechanism to escape innate immune detection and that the interplay between oncogenic viruses and APOBEC3 enzymes may be more complex than previously anticipated.

15. "Is Endogenous APOBEC3H a Transmission Barrier against HIV-1?"
Jiayi Wang, Allison M. Land, Brian J. Hoium, Eric W. Refsland, Elizabeth M. Luengas, Romel D. Mackelprang, William L. Brown, Michael Emerman, Jairam Lingappa and Reuben S. Harris

Several members of the APOBEC3 family of DNA cytosine deaminases can potently inhibit HIV-1 replication by catalyzing extensive cytosine deamination in viral cDNA during reverse transcription (1). HIV-1 counteracts restriction with the virally encoded Vif protein, which adapts the APOBEC3 proteins to a cellular ubiquitin ligase to target them for proteasomal degradation. In humans, A3H is the most polymorphic member of the family and includes seven haplotypes with three encoding for stable proteins and the rest unstable. Stable A3H proteins contribute to HIV-1 restriction and can only be counteracted by hyper-functional but not hypo-functional Vif variants (dictated by amino acids at key positions) (2). We hypothesize that stable A3H enzymes provide a transmission barrier against HIV-1 isolates harboring hypo-functional Vif alleles. To test this hypothesis, we have determined the A3H and viral Vif genotypes of a large cohort of African HIV-1 serodiscordant couples. We are in the process of functionally testing each isolated Vif variant. Current results will be presented. If the hypothesis is correct, a significantly greater percent of hypo-functional Vifs will be evident in the infected index patients of the non-transmitting serodiscordant subgroup.

16. "Whole transcriptome analysis of young and adult host porcine macrophages infected by PRRSV"
Xiong Wang, Sally R. Robinson, Diem K. Ngo and Michael P. Murtaugh

Whole transcriptome analysis of young and adult host porcine macrophages infected by PRRSV Xiong Wang, Sally Robinson, Michael Murtaugh Veterinary and Biomedical Sciences, College of Veterinary

Medicine, University of Minnesota 1971 Commonwealth Ave, Saint Paul, Minnesota, 55108 Different outcomes occur when pigs are infected with Porcine reproductive and respiratory syndrome virus (PRRSV); adult pigs often show a non-significant to mild syndrome while young pigs (including neonates, sucking and nursery pigs) suffer much more serious respiratory disease, secondary infections, and significantly higher morbidity and mortality. Porcine alveolar macrophages (PAMs), an early and key target of PRRSV intrusion, are likely to contribute to this age-dependent infection outcome. In vitro experiments have shown that PRRSV grows better in PAMs from young pigs. Previous research demonstrated no differences in PAM expression of CD163 and CD169, surface proteins that are implicated in PRRSV cell entry between different aged pigs. Therefore, this age-dependent resistance ability towards PRRSV infection could be an intrinsic characteristic of PAMs. The general presence of cellular restriction factors that suppress replication and growth of various viruses led us to propose the age-dependent expression of anti-viral restriction factors. Therefore, we hypothesize that age-dependent presence of intrinsic cellular factors mediating restriction or permissiveness that are responsible for this resistance difference against PRRSV infection. We have examined PAMs from young and adult pigs for susceptibility to PRRSV infection in vitro, and RNA-seq on high-throughput sequencing has been done for the whole transcriptome analysis in order to identify differences in age-dependent restriction factors that are expressed constitutively or are induced following viral infection. This project is expected to provide novel insights towards determining molecular mechanisms of PRRSV resistance.

17. "PCV2 NEUTRALIZING ANTIBODY LEVELS IN COLOSTRUM FROM FARMS WITH HIGH OR LOW LEVELS OF VIREMIA"

CMT Dvorak, BJ Payne, JL Seate, and MP Murtaugh

Porcine circovirus type 2 (PCV2) is the causative agent of porcine circovirus-associated disease (PCVAD). Vaccination against PCV2 at or around weaning effectively controls disease in finishers. Nearly all pigs in the US are vaccinated and develop PCV2-specific antibodies, but virus often is not eliminated, even in the presence of PCV2-specific antibodies. It is thought that antibody titers may not be indicative of a protective immune response, but that anti-PCV2 neutralizing antibodies (NA) are a more effective indicator of a controlled infection. Four sow herds were evaluated on vertical transmission of PCV2, sow PCV2 ELISA and colostrum PCV2 NA to gain information on antibody control of infection. Pre-farrow sow serum samples were tested by PCV2 ELISA. Placental umbilical cord serum (PUCS) and colostrum samples were collected and tested for antigen. For the study, a PCV2 NA assay using VR1BL cells was developed. This assay was optimized for use with both serum and colostrum samples. PCV2 NA titers were determined for colostrum samples. Significant differences in PUCS and colostrum PCV2 PCR were seen between Farms A&B and Farms C&D. No significant differences were observed in sow PCV2 ELISA. There was a broad range of 50% neutralizing antibody titers, from 1:159 to 1:1x106. A significant difference in average neutralizing antibody titers was observed between Farms A&B and Farms C&D. PUCS is more sensitive for PCV2 detection than colostrum in paired samples. PUCS sample cannot be saved and resampled therefore colostrum was used for the NA testing. Sow ELISA results do not indicate differences in sow herd stability. However, in this study, NA titers differed significantly between stable (A&B) and unstable farms (C&D); higher NA titers observed in farms with no to low virus levels compared to farms with high levels of vertical transmission. This suggests that sows on farms with higher vertical transmission may develop less protective NA and may be more susceptible to disease due to amounts of replicating virus present. Likely, protective colostrum antibodies need to be produced and passed to piglets in order to protect against disease. Further studies on the effect of vaccination on viral levels and NA levels are being conducted, but high viral levels and low NA titers might suggest additional vaccinations may be useful for those farms.

18. "Hypoxia-induced Lytic Reactivation of EBV Requires Both HIF-1± and Activated Wild-type p53"
Richard J. Kraus, Xianming Yu, Saranya Sathiamoorthi, Shidong Ma, Shannon C. Kenney, and Janet E. Mertz

Jiang et al. reported that incubation of the EBV(+) B-cell line, B95-8, under 2% oxygen tension led to synthesis of the EBV IE Zta in B95-8 cells (J Clin Virol 37:98, 2006). We report here that hypoxia mimics induce lytic EBV reactivation in some, but not all EBV(+) epithelial and B-cell lines; they primarily do so by inducing accumulation of HIF-1± which directly binds to a hypoxia-responsive element (HRE) located within the BZLF1 gene promoter, Zp. Incubation of EBV(+) Burkitt lymphoma (BL) Sal cells, gastric carcinoma SNU-719, or AGS-Akata cells with deferoxamine (DFO), an iron-chelator, induced EBV lytic-gene expression. ChIP assays indicated HIF-1± bound Zp within the context of whole EBV genomes. ShRNA knockdown of HIF-1± reduced DFO-mediated lytic reactivation. HIF-1± addition induced both Zta and Rta synthesis in cells harboring WT virus, but neither IE protein in Zp HRE and Zta mutant-infected cells despite HIF-1± targeting the Rta homolog ORF50 in KSHV. DFO failed to reactivate EBV BL MutuI cells which contain a mutant p53; shRNA knockdown of p53 in Sal cells inhibited DFO-induced reactivation. Along with stabilizing HIF-1±, DFO also signaled accumulation of p53 phosphorylated at ser15. KU55933, an inhibitor of the protein kinase ATM, blocked both this p53 phosphorylation and DFO-induced EBV reactivation in Sal and AGS-Akata cells without affecting HIF-1± accumulation. Nutlin-3, an mdm2 inhibitor which also promotes accumulation of this phosphorylated p53, synergized with DFO in inducing EBV reactivation. FG4592, a selective inhibitor of propyl hydroxylases, stabilized HIF-1± but did not induce either phosphorylated p53 or Zta synthesis; however, it, too, synergized with nutlin-3 in inducing EBV reactivation. MNL4924, an inhibitor of NEDD8-activating enzyme (NAE), also induced accumulation of HIF-1±, phosphorylated p53 and Zta. Thus, we conclude that hypoxia-induced EBV lytic reactivation requires both phosphorylated wild-type p53 and HIF-1± binding to an HRE within Zp.

19. "1.99 angstrom zinc-free APOBEC3F catalytic domain crystal structure"
Nadine M. Shaban, Ke Shi, Ming Li, Hideki Aihara, Reuben S. Harris

1.99 angstrom zinc-free APOBEC3F catalytic domain crystal structure Nadine M. Shaban, Ke Shi, Ming Li, Hideki Aihara, Reuben S. Harris Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455 ABSTRACT The APOBEC3 cytosine deaminases play a crucial role in the antiviral activity against HIV-1. Here we report a 1.99 angstrom resolution crystal structure of the Vif-binding and catalytic domain of APOBEC3F. This structure is distinct from previously published APOBEC and phylogentically related deaminase structures, as it is the first without zinc bound in the active site. We determined an additional structure containing zinc in the identical space group that allows direct comparison with the zinc-free structure. In the absence of zinc, the conserved active site residues that normally participate in zinc coordination show unique conformations, including a 90-degree rotation by His249 and disulfide bond formation by Cys280 and Cys283. In addition, we found that low pH treatment is sufficient to remove zinc from the enzyme. Zinc coordination and catalytic activity is reconstituted with the addition of zinc only in a reduced environment likely due to the two active site cysteines readily forming a disulfide bond when not coordinating zinc. Only zinc and cobalt, but not other tested metals, are able to reconstitute activity.

20. "Discovery, characterization, and ecology of a Novel Hepatitis A-like virus in wild Olive Baboons (Papio anubis), Uganda"
Andrew J. Bennett, Samuel D. Sibley, Michael Lauck, Geoffrey Weny, David Hyeroba, David H. OConnor, Tony L. Goldberg

Hepatitis A (HAV; family Picornaviridae; genus Hepatovirus) is an RNA virus that causes acute inflammatory disease of the liver in humans and nonhuman primates, and is commonly transmitted through the fecal-oral route. Most often associated with food-borne outbreaks resulting from fecal-contamination, more rarely humans have acquired HAV from the handling of infected non-human primates in captivity. Conversely, recent studies discovering high HAV antibody seroprevalence in wild

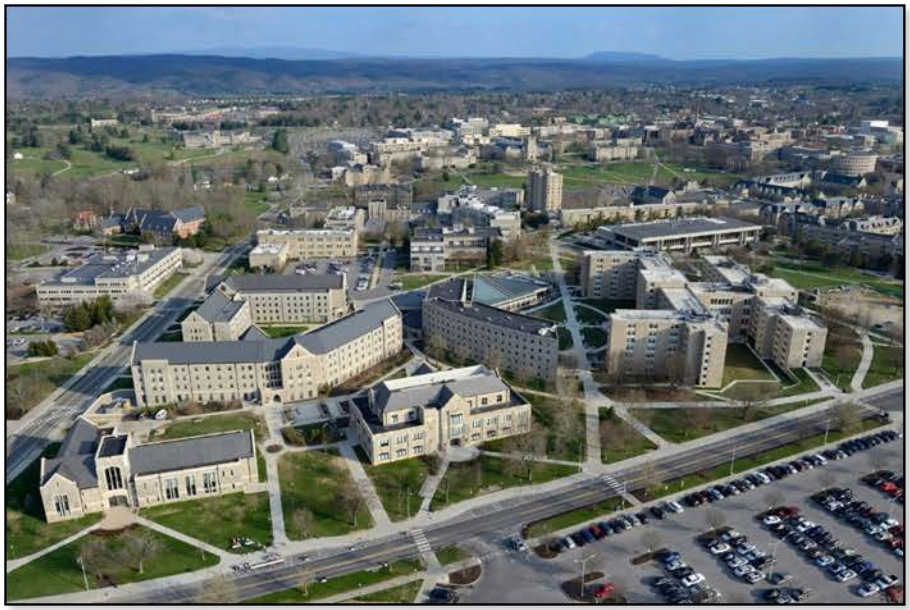
non-human primates have implicated reverse zoonotic transmission in areas of sub-Saharan Africa where human-nonhuman primate contact and conflict occur frequently. We discovered and characterized by Next-Generation Sequencing (NGS) a novel Simian Hepatitis A-like virus in the blood of a wild olive baboon (Papio anubis) in Kibale National Park, Uganda. Furthermore, RT-PCR diagnostics detected viral RNA in the feces of 40% of baboons sampled at the time of blood collection, suggesting the shedding of potentially infectious viral particles into the environment by wild baboons in western Uganda. Additional screening by field-deployable PCR shows non-random distribution of the virus among individuals and groups. Our results implicate this nonhuman primate as a potential zoonotic source of Hepatitis A-like viruses. This study demonstrates the value of NGS for discovering potential reservoirs of zoonotic pathogens, and supports the supposition that HAV-like viruses circulate naturally in wild nonhuman primates.

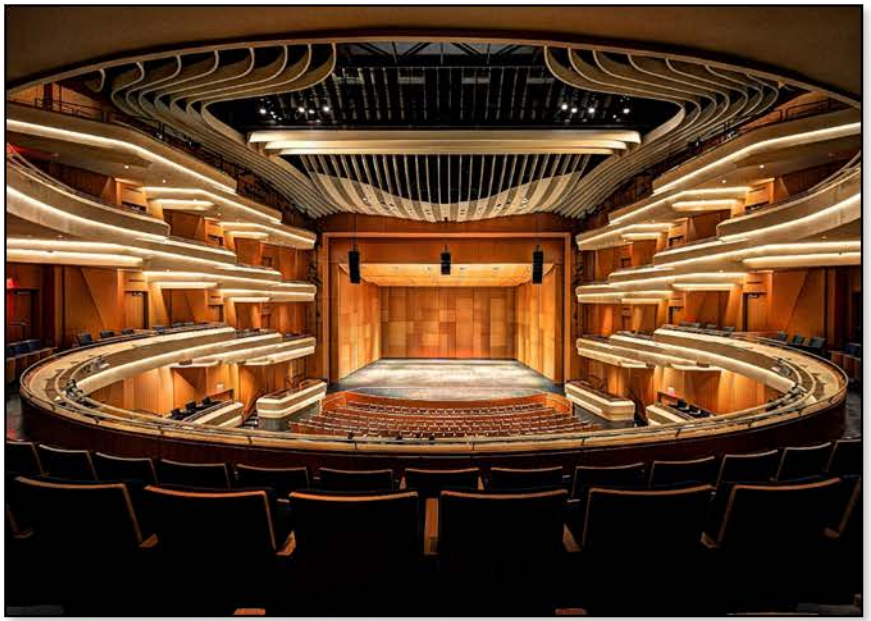
21. "Viewing the biogenensis of HTLV-1 and HIV-1 particles in living cells"
John Eichorst, Joachim Mueller and Louis Mansky

The assembly of human T-cell leukemia virus type 1 (HTLV-1) and human immunodeficiency virus type 1 (HIV-1) particles at the plasma membrane is driven by the Gag protein. Gag oligomerizes at the plasma membrane to form a lattice that is incorporated into the budding virus particle. The pathway by which the Gag proteins are recruited in the budding viral particle (e.g., cytoplasmic versus membrane-associated) is not well understood. In order to determine the recruitment pathway of Gag, we have tagged HTLV-1 Gag and HIV-1 Gag with the photoconvertible fluorescent protein mEos2. The protein mEos2 changes from a green-fluorescing protein to a red-fluorescing protein after being illuminated with a short pulse of violet light. With total internal reflection microscopy (TIRFm), the color of the Gag proteins at the membrane can be made different than the color of the Gag proteins in the cytosol. The recruitment pathway of either HIV-1 Gag or HTLV-1 Gag can be determined by observing the color of the resulting particle. The results presented here indicate that HTLV-1 Gag is recruited into budding sites of virus particles from the plasma membrane while HIV-1 Gag is recruited primarily from the cells cytosol. In addition, the experimental approaches developed for this project demonstrate many of the intricacies and procedures for analysis needed when quantitatively applying photoconvertible fluorescent proteins to examine live samples.

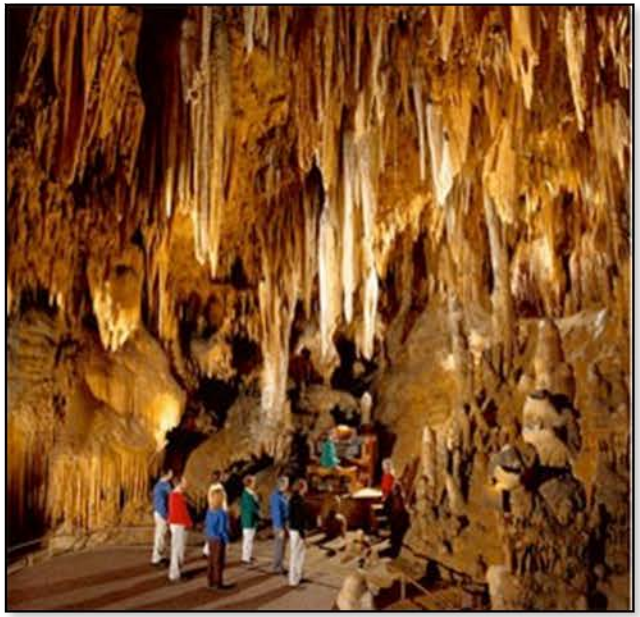
22. "Factors driving porcine B cell proliferation and differentiation dynamics"
Michael C Rahe and Michael P Murtaugh

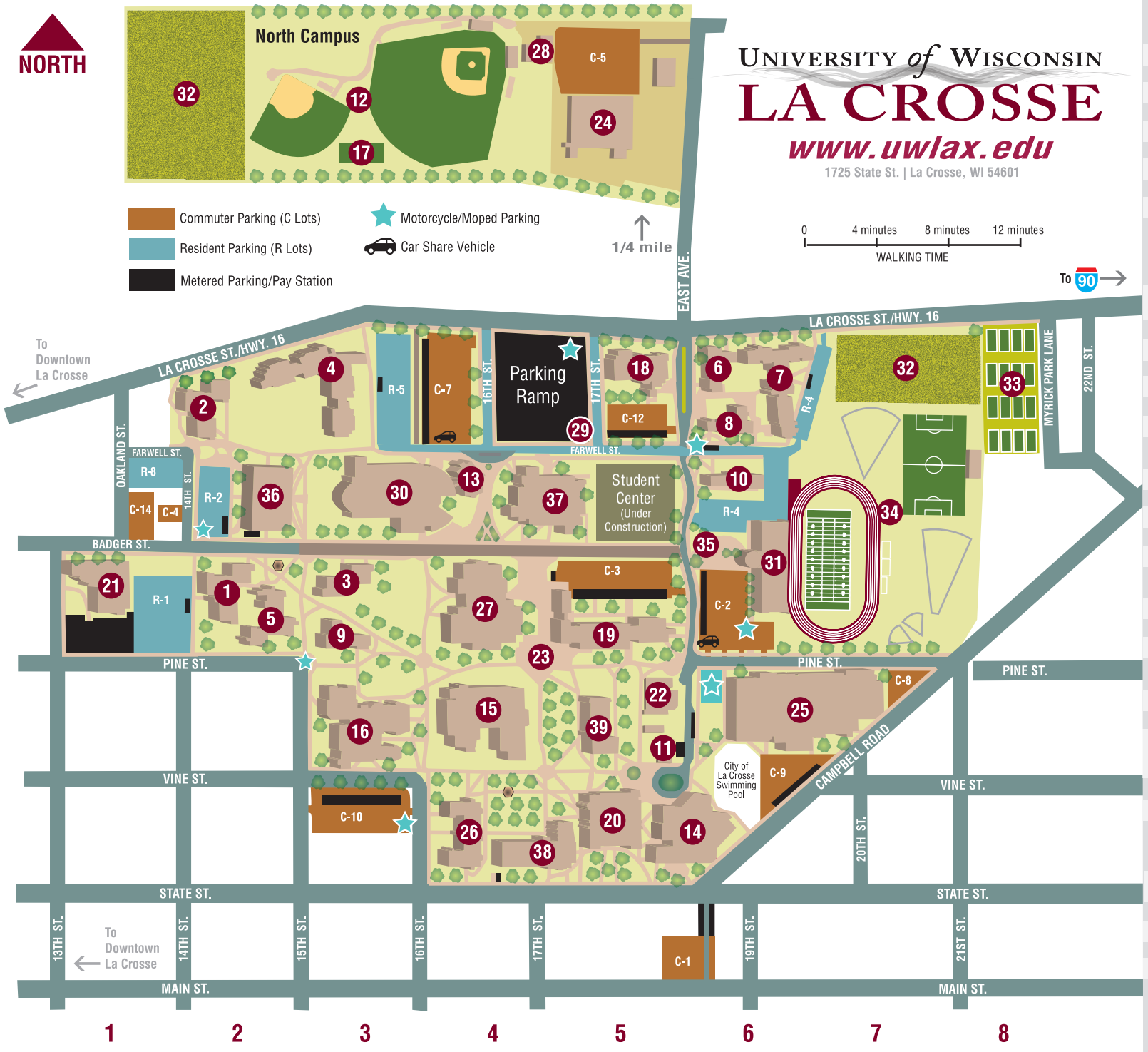
The B cell is the driving force behind the humoral immune response to infection. Terminal differentiation of B cells into immunoglobulin secreting plasma cells is crucial for the neutralization, opsonization, and complement fixation of many pathogens. CD21 enriched porcine B cells were activated in vitro with CD40L and then stimulated with various cytokines to screen for factors causing cellular proliferation and differentiation. IL-21 was a potent inducer of proliferation on activated B cells when compared to unstimulated cells and other purported stimulatory cytokines. Additionally, IL-21 induced the terminal differentiation of activated B cells to immunoglobulin secreting plasma cells. The addition of BAFF and APRIL to CD40L and IL-21 treated B cells enhance B cell viability while also leading to an increase in the differentiation of B cells into antibody secreting plasma cells. These results set the stage for future delineation of anti-viral immune response in swine that will help advance the study of the porcine B cell roles in vaccinology, immunology, and immunotherapeutics.





ASV 2016, June 18-22, 2016
Virginia Tech, Blacksburg, VA





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