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MEETING REPORT

2019 Colorado Alphaherpesvirus Latency Society symposium

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Abstract
Meeting Report on the 9th Annual Symposium of the Colorado Alphaherpesvirus Latency Society (CALS) held on May 8–11, 2019, in Vail, CO.

Keywords Alphaherpesvirus · Herpes simplex virus · Varicella zoster virus · Prion biology · Latency · Reactivation · Pathogenesis

Introduction

The 9th annual symposium of the Colorado Alphaherpesvirus Latency Society (CALS) was held on May 8–11, 2019, in Vail, CO, and was attended by 71 investigators (Fig. 1) who when combined have authored over 2748 PubMed-listed publications and had traveled some 121,825 miles from three continents, eight countries, and twenty one states to attend the symposium. The two full days of scientific sessions featured 31 oral presentations that showcased recent advances in our understanding of α-herpesvirus latency together with a longer memorial lecture in honor of the late Don Gilden, M.D., esteemed neurologist and CALS symposium founder.

Colleen Amanda Mangold, Esteban A. Engel and Jeffery B. Ostler Jr. contributed equally to this work.

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In addition to the short talks, the program included nine two-min presentations by junior investigators highlighting their own work that was subsequently presented as a formal poster session. A small group of exceptional undergraduates, whose interest in herpesvirology is just beginning, were also active participants in the meeting.

Newly added to the symposium was a special session hosted by *Paul* (*Kip*) *Kinchington*, *University of Pittsburgh*, on improved mentoring through more effective communication. Workshop attendees ranged from undergraduate students to established investigators and all noted the value of improved communications. Also added to the symposium was the inaugural meeting of the CALS Women in Science Committee, whose goal is to provide guidance and mentorship to women as they navigate the demands of a career in academic science. As the meeting progressed, the traditional early spring snowfall gave way to rain but this did little to dampen the lively fireside discussion of the available models of α-herpesvirus latency and participants exchanged more general thoughts on how best to establish causality between a disease state and a candidate etiological agent. The relaxed setting offered by the Christiania and Tivoli Lodges set together in the middle of this quaint mountain town provides the perfect venue for attendees to strengthen established collaborations and for the next generation of clinical/basic research scientists to learn about the current and future directions of the field. A brief summary of each of the talks follows.

The 3rd Don Gilden Memorial Lecture was given by Nobel Laureate Stanley B. Prusiner, Institute for Neurodegenerative Diseases, University of California, San Francisco. The lecture was introduced by Charles Grose, Children's Hospital, University of Iowa, who recounted fond memories of his friend and fellow neurologist Dr. Don Gilden and reminded

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Fig. 1 Attendees of the 9th annual symposium of the Colorado Alphaherpesvirus Latency Society (CALS). (Row 1, left to right) Esteban Engel, Julianna Pieknik, Colleen Mangold, Nicholas Baird, Stanley Prusiner, Randall Cohrs, Maria Nagel, Adriana Weinberg, Myron Levin, Rave Mahalingam, Andrew Bubak, Sara Bustos Lopez, and Faith Osinaga; (row 2, left to right) Angus Wilson, Sierra Vigil, Darrick Moore, Christy Niemeyer, David Bloom, Michael Oxman, Ilakkiya Arumugam, Donna Neumann, Trine Mogensen, Paul R Kinchington, Vicki Traina-Dorge, Igor Jurak, and Patrick Lomonte; (row 3, left to right) Emilia Vanni, Caroline Kulesza, Martine Aubert, David Koelle, Gerald Bush III., David Knipe, Todd Margolis, Joshua Ames, Peter Kennedy, Joel Rovnak, Andrew Harrington, and Charles Neff; (row 4, left to right) Andrea Bertke, Stacey Efstathiou, Phillip Krause, Moriah Szpara, Anna Cliffe, and Rafael Harpaz; (row 5, left to right) Abel Viejo-Borbolla, Edouard Cantin, Matthew Taylor, Simon Fletcher, Clinton Jones, Oscar Haigh, Jeffery Ostler, Robert Kalejta, Jon Suzich, Scott Schmid, Leonardo D'Aiuto, Greg Smith, David Davido, Océane Sorel, John Blaho, Klaus Osterrieder, Daniel Depledge, Tony Huang, Victor Hsia, Charles Grose, Vishwajit Nimgaonkar, Tejabhiram Yadavalli, and Seth Frietze. Not pictured: Nadine Jarousse and Jia Zhu

the audience that Dr. Prusiner spent his formative years in his own beloved home state of Iowa. In his talk, Dr. Prusiner described the development of anti-prion therapeutics as potential treatments for Alzheimer's disease and Parkinson's disease. His recent collaborative studies, as well as work from other investigators, have shown that Alzheimer's disease is a double-prion disorder, in which the Aβ and tau proteins become prions and accumulate as plaques and tangles, respectively, leading to neurodegeneration and the observed pathology. The development of cell culture and transgenic mouse models expressing Aβ and tau has allowed the identification of these two proteins as prions. In parallel studies, α-synuclein prions were discovered to be the cause of neurodegeneration in multiple system atrophy. Dr. Prusiner concluded that the ability to study cultured cells and mouse models producing Aβ, tau, or α-synuclein prions is a prerequisite for identifying and testing effective therapeutics for these three important diseases.

Session 1: Clinical

Todd P. Margolis, *Washington University in St. Louis*, opened the session with an overview of herpes simplex virus (HSV) and varicella zoster virus (VZV)–associated disease in the human eye based on his own experiences in the clinic. HSV can cause a number of different patterns of ocular disease each requiring a slightly different approach to therapy. HSV

blepharitis, conjunctivitis, and epithelial keratitis all represent productive viral replication in the skin, conjunctiva, and cornea, respectively. These infections are self-limiting and generally heal without scarring. Treatment with antivirals reduces the healing time. Less often, HSV causes disease of the corneal stroma and the pathology is immune-mediated. Stromal keratitis is often chronic and, if untreated, may lead to corneal scarring with some degree of visual loss. Sometimes, eyes with HSV stromal keratitis will develop corneal neovascularization, which makes the immune-mediated stromal disease more difficult to manage. Effective management of HSV stromal keratitis requires a topical corticosteroid and oral antiviral prophylaxis. HSV corneal endotheliitis reflects active viral replication in the iris and anterior chamber of the eye with secondary functional decompensation of the corneal endothelium, resulting in reversible corneal edema. Management with a treatment dose of oral antivirals plus a topical corticosteroid is very effective, quickly resolving the corneal edema. Dr. Margolis emphasized that there is both an art and science to balancing the use of antivirals and corticosteroids for stromal keratitis, corneal endotheliitis, and iritis, but in the hands of experienced and competent providers, most compliant patients will lose little vision from their episodes of HSV eye disease. Chronic antiviral prophylaxis is one reason for this. Antiviral resistance is generally not a problem, largely because active viral replication in the eye is self-limited, except if a patient is immune compromised. As with HSV, infection by VZV can cause different patterns of ocular disease each requiring a different approach to therapy. Herpes zoster ophthalmicus is typically a much more devastating disease than ophthalmic HSV, with a greater array of complications, including palsies, neurotrophic keratopathy, and post-herpetic neuralgia. Oral antivirals and topical corticosteroids are the mainstays of treatment. No animal or human samples were used in these experiments.

Vishwajit L Nimgaonkar, *University of Pittsburgh*, described studies on the potential association of persistent herpes simplex virus, type 1 (HSV-1) infection and sleep disturbances. HSV-1 infection includes spread to, and lifelong latency in, the nervous system. Persistent HSV-1 infection has been consistently associated with cognitive impairment. Other viral and non-viral infectious agents, particularly those infecting the central nervous system (CNS) can affect sleep and cause cognitive dysfunction. Thus, sleep abnormalities could explain the cognitive dysfunction. A total of of 311 older adults with or without insomnia (Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria IV) participated in the study. Detailed sleep-related data were collected from all participants, of which 145 completed polysomnographic and actigraphy studies. For comparison, Dr. Nimgaonkar and colleagues also evaluated infections with three additional agents associated with cognitive impairment in humans: herpes simplex virus, type 2 (HSV-2), human cytomegalovirus, and toxoplasma gondii. Nominally significant associations were noted with 6.1% of the sleep-related variables, but none remained significant following corrections for multiple comparisons. Similar patterns were observed with the other infectious agents. To conclude, few associations between persistent HSV-1 infection and sleep-related variables were observed. Thus, sleep dysfunction is unlikely to explain the HSV-1-associated cognitive dysfunction. However, the lack of association is striking in view of the diverse cognitive abnormalities reported to be associated with herpesvirus infections. All studies using human subjects or tissue samples have been either approved or deemed non-human subject research by the Institutional Review Board of the University (protocol number PRO10040100).

Jia Zhu, *University of Washington*, *Laboratory Medicine*, presented her work on deciphering peripheral nerve regeneration in genital skin in association with HSV-2 reactivation. HSV-2 establishes latent infections in the peripheral sensory nervous system for the lifetime of its human host. Despite frequent viral reactivation in symptomatic or asymptomatic forms, peripheral nerve damage or neuropathy is rare. HSV-2 reactivation is associated with increased density of neurite networks innervating the affected skin. To understand the molecular mechanisms underlying this HSV-related nerve protection and regeneration, Dr. Zhu and colleagues characterized expression of the p75 neurotrophin receptor (p75NTR), also known as the low-affinity nerve growth factor receptor (LNGFR), in human skin biopsies. Sequential biopsy tissue was obtained from six individuals during an ulcerative HSV-2 recurrence, from the active lesion through to 8 weeks post-healing, as well as normal arm skin from nine individuals seronegative for HSV. These biopsies were evaluated for p75NTR and NCAM expression using dual immunofluorescence staining. Data indicated that p75NTR expression on nerve fibers was increased in healed and post-healed genital biopsy tissues, compared with HSV lesion tissues and arm skin from individuals without HSV infection. Schwann cells associated with myelinated axons and with unmyelinated Remak bundles and free nerve endings, exhibited elevated levels of p75NTR expression in post-healed tissue. The transcriptomes of p75NTR-positive nerve fibers isolated by laser microdissection from HSV-affected skin and from contralateral uninfected tissue from the same individuals were compared. Nerve fibers isolated from post-healed biopsies showed upregulation of genes involved in axon guidance and formation of the extracellular matrix compared with those from lesions. Nerve fibers from both lesion and post-healing samples showed upregulation of genes involved in angiogenesis. Single-cell RNA-seq analysis of control and post-healed skin biopsies suggested that endothelial and fibroblast cells exhibit phenotype changes post-healing. These patterns of gene expression are being verified by in situ staining. Together, the data suggest that temporary nerve damage might

occur during HSV lesion formation and that the tissue microenvironment promotes nerve repair during healing. All studies using human tissue samples have been approved by the University of Washington Human Subjects Review Committee (protocol number STUDY00002443).

Trine H. Mogensen, *Aarhus University*, presented a novel innate immunodeficiency predisposing to VZV infection in the CNS. In most individuals, primary VZV infection causes varicella and zoster during reactivation. However, in a subset, VZV may cause more severe disease, including infection of the CNS. Recent studies by the Mogensen group have demonstrated that defects in the immunological DNA sensor RNA polymerase III (POL III) confers impaired antiviral interferon responses and selectively increased susceptibility to severe VZV infection, thus providing fundamental new insight into VZV immunity during primary infection and reactivation. Dr. Mogensen presented data on the identification of functionally defective genetic variants in POL III in children and adults with CNS disease manifestations, including encephalitis, vasculitis, and stroke. The contribution of POL III to promoter-dependent tRNA and rRNA transcription and immune surveillance during VZV infection were also described, and the current knowledge on POL III and DNA sensing in VZV infection was discussed. Finally, she highlighted remaining questions related to the role of POL III in immunity to VZV during primary infection and in reactivation from latency and how these new insights might be translated into clinical medicine. All studies using human subjects or tissue samples have been approved by the Institutional Review Board of Aarhus University and the Danish National Committee on Health Research Ethics (protocol number #1-10-72-275-15).

Ilakkiya Arumugam, *Sri Ramachandra Medical College and Research Institute*, described the studies on the potential use of open reading frame 68 (ORF68) encoding viral glycoprotein-E (gE), as a marker of VZV reactivation in clinical samples. VZV can establish latency in neurons of the enteric nervous system and can reactivate in immunocompromised/immunocompetent individuals. We have investigated the presence of VZV in participants suspected with possible reactivation of VZV by PCR targeting ORF68. We compared VZV strains from reactivation and primary infection as typical (presence of vesicle) and atypical presentation (absence of vesicle). Participants ($n = 16$) suspected of showing VZV reactivation who either manifested vesicles (typical, $n = 2$) or did not (atypical, $n = 14$) were enrolled in the study. Conventional PCR using primers to ORF68 sequences was performed. Among the participants with atypical presentation, the VZV ORF68 gene was detected in 42.8% ($n = 6$), in which four corresponded to colon biopsies from participants with inflammatory bowel disease and two were cerebrospinal fluid (CSF) samples from participants with CNS manifestations. Among the participants with typical presentation, VZV was detected in 100% ($n = 2$), one from a vesicular lesion and the other from CSF collected from a participant with encephalitis. For two participants (one atypical and the other typical), saliva samples were also tested and found to be positive for the ORF68 gene and further analyzed by DNA sequencing. When compared with the VZV Dumas reference genome, a single amino acid substitution (T40I) was detected in three samples. Residues 1–187 at the amino terminus of gE mediate gI binding and are essential for endocytosis in T cells and skin. Dr. Padma Srikanth and colleagues have previously detected VZV by ORF8 sequencing in atypical presentations arising from reactivation in the CNS and GI tract in renal transplant recipients. This current study is the first to report detection of VZV ORF68 in samples of vesicular fluid, colon biopsy, CSF, and saliva, corroborating prior in vitro studies. Because routine immunization against VZV is not practiced in India, none of the participants had received a VZV vaccine, indicating that VZV can reactivate in different tissues and organs in those who had history of childhood chickenpox. This study using human subjects or tissue samples has been approved by the Institutional Ethics Committee of (other than clinical evaluation of drugs/procedures/devices/diagnostics/vaccine/herbal remedies) (IEC-NI/17/JUN/60/64) Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, India. No animal studies were performed.

Session 2: Epigenetics

Patrick Lomonte, *CNRS-University of Lyon*, *France*, described how during the establishment of latency, HSV-1 genomes associate with promyelocytic leukemia (PML) nuclear bodies (PML NBs) and are chromatinized with constitutive heterochromatin marks but are not definitively silenced. Latency establishment is tightly controlled by PML NBs, although the exact nature of their contribution remains elusive. Interactions between latent viral genomes and PML NBs leads to the formation of viral DNA-containing PML NBs (vDCP NBs) in which the viral chromatin selectively incorporates histone variant H3.3 and two H3.3 chaperone complexes, DAXX/ATRX and HIRA, are shown to be involved in the latent HSV-1 chromatinization. In vivo two repressive histone marks, H3K9me3 and H3K27me3, were also found to be associated with latent HSV-1 genomes. Dr. Lomonte described ChIP and re-ChIP-qPCR studies demonstrating that only the H3K9me3 mark is associated with the latent genomes present in the vDCP NBs. Studies to identify the histone methyltransferase responsible for this histone modification are in progress. Latent HSV-1 genomes present in the vDCP NBs are transcriptionally silenced and do not express LAT; however, destabilization of vDCP NBs by ICP0 restores transcriptional competence leading to expression of lytic genes and as a consequence, viral replication. These studies demonstrate specific

epigenetic regulation of PML-associated latent HSV-1 by H3.3K9me3-dependent HSV-1 chromatinization, without definitive silencing of the viral genome. This anticipates a complex program of epigenetic regulation of latent HSV-1, not just within the whole trigeminal ganglia but also within a single latently infected neuron. No animal or human samples were used in these experiments.

Anna R. Cliffe, *University of Virginia*, described studies of HSV-1 reactivation in response to neuronal hyper-excitability. The exact physiological triggers of HSV-1 reactivation and the resulting changes in neuronal signaling pathways that converge on the viral genome to induce lytic gene expression are not understood. Previously, Dr. Cliffe and colleagues identified a role for Dual leucine zipper kinase (DLK)–mediated JNK activity in reactivation triggered by phosphatidylinositol 3-kinase (PI3K) inhibition. Reactivation following PI3K inhibition was also associated with a JNK-dependent histone phospho/methyl switch on lytic gene promoters. In cortical neurons, the same histone phospho/methyl switch has been found to occur following forskolin treatment, prompting them to examine the contribution of JNK and histone phosphorylation to HSV-1 reactivation triggered by forskolin. In a primary neuronal model of HSV-1 reactivation using murine sympathetic neurons, they found that forskolin-induced reactivation progressed via a phase I-like wave of lytic gene expression that was dependent on DLK/JNK activity, but that was independent of histone H3K9 and H3K27 demethylase activity. As shown previously in cortical neurons, forskolin treatment also triggered a transient histone phospho/methyl switch in sympathetic neurons. To determine the mechanisms of viral gene induction in response to forskolin, they examined the downstream targets of cAMP and identified a role for hyperpolarization-activated cyclic nucleotide–gated (HCN) channels. HCN channels can be bound by cAMP to trigger neuronal hyper-excitability. Both voltage-gated sodium and potassium channel activity was found to be required for HSV-1 reactivation, indicating a link to the excitation state of the neuron. Hyper-excitability was mimicked by removal of a tetrodotoxin block and this resulted in DLK/JNK-dependent reactivation. Despite the similarities between forskolin and PI3K inhibition–induced reactivation, they found that the two triggers could synergize to promote increased reactivation. In addition, forskolin treatment did not result in loss of AKT phosphorylation (which has previously been linked to reactivation following multiple triggers). Dr. Cliffe closed with a model in which neuronal hyper-excitability triggers HSV-1 reactivation via a mechanism that is distinct from NGF deprivation yet still involves a JNK-dependent phase I wave of gene expression. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Virginia (protocol number 4134).

Donna M. Neumann, *University of Wisconsin-Madison*, presented evidence that cellular cohesins, implicated in gene regulation and spatial organization of chromatin in mammalian genomes, also play a role in the control of HSV-1 latency in neurons. Work in her laboratory has shown that subunits of the cohesin complex colocalize with CCCTC-binding factor (CTCF) on viral genomes in a manner similar to mammalian genomes, and hypothesized that this is a mechanism by which DNA loops in viral genomes are anchored. Dr. Neumann and colleagues have previously shown that CTCF differentially occupies the latent HSV-1 genome and reported preliminary data indicating that during latency, CTCF-mediated DNA loops regulate viral lytic gene expression and hypothesize that these loops are anchored by cohesins. To test this, they infected mice and allowed them to establish latent infections. Ganglia were harvested and ChIP assays combined with qPCR were used to show that cohesin localized to some of the CTCF binding sites. Moreover, CTCF sites involved in loop formation were significantly more enriched in cohesin than CTCF binding sites that were not associated with loop formation. Deletion of a CTCF site involved in DNA loop formation abrogated cohesin recruitment, consistent with the idea that cohesin complexes anchor CTCF-mediated DNA loops on the latent HSV-1 genome. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Wisconsin) protocol number M006151).

Colleen A. Mangold, *Pennsylvania State University*, described studies on the identification of strain-specific differences in both host and virus transcriptomes following neuronal infection with HSV-1. Repeated cycles of HSV-1 latency and reactivation, especially later in life, may trigger CNS inflammation and/or neurodegeneration. Understanding how HSV-1 can invade and establish latency in the CNS to bring about localized damage is therefore of the utmost importance. Previous studies have identified host neuronal processes that are impacted by HSV-1 infection including alterations to cell cytoarchitecture, intracellular transport, extracellular matrix, and immune responses. These data provide a strong foundation from which to infer neuronal responses to HSV-1 that may impact neurovirulence, neuroinvasion, and latency. However, these studies lack a species-matched neuronal model that can be combined with RNA sequencing to simultaneously assess both the host and virus transcriptomes. To identify host- and virus-specific factors that contribute to neuroinvasion and neurovirulence, Dr. Mangold examined global transcriptomic changes in differentiated human SH-SY5Y neuroblastoma cells using three different strains of HSV-1 (F, KOS, and McKrae). RNA was isolated and sequenced at 12 and 24 h post-infection. Analysis of viral gene expression demonstrated potential strain-specific differences in the expression of viral transcriptional units. A comparison of differential host gene expression demonstrated strain-dependent effects on the expression of factors involved in adherens junctions, intracellular transport, mitochondrial

dysfunction, and oxidative stress. Additional studies to confirm these findings and integrate both viral and host datasets are ongoing. Overall, these findings provide novel insights into the specificity of host-HSV-1 interactions, and highlight the impact of virus strain on host neuronal responses. No animal or human samples were used in these experiments.

Session 3: Gene function

Igor Jurak, *University of Rijeka*, presented a comprehensive analysis of HSV-1 microRNAs (miRNAs) in various models and latently infected human neurons. HSV-1 has been found to expresses numerous miRNAs in both productive and latent infections; however, the function of these small non-coding RNAs has not been revealed. Interestingly, HSV-2, a closely related virus, expresses many positional homologs (i.e., conserved locus but divergent sequence) indicating that these miRNAs likely regulate viral rather than host transcripts. Regardless, knowledge of the exact sequence of each miRNA is prerequisite for the functional studies. Dr. Jurak's laboratory has observed frequent discrepancies between miRNA sequences in different data sets coming from different laboratories or sequencing platforms, which might misguide functional studies. To address this, they have put together more than 500 million sequence reads obtained by different laboratories using a variety of virus strains and from different productive versus latency models (in vitro and animal models) and from latently infected human trigeminal ganglia. This comprehensive analysis showed that most of abundantly expressed miRNAs (miR-H1-H8) satisfy very stringent criteria for miRNA biogenesis, whereas others (H11-13 etc.) might arise through a non-canonical pathway, and others might require a reevaluation as bona fide miRNAs. Furthermore, the pattern of miRNAs expressed in human samples (limited to only miR-H2-H7) is significantly different from miRNAs expressed in different models in which a large number of miRNAs are usually detected. In addition, the frequency of isomiRs (miRNAs with variations at 5′ or 3′ end) is rather high for HSV-1 miRNAs, and that the strand bias is less pronounced for virus-derived miRNAs as compared with a generic host miRNA. Evidence was presented for the first time showing significant post-transcriptional editing (A to I transition) of miR-H2 in latently infected human neurons but not when produced during productive infection in cultured cells. This suggests that HSV-1 uses a cellular deaminase to increase the targeting repertoire of miR-H2. All studies using human samples have been approved by the Bioethical committee of the Faculty of Medicine, University of Rijeka (2170-24-01-17-03).

Julianna R. Pieknik, *US Food and Drug Administration*, presented in vivo evidence for a role of viral microRNAs (miRNAs) in control of HSV latency. The two most abundant HSV-2 miRNAs, miR-I and miR-II, are expressed during both acute infection and latency. Both miRNAs inhibit ICP34.5 expression and can be processed from the viral latency-associated transcript (LAT) during latency. At unknown times, miR-I and miR-II can also be made from the L/S junction-spanning transcripts (L/ST), but not when ICP4 is present to repress L/ST expression. Loss-of-function in vivo studies evaluating mutations in HSV-2 miRNAs (including miR-I and miR-II) and their HSV-1 homologs have not yet revealed a miRNA phenotype in vivo. In this study, Dr., Pieknik and colleagues evaluated the function of miR-I and miR-II in an in vivo overexpression model using a virus with a mutation in the L/ST ICP4 binding site. To determine whether any phenotypes were in fact due to overexpression of the miRNAs, they rescued this mutation in two ways: first by reintroducing the ICP4 binding sequence into the mutant virus (i.e., traditional rescue), and second, by introducing additional mutations in both miR-I and miR-II in the context of the L/ST ICP4 binding site mutant. In a guinea pig genital infection model, acute and (spontaneous) recurrent infections were both attenuated by introduction of the L/ST ICP4 binding site mutations, to a level similar to that observed in an ICP34.5 deletion mutant virus. Acute and recurrent infections were not attenuated in the rescued version of the ICP34.5 deletion mutant or in either the traditional or the miR-I/II deletion rescues of the L/ST ICP4 binding site mutation. These findings provide strong evidence that ICP4 repression of L/ST transcripts at immediate-early timepoints is essential for viral pathogenesis and that miR-I and miR-II processed from L/ST function in vivo to suppress ICP34.5. This is the first in vivo demonstration of an effect by HSV miRNAs on viral pathogenesis. All animal studies were performed following guidelines and protocols approved by the White Oak Consolidated Institutional Animal Care and Use Committee (protocol #2018-3).

Esteban A. Engel, *Princeton University*, presented work about the improvement of viral vectors, essential tools in neuroscience research. They are used to define the connectivity and function of the central nervous system (CNS) in animal models and as gene therapy vectors to treat inherited and acquired CNS diseases in humans. Recombinant adeno-associated virus is the most widely used gene delivery vector due to its safety, broad tropism, and ease of production. The main pitfalls of AAV vectors are their limited payload capacity of less than 5 Kb, and the lack of small gene promoters that can provide stable, long-term, pan-neuronal expression in the CNS. Most promoters are large in size and get eventually shut down by host silencing mechanisms. To begin to address, these limitations Dr. Engel and colleagues have identified small regions of α-herpesvirus latency–associated promoters that are able to drive stable and long-term transgene expression in the mouse brain and spinal cord. All animal studies were performed following guidelines and protocols approved

by the Institutional Animal Care and Use Committee of Princeton University (protocol number 1943-16 and 1047). No human studies were performed.

David J. Davido, *University of Kansas*, presented results from a high-throughput screen to examine HSV-1 ICP0 transactivation function during acute infection and reactivation. The success of HSV-1 as a near-ubiquitous virus that persists for life reflects its ability to cycle between lytic replication and latency. Cellular factors involved in the switch between lytic and latent infections are largely unknown. Infected cell protein 0 (ICP0), an immediate-early regulatory protein, plays a critical role in regulating the HSV-1 life cycle by enhancing the transcription of all classes of HSV-1 genes. The Davido laboratory has developed a high-throughput cell culture assay that can be combined with small molecule inhibitor screening to examine ICP0 transactivator function. Two HSV-1 reporter viruses, KOS6β (wt) and *dl*x3.1-6β (ICP0 null mutant), were used to monitor ICP0 transactivation of the HSV-1 ICP6 promoter driving lacZ (β-galactosidase) expression. This approach was developed in a 384-well plate format. A ≥ 10-fold difference in β-galactosidase activity was observed in cells infected with KOS6β (ICP0+) compared with *dl*x3.1-6β (ICP0-), demonstrating that ICP0 potently transactivates the ICP6 promoter. This study established the robustness and reproducibility with a *Z*′-factor score of ≥ 0.69, an important criterium for high-throughput analyses. The sensitivity of this reporter system was validated using roscovitine, an inhibitor of HSV-1 gene expression and cyclin-dependent kinases. It is hoped that use of this assay will lead to the discovery of novel cellular mechanisms and pathways associated with HSV-1 ICP0 transactivating activity, which can be tested in reactivation model(s). Furthermore, this high-throughput assay can be employed to rapidly analyze chemical libraries and identify promising inhibitors of HSV-1. No animal or human samples were used in these experiments.

Paul R. Kinchington, *University of Pittsburgh*, presented studies of how two VZV virion–associated transactivators contribute to neuronal VZV growth and spread, and how growth-regulatable VZV can be used to establish latency in neurons without using antivirals. It is known that virion tegument activators affect lytic/latent decisions. A classic example is HSV VP16, which activates IE gene expression. VP16 not only influences whether or not stimulus-induced genomic derepression ("animation") progresses to full reactivation. Virion-derived VP16 may also determine whether or not infections at neuron axonal tips result in latency, depending on whether VP16 is transported to the neuronal nucleus. VZV virions have two abundant activators, the ORF10 IE transactivator homologous to VP16 and the virion form of IE62 (v62), the VZV main transcriptional regulator. Incorporation of ORF10 into the virion can be blocked by deletion of the gene and this does not affect growth in culture, although it does influence infection of organized skin. Incorporation of v62 into virions can be blocked either by deleting the ORF66 protein kinase or by mutating the codon for serine 686 (S686), a phosphorylation target of the ORF66 kinase. A VZV mutant functionally lacking both virion transactivators was constructed by deleting ORF10 in the background of VZV IE62 with S686A. Surprisingly, the mutant replicated efficiently in multiple VZV permissive lines and could also spread in hESC-derived neuron cultures, suggesting that the two virion-associated transactivators are not critical for neuron-to-neuron spread. The second part of the presentation demonstrated an efficient means to establish VZV latency without using antivirals to block lytic infections in neurons but instead by using VZV mutants carrying drug-controlled degron tags within essential viral proteins. This permitted conditional growth of VZV and efficient establishment of latency (as well as reactivation from latency) without the use of acyclovir. No animal or human samples were used in these experiments.

Emilia Vanni, *Harvard Medical School*, presented her recent studies on the regulation of ICP34.5 expression by elements within the 5′UTR of the mRNA. HSV-1 expresses many miRNAs during latency including miR-H4, which is complementary to sequences present in the ICP34.5 mRNA and miR-H4 has previously been shown to downregulate ICP34.5 expression in co-transfection assays. Furthermore, deleting sequences encoding miR-H4 from the virus leads to increased ICP34.5 expression in infected cells and to decreased neurovirulence in mice. Investigating the regulation of ICP34.5 expression by miR-H4, they have found that mutations in miR-H4 also affect a hairpin and an upstream AUG in the complementary ICP34.5 mRNA. Mutations and deletions of these elements affect ICP34.5 protein expression in transfection assays. From these observations, they hypothesize that elements within the 5′UTR of the mRNA regulate ICP34.5 expression in vivo. No animal or human samples were used in these experiments.

Session 4: Mechanism

Gregory A. Smith, *Northwestern University*, described his laboratory's work on the delivery of the HSV-1 genome into the nucleus of host neurons. He reported that the trafficking of these viruses along peripheral nerves to the ganglia by retrograde axonal transport delivers the incoming capsids to the centrosome, but is not sufficient for delivery into the nucleoplasm itself. Following the sustained dynein-based microtubule transport step that drives long-distance retrograde axonal transport, the capsid switches to a kinesin-based centrosome-to-nucleus trafficking paradigm. Remarkably, the kinesin motor responsible for nuclear delivery originates from the incoming viral particle rather than the cell being infected. All animal studies were performed following guidelines and protocols

approved by the Institutional Animal Care and Use Committee of Northwestern University (protocol number A3283-01); no human samples were used in these experiments.

Abel Viejo-Borbolla, *Hannover Medical School*, presented studies performed with the laboratory of Antonio Alcami (Centro de Biología Molecular Severo Ochoa, Madrid, Spain) on the role of HSV-2 glycoprotein G in neurite outgrowth and infection of sensory neurons. During primary infection, HSV-2 replicates in epithelial cells of the skin and mucosa before entering nerve endings to establish latency in neurons. The viral and cellular proteins required for neuronal infection and the factors determining whether this proceeds to lytic or latent replication are not fully characterized. Nerve endings are dynamic structures whose growth is regulated by positive and negative cues such as from nerve growth factor (NGF) and semaphorin 3A, respectively. HSV-2 enhances the expression and activity of growth factors that can increase neurite outgrowth. The Alcami Laboratory has previously shown that purified and soluble preparations of recombinant HSV-2 glycoprotein G (gG2) enhance NGF activity increasing axonal growth. Whether gG2 plays a similar role during infection is not known. To address this, they prepared a recombinant, HSV-2 reporter virus that cannot express gG2 and showed that infection of HEK-293T cells, which secrete soluble factors that inhibit neurite outgrowth, partially reverted their repelling phenotype in a gG2-dependent manner. Moreover, lack of gG2 expression reduced the infection of explanted mouse dorsal root ganglia compared with the parental virus. The use of microfluidic devices showed that gG2 was required for infection through the neurite end but not through the cell bodies. As previously shown, infection through the neurite end resulted in very low number of reporter positive cells and low viral gene expression, indicative of gene silencing and probably latency. Lack of gG2 or NGF further reduced reporter and viral gene expression despite similar delivery of viral genomes to the cell bodies. Taken together, these results indicate that HSV-2 gG and NGF contribute to a basal level of viral gene expression in sensory neurons following infection through neurite ends. No animal experiments were performed. Animal samples were obtained following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Hannover Medical School (Tierschutzgesetz §4 of the German Animal Welfare Law).

Andrea S. Bertke, *Virginia Tech*, discussed her laboratories recent work on how neurotrophic factors (NTFs) contribute to the maintenance of HSV latency in neurons. They had previously reported that depriving adult sensory neurons of either glial cell–derived neurotrophic factor (GDNF) or neurturin (NTN)-induced reactivation. Following binding to GDNF family receptors (GFRs), these NTFs activate Ret, a receptor tyrosine kinase that in other cell types regulates intracellular signaling pathways involved with cell proliferation and differentiation. These include the phosphoinositide 3-kinase (PI3K)/AKT pathway, which is initiated by Ret autophosphorylation at the tyrosine-1062. This signal has been implicated in maintaining HSV-1 latency in embryonic sympathetic neurons. Therefore, they aimed to establish the link between Ret Tyr1062–associated NTF signaling and HSV latency in primary adult sensory neurons. To determine if Ret signaling is important in maintaining latency, the effects of three novel compounds that activate Ret in the absence of GDNF and NTN were investigated, revealing that one of these compounds can prevent NTF deprivation–induced reactivation of HSV-1 but not HSV-2. Unexpectedly, immunoblotting showed that Ret is not phosphorylated at Tyr1062, even in uninfected cells and that multiple other sites (Tyr1016, Tyr1096, Tyr905, and Tyr981) remain unphosphorylated as well. Because GDNF and NTN can also signal through neural cell adhesion molecule (NCAM), the possibility of Ret-independent GDNF signaling through NCAM was investigated as a potential mechanism of maintaining viral latency. Preliminary studies show that treatment with an NCAM function-blocking antibody in the presence of NTFs induces reactivation of HSV-2, providing evidence that other NTF-associated mechanisms maintain latency in adult sensory neurons. Together, these findings show that NTF signaling contributes to maintaining HSV latency in adult sensory neurons, but not necessarily through the PI3K/AKT pathway. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Virginia Tech (protocol number 18-274).

Jeffery B. Ostler, *Oklahoma State University Center for Veterinary Health Sciences*, presented evidence that stress-induced host transcription factors can activate HSV-1 genes. Increased HSV-1 reactivation from latency is correlated with exposure to stressful stimuli. Stress generally leads to increased corticosteroid levels, which activate the glucocorticoid receptor (GR) and this has the potential to stimulate viral gene expression. In experimentally infected mice, the synthetic corticosteroid dexamethasone can stimulate HSV-1 productive infection as well as explant-induced reactivation. Altered expression of several stress-induced transcription factors have also been identified in infected trigeminal ganglia (TG) during reactivation, including Kruppel-like factor 15 (KLF15). GR and KLF15 cooperatively transactivate the viral ICP0 promoter in transfected Neuro-2A and Vero cells. Subsequent studies have identified key regions of the promoter important for transactivation. The ICP0 promoter does not possess canonical GR response elements (GRE); however, it contains several "half-GREs." Deletion of one or more of these "half-GREs" significantly decreased GR and KLF15-dependent transactivation of the ICP0 promoter. Recruitment of GR and KLF15 to the ICP0 promoter occurred by 4 h post-infection. Conversely, GR and KLF15 did not occupy ICP4 or

ICP27 promoter sequences until 8 to 16 h after infection. These studies suggest stressful stimuli can directly stimulate viral promoters and increase viral replication. While the immunosuppressive properties of stress and GR activation will also enhance viral spread, this would appear to occur later during reactivation from latency. No animal or human samples were used in these experiments.

Tony T. Huang, *New York University School of Medicine*, described recent work from his laboratory on the role of nuclear DNA damage in regulating HSV-1 latency. The mTOR pathway integrates both extracellular and intracellular signals and serves as a central regulator of cell metabolism, growth, survival, and stress responses. Neurotropic viruses such as HSV-1 also rely on cellular AKT-mTORC1 signaling to achieve viral latency. Dr. Huang and colleagues have defined a novel genotoxic response whereby spatially separated signals initiated by extracellular neurotrophic factors and by nuclear DNA damage are integrated by the AKT-mTORC1 pathway. They can demonstrate that endogenous DNA double-strand breaks (DSBs) mediated by Topoisomerase 2b-DNA cleavage complex (TOP2βcc) intermediates are required to achieve AKT-mTORC1 signaling and to maintain HSV-1 latency in neurons. As such, suppression of host DNA repair pathways that remove TOP2βcc trigger HSV-1 reactivation. Moreover, perturbation of AKT phosphorylation dynamics by downregulating the PHLPP1 phosphatase led to AKT mislocalization and disruption of DSB-induced HSV-1 reactivation. These findings demonstrate that both cellular genome integrity and environmental inputs are consolidated and co-opted by a latent virus to balance lifelong infection with transmission. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee at NYU School of Medicine (protocol number IA16-00717).

Joshua Ames, *University of Illinois at Chicago*, described studies on the potential role of endoplasmic reticulum–associated degradation (ERAD) in HSV-1 latency and reactivation. A number of cellular pathways are tightly regulated by HSV-1 during its life cycle and this includes the unfolded protein response (UPR) to endoplasmic reticulum (ER) stress pathway that modulates the synthesis and folding of viral proteins. The cellular CREB3/LZIP/Luman proteins are structurally similar to the viral VP16 protein and as such have been considered to play a role in neuronal reactivation. CREB3 is a member of the leucine zipper family of DNA binding protein and is responsible for the upregulation of ER-associated misfolded protein degradation (ERAD) factors such as EDEM1 and HERPUD1. This study found that CREB3 is upregulated during primary HSV-1 infection but without upregulation of downstream ERAD pathway proteins. The addition of a chemical chaperone to non-infected cells reduced protein misfolding and the downregulation of CREB3. However, in HSV-1-infected cells, treatment with the chemical chaperone led to attenuation of viral replication. Furthermore, in a murine model of ocular infection, animals treated with vehicle (PBS) showed robust reactivation in trigeminal ganglia 28 days post-infection, while those treated with the chemical chaperone or with acyclovir failed to reactivate. This raises an interesting question about whether HSV-1 exploits the ERAD pathway or CREB3 to establish latency in the trigeminal ganglia and whether modulation of this pathway could be used to suppress reactivation or even eradicate latent virus. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago (protocol number 17-077).

Oscar Haigh, *CEA-Université Paris Sud*, presented studies of immune responses to HSV-1 mutants that elicit protection against disease and prevent reactivation from trigeminal ganglia (TG). In humans, HSV-1 infection leads to the establishment of latency in sensory neurons of both right and left TG, which innervate the face and cornea. Reactivation events that lead to ocular herpetic disease are potentially sight-threatening and occur in 12 to 30 people per 100,000. Using a murine orofacial infection and ocular disease (OO) model, Dr. Haigh and colleagues have studied how attenuated HSV-1 infections in the lip could dictate the acute phase and reactivation of superinfections by virulent strains. They demonstrated that a delay between inoculations, first with an attenuated virus and then by a more virulent virus delivered to the opposite sides of the mouth, lead to a complete block to acute replication by the superinfecting virus and absence of disease. However, reactivations were exacerbated in mice receiving this inoculation regime. In contrast, a viral mutant deficient in thymidine kinase activity was likewise protected against acute-phase disease but exhibited no reactivation. Superinfecting virus was found in both left and right TGs regardless of the inoculation scheme. Analysis of immune infiltrate dynamics in the lip infection sites and TGs during both acute phase and latency revealed an immune signature associated with protection or disease outcomes. This mouse model mimics a natural route of HSV-1 infection, allowing studies on how primary infection and host immunity influence superinfection. The data obtained thus far will inform immunization strategies and shine new light on mechanisms associated with disease, protection, and suppression of viral reactivation. All procedures involving experimental animals conformed to ethical standards of the Association for Research in Vision and Ophthalmology (ARVO) Statement for the use of animals in research and were approved by the local ethics committee (CEEA 59).

Océane Sorel, *University of California, Irvine*, presented work on the role of T cells during the dissemination of VZV. It is known that VZV is transmitted from person-to-person by the inhalation of viral particles, but how VZV traffics from the initial site of infection to the ganglia and skin remains unclear.

Data from in vivo studies using severe-combined immunodeficient mice implanted with human fetal tissues strongly suggest that T cells are highly susceptible to VZV infection and may play a critical role in VZV dissemination to the skin and ganglia. Dissemination following intrabronchial inoculation can be modeled using simian varicella virus (SVV), which recapitulates the hallmarks of VZV infection in humans. Dr. Sorel and colleagues demonstrated that lung-resident T cells are susceptible and permissive to SVV infection. Additionally, memory T cells can be detected in the ganglia as early as 3 days post-infection, at the same time as viral DNA, and before the detection of a viral-specific T cell response. Although these observations establish a significant role for T cells in SVV spreading to the ganglia, as has been suggested for VZV, the mechanism by which varicella viruses hijack the host's T cells to disseminate to latency sites remains poorly defined. Studies are underway using high-throughput single-cell RNA sequencing to identify transcriptional changes induced by SVV infection in T cells isolated from the lung during acute infection. This will yield novel insights into viral-host interactions at the single-cell level and serve as a model to investigate the pathogenesis of other T cell tropic viruses. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Oregon National Primate Research Center (protocol number 0779).

Session 5: Models

Daniel P. Depledge, *NYU School of Medicine*, described ongoing studies to interrogate HSV-1 latency at a single cell level in an HSV-1 latency model using neurons obtained from the superior cervical ganglia of prenatal rat pups. In this model, viral gene transcription and replication are restricted, and accumulation of the LAT intron lariat is readily detectable. While the mechanisms and modes governing the periodic reactivation of latent HSV-1 remain poorly understood, recent studies from this consortium indicate that reactivation can be best explained by a two-phase model in which an initial burst of viral transcription (phase I) produces sufficient amounts of viral protein to overcome host epigenetic suppression to produce sequential, higher level transcription of viral genes, and active viral replication (phase II). To better understand this model, Dr. Depledge and colleagues employed single-cell transcriptome profiling to define the variety of cell types present in these ex vivo cultures and to subsequently profile host-virus interactions within and between the distinct cell types during latency and reactivation. To overcome intrinsic challenges of single-cell RNA sequencing such as a comparative lack of sensitivity and bulk sequencing datasets, they also examined changes in the transcriptomes during latency and reactivation. By integrating these datasets, they could establish that sympathetic neurons are the dominant cell type present in the cultures and that expression of the HSV-1 LAT non-coding RNA was restricted to these neurons. Changes in host and viral transcriptomes during phase I and phase II provide additional insights into the mechanisms of reactivation. Specifically, pathway analyses of differentially regulated neuronal genes suggest that successful reactivation, much like lytic infection, relies on the ability of the virus to establish control of the host translational machinery. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the NYU School of Medicine (protocol number IA16-00717). No human samples were used in these experiments.

Leonardo D'Aiuto, *University of Pittsburgh*, described an induced pluripotent stem cell (iPSC)–based system they are using to model the interactions of HSV-1 with neuronal progenitor cells (NPCs). The rationale is that HSV-1 infection can lead to damage in regions of the CNS associated with memory formation, which include the hippocampus and associated limbic structures. Adult neurogenesis occurs in the subgranular zone of the hippocampus and the subventricular zone. The new neurons generated from NPCs residing in these regions contribute to learning and memory. The hippocampus is vulnerable to HSV-1 infection, but the effect of the virus on NPCs has received little attention so far. To begin to address this, human iPSC–derived NPCs were infected with an HSV-1 recombinant expressing EGFP and RFP under the control of ICP0 and GC promoters, respectively. Infections were performed over a range of multiplicities of infection (MOI) beginning at 0.0001 increasing to 0.1. The infections were performed in the presence or the absence of antivirals (E)-5-(2-bromovinyl)-2′-deoxyuridine (5BVdU) and interferon-α (IFN-α). After infection, the cells were cultured either as two-dimensional (2D) monolayers or as three-dimensional (3D) spherical aggregates called neurospheres. They found that 5BVdU and IFN-α can efficiently inhibit HSV-1 infection in these cultures and that in most of the 2D and 3D NPC cultures infected at an MOI of 0.001, infectious virus was undetectable 7 days after the withdrawal of the antivirals. Additionally, it was shown that HSV-1 infection at MOI 0.1 impaired NPCs migration even in the presence of antivirals and that 3D NPC cultures were less permissive to HSV-1 than 2D cultures. Taken together, these results suggest that HSV-1 can establish latency in NPCs and highlight the potential of 3D cultures to model host-pathogen interactions. No animal or human samples were used in these experiments.

David C. Bloom, *University of Florida*, described a new HSV-1 latency model in which human neurons are differentiated in vitro from the Lund human mesencephalic (LUHMES) cells, a human neuronal precursor line that can be maintained and expanded as proliferating cells using a doxycycline regulatable c-myc gene, such that addition of tetracycline, GDNF, and dibutyryl cAMP elicits differentiation into post-

mitotic neurons. The Bloom laboratory has previously shown that a latent HSV-1 infection can be established in differentiated LUHMES cells and virus can be reactivated using PI3-kinase inhibitors. New data was presented on the temporal patterns of viral gene expression and the production of infectious virus upon reactivation using two different PI3K inhibitors (LY294002 or wortmannin) or a stimulator of adenylate cyclase (forskolin). All three compounds increased immediate-early, early, and late viral transcripts and decreased the latency-associated transcript LAT by 6 h post-treatment. In contrast to reactivation in the embryonic rat superior cervical neuron model, where infectious virus is not detected until 36 to 48 h after PI3K inhibitor treatment, infectious virus was detected in the supernatant of reactivated LUHMES cultures by 12 h post-treatment. This earlier production of infectious virus in the LUHMES cells during reactivation is similar to that seen in the reactivation of explanted latently infected mouse trigeminal ganglia (TG) and dorsal root ganglia. These data indicate that the "animation phase" that is required to prime reactivation in some in vitro neuron culture models is not required for HSV reactivation in the human LUHMES cells. This suggests that multiple programs of gene activation that prime HSV reactivation may exist in different types of neurons. No animal or human samples were used in these experiments.

David M. Knipe, *Harvard Medical School*, presented recent work from his laboratory on the use of human sensory neurons derived from inducible pluripotent stem cells (iPSCs) as a system to study HSV-1 lytic and latent infection. To date, the majority of HSV-1 neuronal infection and latency studies have been conducted in vivo in mice and rabbits or in cultured rodent neurons. Development of models to study how HSV-1 establishes latent infection in human neurons is essential. They therefore developed a method to differentiate human iPSCs into sensory neuronal lineages by combining ectopic expression of a novel transcription factor and small molecule treatment. Within 3 weeks, the differentiated neurons uniformly express markers of nociceptive neurons and lose the expression of pluripotent markers. The differentiated neurons are excitable and express multiple functional ion channels. HSV-1 lytic replication occurs in the differentiated neurons in the absence of acyclovir (ACV). Infection in the presence of ACV for several days was necessary to establish a non-permissive infection. Removal of ACV then led to a stable latent infection. Lytic transcripts decreased from day 1 to at least day 14 post-infection while latency-associated transcripts remained constant or increased modestly from day 1 to at least 14 days, consistent with an authentic latent infection. Heterochromatin was loaded by day 1 and maintained stably for 21 days. Inhibition of viral DNA replication prevented further increases in viral gene expression, dilution of heterochromatin, and stable latent infection. Reactivation could also be induced by various agents under certain conditions of infection. In this work, they describe a system for differentiation of scalable amounts of human sensory neurons and establishment of an HSV-1 latent infection with stable genomes bearing heterochromatin. Current studies are underway to understand the phenotype of HSV-1 mutant strains in this system. The Harvard Longwood Medical Area Institutional Review Board has determined that their use of the human iPS cells does not fall under Human Subjects Research and on September 15, 2017, issued a determination that this is not human subjects research (IRB17-1420, Knipe).

S. Victor Hsia, *University of Maryland Eastern Shore*, presented studies on the modulation of voltage-gated sodium channel (VGSC) activity in human dorsal root ganglion (DRG) neurons by herpesvirus quiescent infection. The molecular mechanisms of pain associated with alphaherpesvirus latency are not clear and it is hypothesized that voltage-gated sodium channels (VGSC) of dorsal root ganglion (DRG) neurons have abnormal activity during viral infection. To test this, HD10.6, a human DRG-derived neuronal cell line was differentiated in culture and infected with HSV-1. In the absence of virus, the cells exhibited a robust tetrodotoxin (TTX)-sensitive sodium current that was abolished by acute HSV-1 infection within 2 days. A quiescent state of infection mimicking latency can be achieved by infection in the presence of acyclovir (ACV) for 7 days followed by 5 days of ACV washout and the viruses can remain dormant for another 2 weeks. The loss of VGSC activity associated with acute infection was recovered during this latency establishment period. Surprisingly, electrophysiology studies of individual neurons indicated that neurons in the latently infected cultures exhibited higher VGSC current density compared with the controls. Additional preliminary studies showed that latently infected HD10.6, when treated with the histone deacetylase inhibitor trichostatin A (TSA) showed increased viral gene expression and replication. The TSA treatment, nonetheless, removed the strong VGSC current observed in neurons of both the control and latently infected cultures. Together, these observations demonstrated a very complex pattern of neuronal electrophysiology during HSV infection of DRG cells and this may have implications for better understanding the mechanisms of virus-mediated pain linked to latency and reactivation. No animal or human samples were used in these experiments.

Edouard M. Cantin, *Beckman Research Institute of City of Hope*, presented evidence that the absence of CCR2+ inflammatory monocytes in the CNS precludes HSV reactivation. Following initial invasion, HSV establishes an enduring latent infection in the host. Periodic reactivation results in frequent asymptomatic shedding and spread to new hosts. Mild recurrent diseases like cold sores are not uncommon, but more serious diseases like encephalitis (HSE) are relatively rare. Factors distinguishing asymptomatic from symptomatic reactivation have not been delineated. To address this dichotomy, they have modeled HSV-1 latency in immunodeficient Rag mice. As previously reported, latency established with high-

dose (HD) but not with low-dose (LD) infection resulted in fatal herpes simplex encephalitis (HSE) after heat stress (HS)–induced reactivation. Induction of HSE correlated with the accumulation of CCR2+ inflammatory monocytes (IM) in the CNS of HD-Rag but not LD-Rag mice. Lethal irradiation of latently infected WT mice to deplete IMs in the CNS resulted in insensitivity to HS-induced reactivation and the associated HSE. They then used small molecule inhibitors (SMI) to block IM accumulation in the CNS during latency establishment in Rag mice. This treatment precluded HS-mediated HSE in latently infected HD-Rag mice, confirming that IMs are essential for induction of symptomatic disease. Similarly, to EBV and KHSV, inhibition of the inflammatory mediator COX2 by treatment of HD-Rag mice with carprofen (a nonsteroidal anti-inflammatory) also inhibited HSV reactivation. Importantly, adoptively transferred T cells inhibited IM accumulation in the CNS, revealing a novel mechanism of T cell control of HSV reactivation. Observations on the timing of herpesvirus reactivations in hematopoietic stem cell transplant patients are consistent with IMs being important for clinical reactivation. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the City of Hope (protocol number 07043).

Christy S. Niemeyer, *University of Colorado School of Medicine*, described the characterization of primary VZV infection in guinea pigs. In humans, primary VZV infection produces varicella and establishes a reservoir of latent virus that with aging or immunosuppression can reactivate causing zoster. VZV can infect multiple organs to produce multisystem disease including stroke, myocarditis, and gastritis. A critical barrier in dissecting the mechanisms of VZV-associated disease is the lack of a reproducible, well-characterized small animal model for VZV infection. Previous studies have identified the guinea pig (*Cavia porcellus*) is a candidate model. After inoculation, VZV latency can be established in the cranial and enteric ganglia, and latent virus will reactive upon immunosuppression. Dr. Niemeyer and colleagues have reproduced and optimized these studies for a comprehensive characterization of primary VZV infection in the guinea pig. Using outbred, hairless guinea pigs with jugular vein catheters to facilitate inoculations and blood draws, peripheral blood mononuclear cells (PBMCs) were isolated and infected using a monolayer of VZV-infected guinea pig fibroblasts. The VZV-infected PBMCs were able to transmit infection to other fibroblasts in vitro and when re-injected via the catheter produced a transient viremia within 5 days post-infection (DPI). From 15 to 17 DPI, 3/3 VZV-infected guinea pigs developed clusters of raised, erythematous skin lesions in the cervical dermatomes, corresponding to the inoculation site. Furthermore, the rash in one of the animals continued to spread to the right ophthalmic distribution of the trigeminal nerve. Preliminary experiments looking at synaptic function of guinea pig hippocampus showed robust long-term potentiation. These findings demonstrate a well-defined protocol for establishing VZV infection in a small animal model that holds promise for studying the pathogenesis of primary infection in vivo, as well as for testing new antiviral drugs and vaccines. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Colorado, School of Medicine (protocol number 00433).

Session 6: Prevention

Rafael Harpaz, *Centers for Disease Control and Prevention* (*retired*), reviewed the impact of universal varicella vaccination on the incidence of herpes zoster (HZ) from the perspective of the USA. About 50 years ago, Dr. Edgar Hope-Simpson postulated that VZV latency was under immunological control, which was, in turn, maintained by "endogenous" boosting (due to VZV reactivation) and/or by "exogenous" boosting (due to exposure to varicella). If control of VZV latency is indeed maintained by exogenous boosting, it would follow that reduction in VZV circulation through effective pediatric varicella vaccination programs might lead to unintended increases in HZ incidence. This possibility has made policy-makers in many countries wary of introducing varicella vaccination into their immunization schedules. The USA, with its population of > 300 million, has a highly effective national varicella vaccination program lasting more than 20 years and, with credible sources of data regarding HZ incidence, has empiric evidence to address this question. The US data show that HZ incidence has inexplicably been increasing for decades, well-before the introduction of varicella vaccination; indeed, several studies suggest that HZ rates have plateaued among older adults since varicella vaccination was introduced. Furthermore, HZ rates are not different in states having higher versus lower pre-school varicella vaccination rates. There has certainly been no evidence that the US varicella vaccination program increased HZ incidence in the general adult population over baseline trends. In fact, HZ incidence has been declining in younger individuals born shortly before or since varicella vaccination was introduced, but this shift reflects a different phenomenon. Data from the US experience can inform the development of new generations of mathematical models to better predict HZ trends. More importantly, it provides reassurance for countries considering varicella vaccination that an effective program can reduce varicella morbidity and mortality—without raising HZ incidence among adults. No animal or human samples were used to generate the results described in this overview.

Martine Aubert, *Fred Hutchinson Cancer Research Center*, updated the audience on studies to develop a curative

therapy for HSV infection by targeting the virus in its latent reservoirs. The approach involves the introduction of DNA double-strand breaks (DSBs) into latent HSV genomes using rare-cutting endonucleases such as meganucleases or CRISPR/Cas9. The working hypothesis is that the introduction of DSBs into essential viral genes will result in erroneous host-mediated repair that produces deletions or insertions that functionally disable or eliminate the virus. Using the ocular mouse model of latent HSV-1 infection and an optimized adeno-associated virus (AAV) vector to deliver HSV-specific meganucleases to infected ganglionic neurons, Dr. Aubert showed that using a dual-meganuclease treatment they could reduce the HSV-1 genome load by approximately 90% in the superior cervical ganglia (SCG) and 50% in the trigeminal ganglia (TG). Furthermore, of the remaining viral genomes an average of 4 to 6% was mutated. This represents a dramatic improvement. Previously, they observed a maximum of about 4% gene editing, with no measurable loss of viral genomes. In addition, dual-meganuclease treatment of latently infected mice led to a 55% (TG) or 95% (SCG) reduction in viral genomes synthesized de novo after ganglia explant and dissociation to induce reactivation. Single-cell RNA sequencing of ganglionic neurons isolated from latently infected mice administered with different serotypes of reporter-expressing AAV vectors was used to identify the cells that become infected. This showed that both HSV-1 and individual AAV vector serotypes were non-randomly distributed among neuronal subsets suggesting that delivery into all of the neuronal subsets might lead to complete elimination of latent HSV-1. Surprisingly, CRISPR/Cas9, mediated only weak HSV-1 genome editing in vivo despite being highly efficient in cultured neurons. Further optimization of meganuclease delivery and improvements to their cleavage activity are planned. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center (protocol number 1865).

Charles Grose, *Children's Hospital*, *University of Iowa*, presented three observations on the impact of herpes zoster in young children who have been vaccinated. One of the herpes zoster cases he described was so painful that the child needed to be hospitalized because he refused to walk. Two different VZV genes were sequenced from a skin lesion from this individual and the sequence of VZV IE62 (equivalent to HSV ICP4) matched the published vaccine sequence. The Grose Laboratory then compared their data with results from both the Breuer and the Schmid labs. Both have found IE62 SNP 107797 (L446P) to be a reliable marker of vaccine skin rashes, with this residue being leucine in naturally circulating viruses. Interestingly however, they did not find SNP 107797 in genomes from the rash in this case. They also sequenced the VZV ORF0 gene (equivalent to HSV UL56) and found a missense mutation (reversion from vaccine strain to wild type). They had previously found the vaccine ORF0 allele in the widely used VZV Ellen lab strain (considered to be wild type). In the SCID human skin model, VZV Ellen is more attenuated than VZV Oka vaccine strain. Thus, this ORF0 SNP appears to be a virulence marker as well as a rash marker. The third clue was only obtained after they followed the first case for 2 additional years, during which the patient developed asthma. In a previous study of 277 children with herpes zoster enrolled in the Rochester Epidemiology Project at the Mayo Clinic, 63 had a diagnosis of asthma (23%). This is compared with 13% in a control group of 277 children without zoster. The difference was found to be significant ($P = 0.002$). Further, asthma corticosteroids did not account for this association. The Mayo Clinic cohort will be followed in a larger 4-year study. No animal or human samples were used to generate the results described in this overview.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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