Institute for Molecular Virology

Academic Health Center

2008 Symposium
Monday, May 12th 2008

“Host Cell Proteins and Replication”

Symposium Program
Poster and Talk Abstracts

Talks by 2 World-Renowned Virologists and 14 Local Talents

8:30am  Dr. Jonathan Stoye
         NIMR, London

3:15pm  Dr. Sandra Weller
         University of Connecticut

Images credits from the top of the page: The first and third images have been adapted from Chen et al., Nature, 2008 Mar 6;452(7183):116-9, submitted by Hiroshi Matsuo and Reuben Harris, representing the catalytic domain of APOBEC3G. Second image: Pam Skinner and Terri Mattila. Fourth image: Pete Gillis, Rice lab; Hela cells expressing the HSV-1 ICP27 protein.

Special support for this event provided by the Academic Health Center, the Office of the Vice President for Research and the School of Dentistry.

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4th Annual Institute for Molecular Virology Symposium
“Host Cell Proteins and Replication”
May 12th, 2008, 7:30am – 6pm
Coffman Memorial Union Theater

Symposium Program

Numbers given indicate abstract number

7:30 – 8:20  Registration & refreshments; bagels, pastries, coffee
8:20 – 8:25  Welcome and opening remarks (Louis Mansky)
1.  8:25 – 9:30  Morning Keynote Lecture: Jonathan Stoye
   “Studies of restriction factor-retrovirus interactions”
   Introduction by Mark Stenglein

Morning Session

Convenors: Guylaine Haché and Teresa Mattila

2.  9:30 – 9:45  Rebecca S. LaRue, Stefan R. Jónsson, Kevin Silverstein, Valgerdur
   Andréásdóttir, Tim P. L. Smith and Reuben S. Harris
   “Analyses of the two APOBEC3 genes of artiodactyls indicate that
   the seven human homologs arose by frequent recombination prior
   to primatification”

3.  9:45 – 10:00  Seiga Ohmine, Ryuta Sakuma, Amber A. Mael and Yasuhiro Ikeda
   “Antiviral effects of TRIM5alpha orthologues on lentiviral produc
   tion”

4.  10:00 – 10:15  Anne Meehan, Dyana Saenz, James Morrison, Manuel Llano, Mary
   Peretz, and Eric M. Poeschla
   “LEDGF/p75 proteins with alternative chromatin tethers are
   functional HIV-1 cofactors”
10:15 – 10:45  Morning break and poster viewing; coffee

Convenors:  Jun Han and Andrew Hudacek

5.  10:45 – 11:00  Michael Dapp, Christine Clouser, Steven Patterson, and Louis M.
   Mansky
   “Demonstration of a dual mechanism for mutagenic ribonucleosides
   in decreasing viral infectivity and inducing HIV-1 mutagenesis”

6.  11:00 – 11:15  Patrycja J Lech and Nikunj Somia
   “HIV-1 restriction in rabbit cells”

7.  11:15 – 11:30  Ming Zeng, Timothy Schacker, Daniel Feldman, Steve Wietgrefe,
   John Carlis, Jeffery Lifson, Ashley Haase
   “Destruction of the fibroblastic reticular cell network is associated
   with reduction of interleukin-7 and depletion of the naïve CD8+ T
   cell population in HIV/SIV infection”
8. 11:30 – 11:45  Donna A. MacDuff and Reuben S. Harris  
“The Antibody Gene DNA Deaminase, AID, Can Also Restrict Retroelement Transposition”

11:45 – 1:00  Lunch, Coffman Theater Atrium

**Afternoon Session**

Convenors:  Rebecca LaRue & Casey Dorr

9. 1:00 – 1:15  Fang Li  
“Molecular mechanisms of host adaptations and cross-species infections of SARS coronavirus”

10. 1:15 – 1:30  Vincent H. J. Leonard, Patrick L. Sinn, Gregory Hodge, Tanner Miest, Patricia Devaux, Numan Oezguen, Werner Braun, Paul B. McCray, Jr., Michael B. McChesney, and Roberto Cattaneo  
“Epithelial cell receptor-blind measles virus remains virulent but cannot cross epithelia and is not shed”

11. 1:30 – 1:45  Chanakha K. Navaratnarajah, Sompong Vongpunsawad, Numan Oezguen, Thilo Stehle, Werner Braun, Takao Hashiguchi, Katsumi Maenaka, Yusuke Yanagi, and Roberto Cattaneo  
“Dynamic Interaction of the Measles Virus Hemagglutinin with Its Receptor Signaling Lymphocytic Activation Molecule”

12. 1:45 – 2:00  Nicola J Philpott  
“Adeno-associated Virus Site-specific Integration”

2:00 – 2:30  Afternoon break and poster viewing; coffee, snacks

Convenors:  Rachel Nygaard and Patricia Devaux

13. 2:30 – 2:45  Mark Cannon  
“The KSHV vGPCR and Viral Tumorigenesis”

“Chronic immune activation and long-term neuropathological injury following experimental herpes encephalitis”

15. 3:00 – 3:15  Peter Gillis and Steve Rice  
“Expression of Herpes Simplex Virus Type 1 ICP27 in Uninfected HeLa Cells Activates p38 Signaling and Leads to Apoptosis”

16. 3:15 – 4:15  Afternoon Keynote Lecture: Sandra Weller  
“How herpes simplex virus commandeers the host cell chaperone and DNA damage machinery”  
Introduction by Daniel Kronemann

4:15  Closing remarks (Louis Mansky)

4:15 – 6:00  Reception and poster session, Coffman Theater Atrium
IMV Advisory Committee:
Wade Bresnahan
Roberto Cattaneo
Paul Jardine
Guylaine Hache
Reuben Harris
James Lokensgard
Louis Mansky
Michael Murtaugh
Steve Rice
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Office of the Vice President for Research
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1. Studies of restriction factor – retrovirus interactions

Jonathan Stoye, Division of Virology, MRC National Institute for Medical Research, London, UK

During a lengthy period of co-evolution between retroviruses and their vertebrate hosts, a number of host restriction factors (RFs) capable of blocking retrovirus replication have developed. Examples include the primate gene TRIM5alpha and the murine Fv1 gene. Both RFs are constitutively expressed and bind their target, viral CA, shortly after viral cores enter the cytoplasm of the newly infected cell. However RF binding can have different consequences with TRIM5 preventing reverse transcription and Fv1 preventing nuclear uptake or integration. Restriction specificity is genetically determined thereby allowing a variety of genetic approaches for studying the interaction between RFs and the viral CA molecule. In the course of these studies, we have (i) identified regions of RF and CA important for defining restriction specificity, (ii) demonstrated specific binding between mutimerized RF and assembled CA and (iii) shown that these interactions can have context-dependent consequences allowing restriction by at least two distinguishable mechanisms. These data and resulting models for restriction will form the basis of my presentation.

2. Analyses of the two APOBEC3 genes of artiodactyls indicate that the seven human homologs arose by frequent recombination prior to primatification

Rebecca S. LaRue, Stefan R. Jónsson, Kevin Silverstein, Valgerdur Andrésdóttir, Tim P. L. Smith and Reuben S. Harris

APOBEC3 (A3) proteins deaminate cytosines within single-strand DNA and block the replication of many retroviruses and retrotransposons, and potentially DNA transposons. Each A3 protein has one or two conserved zinc-coordinating motifs (Z1a, Z1b or Z2). The presence of only one A3 gene in mice and seven in humans indicates that a remarkable mammal-specific expansion has occurred (Z1a-Z2 versus Z1b, Z1a-Z1b, Z1a, Z1a-Z1a, Z1a-Z1a, Z1a-Z1b, Z2). To gain insights into the mechanism and timing of A3 gene expansion and into the functional modularity of these genes, we analyzed the genomic sequences, expressed mRNAs and activities of the full A3 repertoire of representative artiodactyls (cattle, sheep, and pigs) positioned phylogenetically between rodents and primates. Cattle and sheep have two A3 genes (A3A and A3F; Z1b and Z1a-Z2, respectively), whereas pigs have one (A3F; Z1a-Z2). Domestic and wild pig comparisons indicated that A3A was lost early in the Suidae branch. Interestingly, conserved alternative splicing and internal transcription initiation produced active A3F variants with a Z1a or Z2 zinc-coordinating motif. The artiodactyl A3 genes show little signs of positive selection in contrast to the extremely high levels observed in primates. We conclude that the common ancestor of artiodactyls and primates had two A3 genes and, importantly, the minimal set of zinc-coordinating motifs required to evolve into the present day human A3 repertoire (Z1b-Z1a-Z2). Our studies suggest that the present day human A3 repertoire largely originated prior to the point that primates branched off the mammalian tree, during a relatively brief and dynamic evolutionary period.
3. Antiviral effects of TRIM5alpha orthologues on lentiviral production

Seiga Ohmine, Ryuta Sakuma, Amber A. Mael and Yasuhiro Ikeda

TRIM5alpha, a representative member of the tripartite motif family of proteins, is a cellular factor that is thought to be a key player in the front line of the host defense against lentiviruses. TRIM5alpha contains a RBCC (RING, B-box and Coiled Coil) domain on the N-terminus and a B30.2(SPRY) domain on its C-terminus. Rhesus monkey TRIM5alpha (TRIM5arh) restricts viral replication by targeting the incoming viral capsid through the B30.2(SPRY) domain at the post-entry stage of the viral life cycle before the end of reverse transcription. Our group has reported that TRIM5arh can also block viral production, where the viral Gag polyprotein is the target for restriction. Here we examined the late-phase antiviral potency of TRIM5alpha orthologues on lentiviral production. When compared to the restriction of HIV-1 production observed in the presence of TRIM5arh, the human orthologue of TRIM5alpha did not affect the production of a panel of human immunodeficiency viruses (Group M: NL4-3, 89.6 and 94UG, Group O: CMO2.41 and CMO 2.5), whereas African green monkey and cynomolgus monkey orthologues exhibited an intermediate restriction potency. All TRIM5alpha orthologues appeared to be ineffective at restricting simian immunodeficiency viruses (SIVmac1A11, SIVagmTan-1 and SIVagmSAB-1) during this late phase of the SIV life cycle. Chimeric TRIM5alpha constructs were made between the RBCC domains of these TRIM5alpha orthologues, and the potency of this post-integration restriction was determined by RBCC domain, while the B30.2(SPRY) domain appeared to be insignificant. We are currently conducting studies to further characterize the mechanism of this late-phase restriction mediated by these TRIM5alpha orthologues.

4. LEDGF/p75 PROTEINS WITH ALTERNATIVE CHROMATIN TETHERS ARE FUNCTIONAL HIV COFACTORS

Anne Meehan, Dyana Saenz, James Morrison, Manuel Llano, Mary Peretz, and Eric M. Poeschla
Department of Molecular Medicine, Mayo Clinic College of Medicine, Rochester MN

LEDGF/p75 (p75), a chromatin-interacting protein involved in transcription, is an HIV replication cofactor but the mechanism is uncertain. Outside the viral context, over-expressed HIV IN protein becomes chromatin-trapped through a p75 bridge, or tether: a C-terminal integrase binding domain (IBD) binds to IN and a complex ensemble of N-terminal domains (PWWP domain, A/T-hooks, and intervening charged regions) mediates binding to chromatin. p75_s lentiviral cofactor activity requires the N-terminal domain ensemble but whether it is tethering to chromatin per se that is required versus other emergent properties of intact p75 has been experimentally elusive. Here we have tested the tethering model explicitly. We replaced the entire region containing the chromatin binding domains of p75 with two alternative links to chromatin: the human linker histone H1 or a short peptide (32 amino acids) derived from Kaposi_s sarcoma herpes virus latency-associated nuclear antigen (LANA). These chromatin-binding modules differ widely in evolutionary origin, natural role, and structure. Unlike p75, their chromatin ligands are known and differ sharply. H1 binds outside the nucleosome, to DNA, while LANA binds inside the nucleosome core, to protein. However, both kinds of chimeric proteins (H1-p75CTD (C-terminal domain) and LANA32-p75CTD) specifically rescued HIV infection and HIV integration in p75-deficient cells. Remarkably, simply fusing the LANA32 peptide to GFP-IBD (p75 amino acids 349-427) converts this integration-inhibiting DI protein into a fully functional p75-equivalent integration cofactor. Moreover, in cells that are both stringently p75-deficient and express GFP-IBD (TL4 cells), LANA32-GFP-IBD reproducibly effected a two log rescue of HIV-1 infection. These data provide direct evidence that chromatin tethering per se is the core mechanism for LEDGF/p75 in the HIV life cycle.
5. Demonstration of a dual mechanism for mutagenic ribonucleosides in decreasing viral infectivity and inducing HIV-1 mutagenesis

Michael Dapp, Christine Clouser, Steven Patterson, and Louis M. Mansky

Abstract: The antiviral drugs that comprise highly active antiretroviral therapy (HAART) have been a key success in the clinical management of HIV-1 infection. Clinical limitations, e.g., HIV-1 drug resistance, provide the impetus for the development of new targets and new antiretroviral drugs. Here we report the characterization of a novel nucleoside analog (Vidaza, which is used in the treatment of myelodysplastic syndrome) that has anti-HIV activity and exploits two different steps in the viral life cycle. Using a single round replication assay, we show that Vidaza impacts both the early (i.e., reverse transcription) and late (i.e., transcription) phases of the HIV-1 life cycle in a concentration-dependent manner, and that reductions in viral infectivity coincided with increased viral mutagenesis. Analysis of the mutation spectra implicates that the ribonucleoside is converted to the deoxynucleoside form by ribonucleotide reductase and is then incorporated into the viral DNA, leading to a specific increase in G-to-C transversion mutations. Our data also supports the direct incorporation of the ribonucleoside into viral RNA, which also leads to an increase in the opposite C-to-G transversion mutations in proviral DNA. Our findings provide a proof-of-principle that a ribonucleoside analog can act in a bifunctional manner to affect two steps in the HIV-1 life cycle (reverse transcription and transcription) and ultimately reduce viral infectivity. In each antiviral mechanism, enhanced viral mutagenesis is implicated, suggesting a role for lethal mutagenesis in decreasing viral infectivity. We suggest that such bifunctional inhibitors may possess greater clinical durability.

6. HIV-1 restriction in rabbit cells

Patrycja J. Lech and Nikunj Somia

Understanding the relationship between HIV-1 and cellular host cell factors that inhibit or support infection will add to our knowledge of the HIV-1 life cycle and aid in the development of therapeutics to combat viral infection. Rabbit SIRC cells are highly resistant to lentiviral infection, at least in part, by a rabbit TRIM5 mediated restriction. We have elucidated HIV-1 restriction in SIRC cells to occur via a mechanism that differs to that proposed for rhesus TRIM5± mediated restriction of HIV-1. Here we show that HIV-1 is intercepted at or before reverse transcription resulting in the generation of ~10 fold fewer viral reverse transcriptase products of which only 1/10 _ 1/100 successfully integrate (depending on cell type). Treatment with the proteasome / protease inhibitor MG132 results in a further 3-fold decrease in infection even though the amount of viral DNA transcripts in the cytoplasm and the nucleus are noticeably enhanced. Conversely, treatment with cyclosporine A (CSA) through out the course of HIV-1 infection increases infectivity by 10-fold by a mechanism that decreases the rate of reverse transcription. CSA treatment of SIRC cells during the first four hours of infection does not recapitulate the same phenotype suggesting that CSA plays a role during a later stage in infection. These results support previous works that have shown that the mechanism behind TRIM5 mediated restriction is cell type and species dependent and also suggests that SIRC cells block HIV-1 infection at two stages, at or before reverse transcription and post reverse transcription.
7. Destruction of the fibroblastic reticular cell network is associated with reduction of interleukin-7 and depletion of the naïve CD8+ T cell population in HIV/SIV infection

Ming Zeng, Timothy Schacker, Daniel Feldman, Steve Wietgrefe, John Carlis, Jeffery Lifson, Ashley Haase

The network of fibroblastic reticular cells (FRCs) in the T cell area, which supports the complex microarchitecture of the lymph nodes, is critical for the homeostasis of T cells, especially naïve T cells by providing survival factors such as interleukin-7 (IL-7). How the FRC network changes during human immunodeficiency virus (HIV) infection and pathogenic simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs) is not understood. Here we show that the FRC network is progressively destroyed in SIV infection of RMs. The destruction of the FRC network is temporally and spatially associated with a decrease in IL-7 level and an increase in cellular apoptosis level in lymph nodes in HIV/SIV infection. Importantly, the reduction of IL-7 is strongly associated with reduction of naïve CD8+ T cells in lymph nodes in HIV infection. Supporting the concept that the destruction of the FRC network plays a pathogenic role in HIV/SIV infection, the FRC network remains intact in the chronic nonpathogenic SIV infection of sooty mangabeys (SMs), along with normal levels of naïve CD4+ and CD8+ T cells in the lymph nodes. Our data suggest that the destruction of the FRC network in HIV/SIV infection may impair the T cell homeostasis through reduction of IL-7 production. Furthermore, as the first study showing that naïve CD8+ T cells are severely reduced in lymphoid tissues in HIV+ infection, this result provides an insight into a new mechanism underlying the immunodeficiency of HIV-infected individuals.

8. The Antibody Gene DNA Deaminase, AID, Can Also Restrict Retroelement Transposition

Donna A. MacDuff and Reuben S. Harris

Endogenous retroelements are mobile copy and paste transposons, present in the genomes of all eukaryotic cells. New transposition events can disrupt or alter gene function and the presence of multiple copies of the same retroelement can lead to duplications, deletions and genomic rearrangements through homologous recombination. While such events may be beneficial to evolution if they occur at relatively low frequencies within the germ line, they can also be deleterious if they occur in the somatic cells of a multicellular organism by promoting carcinogenesis. Clearly, a variety of mechanisms must exist to limit the mobilization of such elements. Several members of the APOBEC3 family of DNA cytosine deaminases have been shown to restrict the ability of endogenous retroelements to transpose around the genomes of both yeast and mammalian cells. The antibody gene deaminase, Activation-Induced cytosine Deaminase (AID), is also a member of this protein family. Curiously, whereas AID and antibody diversification reactions seem to be present in all vertebrates, the APOBEC3 proteins are only apparent in mammals and they likely arose by an ancient duplication of the AID gene. Because retroelements and AID are ubiquitous in vertebrates, we hypothesized that AID might also possess the ability to block retrotransposition (MacDuff & Harris 2006, Current Biology). We have tested this hypothesis by pitting AID from a wide variety of species against model retrotransposons in yeast and mammalian cells. Transposition of the yeast Ty1 and mouse MusD retroelements confer Histidine-prototrophy and Neomycin-resistance, respectively. We are therefore able to monitor inhibition of transposition as a reduction in the frequency of His+ or NeoR colonies plated from a culture of cells. Our results indicate that AID is indeed able to inhibit the transposition of a mammalian retrotransposon and we are working to elucidate the mechanism of restriction. Funding: Searle Scholarship, Leukemia Research Fund, University of Minnesota, National Institutes of Health (GM080437) and University of Minnesota Graduate School Doctoral Dissertation Fellowship.
9. Molecular mechanisms of host adaptations and cross-species infections of SARS coronavirus

Fang Li

It is believed that a novel coronavirus, SARS-CoV, was passed from palm civets to humans and caused the epidemic of Severe Acute Respiratory Syndrome (SARS) in 2002-2003. The major species barriers between humans and civets for SARS-CoV infections are the specific interactions between a defined receptor-binding domain (RBD) on a viral spike protein and its host receptor, angiotensin-converting enzyme 2 (ACE2). This study has determined crystal structures of the RBD from various SARS-CoV strains in complex with human or civet ACE2. These structures have revealed structural basis for the major species barriers between humans and civets for SARS-CoV infections. They enhance our understanding of host adaptations and cross-species infections of SARS-CoV and other emerging animal viruses.

10. Epithelial cell receptor-blind measles virus remains virulent but cannot cross epithelia and is not shed

Vincent H. J. Leonard, Patrick L. Sinn, Gregory Hodge, Tanner Miest, Patricia Devaux, Numan Oezguen, Werner Braun, Paul B. McCray, Jr., Michael B. McChesney, and Roberto Cattaneo

The current model of measles virus (MV) pathogenesis implies that apical infection of airway epithelial cells precedes systemic spread. An alternative model suggests that primarily infected lymphocytes carry infection to epithelia basolaterally. This model predicts that a mutant MV unable to enter cells through the unidentified epithelial receptor (EpR) would remain virulent but not be shed. To test this model, we identified residues of the MV attachment protein sustaining EpR-mediated cell fusion. These non-polar or uncharged polar residues define an area located near the binding site of the signaling lymphocytic activation molecule (SLAM, CD150), the MV lymphatic cell receptor. We then generated an EpR-blind virus maintaining SLAM-dependent cell entry, and inoculated rhesus monkeys intranasally. Hosts infected with the selectively EpR-blind virus developed rash and anorexia while averaging slightly lower viremia than hosts infected with wild type, but did not shed virus in the airways. The mechanism restricting shedding was characterized using primary well-differentiated human airway epithelia. Wild type MV infected columnar epithelial cells bearing tight junctions only when applied basolaterally, while the EpR-blind virus did not infect these epithelia. These data suggest that EpR may be a basolateral tight junction protein.

11. Dynamic Interaction of the Measles Virus Hemagglutinin with Its Receptor Signaling Lymphocytic Activation Molecule

Chanakha K. Navaratnarajah, Sompong Vongpunsawad, Numan Oezguen, Thilo Stehle, Werner Braun, Takao Hashiguchi, Katsumi Maenaka, Yusuke Yanagi, and Roberto Cattaneo

The interaction of measles virus with its receptor signaling lymphocytic activation molecule (SLAM) controls cell entry and governs tropism. We predicted potential interface areas of the measles virus attachment protein hemagglutinin to begin the investigation. We then assessed the relevance of individual amino acids located in these areas for SLAM-binding and SLAM-dependent membrane fusion, as measured by surface plasmon resonance and receptor-specific fusion assays, respectively. These studies identified one hemagglutinin protein residue, isoleucine 194, which is essential for primary binding. The crystal structure of the hemagglutinin-protein localizes Ile-194 at the interface of propeller blades 5 and 6, and our data indicate that a small aliphatic side chain of residue 194 stabilizes a protein conformation conducive to binding. In contrast, a quartet of residues previously shown to sustain SLAM-dependent fusion is not involved in binding. Instead, our data prove that after binding, this quartet of residues on propeller blade 5 conducts conformational changes that are receptor-specific. Our study sets a structure-based stage for understanding how the SLAM-elicited conformational changes travel through the H-protein ectodomain before triggering fusion protein unfolding and membrane fusion.
12. Adeno-associated Virus Site-specific Integration

Nicola J Philpott

Adeno-associated virus (AAV) is a non-pathogenic vector that transduces human cells and as such has generated a lot of interest as a gene therapy vehicle. Wild type AAV has the ability to target the integration of its genome to a specific site in the human genome and we believe that this characteristic could be used to improve the efficiency and longevity of currently used vectors. AAV integration is targeted to the AAVS1 site on human chromosome 19 and the integration event is mediated by the AAV Rep protein binding to Rep-binding elements within the AAV genome and the AAVS1 site. We have previously identified a 138 bp AAV p5 integration efficiency element (p5IEE) that is sufficient in cis for Rep-mediated integration. We also found that the AAVS1 is located in a transcriptionally active region of the genome and integration of a transgene into the AAVS1 site allows long-term transgene expression in dividing cells. The targeted integration of a therapeutic transgene into a specific site in the human genome, that is not located near to oncogenes or tumor suppressors, would minimize the significant hazards associated with the non-specific integration of other viral vectors. Therefore we are developing self-complementary AAV vectors that utilize the targeted integration pathway of wt AAV to insert a therapeutic transgene into the human AAVS1. For gene therapy protocols to benefit from the ability of AAV to site-specifically integrate more needs to be understood about the precise mechanism of AAV integration. Our goal is to identify cellular proteins involved in the mechanism of AAV targeted insertion and to determine whether regulated expression of cellular proteins can be used to enhance AAV integration for gene therapy. Such knowledge is required in order to take full advantage of AAV biology and to design gene transfer vectors of maximum efficiency, longevity, and safety.

13. The KSHV vGPCR and Viral Tumorigenesis

Mark Cannon

Kaposi's sarcoma-associated virus (KSHV) is the etiologic agent of Kaposi's sarcoma (KS). KS is the fourth most common malignancy of an infectious origin and is the leading cancer throughout large parts of Africa. Although KS was described in 1872, it wasn't until 1994 that KSHV was discovered in an AIDS-related KS lesion. KSHV is the first gamma-2-herpesvirus (genus Rhadinovirus) associated with human disease and although it has many close relatives that infect lower primates, the closest related human pathogen is EBV, a gamma-1-herpesvirus (genus lymphocryptovirus). KS lesions begin as a polyclonal hyperproliferation of endothelial cells but with time can become oligo- or even monoclonal. The lesions display a complex mix of inflammatory cells, proliferating endothelial cells, and aberrant angiogenesis. The KSHV vGPCR is a chemokine receptor that KSHV pirated from the human genome and has the potential to contribute to all of these processes. For that reason it is considered a promising anti-viral target. The KSHV vGPCR is a constitutively active homologue of the IL8 receptors that signals very broadly and when expressed in transgenic mice causes KS-like lesions and can potentiate the tumorigenicity of other KSHV gene products. Our lab studies how vGPCR dysregulates normal cell signaling with the long-term goal of identifying key signaling intermediates that could be targeted as part of an anti-KSHV therapeutic strategy. We have recent data arguing that the protein phosphatase, Shp2 and the focal adhesion kinase (FAK) are both required for at least some aspects of vGPCR signaling. Both these proteins serve scaffolding as well as enzymatic functions and both are dysregulated in many malignant and infectious processes. Furthermore, both are increasingly receiving attention from medicinal chemists and great strides have been made in developing pharmacologic inhibitors of these enzymes. We will discuss the role of Shp2 and FAK in vGPCR signaling and argue that they are promising signal integration targets in the rationale design of an anti-KSHV and anti-KS therapy.
14. Chronic immune activation and long-term neuropathological injury following experimental herpes encephalitis

Maxim C-J. Cheeran, Anibal G. Armien, Cristina P. Marques, Joseph M. Palmquist, Shuxian Hu, and James R. Lokensgard

Herpes simplex virus (HSV)-1 encephalitis, like many other viral encephalitides, leads to long-term neurologic deficits in surviving patients. We have shown that experimental murine HSV-1 brain infection stimulates a robust microglial cell-driven pro-inflammatory response which precedes the presence of brain-infiltrating systemic immune cells early during the infection. In the present study, we evaluated neuropathological alterations ensuing intranasal HSV-1 infection in a mouse model and investigated the phenotype and infiltration kinetics of leukocyte trafficking into HSV-infected murine brains. We demonstrated that the progression of inflammatory lesions began at the nasal mucosa, but extended through the olfactory nerve and the cranial nerve pathways into the brain. The olfactory bulb, the piriform cortex, and the thalamus showed focal necrotizing lesions as early as 5-7 d p.i. The ventral nerve roots of the trigeminal, facial and vagus nerve and their associated nuclei were also damaged during this infection. Immunohistochemical analyses of CNS lesions, which were sustained up to 60 d p.i., demonstrated specific qualitative differences in the inflammatory cells populating the lesioned region. Flow cytometric analysis of these brain-infiltrating leukocytes at 5, 8, 14, and 30 d post-infection (p.i.), demonstrated a predominantly macrophage (CD45hiCD11b+Ly6Chi) and neutrophil (CD45hiCD11b+Ly6G+) infiltration early during infection, with elevated levels of TNF-a mRNA expression. By 14 d p.i., the immunophenotype of the brain leukocytes shifted to a predominantly lymphocytic (CD45hiCD3+) infiltrate, which was sustained until at least 30 d p.i. at a ratio of 3:1 CD8+:CD4+ T-cells. This T lymphocyte infiltration paralleled increased IFN-gamma mRNA expression in the brain. Activation of resident microglia (CD45intCD11b+) was also detected until 30 d p.i., as assessed by MHC class II expression. In addition, activated Mac2 expressing cells were demonstrated in the brain at 60 d p.i. Furthermore, infected mice given activated immunocytes at 4 d p.i. showed a significant increase in mortality. Further studies are under way to characterize the distribution, kinetics, and location of these inflammatory cells in different brain regions ensuing HSV-1 infection. Taken together, these results demonstrate that intranasal infection results in early macrophage and neutrophil infiltration into the brain followed by prolonged microglial activation and T lymphocyte retention. This prolonged neuroimmune activation may contribute to the neuropathological sequelae observed following herpes encephalitis.

15. Expression of Herpes Simplex Virus Type 1 ICP27 in Uninfected HeLa Cells Activates p38 Signaling and Leads to Apoptosis

Peter Gillis and Steve Rice

The immediate-early regulatory protein ICP27 has been implicated in a variety of functions important for herpes simplex virus type 1 (HSV-1) replication, including viral gene induction, host shut-off, activation of mitogen-activated kinases (MAPKs) p38 and JNK, and apoptosis inhibition. In the present study we sought to identify the functions that ICP27 can carry out in the absence of viral infection by creating stably-transfected HeLa cell lines that inducibly express ICP27. We characterized two such cell lines and found that expression of ICP27 is associated with a significant cellular growth defect. The observed defect in growth is caused at least in part by ICP27-induced apoptosis as indicated by caspase-3 activation and characteristic changes in cellular morphology. In an effort to identify the activity of ICP27 which is responsible for inducing apoptosis, we examined MAPK activation after ICP27 induction. We found that ICP27 expression is sufficient to robustly activate p38 MAPK to a level similar to that observed during wild type HSV-1 infection. ICP27 also activates JNK MAPK, but to a lower level than is observed during viral infection, based on the levels of phosphorylated JNK detected using western blotting and immunofluorescent microscopy. Using chemical inhibitors of the MAPKS, we show that p38 signaling is responsible for the induction of apoptosis in the induced cell lines. Our findings suggest that during viral infection, ICP27 activates the p38 signaling pathway leading to a pro-apoptotic signal. This
finding is in contrast to previous research that has implicated ICP27 in apoptosis inhibition. We suggest that during HSV-1 infection, the initial proapoptotic signal of ICP27 is counteracted by robust JNK signaling, which requires ICP27 as well as an additional but as yet unidentified viral factor.

16. How herpes simplex virus commandeers the host cell chaperone and DNA damage machinery

Sandra K. Weller, Molecular, Microbiology and Structural Biology, University of Connecticut Health Center, Farmington CT 06030

Like other nuclear DNA viruses, herpes simplex virus type 1 (HSV-1) manipulates the cellular environment of the infected host cell. Many pathways are affected including host cell chaperones as well as components of the DNA damage response. For instance, HSV infection results in the reorganization of the heat shock cognate protein 70 (Hsc70) and the 20S proteasome into virus induced chaperone enriched (VICE) domains which also contain misfolded proteins and are resistant to detergent extraction. VICE domains are similar in some but not ways to nuclear aggresomes, cytoprotective structures that form following overexpression of misfolded proteins. HSV-1 may induce VICE formation to sequester “dangerous” signals such as misfolded proteins thus inhibiting apoptosis. Interestingly, HSV-1 also manipulates components of the host damage response. The virus apparently utilizes some components of the ATM response while dismantling others. It has been recognized for some time that HSV-1 inactivates the NHEJ response by causing the degradation of DNA-PK in a proteasome dependent manner mediated by the viral immediate-early ubiquitin ligase, ICP0. More recently, we have also shown that HSV-1 infection also inactivates the ATR-mediated damage response by causing the sequestration of hyperphosphorylated RPA and the ATR interacting protein (ATRIP) in VICE domains away from ATR itself which localizes to the nucleolus. We hypothesize that in order to create an environment conducive to viral gene expression and DNA replication, HSV-1 inactivates pathways such as NHEJ and the ATR mediated response while maintaining the ATM-response to help promote recombination-mediated replication.
17. From RT Inhibitor to RT/IN Dual Inhibitor: A Rational Design  
Zhengqiang Wang and Robert Vince

18. Development of a reverse genetics system for the Tupaia paramyxovirus  
Andrew Hudacek, Patricia Devaux and Roberto Cattaneo

19. In Vitro Characterization of the Protease Activity of the PL2 domain of Porcine Reproductive and Respiratory Syndrome Virus Nsp2 Replicase Protein  
Jun Han, Mark S. Rutherford and Kay S. Faaberg

20. Identification and validation of candidate APOBEC3G-interacting proteins  
Keisuke Shindo, Mark D. Stenglein, April J. Schumacher, William L. Brown & Reuben S. Harris

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Claudia S. Antunes Ferreira¹, Marie Frenzke¹, G. Grant Welstead², Christopher D. Richardson², Roberto Cattaneo¹.  
¹Molecular Medicine Program, Mayo Clinic, Rochester, MN 55905;  
²Department of Medical Biophysics, Univ. of Toronto and Ontario Cancer Institute, Toronto, ON, Canada M5G 2C1

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17. **From RT Inhibitor to RT/IN Dual Inhibitor: A Rational Design**

Zhengqiang Wang and Robert Vince

Singly dosed antivirals are rarely used in HIV / AIDS treatment largely due to the quick emergence of resistant viral strains. Standard AIDS chemotherapy requires combining multiple mechanistically distinct drugs to form highly active antiretroviral therapy (HAART). The success of HAART, however, hinges largely on excellent patient adherence, which is extremely difficult to achieve due to complex dosing and severe side effects associated with HAART. These problems could be alleviated by using designed multiple ligands (DMLs) which are compounds with a single structure that engages multiple biological targets. In this study, HIV RT/IN dual inhibitors featuring two distinct pharmacophores were rationally designed and synthesized. The design was based on delavirdine (DLV), a FDA-approved RT inhibitor of the BHAP family. A tolerant region of BHAP type RT inhibitors was identified and a pharmacophore responsible for IN activity was introduced to generate dual inhibitory activities. The results show that dual activity was achieved by introducing a diketoacid (DKA) functionality to the tolerable C-5 site of DLV; that activity can be balanced by functionalizing the C-3 site; and that compounds with balanced activity show low fold-resistance. The detailed design and chemical synthesis are described, and results of enzymatic assays against RT and IN, as well as cell-based assay against HIV are presented.

18. **Development of a reverse genetics system for the Tupaia paramyxovirus**

Andrew Hudacek, Patricia Devaux and Roberto Cattaneo

We are developing a reverse genetics system for the Tupaia paramyxovirus (TPMV), a negative-sense RNA virus of the tree shrew Tupaia belangeri. The virus is growing in Tupaia baby fibroblasts, but not in any other cell line tested thus far. Its nucleotide sequence revealed that it is most closely related to the morbilli- and henipaviruses of the Paramyxoviridae family. It shares many similarities with measles virus, such as its genome organization with six genes encoding for the production of eight proteins: N, P/V/C, M, F, H and L. The full genome sequence was verified and compared to the GenBank sequence (NC_002199). We identified three conserved nucleotide differences, two of which lead to amino acid substitutions in the H protein. In order to develop the reverse genetics system, we are assembling a full-length cDNA copy of the TPMV genome by joining bicistronic cDNA clones using naturally occurring restriction sites. The genomic leader and trailer were reconstituted in the full-length genomic cDNA. Transcription of the viral genome is under the control of a precisely positioned T7 promoter; cleavage after the last genomic nucleotide is executed by the hepatitis ‘ ribozyme. To rescue the virus, expression vectors have also been constructed for TPMV N, P and L proteins. The N and P constructs, along with the T7 RNA polymerase, will be used to generate a stable cell line for virus rescue. After co-transfection of these cells with the full-length cDNA and expression plasmid for the L protein, virus will be rescued by overlay onto Tupaia baby fibroblasts. The reverse genetics system for TPMV will provide a tool for characterizing the biology of the virus and the determinants of its tropism. We are particularly interested in the host proteins supporting cell entry and that interfere with viral replication.
19. In Vitro Characterization of the Protease Activity of the PL2 domain of Porcine Reproductive and Respiratory Syndrome Virus Nsp2 Replicase Protein

Jun Han, Mark S. Rutherford and Kay S. Faaberg

Porcine reproductive and respiratory syndrome virus (PRRSV) is a positive stranded RNA virus and the etiological agent of porcine reproductive and respiratory syndrome, the most economically cost infection of production pigs worldwide. A cysteine protease PL2 domain has been projected in the N-terminus of the nonstructural protein 2 (nsp2) replicase protein of PRRSV. Nsp2 is a multidomain protein and has been shown to undergo remarkable genetic variation, primarily in its middle region, while exhibiting high conservation in the N-terminal putative protease domain and the C-terminal predicted transmembrane region. Our previous study using deletion mutagenesis showed the PL2 domain is critical for viral replication. Here, we report biological aspects concerning the protease activity of PRRSV nsp2 PL2 domain as follows. (i) The nsp2-3 precursor protein is efficiently processed into one predominant product in vitro and the N-terminal PL2 cysteine protease domain mediates the cleavage. (ii) Both the PL2 domain and nsp2-3 precursor possesses trans-cleavage activity. The PL2 catalytic core required to maintain maximal enzymatic activity is about 200aa (nsp2 aa.47-240), and a further deletion would abolish trans-cleavage activity. (iii) The PL2 protease appeared to have a much stricter requirement for some amino acids for trans-cleavage activity than that of the monocistronic condition. The critical amino acids for enzymatic activity include the predicted Cys-55-His-124 dyad, Cys-111, Trp-125 and Cys-142; (iv) Comparative analysis and site-directed mutagenesis suggested that the cleavage most likely takes place at the site 1196G|G

20. Identification and validation of candidate APOBEC3G-interacting proteins

Keisuke Shindo, Mark D. Stenglein, April J. Schumacher, William L. Brown & Reuben S. Harris

APOBEC3G (A3G) has been shown to interact with many cellular proteins [Kozak et al. (2006) JBC 281, 29105-29119, Chiu et al. (2006) PNAS 103, 15588-15593, Gallois-Montbrun et al. (2007) J. Virol. 81, 2165-2178]. Many of these associations were RNA-dependent. Some may help explain high molecular mass and low molecular mass A3G cellular complexes. However, details of specific A3G-interacting proteins have yet to be understood. Therefore, to investigate potential functional associations, we examined 26 candidate A3G-interacting proteins reported by prior studies and identified through our own tandem affinity purification and yeast two hybrid experiments. First, we asked whether siRNA-mediated depletion of each candidate could affect the predominantly cytoplasmic subcellular localization pattern of A3G. The localization of GFP-tagged A3G was not obviously altered by depleting any of the 26 candidates, suggesting that these proteins are not required for the subcellular distribution of A3G or that the knockdown was not strong enough to cause an effect. Second, we asked whether siRNA-mediated depletion of each candidate could affect A3G-dependent HIV-1 restriction. 293T cells were transfected with a pool of 4 siRNAs per candidate and then transfected with HIV-GFP reporter virus constructs with or without A3G. Virus-containing supernatants were harvested and used to infect fresh 293T cells and virus infectivity was subsequently measured by flow cytometry. The resulting HIV infectivity data enabled the candidate genes to be grouped into one of four categories: (i) minimal effect---20 genes, (ii) impaired virus production---3 genes, (iii) increased virus production---1 gene or (iv) increased A3G activity---1 gene. Although most candidates proved non- or modestly interesting, the gene in category iv may be functionally connected to A3G-dependent HIV restriction.
21. Cytomegalovirus Infection and IFN-gamma Modulate MHC Class 1 Expression on Neural Stem Cells

Maxim C-J. Cheeran, Shuxian Hu, Zibing Jiang, Genya Gekker, Thomas Bakken, Joseph M Palmquist, and James R. Lokensgard

Cytomegalovirus (CMV) is the leading cause of congenital brain abnormalities in children and ventriculoencephalitis in AIDS patients. Many of the pathologies associated with CMV brain infection are seen predominantly in the periventricular region, which is known to harbor neural stem cells (NSCs). In the present study, using an adult murine model for CMV brain infection, we demonstrated that nestin-positive NSCs in the subventricular zone were positive for the virus. This infection was further characterized using monolayer NSC cultures. NSCs supported productive murine CMV (MCMV) replication; demonstrating 3-4 Log increases in viral titers and peak gB expression at 3 days postinfection (d p.i). In addition, MCMV infection induced a robust infiltration of peripheral immunocytes into the brain at 5 d p.i. RNA expression analysis of FACS sorted brain leukocyte populations demonstrated interferon (IFN)-gamma expression in CD45(hi)CD11b(dim) cells. To further characterize the effects of neuroinflammation and viral infection on NSCs, MHC class I expression and cell proliferation characteristics were studied. IFN-gamma and tumor necrosis factor (TNF)-alpha increased MHC I expression on NSCs. On the other hand, viral infection inhibited IFN-gamma-induced MHC-I expression. Furthermore, IFN-g, but not TNF-a, IL-1b, or IL-10, suppressed NSC proliferation in vitro. Extracellular release of lactate dehydrogenase (LDH) was not altered in IFN-gamma treated cultures suggesting that this cytokine may inhibit cell division. It can be concluded from these studies that NSCs are susceptible to MCMV infection and that inflammatory mediators, such as IFN-gamma, alter stem cell function.

22. Activation of reovirus virions by respiratory proteases

Rachel M. Nygaard, Joseph W. Golden, Jessica A. Bahe, Leslie A. Schiff

Mammalian reoviruses naturally infect hosts via the enteric and/or respiratory routes. Productive infection depends upon host-expressed proteases to remove the outer capsid protein sigma-3 and expose the underlying membrane penetration protein, mu-1. In murine L929 cells, endosomal acid-dependent cysteine proteases cathepsin (cat) L and cat B degrade sigma-3. In other cell lines, distinct proteases (including cat S and neutrophil elastase), can mediate productive uncoating. These cell culture studies indicate that reovirus capsid processing can be achieved by a wide variety of proteases. Despite these findings, the proteases that mediate virion uncoating during natural reovirus infections have yet to be completely defined. In a murine model of enteric infection, inhibitor studies reveal a role for secreted pancreatic serine proteases in sigma-3 removal. To identify proteases that might be involved in respiratory reovirus infections, we examined the ability of several respiratory proteases to promote productive virion disassembly. Using in vitro uncoating assays, we found that endogenous respiratory proteases, including human airway trypsin-like protease (HAT), and the inflammatory proteases cat G and chymase, can facilitate sigma-3 removal and generate ISVP-like particles. Subviral particles generated with the inflammatory proteases were infectious, replicated with the same kinetics as chymotrypsin-generated ISVPS, and did not require additional proteolytic processing by cysteine or acid-dependent proteases. We also showed that ectopic expression of HAT and other human airway proteases in cell culture promoted reovirus entry. These findings further demonstrate that reovirus capsid processing can be mediated by a variety of proteases and they provide a foundation for our studies in mouse models of respiratory reovirus infection.
23. **Attenuation of V- or C-defective measles viruses: infection control by the inflammatory and interferon responses of rhesus monkeys**

Patricia Devaux, Gregory Hodge, Michael B. McChesney and Roberto Cattaneo

Patients recruited in virus-based cancer clinical trials, and immunocompromised individuals in need of vaccination, would profit from viral strains with defined attenuation mechanisms. We generated measles virus (MV) strains defective for the expression of either the V protein, a modulator of the innate immune response, or the C protein, which has multiple functions. The virulence of these strains was compared with that of the parental wild type MV in a natural host, Macaca mulatta. Skin rash, viremia, and the strength of the innate and adaptive immune responses were characterized in groups of six animals. Replication of V- or C-protein defective viruses was short-lived and reached lower levels in peripheral blood mononuclear cells and lymphatic organs; none of the mutants reverted to wild type. Neutralizing antibody titers and MV-specific T-cell responses were equivalent in monkeys infected with any viral strain, documenting strong adaptive immune responses. In contrast, the inflammatory response was better controlled by wild type MV, as revealed by inhibition of interleukin-6 and tumor necrosis factor-alpha transcription. The interferon response was also better controlled by the wild type than the defective viruses. Since V- and C-defective MV induce strong adaptive immune responses while spreading less efficiently, they may be developed as vaccines for immunocompromised individuals. Moreover, MV unable to interact with single innate immunity proteins may be developed for preferential replication in tumors with specific contexts of vulnerability.

24. **Mutational analysis of the predicted viral glycoproteins encoded by Guinea Pig Cytomegalovirus (GPCMV) & Replacement of GPCMV gB with human cytomegalovirus (HCMV) gB in a viable chimeric GPCMV.**

Alistair McGregor, Joseph Sweet, Adam Gilbertson, Jodi Anderson, Mark Schleiss.

Species specificity precludes the direct study of HCMV in an animal model. Hence, preclinical study of intervention strategies, such as vaccines, requires the use of an animal CMV studied in its respective host. The guinea pig is the only small animal model that allows the study of congenital CMV infection. Analysis of the recently completed GPCMV genome sequence verifies that the virus encodes predicted homologs to all of the glycoproteins involved in the three distinct glycoprotein complexes found on the surface of HCMV. We had previously identified GPCMV glycoproteins gB, gH and gL and sequence analysis confirmed the existence of gO, gM and gN homologs. Using GPCMV BAC mutagenesis we were able to confirm the essential nature of GPCMV gB (GP55), gH (GP75), gL (GP115), gM (GP100) and gN (GP73) that form the GPCMV gCIII glycoprotein complexes. The various GPCMV mutants were unable to grow in tissue culture unless co-transfected with a rescue plasmid or grown on a complementing cell line. Only the gO homolog (GP74) that should complex with the gH and gL to form gCIII complex was not required for GPCMV replication in tissue culture. We next investigated the ability of HCMV gB (UL55) to functionally substitute for GPCMV gB (GP55) in a chimeric GPCMV via insertion of the UL55 ORF under GP55 promoter control in a chimeric GPCMV BAC. Transfection of the chimeric GPCMV BAC onto GPL cells resulted in the generation of viable virus that expressed HCMV gB. The confirmation of the essential nature of the GPCMV glycoproteins and the ability of HCMV gB to functionally replace GPCMV gB in a chimeric virus has implications for the further development CMV vaccines in the guinea pig model.
25. Guinea Pig Cytomegalovirus (GPCMV) encodes a functional homolog to UL84 that can complement the growth of a HCMV UL84 knockout mutant.

Alistair McGregor, Joseph Sweet & Mark Schleiss.

The GP84 gene in GPCMV is the co-linear homolog of HCMV UL84. In past studies we have demonstrated that the GPCMV co-linear genes to UL82 and UL83 encode functional homolog proteins equivalent to pp71 and pp65 respectively. In this present study we investigated if the GP84 encoded protein (pGP84) had properties similar to pUL84. Preliminary analysis of the predicted protein sequence by Davison and Stow (J.Virol. 79, 12880-92) indicated that it shared conserved motifs found in pUL84. Transient expression studies of pGP84 demonstrated that it exhibited similar properties to the UL84 protein. The pGP84 targeted the cell nucleus but also appeared to shuttle between the nucleus and cytoplasm. Additionally, pGP84 had a transdominant inhibitory effect on GPCMV growth. The transdominant inhibitory domain could be localized to a minimal peptide sequence (99 aa in length). Unlike HCMV, we found that a knockout of the GP84 gene in GPCMV via BAC mutagenesis did not affect the ability of the virus to replicate in tissue culture. However, GP84 was capable of complementing for the loss of the essential UL84 gene in a chimeric HCMV (Towne) expressing pGP84. We concluded that GP84 is a functional homolog to UL84. This makes GPCMV similar to HCMV, but less like other rodent CMV, by encoding single copy co-linear functional homologs of HCMV UL82 to UL84.

26. Novel antiviral chemotherapy that targets the HIV mutation rate

Jay Chauhan, Christine L. Clouser, Louis M. Mansky, and Steven E. Patterson

Rapid emergence of drug resistance and poor therapeutic index are significant problems with HIV chemotherapy. Therapies that exploit novel drug targets are needed for better management of HIV clinically. However, drug development is an expensive and lengthy process. To expedite this process, we have synthesized and characterized both novel and naturally occurring compounds for their anti-HIV activity. Our data demonstrate that myricetin, a naturally occurring flavonoid significantly decreased HIV infectivity without cytotoxicity. The novel compound JC1908 was also shown to have potent anti-HIV activity with minimal toxicity. We are now characterizing the ability of these drugs to be used as part of a combination drug therapy to target the mutation rate of HIV. These drugs may inhibit HIV infectivity by a novel mechanism that could be clinically useful and relevant for HIV infected individuals failing current drug therapy.

27. Humoral immune response to Porcine Circovirus 2 in experimentally infected pigs

Sumathy Puvanendiran, Craig Johnson, Tanja Opriessnig and Michael P. Murtaugh

Porcine Circovirus 2 (PCV2) is a small, non-enveloped virus with a single-stranded DNA genome. PCV2 was first isolated from pigs with post-weaning multi-systemic wasting syndrome (PMWS). PMWS has been identified in most swine-producing countries worldwide. The disease has resulted in significant health challenges and economic damage to the swine industry. While PMWS is the most serious and significant clinical presentation of PCV2 infection, several additional syndromes are associated with PCV2 infection and are collectively referred to as Porcine Circovirus Associated Disease (PCVAD). The pathognomonic histological lesions which characterize PCVAD are depletion of lymphoid cells, inflammation of multiple organs such as liver, spleen, lung
and kidney, and a high viral load in lymphoid tissues. However, the mechanisms of pathogenesis are not known. The PCV2 genome contains two major open reading frames (ORFs), ORF1 and ORF2. ORF1 encodes two replication-associated proteins Rep and Rep' and ORF2 encodes the capsid. In addition, a third ORF putatively encodes a protein with apoptotic activity. The capsid is the major immunogenic protein. There are two genotypes of PCV2 that share 90% sequence homology in ORF2. Although there are some controversial reports, it is believed that genotype 1 is more pathogenic than genotype 2. However, no studies have examined the antibody response to other PCV2 proteins or any biological differences between the two genotypes. Our objective here was to study the antibody response to all viral proteins in pigs experimentally with PCV2 genotype 1 or genotype 2. PCV2 capsid, rep and the possibly apoptotic protein were expressed in E. coli strain BL21 RP, purified by Ni-NTA agarose bead affinity chromatography (Qiagen) and used as antigens for indirect ELISA. Serum samples were obtained from uninfected and infected pigs at weekly intervals and assayed for the presence of antibodies. Our results indicated that animals started to show antibody response to capsid 14 days after infection. There was no significant difference between two genotypes. The antibody response to rep was variable, and no antibody response was observed to the putative apoptosis protein. These results indicate that capsid is the major target of the humoral immune response and it is directed against epitopes conserved in both genotypes.

28. An oncolytic measles virus with a retargeted canine distemper virus envelope circumvents neutralizing antibodies

Koon-Chu Yaia, Guy Ungerechts, Johanna Lampe, Marie E Frenzke, Christoph Springfield, Veronika von Messling and Roberto Cattaneo

Measles virus (MV) is a promising vector for cancer therapy, but high neutralizing antibodies titers of cancer patients might interfere with its oncolytic efficacy. To generate a virus capable of infecting cancer cells in the presence of MV-neutralizing antibodies, we enclosed the MV replicative unit in the envelope of another Morbillivirus, canine distemper virus (CDV). The fusion and attachment proteins of MV and CDV have 66 and 37% identity, respectively, and they have minor serological cross-reactivity. To target the CDV attachment protein (hemagglutinin, H) to cancer cells we displayed on it a single-chain antibody specific for human carcinoembryonic antigen (CEA). The CDV H protein displaying this single chain antibody, coexpressed with the homologous fusion (F) protein, mediated fusion of murine adenocarcinoma cells stably expressing human CEA (MC38cea), proving targeting competency of the CDV envelope. A genome coding for the MV replicative unit and the targeted CDV envelope was engineered and the chimeric virus MV-FCDVHCDVantiCEA generated. This virus grew in Vero cells to titers similar to the parental MV, and in rodent MC38cea cells it maintained the MV cytopathic effect. Its neutralization characteristics were assessed using antisera of six individuals with a history of MV infection or vaccination. In the serum of four individuals no cross-reactive antibodies were detected, whereas the sera of six individuals neutralized the chimeric virus 8-40 times less efficiently than MV. Similarly, sera of mice vaccinated with MV showed neutralizing activity only against MV but not against the chimeric virus. To assess the oncolytic efficacy of the chimeric virus in the presence of anti-MV antibodies, we will rely on an immunocompetent murine model (Ungerechts et al., 2007. Mol Ther. 15: 1991-1997). Toward this, we are arming the targeted chimeric virus with a prodrug convertase gene (purine nucleoside phosphorylase, PNP).
29. **Leukocyte infiltration into cytomegalovirus infected brain**

M.C-J. Cheeran, S. Hu, T. Bakken, J.M. Palmquist, and J.R. Lokensgard

Cytomegalovirus (CMV) infection induces a transient but protective neuroinflammatory response. We have previously shown that the absence of interleukin (IL)-10 results in dysregulated cytokine responses which turns a benign CMV infection lethal. In addition, infiltrating CD8(+) T cells protect against CMV brain infection through a perforin-dependent mechanism. Longitudinal analysis of immune cell migration into CMV infected brains, using a bioluminescence imaging system and adoptive transfer of splenocytes obtained from MCMV primed beta-actin-luciferase transgenic mice, showed maximal infiltration at 5 days post infection (d p.i.). Infiltration of splenocytes in virus-infected animals was significantly increased compared to sham-infected controls. Further examination of leukocytes isolated from infected and sham-infected brains showed two populations of CD45(+) cells, one expressing intermediate levels [CD45(int)] and the other with high CD45 (hi) expression. CD45(int) cells expressed CD11b and represents the resident microglial cell population. The infiltrating CD45(hi) cells were dramatically increased in virus-infected brains. The infiltrating cells expressed T cell, CD3(+); macrophage, CD11b(+); and NK cell, CD49b(+) markers. Among the T lymphocytes, there was a higher proportion of CD8(+) T cells, than CD4(+) cells at 5 d p.i. MCMV infection increased MHC class II expression on both the macrophages and microglial cell populations. Furthermore, RNA analysis of FACS sorted brain leukocyte populations demonstrated that the predominant contribution of iNOS expression was from the macrophage population. Interestingly, we also found four-fold higher levels of iNOS expression in IL-10 deficient mice than wild-type controls. Studies are ongoing to determine if IL-10 deficiency alters the patterns of leukocytes infiltration and corresponding cytokine expression in CMV infected brains.

30. **Discovery of Novel Plant-Derived Compounds That Block HIV-1 Maturation**

Casey Dorr, Oksana Kolomitsyna, Sergiy Yemets, Pavel Krasutsky and Louis Mansky

Clinical concerns regarding HIV-1 drug resistance and side effects emphasize the need to identify new drug targets and develop new anti-HIV-1 drugs. A high-throughput single-cycle HIV-1 assay was developed to screen more than 400 novel triterpene compounds derived from northern Minnesota birch trees for anti-HIV-1 activity. We report the discovery of triterpene compounds that block the late stages of HIV-1 replication. In particular, our findings implicate the mechanism of action as the perturbation of Gag protein processing, specific blocking of the p25 to p24 conversion. This leads to a specific defect in virus maturation and not a general defect in HIV-1 protein processing. These novel triterpene compounds have a similar mechanism of action to that of PA457, a maturation inhibitor currently in clinical trials. Our findings indicate that these triterpene compounds are viable candidates for antiviral drug development.

31. **A novel chemical class of quinazolines with anti-cytomegalovirus effects directed against the inhibition of protein kinase activity**

K. Yeon Choi, Mark Schleiss, Michael Leviton, Jodi Anderson, Manfred Marschal

Cytomegalovirus infection is associated with severe disease in immunocompromised individuals, especially transplant patients, AIDS patients and newborns. Current antiviral therapy faces limitations, such as the induction of adverse effects and drug resistant viruses. In a search of novel drug candidates, we describe here the anti-cytomegaloviral
properties of a chemical class of quinazoline, gefitinib (Iressa®). This compound showed strong inhibitory effects in vitro against human and guinea pig CMV with IC50 in a low micromolar range. Cytotoxicity did not occur at these effective concentrations. The antiviral mode of action was based on the inhibition of protein kinase activity, mainly directed to a viral target kinase (UL97/GP97) in addition to cellular target candidates. In a guinea pig model, gefitinib therapy reduced the magnitude of viral load in blood and lung tissue as detected by real time PCR. Importantly, the rate of mortality of infected animals was reduced by gefitinib treatment.

32. **Novel combination antiviral therapy that targets the HIV-1 mutation rate**

Christine L. Clouser, Steven E. Patterson, Irene J. Dorweiler and Louis M. Mansky

Successful HIV-1 therapy is threatened by the emergence of drug resistance. Therapies that exploit novel drug targets are needed for better management of HIV clinically. In this study, we discovered a novel combination of nucleoside analogs, decitabine and gemcitabine (an anti-metabolite), that synergistically decreased HIV-1 infectivity at nanomolar concentrations without affecting cellular proliferation. The decrease in viral infectivity coincided with a 3.4 fold increase in mutation frequency, implicating increased mutagenesis as a potential mechanism of action. Furthermore, a 40% increase in G-to-C transversion mutations in the proviral DNA were observed and support that decitabine was incorporated into the viral DNA. These findings implicate lethal mutagenesis as playing a role in reducing viral infectivity and suggest that gemcitabine and decitabine, which are already clinically approved for human use, may be a potent anti-HIV therapy that reduces viral infectivity by targeting the HIV-1 mutation rate. This is the first demonstration of a clinically viable combination therapy (ie, viral mutagen and ribonucleotide reductase inhibitor) that extinguishes HIV-1 infectivity by lethal mutagenesis.

33. **Pathways of lymphatic dissemination of wild type measles virus in SLAM-expressing mice**

Claudia S. Antunes Ferreira¹, Marie Frenzke¹, G. Grant Welstead², Christopher D. Richardson², Roberto Cattaneo¹.

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Recent studies in primate models have indicated that the primary replication of wild type measles virus (wt-MV), as that of the animal morbilliviruses, occurs in lymphatic cells expressing the signaling lymphocyte activation molecule (SLAM; CD150), the primary MV receptor. We have generated mice expressing human SLAM with human-like tissue specificity. These mice were bred in an interferon type I deficient background (interferon A receptor knock out, Ifnarko) to allow more extensive MV replication. We hypothesize that SLAM-expressing lymphatic cells may support robust primary viral replication. Groups of three Ifnarko-huSLAM mice were inoculated intranasally (IN) with 106 TCID50 of wt-MV and sacrificed at one, two and three days after inoculation. Serial cryosections of lymphatic organs and lungs were obtained and analyzed by immunohistochemistry and immunofluorescence. We show here that, one day after IN inoculation, wt-MV was detected in upper respiratory lymph nodes (mandibular and mediastinal lymph nodes), in medullary area of thymus and in lungs. We found that MV colocalizes with alveolar macrophages and we are characterizing the cells that support MV replication in the lymph nodes. We previously found that intraperitoneal inoculation (IP) allows MV to
spread more efficiently compared to IN inoculation, so we inoculated groups of six animals IP with 1.5 x 10^6 TCID50 of wt-MV. Three days after inoculation, wt-MV was detected by immunohistochemistry in the mediastinal and mesenteric lymph nodes as well as in spleen and reached titers of 101.5 to 103 TCID50 per 106 mononuclear cells. MV was confined to splenic white pulp and immunostaining of consecutive sections identified T lymphocytes as the main target cell in this organ. Thus, Ifnarko-huSLAM mice are beginning to give insights in the early phases of wt-MV spread.

34. **The Role of SIV-specific CD8+ T cells in the Establishment of Persistent SIV Infection**

Teresa L. Mattila, Joy Folkvord, Elizabeth Connick, Pamela J. Skinner

CD8+ T cells are important in controlling viral infections, including HIV infections. Although the appearance of HIV-specific CD8+ T cells does initially correlate with reduced viral load during HIV infection, for unknown reasons this response is ultimately insufficient, and HIV is never fully cleared from the body. Because the CD8+ T cell response is important in controlling viremia, yet unable to completely eliminate virus from the system, it is important to determine why this vital component of the adaptive immune system fails to completely control HIV infection. The goal of this study is to gain understanding of why virus-specific CD8+ T cells cannot entirely eliminate virus-producing cells during HIV and SIV infections. Our central hypothesis is that B cell follicles are an immune privileged site in which virus-producing cells are protected from virus-specific CD8+ T cells. Our recent findings show that HIV-producing cells concentrate in B cell follicles and HIV-specific CD8+ T cells are excluded from areas within B cell follicles in lymph node sections from HIV-infected individuals. Because many similarities exist between HIV infection in humans and simian immunodeficiency virus (SIV) infection in macaques and SIV model systems are essential tools to understanding HIV/SIV infections and for the development of HIV vaccines, we set out to determine whether the exclusion of virus-specific CD8+ T cells from B cell follicles and the concentration of virus-producing cells in follicles also occurs in the SIV/rhesus macaque model system. We also set out to determine whether during the very early stages of infection prior to the establishment of detectible virus-specific CD8+ T cells, virus-producing cells are concentrated in B cell follicles or are found abundantly in extrafollicular regions as well. For these studies we are determining the spatial localization of SIV-specific CD8+ T cells and SIV-infected cells relative to B cell follicles in the lymph nodes of SIV-infected rhesus macaques, using immunohistochemistry, in situ tetramer staining, in situ hybridization and quantitative image analysis. Our preliminary data shows that there are similar amounts of virus inside and outside of B cell follicles at early time points after infection, but at later time points, virus is more concentrated inside follicles than outside, while at later time points SIV-specific CD8 T cell were detected and were excluded from areas within B cell follicles. These data support the hypothesis that B cell follicles are an immune privileged site in which SIV-producing cells are protected from SIV-specific CD8+ T cells. These data have important implications for the development of a successful HIV vaccine and treatments developed to eradicate HIV.

35. **Oncolytic measles viruses encoding interferon β and the thyroidal sodium iodide symporter for mesothelioma therapy**

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Mesothelioma is a devastating malignancy, typically related to asbestos exposure and
leading to death within 8 to 14 months of diagnosis. Based on promising results in murine mesothelioma models, a nonreplicating adenoviral vector coding for human IFN-β (Ad-IFN-β) was evaluated in a clinical trial, leading to long term remissions in two of the treated patients (Clin Cancer Res 2007;13(15) August 1, 2007). Possible mechanisms underlying the antitumor activity of IFN-β include its antiproliferative actions, anti-angiogenic activity and recruitment of host effector cells to sites of expression. In order to increase the potency of oncolytic measles viruses for mesothelioma therapy, we inserted the IFN-β gene into MV-Edm and MV-NIS vector backbones. The rationale for a virus expressing both IFN-β and the human thyroidal sodium iodide symporter (NIS) was to facilitate noninvasive in vivo monitoring of virus propagation by radioiodine imaging. Human and mouse interferon-β genes were amplified by polymerase chain reaction (PCR) and cloned upstream of the N gene in MV-Edm or MV-NIS backbones. The corresponding viruses, MV- mIFN β and MV- mIFN β-NIS, were rescued by standard methods. The viruses were amplified on Vero cells and their one-step growth curves were found to be comparable to other oncolytic measles strains. Mesothelima cell lines REN, M30 and MSTO-211H were infected with the recombinant viruses at MOIs of 0.1 or 1. Cell viability, IFN-β release and radioiodide uptake were evaluated at various timepoints after infection. Both viruses successfully propagated in the human mesothelioma cells leading to intercellular fusion and cell death. High levels of mouse IFN-β were detected in the supernatants of the infected cells and radioiodine uptake was substantial in the cells infected with MV-mIFN β-NIS. It is expected that these new oncolytic MVs that code for IFN β and NIS will prove to be potent and versatile agents for the treatment of human mesothelioma. In vivo studies are ongoing.

36. Structure of the DNA deaminase domain of the HIV-1 restriction factor APOBEC3G

Kuan-Ming Chen, Elena Harjes, Phillip J. Gross1, Amr Fahmy, Yongjian Lu1, Keisuke Shindo, Reuben S. Harris & Hiroshi Matsuo

Human APOBEC3G is a DNA deaminase that potently inhibits the replication of HIV-1, other retroviruses and retrotransposons. Scant structural information was available for it or related polynucleotide cytosine deaminases, including the mRNA editing protein APOBEC1 or the antibody gene mutator AID. Here, we report the solution structure of the catalytic domain of APOBEC3G. Mutants were screened for improved solubility and enhanced activity to identify a monomeric variant (A3G-2K3A) with significant improvement in both assays. Structural and functional data combined to suggest a DNA deamination mechanism in which a brim of positively charged residues coordinates single-stranded DNA and positions the target cytosine for catalysis.

37. 454 Pyrosequencing of the Lawsonia intracellularis genome

Sushmita Singh and Zheng Jin Tu

Lawsonia intracellularis, an obligate intracellular parasite, is the causative agent of proliferative enteropathy in pigs and a variety of animal species including primates. The complete genome sequence of L. intracellularis PHE/MN1-00 strain was obtained using Sanger sequencing (gi:94730694). Approximately 12,000 clones were sequenced from both ends using Dye-terminator chemistry on ABI 3700 and 3100 Genetic Analyzers (Applied Biosystems, Foster City, CA) at the BMGC DNA Sequencing and Analysis Facility. A total of over 23,000 sequences were used to generate the final assembly, representing a ten-fold coverage of the 1.7Mb genome. Sequence assembly and verification were accomplished with PhredPhrap (http://genome.washington.edu). Several methods including primer walking, homology-based comparisons of gap ends, and multiplex random PCR were used to close the approximately 100 gaps at the end of the shotgun phase. Resequencing of L. intracellularis PHE/MN1-00 using 454
Pyrosequencing technology was performed to confirm the presence of two 5kb (5444 bp) identical spans of DNA fragments identified in the Lawsonia genome from the Sanger data. Using GS FLX (Roche Applied Science, Indianapolis, IN) a total of 43,343,099 base pairs of sequence data were generated providing almost 25X coverage of the Lawsonia genome. The data thus generated were assembled using 454_s GS Assembler program separately and then in conjunction with the Sanger data. We will discuss our findings on Lawsonia genome structure and present a comparative analysis of different assembly programs used in the study.

38. Analyses of the two APOBEC3 genes of artiodactyls indicate that the seven human homologs arose by frequent recombination prior to primatification

Rebecca S. LaRue, Stefan R. Jónsson, Kevin Silverstein, Valgerdur Andrésdóttir, Tim P. L. Smith and Reuben S. Harris

APOBEC3 (A3) proteins deaminate cytosines within single-strand DNA and block the replication of many retroviruses and retrotransposons, and potentially DNA transposons. Each A3 protein has one or two conserved zinc-coordinating motifs (Z1a, Z1b or Z2). The presence of only one A3 gene in mice and seven in humans indicates that a remarkable mammal-specific expansion has occurred (Z1a-Z2 versus Z1b, Z1a-Z1b, Z1a, Z1a-Z1a, Z1a-Z1a, Z1a-Z1b, Z2). To gain insights into the mechanism and timing of A3 gene expansion and into the functional modularity of these genes, we analyzed the genomic sequences, expressed mRNAs and activities of the full A3 repertoire of representative artiodactyls (cattle, sheep, and pigs) positioned phylogenetically between rodents and primates. Cattle and sheep have two A3 genes (A3A and A3F; Z1b and Z1a-Z2, respectively), whereas pigs have one (A3F; Z1a-Z2). Domestic and wild pig comparisons indicated that A3A was lost early in the Suidae branch. Interestingly, conserved alternative splicing and internal transcription initiation produced active A3F variants with a Z1a or Z2 zinc-coordinating motif. The artiodactyl A3 genes show little signs of positive selection in contrast to the extremely high levels observed in primates. We conclude that the common ancestor of artiodactyls and primates had two A3 genes and, importantly, the minimal set of zinc-coordinating motifs required to evolve into the present day human A3 repertoire (Z1b-Z1a-Z2). Our studies suggest that the present day human A3 repertoire largely originated prior to the point that primates branched off the mammalian tree, during a relatively brief and dynamic evolutionary period.

39. Demonstration of a dual mechanism for mutagenic ribonucleosides in decreasing viral infectivity and inducing HIV-1 mutagenesis

Michael Dapp, Christine Clouser, Steven Patterson, and Louis M. Mansky

The antiviral drugs that comprise highly active antiretroviral therapy (HAART) have been a key success in the clinical management of HIV-1 infection. Clinical limitations, e.g., HIV-1 drug resistance, provide the impetus for the development of new targets and new antiretroviral drugs. Here we report the characterization of a novel nucleoside analog (Vidaza, which is used in the treatment of myelodysplastic syndrome) that has anti-HIV activity and exploits two different steps in the viral life cycle. Using a single round replication assay, we show that Vidaza impacts both the early (i.e., reverse transcription) and late (i.e., transcription) phases of the HIV-1 life cycle in a concentration-dependent manner, and that reductions in viral infectivity coincided with increased viral mutagenesis. Analysis of the mutation spectra implicates that the ribonucleoside is converted to the deoxynucleoside form by ribonucleotide reductase and is then incorporated into the viral DNA, leading to a specific increase in G-to-C transversion mutations. Our data also supports the direct incorporation of the ribonucleoside into viral RNA, which also leads to an increase in the opposite C-to-G transversion mutations in
proviral DNA. Our findings provide a proof-of-principle that a ribonucleoside analog can act in a bifunctional manner to affect two steps in the HIV-1 life cycle (reverse transcription and transcription) and ultimately reduce viral infectivity. In each antiviral mechanism, enhanced viral mutagenesis is implicated, suggesting a role for lethal mutagenesis in decreasing viral infectivity. We suggest that such bifunctional inhibitors may possess greater clinical durability.

40. **Destruction of the fibroblastic reticular cell network is associated with reduction of interleukin-7 and depletion of the naïve CD8+ T cell population in HIV/SIV infection**

Ming Zeng, Timothy Schacker, Daniel Feldman, Steve Wietgrefe, John Carlis, Jeffery Lifson, Ashley Haase.

The network of fibroblastic reticular cells (FRCs) in the T cell area, which supports the complex microarchitecture of the lymph nodes, is critical for the homeostasis of T cells, especially naïve T cells by providing survival factors such as interleukin-7 (IL-7). How the FRC network changes during human immunodeficiency virus (HIV) infection and pathogenic simian immunodeficiency virus (SIV) infection of rhesus macaques (RMs) is not understood. Here we show that the FRC network is progressively destroyed in SIV infection of RMs. The destruction of the FRC network is temporally and spatially associated with a decrease in IL-7 level and an increase in cellular apoptosis level in lymph nodes in HIV/SIV infection. Importantly, the reduction of IL-7 is strongly associated with reduction of naïve CD8+ T cells in lymph nodes in HIV infection. Supporting the concept that the destruction of the FRC network plays a pathogenic role in HIV/SIV infection, the FRC network remains intact in the chronic nonpathogenic SIV infection of sooty mangabeys (SMs), along with normal levels of naïve CD4+ and CD8+ T cells in the lymph nodes. Our data suggest that the destruction of the FRC network in HIV/SIV infection may impair the T cell homeostasis through reduction of IL-7 production. Furthermore, as the first study showing that naïve CD8+ T cells are severely reduced in lymphoid tissues in HIV+ infection, this result provides an insight into a new mechanism underlying the immunodeficiency of HIV-infected individuals.

41. **The Antibody Gene DNA Deaminase, AID, Can Also Restrict Retroelement Transposition**

Donna A. MacDuff and Reuben S. Harris

Endogenous retroelements are mobile _copy and paste_ transposons, present in the genomes of all eukaryotic cells. New transposition events can disrupt or alter gene function and the presence of multiple copies of the same retroelement can lead to duplications, deletions and genomic rearrangements through homologous recombination. While such events may be beneficial to evolution if they occur at relatively low frequencies within the germ line, they can also be deleterious if they occur in the somatic cells of a multicellular organism by promoting carcinogenesis. Clearly, a variety of mechanisms must exist to limit the mobilization of such elements. Several members of the APOBEC3 family of DNA cytosine deaminases have been shown to restrict the ability of endogenous retroelements to transpose around the genomes of both yeast and mammalian cells. The antibody gene deaminase, Activation-Induced cytosine Deaminase (AID), is also a member of this protein family. Curiously, whereas AID and antibody diversification reactions seem to be present in all vertebrates, the APOBEC3 proteins are only apparent in mammals and they likely arose by an ancient duplication of the AID gene. Because retroelements and AID are ubiquitous in vertebrates, we hypothesized that AID might also possess the ability to block retrotransposition (MacDuff & Harris 2006, Current Biology). We have tested this hypothesis by pitting AID from a wide variety of species against model retrotransposons in yeast and mammalian cells.
Transposition of the yeast Ty1 and mouse MusD retroelements confer Histidine-prototrophy and Neomycin-resistance, respectively. We are therefore able to monitor inhibition of transposition as a reduction in the frequency of His+ or NeoR colonies plated from a culture of cells. Our results indicate that AID is indeed able to inhibit the transposition of a mammalian retrotransposon and we are working to elucidate the mechanism of restriction. Funding: Searle Scholarship, Leukemia Research Fund, University of Minnesota, National Institutes of Health (GM080437) and University of Minnesota Graduate School Doctoral Dissertation Fellowship.

42. Molecular mechanisms of host adaptations and cross-species infections of SARS coronavirus

Fang Li

It is believed that a novel coronavirus, SARS-CoV, was passed from palm civets to humans and caused the epidemic of Severe Acute Respiratory Syndrome (SARS) in 2002-2003. The major species barriers between humans and civets for SARS-CoV infections are the specific interactions between a defined receptor-binding domain (RBD) on a viral spike protein and its host receptor, angiotensin-converting enzyme 2 (ACE2). This study has determined crystal structures of the RBD from various SARS-CoV strains in complex with human or civet ACE2. These structures have revealed structural basis for the major species barriers between humans and civets for SARS-CoV infections. They enhance our understanding of host adaptations and cross-species infections of SARS-CoV and other emerging animal viruses.

43. Epithelial cell receptor-blind measles virus remains virulent but cannot cross epithelia and is not shed

Vincent H. J. Leonard, Patrick L. Sinn, Gregory Hodge, Tanner Miest, Patricia Devaux, Numan Oezguen, Werner Braun, Paul B. McCray, Jr., Michael B. McChesney, and Roberto Cattaneo

The current model of measles virus (MV) pathogenesis implies that apical infection of airway epithelial cells precedes systemic spread. An alternative model suggests that primarily infected lymphocytes carry infection to epithelia basolaterally. This model predicts that a mutant MV unable to enter cells through the unidentified epithelial receptor (EpR) would remain virulent but not be shed. To test this model, we identified residues of the MV attachment protein sustaining EpR-mediated cell fusion. These non-polar or uncharged polar residues define an area located near the binding site of the signaling lymphocytic activation molecule (SLAM, CD150), the MV lymphatic cell receptor. We then generated an EpR-blind virus maintaining SLAM-dependent cell entry, and inoculated rhesus monkeys intranasally. Hosts infected with the selectively EpR-blind virus developed rash and anorexia while averaging slightly lower viremia than hosts infected with wild type, but did not shed virus in the airways. The mechanism restricting shedding was characterized using primary well-differentiated human airway epithelia. Wild type MV infected columnar epithelial cells bearing tight junctions only when applied basolaterally, while the EpR-blind virus did not infect these epithelia. These data suggest that EpR may be a basolateral tight junction protein.
Dynamic Interaction of the Measles Virus Hemagglutinin with Its Receptor Signaling Lymphocytic Activation Molecule

Chanakha K. Navaratnarajah, Sompong Vongpunsawad, Numan Oezguen, Thilo Stehle, Werner Braun, Takao Hashiguchi, Katsumi Maenaka, Yusuke Yanagi, and Roberto Cattaneo

The interaction of measles virus with its receptor signaling lymphocytic activation molecule (SLAM) controls cell entry and governs tropism. We predicted potential interface areas of the measles virus attachment protein hemagglutinin to begin the investigation. We then assessed the relevance of individual amino acids located in these areas for SLAM-binding and SLAM-dependent membrane fusion, as measured by surface plasmon resonance and receptor-specific fusion assays, respectively. These studies identified one hemagglutinin protein residue, isoleucine 194, which is essential for primary binding. The crystal structure of the hemagglutinin-protein localizes Ile-194 at the interface of propeller blades 5 and 6, and our data indicate that a small aliphatic side chain of residue 194 stabilizes a protein conformation conducive to binding. In contrast, a quartet of residues previously shown to sustain SLAM-dependent fusion is not involved in binding. Instead, our data prove that after binding, this quartet of residues on propeller blade 5 conducts conformational changes that are receptor-specific. Our study sets a structure-based stage for understanding how the SLAM-elicited conformational changes travel through the H-protein ectodomain before triggering fusion protein unfolding and membrane fusion.

Defining the Mechanism of Adeno-Associated Virus Site Specific Integration at the AAVS1 Site

Michael P. Jarcho and Nicola J. Philpott

Adeno-associated virus (AAV) has great potential as a gene therapy vector. AAV integration into a host genome is specific to the AAVS1 site, thus avoiding the problems associated with randomly integrating vectors. To make use of this characteristic we must first fully identify and understand the mechanisms by which AAV integrates into the AAVS1 site. AAV site specific integration is mediated by the AAV Rep protein and the AAV p5 promoter element. The AAVS1 site is 8.2 kb and located on human chromosome 19. We are endeavoring to sequence the specific sites of insertion to better understand p5-Rep-mediated integration. Cells were transfected with plasmid DNA containing combinations of Rep, the p5 promoter and eGFP transgene. Clonal cell lines were generated, the genomic DNA isolated and insertion sites are being analysed.

In order to decipher the mechanism of AAV integration our goal is to identify the cellular proteins involved. We are using two methods to do this; siRNA knockdown of cellular proteins that bind Rep and knockout of genes involved in DNA repair. ANP32B is a protein known to bind to Rep. Using ANP32B siRNA plasmids generated at the U of M core facility we were able to develop cell lines that express decreased levels of ANP32B. We have transfected these knock down cell lines with plasmid DNA containing the p5 promoter and Rep. The rate of integration will allow us to determine whether ANP32B affects the rate of Rep-mediated integration. Rep-mediated integration occurs by a mechanism of non-homologous recombination; therefore, we hypothesize that DNA repair proteins play a role in integration. DNA-PKcs and Ku70 are components of the human DNA-PK complex that regulates DNA double strand break repair and have both been shown to bind Rep. We have acquired several cell lines where these (and other repair proteins) have been knocked out (kind gift of Eric Hendrickson, BMHH). By transfecting these cell lines with plasmid DNA containing the p5 promoter and Rep we will be able to determine how these proteins affect integration. Once these questions are answered we will be one step closer to using AAV as a viable gene therapy vector.