

The 13th Annual Meeting of the Rocky Mountain Virology Association: Current Advances in Virology and Prion Biology in the Rocky Mountain region

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Virology and Prion biology has significantly impacted basic research in molecular biology, neurology and neuropathology, immunology, epidemiology, epigenetics and gene regulation, pathogenesis. In addition, many technical improvements ranging from trans-gene expression to high-throughput screening have their roots in virology/prion research. Translational medicine has benefitted not only from the technical advances, but also from recent advances in cell biology stemming from basic virology/prion biology. With this in mind, it would benefit those interested in translational medicine to keep abreast of current topics in virology.

The 13th annual meeting of the Rocky Mountain Virology Association

(www.RockyMountainVirologyClub.org) was held Sept 27-29, 2013 despite unprecedented flooding that caused severe regional damage and compromised road access to the forestry extension campus of Colorado State University at Pingree Park. Our annual meeting has grown significantly since its 2001 inauguration; however, the focus has remained the same. Our aim remains to gather local, regional and national virologists to share and discuss scientific data and ideas, to foster graduate student education, and to help early stage investigators in their career development. This year, approximately 80 students, post-docs, early-stage and established scientists (basic, clinical and veterinary) navigated washed-out unimproved mountain roads, misting rain and snow squalls to attend 40 presentations highlighting advances in virology and prion biology. In attendance were individuals from Montana, Wyoming, Colorado, Iowa and Tennessee along with one moose and two mountain lions. Our professionally managed on-site child day care was completely utilized and added a unique poster to our poster session that displayed a child's-eye view of virology. Dr. Barry T. Rouse, BVSc, Ph.D., DSc, University of Tennessee presented the plenary talk describing factors host and virus factors involved in virus infection. In addition, Susan Carpenter, Ph.D. Iowa State University, described strategies of Lentivirus persistence, Mario L. Santiago, Ph.D. University of Colorado School of Medicine described immunobiology of retrovirus restriction factors and Candace K. Mathison, Ph.D. Colorado State University talked about maternal transmission of chronic wasting disease. Taken together, this year's meeting was a success in quality of presentations, collaborations established, and careers advised all in the picturesque setting of golden Aspen, white capped peaks and clear nights filled by the Milky Way. Following is a brief summary of the presentations provided by the contribution author (**identified by number in the figure**) listed in alphabetical order.



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Figure. Attendees at the 2013 Rocky Mountain Virology Society meeting.

- 1) Donal O'Toole;
- 2) Neeltje van Doremalen;
- 3) Claire Birkenheuer;
- 4) Nisha Duggal;
- 5) Charlie Calisher;
- 6) Hannah Romo;
- 7) Amber Rico;
- 8) Nunya Chotiwan;
- 9) Penny Clarke;
- 10) Joel Rovnak;
- 11) Dieandra Walker;
- 12) Jacqueline Bowlin;
- 13) Stephanie James;
- 14) Anna Blackmon;
- 15) Ann Hawkinson;
- 16) Aimee Ortega;
- 17) Amy Stone;
- 18) Danielle Adney;
- 19) Carol Blair;
- 20) Angela Mitchell;
- 21) Maria Nagel;
- 22) Myrna Miller;
- 23) Mario Santiago;
- 24) Rushika Perera;
- 25) Selene Marysol Garcia-Luna;

- 26) Stephanie Moon;
- 27) Amanda McGuire;
- 28) Brian Niemeyer;
- 29) Eamon Quick;
- 30) Scott Seitz;
- 31) Yonghua Zhuang;
- 32) Olve Peerson;
- 33) Seth McDonald;
- 34) Jeff Christiansen;
- 35) Grace Campagnola;
- 36) Blair DeBuyscher ;
- 37) Keriann Tremblay;
- 38) Sandy Quackenbush;
- 39) Wendy Maury;
- 40) Dan Konet;
- 41) Krista Latta;
- 42) Candace Mathiason
- 43) Kejun Guo;
- 44) Elizabeth Dietrich;
- 45) James DeMartini;
- 46) Jeff Wilusz;
- 47) Phillida A Charley;
- 48) Barry Rouse;
- 49) Albert Jeon;
- 50) Elliott Chiu;

- 51) Nicholas L. Baird;
- 52) Lauren Oko;
- 53) Michelle Deaton;
- 54) Andrew Block;
- 55) Joseph Fauver;
- 56) Joseph Prescottt;
- 57) Kevin Cavanaugh ;
- 58) Brad Barrett;
- 59) Susan Carpenter;
- 60) Geoff Letchworth;
- 61) Niklaus Mueller;
- 62) Timothy Wanninger;
- 63) Glenn Capodagli;
- 64) Tony Schountz;
- 65) Randall Cohrs;

Not pictured

Becky Gullberg; Carmen Ledesma Feliciano; Craig Miller; Farah Vera Maloof; Glenn Telling; Ken Olson; Patrick Brennan; Ryan Troyer.

Experimental infection of goats with MERS CoV.

**Danielle Adney, Vienna Brown,
Helle Bielefeldt-Ohmann and Richard Bowen**

Colorado State University.

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Danielle Adney

Abstract

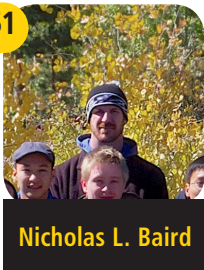
The World Health Organization recently announced the discovery and initial characterization of the Middle East respiratory syndrome coronavirus (MERS-CoV). This zoonotic β -coronavirus manifests a clinical disease in humans closely resembling severe acute respiratory syndrome (SARS), boasts a case-fatality rate of >50%, and is regarded as a substantial threat to global public health. Preliminary case studies and serological surveys suggest that the natural reservoir may be bats, dromedary camels, or goats, although this connection is still vehemently disputed. Currently, disease pathogenesis and transmission are essentially undefined, and understanding such patterns is considered a top priority. Here, we present initial characterization of clinical disease, pathology, viral shedding, and seroconversion of Nubian goats experimentally infected with MERS-CoV as well as two co-housed contact controls.

Neurons are not killed by Varicella Zoster Virus

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Departments of Neurology¹ and Microbiology², University of Colorado School of Medicine, Aurora, Colorado

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Nicholas L. Baird

Abstract

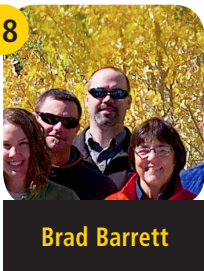
Varicella zoster virus (VZV), an exclusively human neurotropic alphaherpesvirus, causes varicella (chickenpox). After primary infection, VZV becomes latent in ganglionic neurons along the entire neuraxis. VZV reactivation usually results in zoster (shingles), often complicated by postherpetic neuralgia, and less frequently by VZV meningitis, vasculopathy, myelopathy and ocular disease. VZV produces a cytopathic effect in cells derived from viscera and skin. Thus, attempts to study VZV infection of neurons *in vitro* has been difficult, primarily because “contaminating” non-neuronal cells become lytically infected leading to destruction of the culture. During lytic VZV infection, VZV DNA replicates, viral genes are transcribed and translated, and high titers of infectious particles are produced leading to cell death in a week or less. Herein, we show that differentiated neurons (>95% β III-tubulin positive) infected with VZV do not develop a cytopathic effect and lack markers of apoptosis (cleaved caspase-3 and -9). We hypothesize that there is an inherent difference in the growth of VZV in neurons compared to all other cells, thereby allowing neurons to survive VZV infection. VZV-infected fibroblasts and neurons were monitored over time for viral DNA, RNA and virus production. VZV DNA accumulated by ~30 fold in fibroblasts, but not in neurons. Viral transcripts accumulated to similar levels in both cultures, although fibroblasts produced 200-fold more infectious virus than neurons. Electron microscopy revealed high numbers of aberrant viral particles that lacked DNA cores. Together, these data suggest that a critical factor responsible for neuronal survival is that VZV DNA replication, but not viral transcription, is blocked. Our successful development of a model of non-lytic VZV infection of neurons will allow studies that elucidate molecular mechanisms involved in virus growth and reactivation from neurons.

TLR7 and the Antibody Response to Pathogenic Retrovirus Infection

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University of Colorado - Denver, Anschutz Medical Campus.

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Abstract

Toll-like Receptor 7 (*TLR7*) is considered a central mediator of the retrovirus specific IgG response based on data on the C57BL/6 (B6) genetic background, which do not develop severe splenomegaly due to *Fv2* resistance. Thus, it is unclear to what extent the *TLR7*-mediated IgG response influences recovery from pathogenic retrovirus infection. The X-chromosome location of *TLR7* also implicated *TLR7* in sex-dependent differences in viral infection outcomes, since 2 copies are present in females while only 1 in males. We employed an F_1 hybrid approach to investigate the role of *TLR7* in pathogenic retrovirus infection. Male *Fv2* susceptible ($B6 \times BALB/c$) F_1 hemizygous or null for *TLR7* were generated and infected with FV. Severe splenomegaly developed by 28 days post-infection (dpi) regardless of *TLR7*, but *TLR7*-null F_1 mice had significantly higher viremia and cellular infection levels. However, in contrast to B6 mice, total FV-specific IgG responses were similar between *TLR7* hemizygous and null F_1 mice. *TLR7* significantly influenced FV-specific IgG2a and IgG2c, but not IgG1, IgG2b and IgG3 titers. In female F_1 mice, the presence of one or two copies of *TLR7* had no significant impact on FV replication, splenomegaly and IgG2a and IgG2c responses. These findings narrow the role of *TLR7* to a specific IgG subclass, suggest that this subclass composition possess significant antiviral effector properties, and implicate other sensors in the overall IgG response during pathogenic retrovirus infection. The results also suggest that 1 or 2 copies of *TLR7* does not significantly impact the outcome of pathogenic retrovirus infection and immunity.

RV-cyclin and CDK8: teasing apart transcription regulation

Claire Birkenheuer, Sandra L. Quackenbush,
and Joel Rovnak

Colorado State University, Fort Collins, CO

03



Claire Birkenheuer

Abstract

Walleye dermal sarcoma virus infection in walleye fish provides an accessible model for studying tumor development. Retroviral cyclin (RV-cyclin), a protein encoded by WDSV, interacts with and enhances the kinase activity of cyclin-dependent kinase 8 (CDK8). CDK8 is an under-studied oncogene, important in human colon cancer and melanoma. Due to the nature of the CDK8/RV-cyclin interaction, we hypothesized that RV-cyclin aids in tumor development by altering expression of a certain set of oncogenes—the serum response genes (*FOS*, *EGR1*, and *JUN*). These genes are regulated by CDK8. Using q-RT-PCR analysis, we show that RV-cyclin up-regulates expression these genes. Nuclear run-on experiments indicate RV-cyclin enhances the rate of transcription elongation of *EGR1*. This result is supported by CHIP experiments, analyzing the levels of RNA Pol II occupancy across the *EGR1* gene locus. RNA Pol II levels at *EGR1* are not altered by RV-cyclin under serum-starved conditions. After serum-stimulation, RV-cyclin-expressing cells have greater levels of RNA Pol II in the open reading frame of *EGR1* than control cells, suggesting an enhancement of transcription elongation. Additionally, RV-cyclin does not increase phosphorylation events in the mitogen-activated-protein kinase pathway, or decrease the rate of mRNA decay of the transcripts. In conclusion, RV-cyclin's interaction with CDK8 is part of a previously un-described mechanism of retro-viral induced oncogenesis, and could serve to further delineate CDK8 function in human cancers.

Experimental models

C.H. Calisher

CSU/CVMBS

05



Charlie Calisher

Abstract

“Model” experimental host systems with respect to viruses or microorganisms are expected to be representative, typical, or illustrative of the host and of the results one would observe under non-experimental (natural) conditions. Do they? Always? Recent evidence suggests that at least some model systems do not reflect what occurs under natural conditions. That means they are not modeling anything. So, why use them? Better to have a poor model than no experimental system? Really?

Nuclear Magnetic Resonance Assignment of CCHF vOTU to Aid in Drug Discovery

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Elan Z. Eisenmesser², and Scott D. Pegan¹§

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Aurora, CO 80045, USA

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Glenn Capodagli

Abstract

The Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a tick-borne nairovirus with mortality rates reaching as high as 80% for which there is currently no substantial treatment. CCHFV contains a viral Ovarian Tumor Domain (vOTU), a class of deubiquitinating enzymes so-called due to its ability to cleave Ubiquitin (Ub) from host proteins. Ub is a protein that is attached to other proteins, thereby acting as a signal for the cell. Typically, proteins are not attached to a single Ub moiety, but several through the attachment of additional Ub proteins to one of seven lysine residues found on the originating Ub. These seven different linkage forms of poly-Ub have been shown to play distinct key roles in protein trafficking and stabilization, as well as the regulation of cellular processes to include cellular defense and division. The crystal structure of CCHFV's vOTU has been resolved, but is limiting in its effectiveness as a platform for drug discovery and design. In order to allow for a better platform to monitor the interaction between CCHFV's vOTU and its substrates, including possible drug candidates, the solution structure of the vOTU through Nuclear Magnetic Resonance was pursued. Both single-labeled N15- and double-labeled C13/N15-CCHFV-vOTU were purified and their enzymatic activity verified in order to allow for the complete backbone assignment of CCHFV's vOTU.

Strategies of Lentivirus Persistence: Immune Evasion vs Viral Fitness

Susan Carpenter¹, Hyelee Loyd¹, Wuwei Wu¹, Karin S. Dorman¹, Sandra D. Taylor², and Robert H. Mealey²

1 Iowa State University and

2 Washington State University

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Susan Carpenter

Abstract

The lifelong persistence of lentiviruses is a function of their ability to evade immune recognition and elimination as well as the ability to replicate (replicative capacity). In vivo populations of virus must maintain a balance between positive selection for genetic change to escape virus-specific CTL and neutralizing antibody, and purifying selection that maintains optimal structure and function of viral proteins to maximize replicative capacity. We have undertaken longitudinal studies in horses experimentally infected with equine infectious anemia virus (EIAV) to determine if there is an evolutionary tradeoff between immune evasion and 'fitness', i.e. replication phenotype in the absence of an immune response. Our early studies in a limited number of horses suggested that genetic and phenotypic variation in Rev contributes to variant selection in vivo. More recently, we examined the effect of genetic changes in SU on immune escape and replication phenotype. Over time viral genotypes evolved that were increasingly resistant to broadly neutralizing antibody, often resulting in recrudescence of clinical disease. When tested in growth competition and infectivity assays, EIAV variants resistant to broadly neutralizing antibody were 4-40 fold-less infectious in vitro than founder virus; however, small differences in cell-free infectivity could be overcome by efficient cell-to-cell spread. In some cases, variation in SU was accompanied by changes in Rev and in U3, which significantly reduced nuclear export activity and LTR promoter activity as compared with founder virus. Overall, our studies provide evidence of a trade-off between immune evasion and virus fitness, and suggest multiple viral genes can interact and contribute to virus persistence in the presence broadly reactive immune responses.

Interactions between segmented RNA viruses and the RNA decay machinery

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² Utah State University

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Abstract

RNA viruses must navigate their way around the cellular RNA decay machinery in order to establish a successful infection. Previous work from our group has identified the molecular mechanisms for how several positive sense RNA virus families accomplish this task of ensuring stability of their transcripts. However little is known about the interaction between segmented RNA virus and cellular RNA decay machinery. The interaction between the transcripts of these viruses and the RNA decay machinery may be particularly interesting for two reasons. First, unlike positive or negative strand RNA viruses, the ambisense strategy of gene expression used by cytoplasmic segmented RNA viruses requires that both the complimentary genomic and anti-genomic strands venture outside of membrane-associated replication factories and be stabilized for translation. Second, unlike plus sense RNA viruses, segmented RNA viruses theoretically could use differential RNA stability as a means of post-transcriptional regulation to exert fine control of relative transcript levels and protein production. Based on previous work with other virus RNAs, we hypothesize that the 3' untranslated region (UTR) of segmented RNA viruses mediate the interaction of the transcripts of segmented RNA viruses with the cellular RNA decay machinery. To test this hypothesis, we have cloned fragments of the 3' UTRs of all of the mRNAs generated by the two segments of Junin Virus (JUNV) into an expression vector. Interestingly, several of the JUNV 3' UTR fragments appear to be interacting strongly and specifically with a cellular protein as assayed by UV crosslinking. We are currently assessing how these JUNV virus 3' UTR reporter RNAs interact with the various processes that constitute the major pathways of cellular mRNA decay (deadenylation, decapping, 5'-3' exonucleolytic decay and 3'-5' exonucleolytic decay) using a series of in vitro RNA decay assays in cytoplasmic extracts.

Characterization of Moose CWD Prions

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Jeff Christiansen

Abstract

Chronic wasting disease (CWD) is the only known transmissible spongiform encephalopathy (TSE) naturally affecting wildlife. Free-ranging deer and elk have been diagnosed with CWD throughout the Rocky Mountain region and elsewhere. Only recently were CWD infected moose identified, three in Colorado and one in Wyoming.⁽¹⁾ In addition, captive moose have been infected by oral inoculation of CWD infected mule deer brain homogenates.⁽²⁾ Transmission of disease between cervid species has been predicted due to prion protein sequence homology, but experimental evidence of transmission from moose to deer or elk has been lacking.⁽³⁾ Understanding the transmission characteristics of CWD between all of the susceptible species sharing a habitat is crucial for the management of disease. We experimentally transmitted CWD prions from moose to transgenic (Tg) mice expressing either deer or elk prion protein (PrP). All of the inoculated animals showed clinical signs consistent with CWD infection. Mean disease incubation times of ~320 days were observed for Tg(DeerPrP) mice and ~290 days for Tg(ElkPrP)mice. Western blotting confirmed the presence of proteinase K (PK) resistant material in both transgenic lines with glycoform ratios and migration patterns similar to deer derived CWD. Deposition of PrP^{Sc} was observed in brain regions consistent with previous observations of animals infected with type II CWD.⁽⁴⁾ Surveillance of harvested moose should continue in CWD endemic areas, based on the facile transmission between members of the cervidae family. Behavioral differences, such as small social grouping, likely account for the low incidence rates of CWD in moose compared to infection rates observed in the mule deer population.

References

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The vOTU domain of PRRSV displays a unique substrate preference with implications for pathogenicity

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Michelle Deaton

Abstract

The porcine reproductive and respiratory syndrome virus (PRRSV) is an economically devastating disease that presents global concerns to the pork industry, which have been exacerbated by the emergence in 2006/2007 of highly pathogenic PRRSV (HP-PRRSV) in China and Southeast Asia. PRRSV is a positive-sense, single-stranded RNA virus in the genus *Arterivirus* of the family *Arteriviridae*. Within the non-structural protein 2 region of the viral proteome, a de-ubiquitinating enzyme has been identified and classified as a viral Ovarian Tumor Domain (vOTU) protease. vOTUs from various viruses have been characterized extensively, and it has been shown that vOTUs from different species can vary greatly in their preference for substrate. Since various strains of PRRSV have large variations in virulence, the vOTUs of two strains were selected for characterization; the highly pathogenic JXwn06 strain and the North American VR-2332 strain. The substrate specificities of the vOTUs from these strains were compared with each other as well as other vOTUs. While both vOTU domains showed de-ubiquitinating activity on a K48-Ub substrate, only the PLP2 domain from the HP-PRRSV strain JXwn06 displayed activity against K63-Ub substrates. This represents the first report of a biochemical activity unique to HP-PRRSVs that has direct implications for a potential increase in immunosuppression and virulence exhibited by this Asian HP-PRRSV strain.

Defining the mechanisms of pathogenicity of Nipah virus in smooth muscle cells and endothelial cells

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3 Rocky Mountain Veterinary Branch, DIR, NIAID, NIH, Hamilton, MT.

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Blair DeBuysscher

Abstract

Nipah virus (NiV) is a highly pathogenic zoonotic paramyxovirus. NiV infection in humans is characterized by respiratory and neurological complications, with high case fatality rates. During infection, microvascular endothelial cells (ECs) are major targets of the virus. Infected ECs undergo cellular changes such as the formation of multinucleated giant cells by cell-to-cell fusion and these cellular changes contribute to disease. Post-mortem examination shows smooth muscle cells (SMCs) of the vascular system are also targets of NiV. However, little is known about the consequences of SMC infection. The Syrian hamster and African green monkey are reliable models that recapitulate human NiV disease. Using these models, we observed infection of both SMCs and ECs. However, similar to human infection, no pathology is associated with the SMCs, although neighboring ECs show cytopathology. To investigate the differences observed *in vivo* between these cell types, we infected primary human lung ECs and pulmonary artery SMCs with NiV. Both cell types were permissive and NiV replicated to similar levels in the supernatants. ECs develop syncytia promptly following NiV infection, resulting in destruction of the monolayer. After initial infection, NiV spread from infected cells to adjacent cells in the ECs, causing syncytia. In contrast SMCs do not show cytopathic effects, even after 3 weeks of infection, despite continuous progeny virus production. We also observed little cell-to-cell spread of virus with single cells positive for NiV in SMCs. This lack of destruction of NiV-infected SMCs may allow these cells to serve as centers for virus propagation and dissemination.

An ex vivo avian leukocyte culture model for West Nile virus infection

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Elizabeth Dietrich

Abstract

West Nile virus (WNV) replicates in a wide variety of avian species, which act as amplification hosts. In particular, WNV generates high titers and elicits severe pathology in American crows (AMCRs; *Corvus brachyrhynchos*), a species that has been used as a sentinel for WNV circulation. Previous studies have identified a single genetic substitution, T249P in the NS3 helicase protein, which is associated with the capacity for generation of higher viremia by certain lineage 1 WNV strains in AMCRs. The KN-3829 strain, which was isolated in Kenya and contains a threonine residue at NS3 249, elicits an approximately million-fold reduction and two-day delay in viremia onset with an associated limited virulence in AMCRs compared to an infectious clone derived mutant containing an NS3 T249P substitution. Based on preliminary time course studies, we hypothesized that this difference is due to differential replication in avian leukocytes. Therefore, we isolated peripheral blood mononuclear cells (PBMC) from American crows and infected them with WNV in an *ex vivo* culture system. The KN-3829 strain demonstrated no detectable viral growth, while WNV strains and viral mutants containing the NS3 249P residue were able to replicate in *ex vivo* PBMC culture. Thus, the ability to replicate in leukocyte culture is predictive of *in vivo* viremia phenotypes in AMCRs. This *ex vivo* leukocyte culture system may be a useful model for pathologic assessment of WNV strains.

Species Tropism of Middle East Respiratory Syndrome Coronavirus is Restricted by Dipeptidyl Peptidase 4

Neeltje van Doremalen

NIAID/RML.

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Neeltje van Doremalen

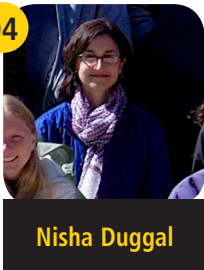
Abstract

Middle East respiratory syndrome coronavirus (MERS-CoV) has caused over 90 human cases with an estimated 50% case fatality rate since mid-2012. MERS-CoV is phylogenetically most closely related to coronaviruses detected in bats. The detection of MERS-CoV neutralizing antibodies in dromedary camels suggests the potential involvement of an intermediate reservoir in the emergence of MERS-CoV in humans. MERS-CoV is able to replicate in variety of cell lines of mammalian origin (e.g. bat, human, non-human primate, pig). In addition, MERS-CoV replicates in the lower respiratory tract of rhesus macaques. However, infection of Syrian hamsters, ferrets and mice with MERS-CoV has been unsuccessful suggesting a species tropism restriction of MERS-CoV. We investigated the role of the recently identified receptor of MERS-CoV, dipeptidyl peptidase 4 (DPP4), in the apparent species tropism of MERS-CoV. Infection of cell lines of human and non-human primate origins resulted in replication of MERS-CoV, whereas hamster, ferret or mouse cell lines were not susceptible. We next cloned the respective DPP4s of different animal species. Transfection of non-susceptible BHK (baby hamster kidney) cell line with either human or rhesus macaque DPP4 and subsequent infection with MERS-CoV resulted in viral replication, whereas transfection of hamster, ferret and mouse DPP4 did not. This suggests that the species restriction of MERS-CoV is at the DPP4 receptor level. In addition, expression of DPP4 of common livestock species in the Middle East (goat, camel, sheep and cow) also supported viral replication of MERS-CoV, suggesting that these species could potentially serve as intermediate animal hosts.

Sequence and phenotypic analyses of 2012 West Nile virus isolates from Texas fail to associate viral genetic factors with outbreak magnitude

Nisha Duggal, Roger Nasci, and Aaron C. Brault.

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Nisha Duggal

Abstract

In 2012, the U.S. experienced the largest outbreak of WNV human encephalitis since 2003. In order to determine whether the increase in WNV transmission in 2012 could have been due to recent sequence changes in the WNV genome, we sequenced 17 full-length isolates made from mosquito pools in Texas in 2012 and compared them to isolates from previous years. We found a similar amount of divergence in the 2012 Texas isolates compared to isolates from previous years, with most of the genome evolving under purifying selection and genetic drift. Further, we compared isolates from Dallas County, that exhibited a 2012 incidence rate of 16 WNV cases per 100,000 population, to isolates from Montgomery County, with a 2012 incidence of 3 WNV cases per 100,000 population. While genetic differences did exist between Dallas and Montgomery County viral populations, weak evidence supports genetic population subdivision or adaptive changes in the Texas isolates. Finally, *in vitro* growth rates of Dallas and Montgomery County WNV isolates with the aforementioned genetic differences were assessed in mammalian and mosquito cells. Results demonstrated that isolates with variable amino acids exhibited indistinguishable replication profiles compared to one another or to the NY99 strain, indicating that these 2012 WNV genetic differences did not afford an *in vitro* replication advantage. Together, these data do not support genetic viral adaptation as an explanation for increased WNV incidence in 2012.

Simian varicella virus reactivation is accompanied by increase in the T cell exhaustion marker PD1

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1 University of Colorado

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Stephanie James

Abstract

Similar to varicella zoster virus (VZV) in humans, simian varicella virus (SVV) establishes latency and reactivates in primates after immune-suppression. Five rhesus macaques were inoculated with SVV and 142 days later (latency) four of them were immunosuppressed. Blood samples were analyzed for T cell phenotypes and programmed death receptor 1 (PD-1; T cell exhaustion). T cells counts decreased during immunosuppression, except CD8+ naïve cells which fluctuated and peaked one week prior to zoster and PD-1 increased on all T cell subsets at reactivation. These data suggest that immunosuppression- induced reactivation is due to decreased T cells and T cell exhaustion.

Role of the phosphatidylserine receptor TIM-1 in enveloped virus entry

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and Wendy Maury

University of Iowa

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Wendy Maury

Abstract

We have previously demonstrated that the cell surface receptor, T cell immunoglobulin mucin domain-1 (TIM-1), enhances filovirus infection of epithelial cells. Here, we identify key residues within the phosphatidylserine (PtdSer) binding pocket of the TIM-1 IgV domain that are critical for Ebola virus (EBOV) entry through direct interaction with PtdSer on the viral envelope. PtdSer is present on the surface of EBOV GP VSV pseudovirions, EBOV VLPs and recombinant VSV expressing EBOV GP that was generated in tissue culture or in vivo. PtdSer liposomes, but not phosphatidylcholine liposomes, compete with TIM-1 for EBOV binding and infection. Further, the PtdSer-binding protein Annexin V (AnxV) substitutes for the TIM-1 IgV domain, providing independent evidence that virion associated PtdSer is involved in filovirion uptake. As further evidence of the critical role of virion-associated PtdSer in TIM-1-mediated uptake, TIM-1 enhanced internalization of pseudovirions and VLPs lacking a glycoprotein, indicating that TIM-1 and PtdSer-binding receptors can mediate virus uptake independent of a glycoprotein. Our findings suggest TIM-1-dependent uptake of EBOV occurs by apoptotic mimicry. Since TIM-1-dependent filovirus infection does not require viral glycoprotein/receptor interactions, we sought to determine if TIM-1 could mediate uptake of a wide range of enveloped viruses and found that TIM-1 enhance entry of alphaviruses and a baculovirus. These results provide evidence for a broad role of TIM-1 as a PtdSer-binding receptor that mediates enveloped virus uptake and utilization of PtdSer-binding receptors may explain the wide tropism of many of enveloped viruses and provide new avenues for controlling their virulenc

Maternal Transmission of Chronic Wasting Disease

Candace Mathison

Colorado State University

Abstract

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Transmissible spongiform encephalopathies (TSEs), or prion diseases, are protein misfolding diseases affecting humans and animals. TSEs can occur spontaneously or result from exposure to infectious prions. The latter has been especially well characterized in bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD) of deer and elk and scrapie of sheep. While still incompletely proven or understood, an additional and potentially highly efficient pathway of transmission exists— that from mother to offspring. Using a new, small cervid animal model (muntjac deer) for transmissible spongiform encephalopathies (TSEs), we report evidence of mother to offspring transmission of prion disease as well as a higher mortality rate of offspring born to TSE-infected mothers. Overall, transmission was seen in 100% of fawns conceived by TSE-infected does. In viable fawns born to infected mothers, we demonstrate not only early prion infection, but also progressive TSE disease. Furthermore, we have detected in utero transplacental passage of prions into fetal tissues, suggesting pre-natal transmission. These findings provide evidence that mother to offspring transmission can occur, may be efficient, and thus may be underestimated for all prion diseases.

Mechanisms of prion evolution Characterization of Viral Excretion and Tissue Tropism of Feline Immunodeficiency Virus in Feline Saliva and Oral Tissues

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Abstract

Feline immunodeficiency virus (FIV) is believed to be transmitted in saliva primarily by bite wounds, although mechanisms associated with salivary transmission have not been well studied. Human immunodeficiency virus (HIV) is also known to be present in the saliva of infected individuals and has been shown to be genetically, structurally, and biochemically similar to FIV. Studies involving HIV salivary pathogenesis have increased the prospect of alternative antiviral therapies and diagnostic methodologies in endemic areas. Therefore, further elucidation of lentiviral salivary excretion and transmission mechanisms may have significant implications in both medical and veterinary research. To characterize the excretion and tropism of FIV in saliva and oral tissues, and to hypothesize mechanisms associated with oral shedding and transmission of FIV, eighteen cats were intravenously inoculated with a well-characterized strain of FIV. Viral RNA and DNA present in saliva and oral mucosal and lymphoid tissues were quantified using real time polymerase chain reaction (RT-PCR) analysis. Microsphere immunoassays (MIAs) were performed to quantitate total IgA and IgG and to detect FIV-specific antibodies in saliva samples at various time points. In addition, histologic evaluation of oral tissues was retrospectively performed by light microscope. We demonstrate that saliva contains significant amounts of both viral RNA and proviral DNA, and that viral and proviral loads in oral lymphoid tissues are significantly higher than oral mucosal tissues. Results from MIAs show that total IgG in saliva of FIV-infected cats increases over time, and that FIV-specific IgG antibodies to FIV-C capsid (CA) and surface protein (SU) are consistently detected in infected individuals, with fluorescence intensity also increasing over time. Anti-SU IgA is consistently detected in saliva of infected cats with fluorescence intensity increasing slightly over time, however, no significant changes are observed in regard to total IgA or the detection of anti-CA IgA; findings that mimicked responses previously noted in plasma. Moderate lymphoid hyperplasia, and mild to moderate, lymphoplasmacytic and mastocytic stomatitis and glossitis were noted histologically. These results suggest multi-organ involvement in viral shedding and infectivity that appears to predominate in oral lymphoid tissues.

Flaviviruses choose heads or tails: dysregulation of cellular RNA decay by viral 5' or 3' untranslated regions

Stephanie L. Moon¹, Shelton S. Bradrick², John R. Anderson¹, Mary K. Dozier¹, Jeffrey Wilusz¹

1 Colorado State University.

2 Duke University Medical Center.

26



Stephanie Moon

Abstract

How viruses interact with the host mRNA decay machinery to preserve their transcripts and potentially dysregulate the host response to infection is an understudied aspect of host-virus interactions. Arthropod-borne Flaviviruses have been shown to generate a unique non-coding RNA during infection called subgenomic flavivirus RNA (sfRNA) from the 3' UTR. sfRNA is a decay intermediate formed from incomplete degradation of the viral genomic RNA by the major host 5'-3' exoribonuclease Xrn1. The sfRNA also acts as a reversible competitive inhibitor of Xrn1, limiting the ability of the enzyme to perform its normal function in degrading host mRNAs. Although other members of the *Flaviviridae* do not generate sfRNAs, we observed significant changes in host mRNA stability during both Hepatitis C virus (HCV) and Bovine Viral Diarrhea virus (BVDV) infections. We hypothesize that the highly structured 5' UTRs of Hepacivirus and Pestivirus RNAs may similarly stall and repress Xrn1. In support of this hypothesis, we have detected several decay intermediates generated as Xrn1 degrades the HCV 5' UTR, and observe several signs of Xrn1 dysfunction in cells and cell-free assays. Intriguingly, Xrn1 dysfunction during HCV infection is associated with increased abundance of intact, translatable mRNAs encoding proteins implicated in pathogenesis. Future studies will seek to determine if other viral IRES elements can inhibit Xrn1 activity, identify the key structural elements that stall the Xrn1 enzyme on the HCV and BVDV 5' UTRs, and determine how Xrn1 suppression feeds back to influence other aspects of cellular mRNA decay and perhaps coordinated transcription.

The 3' untranslated region of the RTA immediate early transactivator is critical for gammaherpesviruses replication

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28



Brian Niemeyer

Abstract

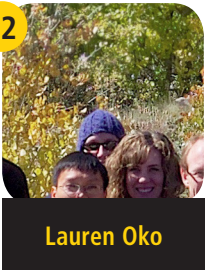
Murine gammaherpesviruse 68 (γ HV68) is related to Epstein-Barr virus and Kaposi's Sarcoma Associated Herpesvirus and serves as a small animal model system. These viruses encode a common immediate-early transactivator, Rta, which is necessary and sufficient to initiate lytic infection and reactivation from latency. We previously identified a region in the left end of the γ HV68 genome that contains eight transcripts that are abundantly expressed and give rise to viral miRNAs. Among the most abundant of the γ HV68 miRNAs is miR-M1-1, which is predicted to target the 3' untranslated region of the *rta* transcript in γ HV68. Dual luciferase assays were used to confirm regulation of Rta by miR-M1-1. Next, two recombinant viruses were generated to contain a 6 base pair mutation in the Rta untranslated region to abolish miR-M1-1 interaction. We expected that loss of miRNA repression of Rta expression in this Rta seed-match mutant virus (γ HV68-Rta-SMM) would result in overexpressed Rta and enhanced lytic replication. Instead, we found that the γ HV68-Rta-SMM results in decreased virus production, with decreased transcription of Rta and Rta-responsive genes during lytic infection. We also found that transfection of an Rta expression plasmid prior to γ HV68-Rta-SMM infection restored virus transcription and production, demonstrating that the defect of the γ HV68-Rta-SMM is specific to Rta expression. Next, we tested whether the 6 base pair change in the Rta 3'UTR conferred RNA instability upon luciferase reporter genes, and found equivalent stability to the wild-type 3' UTR. To test this further, we generated a specific artificial miRNA that is complementary to the mutant seed match region and demonstrated that this artificial miRNA negatively regulated expression of a luciferase gene fused to the SMM mutated 3' UTR of Rta, but not of luciferase fused to the wild-type 3' UTR sequence. We are currently testing a 3kb RNA encoded opposite the RTA gene, which was shown to produce a small peptide that stabilizes the RTA transcript in KSHV. In addition to testing this 3kb region, we are examining if inhibition of the NF- κ B pathway, proteasome activity, or histone deacetylases repairs the defect in the mutant virus. To date, this study demonstrates a critical function for the 6 base pair sequence of the γ HV68 Rta 3' UTR that serves as a target of miR-M1-1. However, our data also suggest that regulation of Rta expression by this 3' UTR region may not be solely attributable to miRNA regulation and may indicate additional complexity in the exquisitely complex regulation of this viral activator.

NASA Gammaherpesvirus lacking small regulatory RNAs is defective in lytic replication and in virulence

Lauren Oko,

University of Colorado AMC

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Lauren Oko

Abstract

Gammaherpesviruses (γ HV) have a complex relationship with their mammalian hosts and exhibit a very specific host range. Gammaherpesviruses are lymphotropic viruses that are associated with a number of lymphoproliferative disorders, lymphoid malignancies and nonlymphoid carcinomas. Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) are a few of the gammaherpesvirus that affect humans. γ HV produce functional small non-coding RNAs. EBV and KSHV encode RNA polymerase (pol) II derived microRNA (miRNA) and EBV also encodes pol III derived non-coding RNAs (EBERs). Murine gammaherpesvirus (gHV68) is a small animal model widely used to study γ HV infection and pathogenesis. Like the human γ HVs, gHV68 encodes functional non-coding RNAs, but these RNAs are unique in that they are pol III derived polycistronic tRNA-miRNA encoded RNAs (TMERs). The regulatory effects of the TMERs are being investigated in response to auto-regulation of viral genes as well as host regulation to effect immune evasion and promote virus infection. We generated and verified two independent TMER total knockout viruses (gHV68 TMER-TKO); to identify the role(s) the TMERs play in infection and pathogenesis. We have demonstrated that the gHV68 TMER-TKO virus has a small deficiency in replication in both a single step and a multiple growth analysis at early points in 3T12 and BHK cells. This early growth defect is overcome by later time points, indicating that the TMERs are not vital to viral production but may assist in initiating a robust lytic replication cycle. Infection of C57Bl/6 mice show enlargement of the spleen and infection of B cell subset similar to wild-type virus at 14 days post-infection, indicating that establishing latency may not require TMERs. Although the gHV68 TMER-TKO mutant viruses affect healthy C57Bl/6 mice equivalently to wild-type, there is significant attenuation, both in delayed in disease signs and mortality, in the acute pneumonia model upon infection of BALB/c IFN γ $^{-/-}$ mice. These data suggest the TMERs play a substantial role in acute pathogenesis. To date, we identified that the TMERs have a minor part in lytic replication early on *in vitro* and a significant role in the acute pneumonia model. Currently, work is continuing on what effects the TMERs have on reactivation, and on dissociating the RNA components (vRNA) and miRNA required in lytic replication, latency, reactivation and pathogenesis.

Potential assay for detection of PrP^{res} in grasses from Rocky Mountain National Park

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2 Department of Bioagricultural Sciences and Pest Management.

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Aimee Ortega

Abstract

Chronic wasting disease (CWD) affects cervids such as elk, deer, and moose. Over the last decade CWD has not only become endemic in parts of Colorado and Wyoming but has continued to spread outside of the endemic zone. The disease is one of many transmissible spongiform encephalopathies which occur due to the accumulation of an abnormally folded, proteinase K resistant, form of the normal cellular prion protein PrP^C. This abnormally folded form, PrP^{res}, seeds conversion of PrP^C into PrP^{res} and eventually forms amyloid fibrils. The exact mechanisms behind transmission and spread of CWD are unknown but research has shown that it can be spread through direct animal to animal contact or via indirect exposure to contaminated feed and water sources. We want to further explore the latter and determine whether prions can be detected in grasses and other plants by use of the protein misfolding cyclic amplification assay (PMCA). Here we describe the optimization of PMCA using rice grass samples spiked with known concentration of prions. We will then test grasses and other plants from Rocky Mountain National Park to establish whether plants could be serving as a vector for CWD.

Alcelaphine herpesvirus 2 induces an MCF-like syndrome in American bison (*Bison bison*)

D. O'Toole, N. Taus, H. Li

01



Donal O'Toole

Abstract

Malignant catarrhal fever (MCF) caused by ovine herpesvirus-2 (OvHV-2) is a major problem for producers of American bison (*Bison bison*) in North America. Our group is interested in developing a vaccine that could protect MCF-susceptible ungulates from disease. Alcelaphine herpesvirus 2 (AIHV-2) derived from topi (*Damascus sp.*), is a member of the MCF virus group and not been reported to cause disease. It has the signal advantage that, unlike OvHV-2, it can be propagated in cell culture. To evaluate whether AIHV-2 might be a practical vaccine candidate, seven yearling American bison were inoculated intranasally (IN) ($n=4$) or intramuscularly (IM) ($n=3$) with AIHV-2 and monitored for infection status using AIHV-2-specific real time PCR and competitive inhibition ELISA, and evidence of disease development. Two additional bison served as in-contact controls. Five bison (2 IN and 3 IM challenged) became infected. One of 3 IM challenged bison developed clinical signs of MCF at 24 days post inoculation (DPI) and was euthanized at 26 DPI. One of 4 IN challenged bison developed clinical signs at 33 DPI and died at 38 DPI. Lesions in both bison were consistent with MCF. The remaining 5 challenged bison were either euthanized at the end of the study at 71 – 74 DPI ($n = 4$), or died of intercurrent disease ($n = 1$; pulmonary abscesses). AIHV-2 is not a good candidate at this time for use in vaccinating bison against OvHV-2.

Invading the Cellular Metabolome: A hallmark of Dengue Virus Infection of the Human Host and Mosquito Vector

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24



Rushika Perera

Abstract

Similar to other positive strand RNA viruses, dengue virus (DENV) relies exclusively on the host to fulfill its membrane and energy requirements. This is especially true since it is an enveloped virus and therefore, must utilize host-derived lipid membranes to enter and bud out of infected cells. In both mammalian and insect cells, DENV causes significant perturbations in lipid biosynthesis and membrane composition during its infectious cycle. We have carried out high-resolution mass spectrometry to quantify the metabolic changes that are induced upon DENV infection. Our results indicate that DENV infection elevates the expression of lipids that have the capacity to change the physical properties of the bilayer such as curvature, permeability, and the recruitment and assembly of protein complexes in the membrane. Several of the identified molecules also function as bioactive messengers that control signaling and membrane trafficking pathways in the cells. We have also identified specific cellular lipid biosynthetic enzymes that are recruited by the viral replicase to sites of viral RNA replication. While several of these enzymes interact with viral proteins, inhibition of their activity is detrimental to DENV replication. A comparative analysis of these events in the human host, mosquito cells and mosquitoes is currently being pursued. Studies on how DENV induced perturbation of the cellular metabolomic environment provide a gateway to understanding normal cellular processes and viral exploitation of the cell will be discussed. Such knowledge will be essential for the future identification of novel biomarkers to support antiviral drug design.

The Immune Response to Sin Nombre Hantavirus in the Novel Rhesus Macaque Model of Hantavirus Cardiopulmonary Syndrome

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Joseph Prescott

Abstract

Pathogenic New World hantaviruses cause a disease termed hantavirus cardiopulmonary syndrome (HCPS). The primary agent of HCPS in North America is Sin Nombre virus. Humans that succumb to infection have high amounts of pro-inflammatory cytokines in the lungs, and although hantaviruses primarily infect endothelial cells, infection is non-cytopathic. Therefore, it is thought that HCPS has an immunopathogenic component. In an effort to develop animal models for HCPS caused by SNV, we inoculated Rhesus macaques and monitored signs of disease. Six of 8 animals developed severe disease strikingly similar to HCPS and were euthanized 16-22 days post-inoculation. Peripheral blood mononuclear cells (PBMC) were isolated throughout the course of infection for flow cytometric analysis. CD8⁺ T-cells increased dramatically during late stages of disease. These cells showed an effector memory phenotype. There was also a large increase in the proportion of CD8⁺ T-cells expressing proliferation and activation markers, and in cell percentages expressing granzyme B. Neutrophilia is often observed in HCPS. Analysis of whole blood and PBMCs showed an increase in neutrophils and granulocytes only in macaques that developed disease. Lymphocytes from infected animals that were stimulated *ex vivo* showed robust production of inflammatory cytokines. Macaques that developed disease expressed high levels of cytokines in the lungs immunohistochemically, primarily originating from alveolar macrophages. The kinetics of immune activation suggests that a robust response is elicited, coinciding with disease onset, and this response might contribute to disease via an immunopathogenic mechanism. This model provides an insight into the immunological events leading to HCPS.

Characterizing West Nile Virus Infection of Ex Vivo Slice Cultures

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Eamon Quick

Abstract

West Nile virus (WNV) infections in the United States are the leading cause of viral encephalitis and acute flaccid paralysis, which can result in life-long sequelae or even death. It is of vital importance to understand the mechanisms of WNV pathology in the central nervous system (CNS), particularly the role of the immune response which can be both beneficial and detrimental to the health of CNS cells. Using current *in vivo* and *in vitro* models, it is difficult to discern the innate immune response profile of the CNS to WNV infection. We now employ a novel slice culture (*ex vivo*) model of mouse brain and spinal cord samples to investigate the response of WNV-infected CNS cells *in situ* and without peripheral immune cell invasion. Our results show that WNV grows in the *ex vivo* cultures and that neurons and astrocytes are the main cell types infected. There is significant expression of several cytokines, especially CCL5 and CXCL10 in infected cultures. In addition, although not infected, microglia are activated in large numbers following WNV infection, and are able to migrate and phagocytose WNV-positive material.

Liposome-Antigen-Nucleic Acid Complexes Protect Mice From Lethal Challenge with Western and Eastern Equine Encephalitis Viruses

Amber Rico

Colorado State University

07



Amber Rico

Abstract

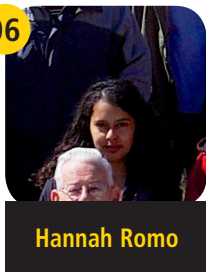
Alphaviruses are mosquito-borne viruses that cause significant disease in animals and humans. Western and eastern equine encephalitis virus (WEEV and EEEV), two New World alphaviruses, can cause fatal encephalitis and EEEV is a select agent of concern in biodefense. However, we have no antiviral therapies against alphaviral disease and current vaccine strategies target only a single alphavirus species. In an effort to develop new tools for a broader response to outbreaks, we designed and tested a novel alphavirus vaccine comprised of cationic lipid nucleic acid complexes (CLNCs) and the ectodomain of WEEV E1 protein (E1ecto). Interestingly, we found that the CLNC component, alone, had therapeutic efficacy, as it increased survival of CD-1 mice following lethal WEEV infection. Immunization with the CLNC-WEEV E1ecto mixture (lipid-antigen-nucleic acid complexes; LANACs) using a prime/boost regimen provided 100% protection in mice challenged with WEEV subcutaneously, intranasally, or via mosquito. In addition, the LANAC immunization protocol significantly increased survival of mice following intranasal or subcutaneous challenge with EEEV. Mice immunized with LANAC mounted a strong humoral immune response, but did not produce neutralizing antibodies. In summary, our LANAC formulation has therapeutic potential and is an effective vaccine that offers protection against two distinct species of alphavirus irrespective of the route of infection.

Identification of avian pathogenic determinants of lineage 2 West Nile viruses

Hannah Romo

Colorado State University

06



Hannah Romo

Abstract

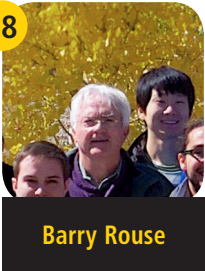
Avian species highly susceptible to West Nile virus infection are crucial for widespread transmission because they serve as highly efficient reservoirs and amplifying hosts. A single positively selected amino acid substitution (NS3-T249P) of a WNV lineage 1 isolate causes increased virogenesis and mortality in experimentally inoculated American crows (AMCRs). The same NS3-249Pro substitution was observed, for the first time, in a lineage 2 WNV isolate [2010 NeaSanta (NS10)] made during a 2010 human WNV encephalitis outbreak in Greece. A His is associated with the NS3-249 loci in all other lineage 2 strains. The phenotypic effects of polymorphisms at the NS3-249 site in two parental lineage 2 WNV strains, WNV-South African 1989 (SA89) and NS10, were assessed in wild-caught AMCRs. Significantly elevated viremia and 100% mortality was observed in AMCRs inoculated with the SA89 and NS10 strains containing a Pro at the NS3-249 site, with complete mortality observed on days 10 and 8, respectively. Strains containing the NS3-249Thr substitution showed significantly reduced viremia and mortality profiles in AMCRs, with the SA89 NS3-249Thr showing no detectable mortality or viremia. The SA89 and NS10 backbones containing an NS3-249His substitution showed only slight or moderate reductions of viremia and mortality, respectively. Phenotypic differences between SA89 and NS10 containing similar mutations at the NS3-249 site suggest a potential for modulation by other genetic differences in additional viral genes.

Immunity or tissue damage upon virus infection – what decides the outcome and how can we exploit such knowledge?

Barry T. Rouse

Lindsay Young Distinguished Professor of Microbiology, Department of Pathobiology, College of Veterinary Medicine, University of Tennessee.

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Barry Rouse

Abstract

Virus infections invariably have a variable outcome in the host they infect. Even during flu pandemics a very minor fraction of the population develop clinical disease and precious few die. When polio was at its peak very few developed neuromuscular consequences. Feline coronavirus infection causes fip only a very rare instance. We shall discuss the many factors of infection and host responses that determine whether or not a virus infection results in significant tissue damage. After a general overview the presentation will focus on unpublished results about how micorRNA expression can influence the outcome of herpetic infection of the CNS. The final phase of the presentation will discuss the role of some host counter-inflammatory events that influence whether or not the herpesvirus infection of the eye causes a blinding chronic lesion. The point will be emphasized that the better management of viral-induced tissue damage will come from a detailed understanding of pathogenesis and that in general the best results of therapy occur when multiple step in pathogenesis are targeted.

Immunobiology of retrovirus restriction factors

Mario L. Santiago, PhD

Department of Medicine, University of Colorado School of Medicine

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Mario Santiago

Abstract

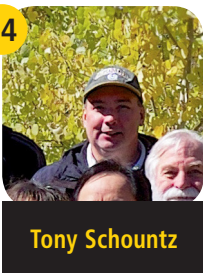
The continuing 'arms-race' between mammalian hosts and retroviruses resulted in the evolution of innate restriction and viral antagonistic mechanisms. Innate restriction factors have the capacity to directly inhibit retroviruses, but these mechanisms would not prevent recurrent infections. In contrast, the adaptive immune system could generate highly specific responses that can be stored as a 'memory' response and subsequently deployed to combat subsequent infections. Thus, it is reasonable to suspect that innate restriction factors may have evolved to not only supplement, but also to prime and shape the adaptive immune response. In this talk, I will present evidence supporting this hypothesis, citing the immunological impact of the APOBEC3 and Tetherin/BST-2 restriction factors in a mouse model of retrovirus infection. APOBEC3, a deoxycytidine deaminase that is counteracted by the HIV-1 Vif protein, can shape the retrovirus-specific neutralizing antibody response. Tetherin/BST-2, a protein that could prevent virion release and is counteracted by HIV-1 Vpu, can shape the antiretroviral cell-mediated immune response. These data suggest that the interplay between innate restriction factors and adaptive immunity may be a key immunological paradigm, with potential applications for HIV-1 vaccine and immunotherapeutics discovery.

Differential Immune Gene Expression in Lymph Node Cell Cultures from Deer Mice Infected with Sin Nombre or Andes Hantaviruses

Tony Schountz^{a,b}, Timothy I Shaw^c, Travis Glenn^{c,d}, Sandra Quackenbush^b, Joel Rovnak^b, Elaine Haddock^e, Heinz Feldmann^e and Joseph Prescott^e

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Tony Schountz

Abstract

Hantavirus cardiopulmonary syndrome (HCPS) is a cytokine-mediated disease with a high case-fatality rate that is caused by several New World hantaviruses. Each virus is naturally hosted by a principal rodent species without conspicuous disease, and once infected the rodents remain infected, perhaps for life. Deer mice (*Peromyscus maniculatus*) are the natural reservoir hosts of Sin Nombre virus (SNV), an etiologic agent of HCPS in North America. Despite a helper T cell response that leads to high titered neutralizing antibodies, deer mice remain persistently infected with SNV. Deer mice are also susceptible to Andes hantavirus (ANDV), which is naturally hosted by long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) and causes HCPS in South America; however, deer mice clear ANDV. We infected deer mice with SNV or ANDV to identify differences in host responses that might account for these different outcomes. We found no differences in hematological parameters, and SNV viral RNA levels were higher in the lungs but not different in heart, spleen or kidneys. Examination of lymph node cell antigen recall responses by real-time PCR array indicated elevated gene expression in deer mice infected with ANDV and suggested maturation towards a Th2 phenotype in some ANDV-infected deer mice. Analysis of MiSeq data identified 80 genes that were differentially expressed in nucleocapsid antigen-stimulated lymph node cell cultures. All but two of the transcripts were more abundant in cultures from ANDV-infected deer mice than those from SNV-infected deer mice. About half of the transcripts have immune functions, while some are not known to have immune function. Several interferon and/or antiviral response genes were all more abundant in ANDV cultures, indicating that such responses occur with greater magnitude in deer mice infected with ANDV than SNV. Together with our previous work, these data suggest the magnitude of the immune response is substantially greater during ANDV infection compared to SNV infection, and this robust response may account for clearance of ANDV.

Evaluating the role of RLR helicases in viral pathogenesis

Scott Seitz

University of Colorado AMC.

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Scott Seitz

Abstract

Rig-I like RNA helicases (RLRs) represent a broad group of proteins necessary to facilitate a Type I interferon response during viral infection. Most notably work with Rig-I and MDA5 has shown that RNA helicases are essential in creating an antiviral response. Two other RLRs: Dhx58 and Ddx60; have been poorly characterized in viral pathogenesis both in vitro and in neuroinvasive infections. To determine the role of these helicases in viral pathogenesis, in vitro and in vivo models were utilized. Helicase function was then compared between Type 3 reovirus, West Nile virus, and Japanese encephalitis virus by microarray, RTPCR, and ICC/IHC. Results suggest the two helicases are highly upregulated following infection and localize with viral components. Further studies are needed to elucidate the importance of these helicases in viral pathogenesis.

Hepatitis C Virus Core protein directly inhibits Interferons through non-productive increases in STAT1 protein in plasmacytoid Dendritic Cells

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1 University of Colorado, Anschutz Medical Campus, Denver, CO.

2 University of Washington, Seattle, WA

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Amy Stone

Abstract

Background: Understanding the basic mechanisms of how Hepatitis C Virus (HCV) interacts with the Interferon system may lead to novel treatments and better viral control. Plasmacytoid dendritic cells (pDCs) act as sentinels for virus infections and make Interferons (IFNs) upon recognition of viral particles. pDCs are targeted by HCV resulting in reduced cell numbers and dysfunctional IFN production. We hypothesized that pDCs were targeted through HCV Core protein to disrupt IFN production and induce cell death, thus attenuating the pDC-driven IFN response during the early stages of infection.

Methods: Using a pDC cell line and recombinant HCV Core protein we characterized the inhibition of Toll-Like Receptor and RIG-I-Like Receptor induced IFN production by HCV Core protein.

Results: Pre-treatment with recombinant HCV Core protein can block the induction of Type I and III IFN mRNA of GEN2.2-pDCs after TLR stimulation (Loxoribine – toll-like receptor [TLR] 7 agonist and CpGA – TLR9 agonist) as well as the robust induction of IFN mRNA after stimulation with the pU/UC RNA (HCV 3' UTR genomic RNA). Additionally, IFN α protein production was significantly inhibited by HCV Core pretreatment followed by stimulation by CpGA or pU/UC RNA. IFNL1 levels were also inhibited by the presence of HCV Core prior to stimulation. Incubation for 24 hours with HCV Core induced a non-productive upregulation of STAT1. Exposure to HCV Core prior to stimulation also induced a decrease of IRF7 but not IRF3 protein levels. The mechanism of this inhibition is not due to cell death, as GEN2.2-pDCs are resistant to HCV Core induced apoptosis and proliferation is not inhibited.

Conclusions: HCV Core directly acts upon pDCs to induce reduced IFN mRNA levels following stimulation through TLRs. Apoptosis in the GEN2.2-pDC cell line was not induced by exposure to HCV Core suggesting that the reduction in IFNs was not due to cell death. Through understanding how HCV Core disrupts the IFN response from pDCs, we can develop novel methods of subverting the virus and inducing an antiviral state in patients. Translationally, blocking HCV Core with an antibody in patients may increase natural interferon production and promote viral clearance.

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Glenn Telling

Prion Research Center (PRC), Colorado State University

Abstract

The parameters controlling prion transmission and evolution among mammalian species are enigmatic. The unpredictable recurrence of prion epidemics, their incurability, lethality, and demonstrated zoonotic potential, make their understanding a highly significant pursuit, and it remains our overarching, long-term goal. Focus on the burgeoning chronic wasting disease (CWD) epidemic remains an important component of this objective because of its inexorable, contagious spread, evolving host range, and uncertain zoonotic potential. It is generally accepted that prion replication occurs by conformational corruption of host-encoded cellular prion protein (PrP^C) by the pathogenic isoform (PrP^{Sc}). Significantly, the prion mechanism is emerging as a ubiquitous means of protein-mediated information transfer and pathogenesis. The undisputed infectious properties of prions provide a peerless system in which to elucidate common mechanistic themes, and to develop integrated therapeutic approaches for a spectrum of neurodegenerative disorders. Two substantial findings from our group challenge basic assumptions about prion biology:

- It is widely accepted that prions adapt to a new species by forming PrP^{Sc} comprised of the new host's primary structure, and that this newly formed PrP^{Sc} is more pathogenic to the new host compared to the old. Unexpectedly, we now detail several remarkable occurrences where prions produced following interspecies transmission, fail to subsequently adapt in the new host, but rather maintain the ability to cause disease in the original host. We are exploring the mechanism underlying such *non-adaptive prion amplification (NAPA)*, which contradicts a fundamental mechanistic assertion about species barriers and adaptation, namely that optimal disease progression occurs when the primary structures of PrP^{Sc} and substrate PrP^C are closely related.
- Prion strains are thought to be composed of a heterogeneous population of PrP^{Sc} conformers. It is assumed that a given PrP^C primary structure is compatible with only a sub-population of PrP^{Sc} conformations, and that this selective property that governs the susceptibility of that species to infection. Contradicting this view, we find that under conditions of co-expression, a "susceptible" PrP^C variant influences the ability of an otherwise "resistant" PrP^C to propagate an unfavorable strain. We are exploring the resulting hypothesis that PrP^C conformational compatibility is not fixed. Rather, dominant and recessive prion traits are epigenetically controlled by conformational templating.

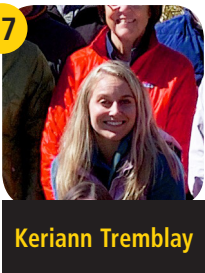
These findings provide novel insights into disease etiology, the means by which prions manifest dominant and recessive traits, and address the enigmatic mechanisms of infectious propagation, adaptation, and strain evolution. Resolution of these issues is a high priority given the frequent, unpredictable occurrence of prion epidemics, their lethality, and their demonstrated zoonotic potential.

A study of interesting and novel transcripts from the VZV genome

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Abstract

Varicella zoster virus (VZV) is a human neurotropic alphaherpesvirus. Primary VZV infection results in varicella (chickenpox) and subsequently establishes lifelong latency in neurons. Reactivation later in life can produce herpes zoster (shingles). The complete DNA sequence has been determined for multiple clinical VZV isolates and computer-assisted DNA sequence analysis has revealed 71 open reading frames (ORFs). Herein is presented two collaborative studies to that describe interesting and novel VZV transcripts. The first study involves the predicted splicing event between ORFs 42-45 and is a collaborative effort with Dr. Ken Miller from Ft. Lewis College. Dr. Miller has isolated a compound from *Bacillus* that inhibits VZV replication in tissue culture, and the suspected mode of action is inhibition of RNA splicing. The second study involves characterization of three previously unannotated ORFs located in the proximity of ORFs 60 and 61. In order to investigate both hypotheses, mRNA was isolated from VZV infected cells and cDNA was synthesized. Real-time PCR, gel electrophoresis, and DNA sequencing were used to map the ORF 42-45 splice junction site and show the presence of transcripts mapping to each hypothetical ORF along with preliminary fine structure mapping of the 3'-terminus.

Identification of novel gammaherpesviruses in felids

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Abstract

Gammaherpesviruses (GHVs) are associated with a variety of disease conditions in numerous animal host species. To identify cat-associated GHVs, we screened domestic cat (*Felis catus*), bobcat (*Lynx rufus*) and puma (*Puma concolor*) blood cell DNA samples from California, Colorado and Florida using a degenerate pan-GHV PCR targeting the conserved glycoprotein B gene. Additional pan-GHV and long-distance PCRs were then used to sequence a contiguous 3.5 kb region of each putative virus species including partial glycoprotein B and DNA polymerase genes. We identified three novel GHVs, each present predominantly in one particular felid species: *Felis catus* GHV 1 (FcaGHV1) in domestic cats, *Lynx rufus* GHV 1 (LruGHV1) in bobcats, and *Puma concolor* GHV 1 (PcoGHV1) in pumas. To estimate infection prevalence, we developed real-time quantitative PCR assays for each virus and screened additional blood cell DNA samples from all three species (n = 282). FcaGHV1 was detected in 16% of domestic cats at U.S. shelters across all study sites. LruGHV1 was detected in 47% of bobcats and 13% of pumas across all study locations. PcoGHV1 was detected in 6% of pumas, all from a specific region of Southern California. Statistical analyses of virus infection related to geographic location, cat age and sex demonstrated multiple significant correlations. Notably, FcaGHV1 PCR positivity was very strongly associated with cats that were adult (odds ratio = 8.2) and male (odds ratio = 21.8).

Varicella-Zoster Virus and Autophagy

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Abstract

Varicella-Zoster virus has been poorly studied in relation to autophagy. Research published by Takahashi et. al. (2009) indicates that VZV infection induces autophagy in both vOka infection of MRC5 cells as well as in vesicle samples from patients with zoster infection. However, this work gives an incomplete picture because only fibroblast cells were studied, leaving out a significant portion of the VZV life cycle, which occurs in sensory neurons. Thusly, we sought to understand the effect of VZV infection on autophagy levels in neuron-like, SH-SY5Y cells. In the studies presented, our initial work demonstrates that there is no difference in autophagy levels between infected and uninfected MRC5 and SH-SY5Y cells. The cell types were infected with Varicella-Zoster virus under serum-rich and serum-free conditions, with no augmentation of autophagy in either case. These results run in contrast to previous work compiled by Takahashi et. al. In comparing the autophagy levels of the two cell types it was also elucidated that SH-SY5Y cells demonstrate higher levels of autophagy per microgram of protein in comparison to MRC5 cells under serum-free conditions. This finding is the opposite of the result of our experiments conducted in the presence of serum. The optimization of the lipofectamine transfection for the overexpression of LC3 was also successful as punctuate expression of LC3-GFP was observed by immunofluorescence microscopy. These data emphasize the need for further experimentation in order to confirm the results both within our own work as well as in comparison with the work of others in relation to VZV and autophagy.

References

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Mitochondrial apoptotic activity of p53 contributes to neuron apoptosis upon reovirus-infection

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Yonghua Zhuang

Abstract

Reovirus infection is a well-characterized experimental system for studies of viral pathogenesis within the central nervous system (CNS). The tumor suppressor p53 plays a critical part in cell fate determination after various exposures including virus infection. It has been reported that p53 and mitochondria-mediated pathway played an important regulatory role in avian reovirus-induced apoptosis in BHK-21 cells and that stabilization and activation of p53 enhanced reovirus oncolysis. However, the role of P53 and mitochondria-mediated pathway in neuronal apoptosis occurring in reovirus-infected brains is still unknown. In the preliminary study, we demonstrate that p53 was upregulated in reovirus-infected brain tissue and accumulated into the mitochondria. Specific inhibition of mitochondrial p53 translocation by Pifithrin μ reduced the caspase 3 activity and alleviated tissue injury induced by reovirus infection on brain slices culture *in vitro*. Administration of Pifithrin μ significantly improved the survival rate of reovirus encephalitis in mice. Overall, our preliminary studies imply that mitochondrial apoptotic activity of p53 contributes to neuron apoptosis upon reovirus-infection.

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