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MEETING REPORT

2018 Colorado Alphaherpesvirus Latency Society Symposium

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Abstract
Meeting Report on the 8th Annual Symposium of the Colorado Alphaherpesvirus Latency Society (CALS), held on May 16–19, 2018, in Vail, Colorado.

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

Introduction

The 8th annual symposium of the Colorado Alphaherpesvirus Latency Society (CALS) was held May 16–19, 2018, in Vail, Colorado, where 70 investigators who have authored over 3009 PubMed-listed publications involving herpesvirology and who traveled 116,701 miles from 3 continents, 6 countries, and 21 states assembled for the 2-day symposium consisted of 31 oral presentations by investigators to discuss advances in the research of alphaherpesvirus latency (Fig. 1). In addition, 12 2-min presentations were given by new investigators to highlight their findings that were subsequently presented at the formal poster session. In addition, a small group of exceptional undergraduates whose interest in herpesvirology is just beginning were also hosted. The Don Gilden Memorial Lecture was presented by Dr. Ana Pombo from the Berlin Institute for Medical Systems Biology at the Max Delbrück Center for Molecular Medicine. This highly technical presentation based on her studies to understand the interplay between genome architecture and transcriptional regulation has already provided insights into control of latent alphaherpesvirus transcription during latency. This year we missed the traditional blizzard and all enjoyed the relaxed setting of The Christiania and Tivoli Lodges in the quaint mountain town nestled in the heart of the Colorado Rocky Mountains where established collaborations were strengthen, new collaborations were formed, and the next generation of clinical/basic research scientists were introduced. A brief summary of the presentations follows in alphabetical order by author's last name.

Andrea Bertke, *Virginia Tech*, presented data on a novel RET agonist that maintains HSV latency. HSV-1 and HSV-2 infect and establish latency in peripheral neurons after ocular or genital infection. This lab had previously shown that deprivation of neurotrophic factors neurturin (NTN) and glial cell derived neurotrophic factor (GDNF) induced reactivation of HSV-1 and HSV-2 in primary adult sensory neurons, while NGF or GDNF deprivation effectively reactivated HSV-1 in adult sympathetic neurons. GDNF and NTN bind to glial family receptors alpha 1 and 2 (GFRα1 and GFRα2), which activate the RET tyrosine kinase to maintain neuronal signaling and functions. They hypothesized that maintaining RET signaling would prevent reactivation induced by neurotrophic factor deprivation. Therefore, this lab tested three novel RET agonists that were previously validated for function in vitro and in vivo. The three agonists differed in their abilities to prevent reactivation when latently infected neurons were deprived of neurotrophic factors, in some cases showing neuron and virus selectivity. However, one of these agonists effectively prevented reactivation. Thus, continuous RET activation can maintain HSV-1 and HSV-2 latency in adult neurons, and selective RET agonists may provide an alternative therapeutic option to prevent recurrent disease. All animal studies were performed following guidelines and protocols approved

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Fig. 1 Participants of the 8th annual symposium of the Colorado Alphaherpesvirus Latency Society. (*Row 1*, *from the left*) Nicholas Baird, Randy Cohrs, Benjamin Warner, Erin Buckingham, Qiaojuan Zhang; (*row 2*, *from the left*) Andrea Bertke, John Blaho, Tao Peng, Anna Wald, Sara Bustos, Peter Baciu, Maria Nagel, Satish Mehta, Ravi Mahalingam, Padma Srikanth, Zeb Hawkins, Michael D. Gershon, Faith Osinaga, Anne Gershon, Rachel Dawson, Ana Pombo; (*row 3*, *from the left*) Alex Greninger, Keith Jerome, Andrew Bubak, David Koelle, Peter Kennedy, Barry Rouse, Ken Jones, David Bloom, Patrick Lomonte, Orkide Koyuncu, Aaron Prattis, Seth Frietze; (*row 4*, *from the left*) Rick Thompson, Mackenzie Shipley, Joshua Schiffer, Moriah Szpara, Ian Hogue, Chiharu Graybill, Hannah Tsingine; (*row 5*, *from the left*) Angus Wilson, Ian Mohr, Srinivas P Kalanghad, Charles Grose, Todd Margolis, Emilia Vanni, Hui-Lan Hu, Victor Hsia; (*row 6*, *from the left*) Preston Neff, Daniel Depledge, Homayon Ghiasi, Werner Ouwendijk, Anna Cliffe, Caroline Kulesza, Leonardo D'Aiuto, Joel Rovnak, Benedikt Kaufer, Dallas Jones, Georges Verjans, Scott Schmid, David Davido, Matthew Taylor, Vishwajit Nimgaonkar, Clinton Jones, Abel Viejo-Borbolla, Edouard Cantin, David Knipe. Not shown: Nancy Sawtell, Jane Dantinne, Brent Palmer

by the Institutional Animal Care and Use Committee of Virginia Tech (protocol number 15-237). No human studies were performed.

David Bloom, *University of Florida*, demonstrated how lund human mesencephalic neuronal cell line supports HSV-1 latency in vitro. Lund human mesencephalic (LUHMES) neuronal cells are human embryonic neuronal precursor cells that can be maintained as proliferating cells due to the expression of a doxcycline regulatable c-myc gene. They can then be differentiated to post-mitotic neurons by the addition of doxcycline, GDNF, and bibutyryl cAMP. The Bloom lab has demonstrated that these cells can be infected with HSV-1 at an m.o.i. of 3 and the majority of the cells survive. By 6–10 days after infection, there is a loss of lytic gene transcription and an increase in numbers of neurons that express the latency associate transcript (LAT). ChIP analyses reveal an increase in H3K27triMe on the genomes by day 10 p.i. Importantly, the virus can then be reactivated by the addition of a PI3K inhibitor, which had previously been shown to reactivate HSV-1 latent infections in rat neuron cultures. While there have previous been other rodent primary culture neuron systems described, these have been limited by the fact that they are not scalable and it is difficult to obtain more than 500,000 cells to work with in a given experiment. Several recent papers have described a human DRG neuron culture model and human iPSC neuron culture models which are scalable, however, require the presence of antiviral suppression be maintained following HSV infection. Therefore, this lab believes that the LUHMES cell model described here may be especially useful for studying a number of aspects of HSV-1 latency and reactivation due to its scalability, that latency can be maintained without the use of antiviral inhibitors, and the latent gene expression profiles are similar to those seen in vivo, including the heterogeneity of cell populations expressing the LAT. No animal or human studies were performed.

Andrew Bubak, *University of Colorado School of Medicine*, presented data on VZV inducing nuclear translocation of neurokinin-1 receptor, promoting lamellipodia formation and viral spread in spinal astrocytes. During reactivation, VZV reactivation can infect the spinal cord and produce a paraparesis (myelopathy) that can recur and is difficult to treat. The pathogenesis of VZV myelopathy is not well-

characterized but infection of spinal astrocytes, the most abundant cell in the spinal cord and key regulators of extracellular homeostasis of neurotransmitters and ions, most likely plays a critical role. Recent studies suggest a role for the neurokinin-1 receptor (NK-1R), which is found on astrocytes, in cytoskeletal alterations that may, in turn, promote viral spread. Thus, this group examined the role of NK-1R in VZV-infected primary human spinal astrocytes (HA-sp) to shed light on the pathogenesis of VZV myelopathy. HA-sp were mock- or VZV-infected and examined for substance P production, NK-1R localization, morphological changes, and viral spread in the presence or absence NK-1R antagonists. HA-sp were permissive to VZV infection, which induced aberrant nuclear localization of the full-length NK-1R isoform in the absence of its endogenous ligand, substance P. VZV-induced nuclear NK-1R was associated with extensive lamellipodia, as well as filipodia formation, which have been previously reported to facilitate VZV spread. The formation of lamellipodia and filipodia, as well as VZV DNA and viral spread, were decreased with the addition of an NK-1R antagonist, aprepitant or rolapitant. Overall, this lab identified a novel pro-viral function of NK-1R associated with aberrant nuclear localization, inducing lamellipodia that facilitates cell-to-cell spread of VZV. This NK-1R-dependent process occurred in the absence of substance P, suggesting that a virus-induced factor(s) binds to NK-1R and initiates this signaling cascade. The ability of the aprepitant and rolapitant, both currently in clinical use as anti-emetics, to inhibit lamellipodia formation and viral spread provides a promising alternative to nucleoside analogs in treating VZV myelopathy and related complications. No animal or human studies were performed.

Erin Buckingham, *University of Iowa*, presented data showing the limited effect of the neurovirulence protein ICP34.5 on reassortment of the LC3 autophagy protein with purified HSV-1 when compared with PRV and VZV HSV-1 produces a well-known neurovirulence protein, ICP34.5. The ICP34.5 protein enhances CNS neurovirulence in the mouse model; in a mouse DRG model, HSV mutant viruses lacking ICP34.5 failed to reactivate from latency. The N-terminus of this protein can inhibit autophagy by binding to Beclin-1. The signature protein in the autophagy pathway is lipidated microtubule-associated protein 1 light chain 3 (MAP1LC3-II); ICP34.5 diminishes the downstream production of LC3-II. The goal of this research is to investigate whether ICP34.5 exerts any effect after secondary envelopment on the herpes exocytosis pathway. This lab recently demonstrated LC3-II in blots of gradient-purified VZ virions. They hypothesized that LC3-II was a component of an exocytosis vesicle encasing VZV. To determine whether LC3-II+ vesicles encased HSV1 (ICP34.5+) or PRV (ICP34.5−), they examined 4 gradient-purified HSV1 strains (KOS, F, McKrae, and wild type) and 2 PRV strains. In every case, the blot for LC3-II was positive. As further control experiments, they tested samples of similarly purified adenovirus, HIV1 and vesicular stomatitis virus for LC3-II and they were negative. These results indicated that (1) LC3-II was not acquired nonspecifically during gradient purification, (2) LC3-II did not nonspecifically attach to viral glycoproteins, and (3) LC3-II was not acquired in the cytosol by enveloped RNA viruses as they bud through the plasma membrane. Further, these results suggested that LC3-II was a component of an exocytosis vesicle that carried at least some VZV, HSV1, and PRV particles from the virus assembly compartment to the plasma membrane and that LC3-II+ remnants were retained by viruses recovered from infected cells, even after centrifugation but not after proteinase treatment. Finally, the Beclin-binding ICP34.5 protein did not appear to inhibit reassortment of LC3-II positive membranes in the autophagy pathway into virus-containing vesicles. No animal or human studies were performed.

Edouard Cantin, *City of Hope*, discussed a role for gut commensal bacteria in herpes stromal keratitis. Immune mechanisms are causally implicated in the pathology of Herpes stromal keratitis (HSK), a common, serious disease resulting from reactivation of HSV-1. The microbiota (mb) is known to play an important role in the development and homeostatic regulation of the immune, nervous and endocrine systems. To determine a role for gut commensal bacteria in development of HSK, this lab used an antibiotic cocktail (Abx) to deplete them in C57BL/6J (B6) mice prior to infection with HSV-1 17+. HSK scores were significantly lower in Abx treated, compared to control saline-treated mice, and this was correlated with significant reductions in absolute immune cell numbers, especially CD4 T cells and B cells in the blood, spleen, and brainstem (BS) as well as infiltrates into the cornea. A sex effect was observed, with control females having higher HSK scores than males. Remarkably, Abx treatment abolished the sex effect but did not alter susceptibility to HSV encephalitis (HSE) in the resistant B6 mice. Because HSV-1 induces hyper-inflammatory responses in susceptible 129S6 (129) compared to B6 mice, they assessed HSK and HSE in B6 and 129 mice reciprocally transplanted with fecal microbiota (mb). HSE mortality was increased significantly in male but not female B6 mice transplanted with 129 mb (B6-129mb) compared to B6-B6mb. Increased HSE in B6-129mb mice correlated with increased BS infiltration by innate cells. However, differences in corneal disease between B6mb and 129mb mice were evident only at late time points, with increased corneal disease in B6 females. Although this data appears confounding, investigation of corneal disease in HSV-KOS infected 129 mice transplanted with B6mb or 129mb revealed that the B6 mb predisposes towards corneal disease, while the129 mb increases HSE symptoms. Thus, the severity of HSK and HSE is determined by the microbiome, sex, and the specific combination of mouse genotype and virus strain. 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). No human studies were performed.

Anna Cliffe, *University of Virginia*, presented data on neuronal hyper-excitability triggering JNK-dependent reactivation of HSV from latency. Reactivation of HSV from a latent infection involves the upregulation of lytic gene expression from promoters assembled into silent heterochromatin. The exact physiological triggers of reactivation, the cell signaling pathways involved and how these signals act on heterochromatin-associated lytic promoters is not understood. Previously, this lab identified a role for DLK/JIP3-mediated activation of JNK in triggering HSV reactivation following PI3-kinase inhibition. JNK activation triggered an initial wave of lytic gene expression (known as phase I) that occurred independently of histone demethylase activity and instead involved a histone phospho/methyl switch. Using a model of HSV reactivation in mouse sympathetic neurons, they investigated how increasing levels of cAMP triggered HSV-1 reactivation. Interestingly, this group found that reactivation triggered by increasing cAMP levels (by treatment with forskolin or a cAMP mimetic) also resulted in a DLK/JNK-dependent phase I-like wave of gene expression. To determine how forskolin triggered JNK-dependent reactivation, they investigated the downstream targets of cAMP. Interestingly, they found that reactivation still occurred following inhibition of the signal transduction proteins CREB, RAPGEF2, or EPAC. In neurons, high cAMP levels can result in increased action potential firing or hyper-excitability. They mimicked hyper-excitability by addition of high concentrations of extracellular KCl, which also resulted in reactivation. cAMP can trigger neuronal hyper-excitability by acting on hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Inhibition of either HCN channels or potassium channels prevented forskolin-mediated reactivation. In addition, they found that HCN channel activity was also required for JNK activation and a global histone phospho/methyl switch triggered by forskolin. Therefore, the lab put forward a model in which neuronal hyper-excitability triggers a JNK-dependent histone phospho/methyl switch, which permits lytic gene expression from heterochromatin-associated promoters. They hypothesize that this mechanism of reactivation occurs in response to certain physiological stimuli that trigger hyper-excitability, such as changes in hormone levels or inflammation. 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). No human studies were performed.

Leonardo D'Aiuto, *University of Pittsburg*, presented data on human brain organoids to model HSV1-CNS interactions. Investigation of the pathogenesis of neurotropic viruses in humans has been hampered by the absence of a satisfactory human system to model virus/CNS interactions. Induced human pluripotent stem cells (iPSC)-based technologies have revolutionized the methodological approaches to model aspects of neurodevelopmental and neurodegenerative diseases. However, the modeling of human CNS infections utilizing iPSC-based systems has been minimally reported in the virology field. Importantly, recent advances in stem cell differentiation strategies are allowing the generation of three-dimensional (3D) cultures that recapitulate features of a developing brain. Here, this lab employ human brain organoids derived from iPSCs to model HSV-1 infections in a 3D system that mimics in vivo tissue architecture. Three-month-old brain organoids were infected with HSV1, strain KOS (1500 pfu/organoid). Immunohistochemistry analysis of paraffin sections generated at 48 hpi showed expression of ICP4 in the nuclei of MAP2+ cortical neurons across the entire area of the sections. These results indicate that cortical neurons are permissive to HSV1. To establish latency, brain organoids were infected in the presence of antivirals (E)-5-(2-bromovinyl)-2′-deoxyuridine and interferon-α for 11 days. In these culture conditions, spontaneous viral reactivation was observed in only 18% of the organoids. The treatment of the remaining organoids with PI3 kinase inhibitor or histone deacetylase inhibitors sodium butyrate and trichostatin A for 22 days after the withdrawal of the antivirals did not induce viral reactivation. These results parallel the low efficiency of HSV1 reactivation in the CNS (as opposed to peripheral ganglia) of animal models. Collectively, these results indicate that brain organoids offer an extraordinary opportunity for modeling the interaction of HSV-1 with the human CNS. No animal or human studies were performed.

Daniel P. Depledge, *New York University School of Medicine*, revisited HSV-1 latency at single cell resolution. Following primary infection, HSV-1 establishes life-long latency in peripheral ganglia. Latency is evidenced by restricted viral gene transcription, minimal viral protein production, and the absence of viral DNA replication. These features are faithfully recapitulated in this lab's experimental model which selectively enriches for sympathetic neurons during ex vivo culturing of dissociated superior cervical ganglia (SCG) excised from rat pups. While the mechanisms and modes governing the periodic reactivation of latent HSV-1 remain poorly understood, this group's recent studies suggest reactivation is best explained by a two-phase model in which an initial burst of viral transcription (phase I) produces sufficient viral protein to overcome host epigenetic suppression and results in the temporal transcription of viral genes (phase II) necessary for productive replication. To further this lab's understanding of this SCG model and to tease out the molecular mechanisms that govern HSV-1 latency and reactivation, this lab has employed single cell transcriptome profiling to explore and 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 viral latency and reactivation. While sympathetic neurons dominate the cultures, the

presence of support cells, including Schwann cells and satellite glial cells, adds complexity to the system. This provides a unique opportunity to explore interactions between discrete cell types in the presence and absence of HSV-1. Pathway analyses based on differentially regulated genes in each cell type suggests that successful reactivation, much like lytic infection, rests on the virus successfully establishing 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_AR2). No human studies were performed.

Anne A. Gershon, *Columbia University*, presented data on enteric zoster, diagnosis with saliva, and use of a guinea pig model to study the role of innate immunity in protection against VZV infection. Varicella is a disseminated condition that occurs in naïve hosts, whereas zoster is usually localized because it occurs when VZV reactivates from latency in a setting of at least partial immunity. Latency is established in dorsal root (DRG), cranial nerve (CNG), and autonomic ganglia, including the enteric nervous system (ENS). Transport in infected axons from DRG/CNG transmits VZV to epidermal regions that these neurons innervate, causing rash. In contrast, VZV reactivation in enteric ganglia, which do not project to the epidermis, causes no rash. VZV DNA appears in saliva when VZV infection is active (varicella or zoster) but not when latent. Detection of salivary VZV DNA may thus enable occult zoster (VZV reactivation without rash) to be identified. Cutaneous symptoms without rash (zoster sine herpete) occurs when VZV reactivates in DRG/CNG neurons that fail to transmit VZV to the epidermis. Visceral pain accompanies reactivation of VZV in the ENS, which transmits VZV to enteric targets, which include enteroendocrine cells of the gastrointestinal (GI) mucosa. The discovery of salivary VZV in patients with otherwise unexplained visceral pain thus suggests enteric zoster, which this group has confirmed by demonstrating VZV DNA, RNA, and protein in GI biopsies. Valacyclovir-treatment relieved visceral pain in 9/9 patients with salivary VZV DNA, but may mask biopsy confirmation. This lab developed a guinea pig model that utilized Intravenous injection of VZV-infected T cells to infect the animals. Varicella did not occur in immunocompetent guinea pigs; however, latency was established. Subsequent immunosuppression with tacrolimus and mimicry of stress with corticotrophin releasing hormone (CRH) reactivated VZV to give rise to disseminated zoster. When VZV-infected T cells were given to tacrolimus/CRH-treated guinea pigs, rapidly lethal disseminated varicella resulted. These guinea pig data suggest that a tacrolimus-sensitive component of innate immunity plays a critical role in protecting hosts from VZV. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Columbia University (protocol number AAAT 4451); all studies using human subjects or tissue samples have been either approved or deemed non-human subject research by the Institutional Review Board of Columbia University (protocol number AAAJ7264).

Homayon Ghiasi, *Cedars-Sinai Medical Center*, discussed the role of IFN-γ over-expression in HSV-1 latency-reactivation. The role of IFN-γ in HSV-1 latency-reactivation is controversial. This study was designed to answer two questions: (1) If IFN-γ play any role in HSV-1 latency-reactivation? and (2) If assuming latency in a latency associated transcript (LAT)-minus virus is a threshold, can we further reduce this threshold by using recombinant viruses expressing stimulatory molecules in a LAT-minus background and without changing the dose of infection? To answer these two questions, C57BL/6 mice were ocularly infected with 2 X 10^5 PFU/eye of a recombinant HSV-1 expressing two copies of murine IFN-γ under the control of the latency associated transcript (LAT) promoter in a LAT-negative recombinant virus (HSV-IFN-γ). Control mice were ocularly infected with parental virus that were derived from an HSV-1 strain McKrae (dLAT2903). Primary virus replication in the eyes and trigeminal ganglia (TG) were similar between HSV-IFN-γ and the parental virus. Latency and explant reactivation was lower in HSV-IFN-γ-infected mice than those infected with the parental virus. Higher latency was correlated with higher levels of CD8 and PD-1 mRNAs. Results suggest that (1) HSV-1 recombinant viruses expressing IFN-γ were capable of significantly reducing latency-reactivation compared with their LAT-minus parental virus; (2) that HSV-IFN-γ, despite expressing IFN-γ constitutively, had lower latency-reactivation than parental viruses suggesting that IFN-γ does not play any role in the increase of latency-reactivation; and (3) that by using immune stimulatory factors such as IFN-γ, we can further reduce the level of latency-reactivation and T cells exhaustion even in a LAT-minus background. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Cedars-Sinai Medical Center (protocol number 5030). No human studies were performed.

Chiharu Graybill, *University of Colorado School of Medicine*, demonstrated that whole genome sequencing and GC-skew plot revealed potential origins of DNA replication in VZV. VZV and other herpesviruses depend on DNA replication for their progeny production. DNA origins of replication, stretches of sequence where replication processes are initiated, are critical elements of the VZV genome not only for lytic infection but also for reactivation from latency. Previous study had suggested the presence of another origin of replication, WR region located between 67,771 and 72,011 nt of VZV32 genome, in addition to the two known origins. However, how many more potential origins exist in the genome and whether WR region functions as a replication origin or not remain to be examined. In this study, this lab identified a potential origin of

replication, A region located between 375 and 4,137 nt of VZV32 genome, by whole genome sequencing and GC-skew plotting, a computation prediction method. This group also developed a GFP reporter system to test if both the WR region and A region could serve as origins of replication. Their results demonstrate that A region, but not WR region, functions as an origin of replication. More importantly, replication proceeded in the total absence of viral proteins, indicating that host proteins are sufficient to initiate VZV DNA replication. These results substantially alter the current view of VZV replication and open new avenues for different replication mechanisms. No animal or human studies were performed.

Alex Greninger, *University of Washington*, showed that ultrasensitive capture of human HSV genomes directly from clinical samples reveals extraordinarily limited evolution in cell culture. HSV is difficult to sequence due to the large DNA genome, high GC content, and presence of repeats. To date, most HSV genomes have been recovered from culture isolates, which may not accurate represent circulating clinical strains. This lab reported the development and validation of an oligonucleotide hybridization panel to recover near-complete HSV genomes at abundances up to 50,000-fold lower than previously reported. Using copy number information on herpesvirus and host DNA background via quantitative PCR, they report a protocol for pooling for cost-effective recovery of more than 50 HSV-1 or HSV-2 genomes per sequencing run. This group demonstrated the ability to recover > 99% of the HSV genome at > 100× coverage in under 72 h at a reagent cost of less than $50 per viral genome. They also reported a new computational pipeline for rapid HSV assembly and annotation. Using the above tools and a convenience sample of 17 HSV-1-positive clinical swabs sent to this lab for viral isolation, they showed limited evolution of HSV-1 during viral isolation in human fibroblast cells from clinical samples. They also demonstrated the ability recover HSV sequence from latently infected ganglia. Their data indicates that many past studies using low-passage clinical isolates of alphaherpesviruses are reflective of the viral sequences present in the lesion and thus can be used in phylogenetic analyses. They also detect superinfection within a single sample with radically different HSV-1 strains from separate oral lesions in an immunosuppressed patient sampled over a 2.5-week period, illustrating the power of direct-from-specimen sequencing of HSV. All studies using human subjects or tissue samples have been either approved or deemed non-human subject research by the University of Washington Human Subjects Division (protocol number is STUDY 408). No animal studies were performed.

Ian Hogue, *Arizona State University*, investigated pseudorabies virus (PRV) egress and spread and reported that PRV uses constitutive secretory mechanisms and does not depend on action potential firing in neurons. Following reactivation from latency, newly assembled herpesvirus particles traffic to the site of egress and then exit from infected cells by exocytosis. Using a live-cell fluorescence microscopy assay of viral egress, this lab previously reported that PRV particles take advantage of constitutive secretory mechanisms, governed by Rab6, Rab8, and Rab11, for exocytosis in non-neuronal cells. However, neurons possess highly specialized secretory mechanisms for regulated exocytosis of synaptic and neuropeptide vesicles, governed by Rab3 and Rab27. Because alphaherpesviruses spread along circuits of synaptically connected neurons in vivo, and infection of neurons is correlated with increased action potential firing in vivo and in vitro, this lab hypothesized that PRV uses regulated secretory mechanisms, dependent on action potential firing, for egress and spread from neurons. To address this hypothesis, this group used a compartmentalized neuron culture system to measure transneuronal spread of PRV, together with pharmacological and optogenetics approaches to modulate neuronal activity. Unexpectedly, they found that transneuronal spread of PRV is not correlated with action potential firing. To better understand why this is the case, they further investigated the molecular mechanisms of viral egress using their live-cell fluorescence microscopy assay. Accordingly, they found that egress of PRV particles is not associated with regulated secretory Rab proteins, Rab3 and Rab27; rather, PRV particles use the constitutive secretory pathway, even in professional secretory cells that do possess regulated exocytosis mechanisms. These findings have important implications for the understanding of alphaherpesvirus spread in the nervous system: while both neurotransmission and viral spread follow synaptic circuits, these processes appear to be mediated by distinct molecular and cellular pathways. It remains to be determined whether the observed elevated firing rate of infected neurons affects the establishment of latency in subsequently infected post-synaptic neurons. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Princeton University (protocol number 1947). No human studies were performed.

Victor Hsia, *University of Maryland Eastern Shore*, discussed electrophysiology of HSV-1 infection of DRG neurons during acute and quiescent phases. Reactivation of alphaherpesvirus may cause excruciating pain and the molecular mechanisms are not clear. This lab hypothesized that the voltage-gated channels (VGC) on neurons controlling electrical impulses may have abnormal activity during viral infections. To investigate the hypothesis, this lab used an in vitro model ND7/23 hybrid cell line, which was generated by crossing mouse neuroblastoma (N18Tg2) with rat dorsal root ganglion neuron. The ND7/23 while differentiated exhibited robust sodium and calcium current and acute HSV-1 infection significantly abolished the activity within 2 days. During the quiescent state of infection achieved by acyclovir at 7 days postinfection, recovery of calcium but not the sodium VGC

activity was recorded. Additional preliminary studies showed that certain cytokine appeared to have effects rescuing calcium VGC activity lost due to infection. These observations displayed a very complex phenomenon of neuronal electrophysiology during herpesvirus infection and may have implication for understanding the mechanisms of virus-mediated pain. No animal or human studies were performed.

Keith R. Jerome, *Fred Hutchinson Cancer Research Center*, presented data showing efficient in vivo gene editing of latent HSV via adeno-associated virus (AAV)-mediated delivery of meganucleases or CRISPR/Cas9. HSV infections remain a common, serious problem associated with significant morbidity. After primary infection, HSV establishes latency in the peripheral nervous system. Latent virus is the source for viral reactivation and the recurrence of clinical disease and is not eliminated by current antiviral therapy. Despite much effort, a vaccine remains elusive. Therefore, there is a need for a novel therapeutic approach that would cure latent HSV infection. This group has evaluated a potentially curative strategy, in which HSV-targeted endonucleases induce mutagenesis of essential HSV genes, thus disabling the virus and rendering it incapable of replication or reactivation from latency. Using a well-established mouse model system, they demonstrated that AAV can efficiently deliver anti-HSV meganucleases or CRISPR/Cas9 to the trigeminal (TG) and superior cervical (SCG) ganglia of mice with latent HSV-1 infection. Gene editing efficiency approached 30% of HSV genomes in SCG for animals treated with meganucleases and nearly 20% in the corresponding TG. Droplet digital PCR analysis suggested that this difference in efficacy may relate to more efficient AAV delivery to SCG, along with lower HSV loads in SCG relative to TG. Interestingly, CRISPR/Cas9, while having very high activity in vitro (> 50% gene editing of latent HSV), showed little efficacy in vivo (gene editing < 1%). This lab ascribed this to the large size of the Cas9 coding sequence, which is close to the packaging limits of AAV and thus limits available optimizations; this contrasts with the very small meganucleases, which can be packaged into more efficiently expressing self-complementary variants of AAV. These results support continued efforts towards gene editing as an anti-HSV strategy, emphasize the importance of efficient delivery for in vivo gene editing applications, and should encourage workers in the field to appreciate the full diversity of targeted nucleases available beyond simply CRISPR/Cas9. 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), Assurance #A3226-01. No human studies were performed.

Clinton Jones, *Oklahoma State University*, discussed work performed with collaborators Fouad S. El-mayet and Laximan Sawant that described regulation of reactivation from latency by stress-induced factors. Bovine herpesvirus 1 (BoHV-1) establishes life-long latency in sensory neurons. The synthetic corticosteroid dexamethasone, which mimics the effects of stress, always induces reactivation from latency in calves and rabbits that are latently infected with BoHV-1. Recently, studies revealed that 102 genes associated with the Wnt/β-catenin signaling pathway are differentially expressed in TG during the latency-reactivation cycle in calves. Wnt agonists were generally expressed at higher levels during latency, but decreased during dexamethasone-induced reactivation. Previous studies also demonstrated Krüppel-like transcription factors (KLF), including KLF4, KLF6, KLF15, and PLZF are induced during reactivation from latency. Most sensory neurons that express bICP0 or VP16 during reactivation also express the glucocorticoid receptor (GR). Based on these observations, this lab hypothesized that cellular factors trigger lytic cycle viral gene expression during early stages of reactivation from latency. They now demonstrate that KLF4 or KLF15 synergistically cooperate with the GR to stimulate productive infection. The immediate early transcription unit 1 (IEtu1) promoter drives bICP0 and bICP4 expression and is synergistically stimulated by dexamethasone and KLF15 because it contains two functional GR response elements (GREs). The bICP0 gene also contains a separate early promoter that ensures bICP0 is expressed throughout productive infection. KLF4 or KLF15 cooperate with the GR to stimulate bICP0 early promoter activity in mouse neuroblastoma cells (Neuro-2A). Sequences necessary for trans-activation contained putative ½ GREs, KLF binding sites, and Sp1 binding sites; however, "whole" GREs with dyad symmetry were absent. KLF4 is a pioneer transcription factor that binds to silent chromatin and remodels chromatin, which may be essential for activating viral promoters during reactivation from latency. In summary, these studies demonstrated that KLF4, KLF15, and the GR have the potential to stimulate viral gene expression and productive infection following stressful stimuli. No animal or human studies were performed.

Dallas Jones, *University of Colorado School of Medicine*, presented data showing that VZV productively infects human PBMCs to modulate expression of immunoinhibitory proteins and blocking PD-L1 enhances virus-specific CD8+ T cell effector function. Varicella zoster virus (VZV) is a lymphotropic alphaherpesvirus that produces varicella on primary infection and causes zoster, vascular disease, and vision loss upon reactivation from latency. VZV-infected peripheral blood mononuclear cells (PBMCs) disseminate virus to distal organs to produce clinical disease. To assess immune evasion strategies elicited by VZV that may contribute to dissemination of infection, human PBMCs and VZV-specific CD8+ T cells (V-CD8+) were mock- or VZV-infected and analyzed for PD-1, PD-L1, PD-L2, CTLA-4, LAG-3, and TIM-3 expression using flow cytometry. All VZV-infected PBMCs (monocytes, B cells, NK, NKT, CD4+, and CD8+ T cells)

and V-CD8+ significantly inducted PD-L1 expression compared to uninfected cells. VZV induced PD-L2 expression in V-CD8+ and B cells, while only T lineage cells upregulated PD-1 expression. Monocytes had a bystander effect for PD-L1 induction and V-CD8+ had a bystander effect for both PD-L1 and PD-1. LAG-3/TIM-3 expression was not induced in all T cell populations; CTLA-4 expression was significantly induced in V-CD8+ and bystander V-CD8+. To test whether PD-L1, PD-L2, or CTLA-4 regulates V-CD8+ effector function, autologous PBMCs were VZV infected and co-cultured with V-CD8+ cell lines in the presence of blocking antibodies against PD-L1, PD-L2, or CTLA-4; ELISA analyses revealed significant elevations in IFN-γ only upon blocking of PD-L1. Together, these results identified 4 immune cells that are permissive to VZV infection (monocytes, B cells, NK, and NKT cells); along with a novel mechanism for inhibiting CD8+ T cell effector function through induction of PD-L1 expression. No animal or human studies were performed.

Srinivas P. Kalanghad, *New York University School of Medicine*, discussed defining the stepwise nature of HSV-1 reactivation. Using a cultured primary neuron model for HSV-1 latency, this lab has established that reactivation in response to physiological stresses proceeds via a novel two-step transcription program. The first step (termed phase I) is defined by generalized transcription of the viral gene repertoire and is likely independent of viral activators and viral DNA replication. Work by Anna Cliffe and colleagues showed that this is achieved by JNK-mediated phosphorylation of histones associated with the viral chromatin rendering the latent episome more permissive to transcription by RNA polymerase II. The second transcriptional wave (termed phase II) resembles the ordered cascade observed during acute (de novo) infection, coincides with the onset of viral DNA replication, and culminates in production of new infectious particles. Phase II is reliant upon viral activators such as VP16 and removal of heterochromatin-associated, repressive histone modifications. To understand why DNA replication appears limited to phase II, this group has analyzed viral transcription using a combination of RNA-seq and mRNA-specific RT-qPCRand has followed the synthesis and localization of selected components of the viral DNA replication machinery. By using immunofluorescence and cycloaddition chemistry to score phase I and phase II in individual neurons, this lab is gaining insights into the hitherto unknown population dynamics of reactivation in this model. These studies argue against a simple on/off binary switch that controls latency. These findings suggest instead that successful reactivation reflects progressive increases in viral gene expression that allow the virus to overcome multiple epigenetic barriers imposed by the host neuron. 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 160713-03). No human studies were performed.

David M. Knipe, *Harvard Medical School*, demonstrated that herpes simplex viral functions promote a poised latent infection. HSV-encoded functions promote the establishment of latent infection, but the silenced genome must be capable of reactivation upon proper stimuli to the neuron. The HSV LAT has been known to promote latent infection and also to promote reactivation, but this apparent paradox has not been resolved. This lab hypothesizes that LAT promotes facultative heterochromatin silencing of the viral genome in a form that allows reactivation of virus. They also found that ICP0 promotes LAT expression and facultative heterochromatin during latent infection. A third viral function, the CTRL2 CTCF binding site, serves as a chromatin insulator to keep heterochromatin away from the LAT promoter and enhancer and promotes reactivation. This group proposes that CTCF binding at CTRL2 brings the open LAT promoter near the VP16 and/or ICP0 promoter to allow them to be activated upon reactivation. Thus, viral functions promote epigenetic silencing of the HSV-1 genome by assembling facultative heterochromatin on viral lytic promoters in trigeminal ganglia, which results in a poised state that allows efficient reactivation. They are further testing these models of poised latent infection. They are also exploring possible ways to lock in the silenced genome, edit the viral genome to a nonfunctional state, and/or destabilize the latent genome. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Harvard University (protocol number 8600021). No human studies were performed.

David Koelle, *University of Washington*, presented the question of CD4 T cell responses to VZV: is gE special? Herpes zoster (HZ) incidence and severity increase with age and declining immunity. HZ prevention vaccines, including the recombinant gE zoster vaccine (RZV), boost CD4 T and B cell responses. The relative importance of gE and the AS01B adjuvant system to RZV clinical activity are unknown. This group's goal was to compare baseline CD4 memory responses between gE and other VZV proteins. VZV-reactive polyclonal CD4 T cell lines expanded from PBMC from 55 VZV subjects, ages 22–69, were screened with 71 VZV proteins. Immunoprevalent ORFs, including gE, were epitope-mapped. HLA restrictions were determined using defined artificial antigen-presenting cells or tetramers. Chemokine receptor and memory subset marker expression were assessed. At baseline, 44 VZV ORFs (62% of ORFs tests) were recognized by CD4 T cells from at least one subject. While CD4 T cell responses to ORF68 (gE, in RZV) and ORF67 (gI, forms heterodimers with gE) were prevalent (27% and 24% of subjects, respectively), responses to ORF9, ORF40, and ORF63 were also detected at $>20\%$. This lab

identified 149 CD4 epitopes and determined HLA restrictions for 125; about 90% were HLA-DR restricted. They compared several CD4 T cell parameters between gE and gI. Within persons with documented responses, up to 9 (median = 2, $n = 44$ persons) CD4 T cell epitopes were found for gE and up to 7 (median = 2, $n = 32$ persons) for gI. Predicted HLA-binding affinities were similar between gE ($n = 28$) and gI ($n = 23$) epitopes. Tetramer-specific CD4 T cells—typically ~ 10 cells/million PBMC per epitope—were predominantly CD45RA(-) and CXCR3hi, with bimodal expression of CXCR5 (consistent with circulating T-follicular helper cells) and CCR6, and did not differ between gE and gI. The similarity of responses to gE and gI suggests that clinical HZ prevention activity, if due to boosting of CD4 T cells, may not be unique to gE. 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 of Washington (protocol number STUDY00001399). No animal studies were performed.

Orkide Koyuncu, *Princeton University*, discussed the alphaherpesvirus switch: escape from silencing. Alphaherpesviruses are common pathogens of mammals. After initial infection of their hosts, their genomes reside as a life-long latent infection in the peripheral nervous system. Multiple times during latency, viral genomes can reactivate to start a disseminated productive infection. Approximately 70% of the adult population has latent alphaherpesvirus infections. Currently, there are no methods to clear viral genomes or latently infected neurons. Replication of alphaherpesviruses is well studied in cultured cells and molecular details of productive replication have been identified. However, questions remain concerning how a productive or a latent infection is established. Regulation of latency and reactivation is challenging to study because, available animal models do not precisely recapitulate the human latency-reactivation episodes; culturing neurons preserving their in situ architecture is difficult; while infections in vivo often result in latency, infections of dissociated neuronal cultures result in a productive infection unless viral replication is suppressed by drugs; and neuronal biology including metabolism, transport, and injury pathways that affect the viral life cycle are not well studied. Recently, this lab developed a reproducible and reactivateable in vitro latency system by infecting axons of compartmented neurons with a low concentration of pseudorabies virus (PRV) without the use of drugs. Since the outcome of infection is always silenced under these conditions, they studied mechanisms that enable escape from silencing. They investigated host and viral factors that prevent establishment of latency and promote productive replication of axonally delivered genomes. Activating neuronal protein kinase A (PKA) and cJun-N-terminal kinase (JNK) in the cell bodies prevented PRV genome silencing. Moreover, if viral tegument proteins are present in the cell bodies, silencing was overcome rapidly, independent of cellular PKA and JNK pathways. Identification of viral proteins and their cellular interaction partners promoting escape from silencing (or reactivation) will lead to novel therapeutics blocking alphaherpesvirus shedding and spread. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Princeton University (protocol number 1947-16). No human studies were performed.

Patrick Lomonte, *CNRS*, *University of Lyon*, discussed about the functional requirement of promyelocytic leukemia nuclear bodies (PML NBs) and histone H3.3 chaperones for the chromatinization of PML NB-associated latent HSV-1 genomes. HSV-1 latency establishment is tightly controlled by PML NBs, although their true implication is still elusive. A hallmark of HSV-1 latency is the interaction between latent viral genomes and PML NBs leading to the formation of viral DNA-containing PML NBs (vDCP-NBs). Using infected human primary fibroblasts reproducing the formation of vDCP-NBs, combined to an immuno-FISH approach developed to detect latent HSV-1, this lab showed that vDCP-NBs contain both histone H3.3 variant chaperone complexes, i.e., DAXX/ATRX and the HIRA complex (HIRA, UBN1, Cabin1, and Asf1a), and H3.3 itself. HIRA and H3.3 were also detected in vDCP-NBs present in trigeminal ganglia (TG) neurons from HSV-1 infected wild-type mice or infected cultured TG neurons issued from transgenic mice expressing a tagged H3.3 (eH3.3). ChIP-qPCR on eH3.3 or eH3.1-expressing infected human primary fibroblasts show that latent viral genomes are chromatinized exclusively with eH3.3 and not eH3.1. DAXX, ATRX, HIRA, and UBN1 were also found interacting with viral loci. Single inactivation of individual components of the H3.3 chaperone complexes significantly affects the formation of vDCP-NBs, but mildly impacts on the association of H3.3 with latent genomes. Only dual inactivation of proteins of either complex has a significant impact on latent HSV-1 chromatinization, suggesting a redundancy of activity between both chaperone complexes. Inactivation of PML by shRNAs significantly impacts on the chromatinization of the latent viral genomes with H3.3 without any overall replacement of H3.3 with H3.1. Similarly, infection of MEF cells from Pml KO mice leads to major decrease of H3.3 association with latent genomes. Consequently, the study demonstrates a specific epigenetic regulation of latent HSV-1 through an H3.3-dependent HSV-1 chromatinization involving the two H3.3 chaperones DAXX/ATRX and HIRA complexes. Additionally, the study reveals that PML NBs are major actors of the H3.3 chromatinization through a PML/histone H3.3/H3.3 chaperones axis. All animal studies were performed following guidelines and protocols

approved by the Institutional Animal Care and Use Committee of the Paris I University (protocol number 2012-0047) and in accordance with European Community Council Directive 2010/63/EU. No human studies were performed.

Maria A. Nagel, *University of Colorado School of Medicine*, discussed VZV-induced disruption of perineurial cells via IL-6: implications in neurotropic viral infections and peripheral nerve injury. VZV vasculopathy occurs after virus reactivates from ganglia, spreads along nerve fibers to arteries, and produces stroke. Temporal arteries from VZV vasculopathy patients contain virus in perineurial cells that form a protective barrier between peripheral nerves and surrounding tissue. This lab hypothesized that during VZV reactivation, VZV disrupts cell adhesion proteins in perineurial cells, potentiating infection of surrounding vascular cells. They mock- and VZV-infected primary human perineurial cells (HPNCs) and compared expression and distribution of cell adhesion proteins (claudin-1, E-cadherin, and N-cadherin) at 3 days post-infection. Results showed no VZV-induced changes in claudin-1 transcripts were seen; however, claudin-1 redistributed from the membrane/cytoplasm to the nucleus in VZV-infected cells compared to mock. Furthermore, while mock-infected cells expressed E-cadherin and not N-cadherin, VZV-infected cells did not express E-cadherin but expressed N-cadherin. These VZV-induced changes were confirmed in vivo in temporal arteries from VZV vasculopathy patients. Addition of conditioned media from VZV-infected cells to uninfected HPNCs revealed a soluble factor that was able to induce the same changes seen in VZV-infected cells, which was absent in conditioned media from mock-infected cells, and also caused an increase in cell migration. Since IL-6 was induced in VZV-infected cells and was a candidate soluble factor for inducing EMT, an anti-IL6 receptor antibody was used to pretreat cells before addition of conditioned media. Indeed, the anti-IL6 receptor antibody prevented VZV-induced alterations in cell adhesion proteins in HPNCs. Overall, this group's findings indicated that VZV-induced redistribution of claudin-1, downregulation of E-cadherin, and upregulation of N-cadherin was mediated by interleukin-6 and may lead to loss of perineurial cell barrier integrity, allowing viral spread from nerve fibers to surrounding vascular cells in VZV vasculopathy. Furthermore, the possibility is raised of a role for anti-IL6 receptor blockade in prevention of neurotropic virus entry and exit from peripheral nerves. No studies using animals, human subjects, or tissue samples were performed.

Vishwajit L. Nimgaonkar, *University of Pittsburgh School of Medicine*, explored HSV-1 exposure and age-related cognitive impairment: relevance for latent infections? Objectives stated that more than 12 studies have documented that persons exposed to HSV-1 (indexed by elevated titers of IgG antibodies in serum/plasma) have significant cognitive impairment, even in the absence of encephalitis. The associations are detectable among healthy individuals and people with psychiatric disorders. The patterns of cognitive impairment vary, but a minority of studies did not detect significant associations. Confounds related to age, which is correlated both with serostatus and cognitive function could explain the inconsistencies. This lab, therefore, combined all their published data and modeled for age-related variation in HSV-1 exposure. Methods included healthy individuals, patients with schizophrenia, and their relatives ($N = 3506$). None of the participants reported prior encephalitis. All participants completed the Penn computerized neurocognitive battery (CNB) for cognitive assessments, with minor differences. Generalized linear model to investigate the association between HSV-1 serostatus and CNB scores was initially used; significantly associated CNB variables were selected for a novel varying coefficient model analysis that included variables for age, age × HSV-1 serostatus interactions, and covariates (gender, affection status, education, and site). Results showed that the initial analysis detected significant associations between HSV-1 serostatus and 10/30 cognitive variables; all effect sizes were in the small to medium range. The study next analyzed these domains with the varying coefficient model. The accuracy indices of face memory, spatial ability (visual object learning), emotion recognition, and abstraction/mental flexibility were impaired in the age ranges of 20–30 years. Spatial ability and face memory (accuracy) were impaired in the 40–55 age range. Conclusions indicated that participants seropositive for HSV-1 have modest impairment in discrete cognitive domains when age-related interactions with HSV-1 serostatus are accounted for, even in the absence of prior encephalitis. The data are consistent with a hypothesis attributing the cognitive impairment to "micro-recurrences" of latent HSV-1 infection in the brain. All studies using human subjects were approved by the University of Pittsburgh IRB (approval nos: PRO14120059, PRO13110493, PRO11120013, PRO12020320, PRO09080146, IRB010218, IRB0309025) and Dr. RML Hospital ethics committee (No. 18-62/06-RMLH(HA-I)/IEC/Vol.II/74 date d16/02/2009, No. 28/2011/IEC/PGIMER/RMLH/11557, PGIMER-RMLH dated 16.11.12, No. 18-7/2005-RMLH(HA-I)/5487 dated 13/05/2005, No. 18-62/06-RMLH(HA-I)/IEC/Vol. II/80 date d16/02/2009, No. 18-10/2002-RMLH(HA-I)/5484 dated 13/05/2005, No. 1/2010/PGIMER-RMLH/EC/Vol. II/346 dated 20/08/2010). No animal studies were performed.

Werner Ouwendijk, *Erasmus Medical Center*, described a non-human primate model of *Varicellovirus* infection of the enteric nervous system. The pathogenesis of enteric zoster, a rare but serious complication of reactivation of latent VZV in the enteric nervous system (ENS), is largely unknown. Infection of monkeys with the closely related *Varicellovirus* SVV mimics VZV disease in humans. Herein, this lab determined the applicability of the SVV model to study

Varicellovirus infection of the ENS. Viral DNA was detected by virus-specific quantitative real-time PCR in intestinal tissues of latently VZV-infected humans and SVV-infected monkeys. In situ analyses showed that enteric neurons expressed SVV-specific RNA but not protein, suggesting establishment of viral latency of the ENS in SVV-infected monkeys. Virus-infected T cells were detected in gut-draining mesenteric lymph nodes and localized near enteric nerves in the gut during acute SVV infection. Furthermore, flow cytometric analysis of blood from acutely SVV-infected monkeys demonstrated that virus-infected T cells expressed the gut-homing receptor $\alpha 4\beta 7$ integrin. Collectively, the data demonstrate that SVV infects and potentially established latency in ENS neurons and supports the role of T cell to transport the virus to the gut. Because SVV reactivation can be experimentally induced, this model holds great potential to study enteric zoster. All animal studies were performed in compliance with European guidelines (EU Directive on Animal Testing 86/609/EEC), Dutch legislation (Experiments on Animals Act, 1997), and the protocol was approved by the independent animal experimentation ethical review committee DCC (27 June 2011, Erasmus Medical Center permit number EMC2374); all studies using human subjects or tissue samples have been either approved or deemed non-human subject research by the Institutional Review Board of Erasmus Medical Center (protocol number MEC-2017-009).

Tao Peng, *University of Washington*, IL-17c, discussed the peripheral nervous system and herpes infection. The mammalian peripheral nervous system (PNS) has significant capacity to regenerate, which is largely dictated by the interactions between peripheral neurons and their targets. HSV is an alphaherpesvirus that establishes latency in sensory ganglia, reactivates frequently in peripheral skin, and is rarely associated with sensory neuropathy; an intriguing question is how PNS maintains their function in spite of chronic reactivation? This lab's recent studies, using human genital skin biopsies from recurrent HSV-2 infection, suggest that IL-17c, an epithelial derived cytokine mediates interaction of epidermal keratinocytes, reactivating viruses and PNS, and promotes peripheral nerve growth in humans. Skin biopsies obtained during asymptomatic human HSV-2 reactivation exhibit a higher density of nerve fibers relative to biopsies during virological and clinical quiescence. Keratinocytes, the initial cells of HSV replication, produce IL-17c during HSV-2 reactivation and IL-17RE, a receptor subunit specifically for IL-17c signaling, is expressed on nerve fibers in human skin and sensory neurons in dorsal root ganglia (DRG). Exogenous human IL-17c provides directional guidance and promotes neurite growth and branching of primary DRG neurons in microfluidic devices. This lab's recent studies using engineered mice over-expressing IL-17c specifically in skin show that IL-17c induced transcriptional profiles to stimulate peripheral nerve growth in skin. In summary, these studies suggest that IL-17c is a neurotrophic cytokine for PNS in humans and mice, and understanding IL-17c pathways may provide novel approaches for treating sensory peripheral neuropathies. All studies using human subjects or tissue samples have been either approved or deemed non-human subject research by the Institutional Review Board of University of Washington (protocol number is STUDY00002443). No animal studies were performed.

Ana Pombo, *Max Delbrüeck Center for Molecular Medicine*, presented the second Don Gilden Memorial Lecture, discussing RNA polymerase II: at the crossroads of gene regulation and genome architecture. RNA polymerase II transcribes protein coding genes into messenger RNAs, and its action occurs with the sequential phosphorylation of serine-5, serine-7, and serine-2 residues on the C-terminal domain, marking transcription initiation, the transition to elongation, and finally elongation, respectively. Polycomb repression in mouse embryonic stem cells (ESCs) is tightly associated with promoter co-occupancy of RNA polymerase II (RNAPII) which is thought to poise genes for activation during early development. However, it is unknown whether RNAPII poising is lost, or a general feature of Polycomb repression during differentiation. This lab has mapped the genome-wide occupancy of RNAPII and Polycomb in pluripotent ESCs, neuronal precursors, and non-dividing functional dopaminergic neurons and has found that poised RNAPII complexes are ubiquitously present at Polycomb-repressed genes. They observed both loss and acquisition of RNAPII and Polycomb at specific groups of genes reflecting their silencing or activation in particular cell types. Strikingly, RNAPII remains poised at transcription factor genes which are silenced in neurons through Polycomb repression and have major roles in specifying other lineages. These findings conclude that RNAPII poising is intrinsically associated with Polycomb repression throughout differentiation and suggests that the tight interplay between RNAPII poising and Polycomb repression not only instructs promoter state transitions but also may enable promoter plasticity in differentiated cells. No animal or human studies were performed.

Barry T. Rouse, *University of Tennessee*, presented pathogenesis of herpes encephalitis-perhaps! Viruses rarely enter the brain but when they do, the effects can be devastating. An example is herpes simplex encephalitis (HSE), a rare disease in adults usually caused by HSV-1 occurring most commonly in persons already latently infected with the virus. Although some cases of HSE occur in persons with genetic problems of the immune system or are heavily immunosuppressed, most affected persons have no apparent problem with their immune systems. An explanation why HSE occurs in such persons is still needed. This lab hypothesize that HSE occurs during a coalescence of events, which included viral reactivation from latency at a time when the immune system is being compromised, perhaps temporarily, by metabolic

changes. These ideas cannot be tested in humans but this lab has established a mouse model in which events leading up to HSE can be evaluated. Thus, this group could show in a mouse model that inhibition of glucose metabolism with the molecule 2-deoxyglucose from the time of local infection with HSV-1 resulted in the majority of animals developing HSE. They anticipate that the 2DG therapy could impair two stages of immunity, which normally protect against infection of the central nervous system. The first is proposed to be a blunting of the function of an innate immune component at the infection site or within the local nerve ganglion. This prevents innate cells from functioning sufficiently to limit the extent of local viral replication and to minimize productive infection of neurons in the local ganglion. The second component is proposed to be an effect on the expansion or effector function of CD8 T cells which in normal circumstances protect neurons and prevent virus from spreading to the brain. Preliminary data will be presented which support these ideas. They also present data indicating that changing the metabolic environment of the latently infected TG influences the speed and efficiency of viral reactivation. They anticipate that their findings could result in changes in diagnostic procedures and therapeutic management of HSE. Thus, in addition to the currently used antiviral drug treatment any detected metabolic abnormalities could be corrected as well, with the combination therapy minimizing the consequences of the HSE syndrome. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Tennessee University (protocol number 1244); 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 of Tennessee (protocol number 1244).

Nancy M. Sawtell, *Cincinnati Children's Hospital Medical Center*, reviewed HSV reactivation and the mechanism of neuronal destruction. A fundamental question in HSV pathogenesis is the consequence of viral reactivation to the neuron. Evidence supporting both post-event survival and demise is published. Here, findings from an in vivo temporal analysis of the resolution and potential role of T cells and cytokines in this process are presented. Induced in vivo reactivation in the trigeminal ganglia is defined spatially and temporally, restricted to 2–3 neurons, and resolved in the ganglia within 48 h post-induction. The infectious virus is contained and inflammatory cells surround the reactivating neuron. Capturing the morphology of reactivating neurons during this time frame revealed fragmented HSV protein-positive neurons. This dramatic phenotype increased with time post-reactivation. The fragmentation and detection of cleaved caspase-3 in fragmented bodies strongly supports the hypothesis that neurons are destroyed post-reactivation. This observation led this lab to reexamine the previously reported non-cytolytic role of T cells in regulating HSV reactivation. Latently infected mice were treated with anti-CD4 and anti-CD8 depleting antibodies. Intriguingly, no phenotypic differences between control and depleted mice at 48 h post-induction were observed. Conventional depletion treatments have been reported to incompletely eliminate tissue resident immune populations, so it remained possible that tissue resident T cells were mediating the response to reactivation. To test this, mice were treated with neutralizing antibodies against inflammatory signaling cytokines IFN-γ, TNFα, and IL-2Rβ, prior to induced reactivation. In contrast to T cell depletion, fragmented neurons were not detected and focalized inflammation was not observed around HSV-positive neurons in cytokine neutralized mice at 48 h post-induction. These data demonstrated that there is neuronal destruction post-reactivation that is mediated by inflammatory cytokines in the ganglion. We further refined our strategy for T cell depletion, achieving well-documented near-complete depletion of tissue resident T cells but surprisingly, again, T cell depletion had no effect on the resolution of reactrivation at 48 h post-induction. Together, our findings indicate that the resolution of reactivation occurs through a delayed apoptosis of individual reactivating neurons which are cleared by Iba1 expressing microglia. Inflammatory cytokines but not specific CD4 and CD8 resident T cells directly orchestrate this event. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Cincinnati Children's Hospital Medical Center (protocol number 2017-0081). No studies using human subjects or tissue samples were performed.

Joshua Schiffer, *Fred Hutchinson Cancer Research Center*, presented data on the bottlenecks to intra-host evolution of HSV-2. Viral evolution is a central feature of several untreated viral infections in humans, including HIV. Continual generation and fixation of new mutations in persons with untreated HIV reflects the high error rate of the HIV reverse transcriptase replication enzyme as well as continual positive selection pressure from the acquired immune response. It is less certain whether human herpesviruses such as HSV-2 evolve over time within infected persons: the viral DNA polymerase is less error prone relative to HIV reverse transcriptase and whole body viral population sizes of HSV-2 are considerably lower than those of HIV. Moreover, viral latency maintains chronic HSV-2 infection in the absence of therapy, rather than uninterrupted viral replication and cell-to-cell transmission. In the majority of HSV-2 infected persons, intense tissue resident immune responses eliminate lytic viral replication in the human genital tract, including any newly mutated strains that may emerge in this environment. Using mathematical models of chronic untreated infection, this lab demonstrated that the latent, lytic cycle represents the most critical bottleneck to intra-host evolution of HSV-2. Their model predicts that re-seeding of the latently infected ganglia throughout the lifetime of an infected person is a prerequisite

for permanent accrual and reactivation of strains with new mutations. Based on the random reactivation pattern of HSV-2, new mutations may accrue in strains which were archived in the ganglia many years prior to contemporaneous sampling. Even if new mutations occur during every high viral load reactivation event in the genital tract, the slow rate of mutation occurring within multiple archived strains is therefore likely to generate a phylodynamic signal of weak positive selection. To avoid underestimation of the true impact of the acquired immune response on strain evolution within a single infected host, autopsy studies involving deep sequencing of latently infected ganglia are required. No animal or human studies were performed.

Mackinzie Shipley, *Pennsylvania State University*, discussed the genome-wide surveillance of genital HSV-1 from multiple anatomic sites over time. It is not yet known to what degree the viral population in a naïve host adapts over time and through multiple cycles of latency and reactivation. This lab presented genomic and in vitro analyses of temporally-separated HSV-1 shedding from a participant who suffers from frequent recurrences of genital HSV-1. Using a targeted oligonucleotide enrichment strategy, they compared viral genomes obtained from uncultured swabs collected on different days and from distinct genital sites. These swabs were compared to three viral cultures spanning a 4-month period. The uncultured and cultured viral genomes were found to share a high level of genomic identity at the consensus level. Deep genome sequencing enables the examination of minority variants in viral populations, and they found over a 2-fold difference in the number of minority variants between lesion, non-lesion sites, and cultured viral isolates. The distribution of minority variants suggested the potential for both a bottleneck and subsequent drift in the viral population in cell culture. When comparing this participant's virus to known HSV-1 genomes, they found that the virus had a unique phylogeny, novel coding variations in 21 viral proteins, and a truncation in the UL11 viral tegument protein. Normal T cell and antibody responses were identified in the participant, suggesting that unique viral genomic features may contribute to the high recurrence rate of this genital infection. These data revealed that viral diversity exists over a short time frame within a person, albeit on the level of minority variants, rather than consensus-level coding changes. Future studies will be targeted to examine the viral population in settings where a greater degree of viral population flux is expected, such as during transmission or over the first year of infection in a new host. All studies using human subjects have been approved by The University of Washington Human Subjects Division, and the participant provided informed consent. No animal studies were performed.

Padma Srikanth, *Sri Ramachandra Medical College and Research Institute*, reviewed the manifold manifestations of VZV. VZV an alphaherpesvirus DNA virus is known to establish latency in cranial nerve root, dorsal nerve ganglia, and enteric neurons. VZV reaches ENS through infected lymphocytes and axonal transport via dorsal root ganglia and become latent in neurons of enteric system. Reactivation and relapse are known to occur. This lab presented spectrum of clinical disorders associated with VZV. Gastro intestinal tract: nine biopsy samples from the colon (eight IBD and one with carcinoma of the rectum) were tested by conventional PCR for VZV targeting ORF 8. Of these four colon biopsy samples tested positive [three (37%) IBD and one CA rectum]. In addition, nine healthy colon/gastric tissue samples were tested for VZV by PCR and found to be negative. Indication for colonoscopy in these patients was for reasons other than IBD. Central nervous system: VZV was detected in two CSF samples, one in an 8-year-old child with overt clinical varicella and in the other a 68-year-old adult with herpes ophthalmicus which later progressed to varicella meningitis. Kidney: 6 months following post renal transplant, two recipients developed VZV-induced graft dysfunction one with clinical varicella and the other without evidence of clinical varicella. Response to therapy: all patients with PCR evidence of VZV were treated with intravenous acyclovir and responded well. In patients with IBD detection of VZV also resulted in alteration in treatment protocol, i.e., dosage of immunosuppression was reduced, e.g., corticosteroids/thiopurines/anti-TNF agents. Sequencing: after conventional PCR, the product was sequenced by Sanger sequencing targeting ORF 8 (275 bp). Bioedit V.7.09 and BLAST (https://blast.ncbi.nlm.nih.gov/) was used for sequence analysis. All sequences have been confirmed to be human herpesvirus 3. It was the conclusion of this lab that detection of VZV in IBD patients alters protocols for therapy. Against the backdrop of immunosuppression PRTR's are more vulnerable to varicella infection. 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 deemed to be University, Porur, Chennai, India. No animal studies were performed.

Moriah Szpara, *Pennsylvania State University*, discussed the genomic analysis of HSV-1 in the niche of genital infection. HSV-1 and HSV-2 cause millions of chronic infections. The epidemiology of genital herpes has undergone a significant transformation over the past two decades, with the emergence of HSV-1 as a leading cause of first-episode genital herpes. This shift raises the possibility that HSV-1 may face new selective pressures and undergo genetic adaptation as it moves into the genital niche. In collaboration with Christine Johnston at the University of Washington, the lab is conducting a genome-wide analysis of HSV-1 variation, to observe how the virus adapts to the human genital niche during the first year of primary infection and to discern how the virus is affected by the laboratory culture required for in vitro

studies. Insights on viral genetic variation are obtained using comparative genomic analyses of longitudinal HSV-1 shedding swabs, which are part of an ongoing clinical study. This group has collected hundreds of swabs from over 50 study participants during their first year of infection. Approximately half of these participants have had one or more viral swabs successfully captured in culture. Thus far, this lab has examined the viral genome population in two individuals, one of whom experienced a highly recurrent pattern of genital HSV-1 shedding, while the other did not. Both participants have been sampled over a period of several months—spanning latency and reactivation in vivo—via oligo-enrichment and deep sequencing of viral swabs and parallel viral cultures. The data revealed a high level of viral genomic identity within-patient, over time. Early data also suggests the potential for a bottleneck and subsequent genetic drift upon viral introduction into cell culture. Based on these pilot data, the next challenges will be to observe how the viral population shifts during transmission between individuals and throughout the first year of infection, when an individual's innate and adaptive immune responses to HSV-1 are first developing. The University of Washington Human Subjects Division approved the clinical study and sample repository (IRB# 46579 and IRB# 23803), and the participants provided informed consent. Viral genomic analyses were designated non-human research by the Pennsylvania State University (PSU) Office for Research Protection. No animal studies were performed.

Matt Taylor, *Montana State University*, discussed quantifying HSV-1 recombination through differential fluorescent protein expression. Intergenomic recombination mediates mutation and diversification of HSV-1. Recombination requires multiple viral genomes present in the same cell, which can occur either during simultaneous co-infection or following superinfection of latently infected neurons. Research efforts to assess HSV-1 recombination are often complicated by the use of attenuating mutations that differentiate viral progeny but unduly influence the outcome of neuronal or animal models of infection. To circumvent the reduced viral replication and spread associated with marked viral genomes, this lab has developed an assay based around fluorescent protein (FP) expression cassettes. They isolated viruses harboring either a cyan or yellow FP expression cassettes inserted into the US or the UL regions of the HSV-1 genome, respectively. These FP cassettes allowed the rapid discrimination of parental and recombinant genomes based the fluorescence profile of viral plaques. The FP expression cassettes had limited effect on viral replication and production of progeny virions. Co-infection of the two viruses resulted in recombinant progeny dependent on MOI and independent of the time post-infection, at a rate similar to previous reports. Sequential passage of mixed viral populations revealed limited change in the distribution of parental and recombinant progeny, supporting the conclusion that the FP cassettes were not unduly influencing recombinant populations. This group then implemented this two virus co-infection experiment in cultures of neurons or mouse model of neuroinvasion to analyze recombination during intercellular spread. Interestingly, the resulting distributions of viral progeny revealed large, seemingly random shifts in parental and recombinant distributions. Together these results highlight the utility and potential for neutrally marked viruses to measure HSV-1 recombination. Importantly, this lab can now go on to identify whether only actively replicating genomes or latent genomes are accessible to recombination during co-infection or superinfection in a host. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of Montana State University (protocol number 2016-31 and 2017-09). No human studies were performed.

Richard Thompson, *University of Cincinnati*, presented data on HSV-2 VP16 regulation and viral pathogenesis. The US and global disease burden resulting from HSV infection includes life-threatening encephalitis, blindness, devastating neonatal infection, increased risk of HIV infection, neurological disease, and a list of other disease outcomes. Life-long latent infections in sensory neurons and periodic reactivation of latent virus are central to the maintenance and propagation of these viruses. This lab sought to capitalize on their recent novel insights into when and how the latent/lytic balance is set and a pivotal role for VP16 and its regulation in HSV-1, by asking if the HSV-2 VP16 promoter regulate these properties as well? Evolving from the same primordial simplex virus, HSV-1 (6 million years (ma) in the human lineage) and HSV-2 (4.5 million years in the chimpanzee lineage (ChHV) and then 1.5 ma in the human line) share ~ 85% AA identity in the VP16 ORF, but their promoter sequences are remarkably different. Importantly, whether the differences between these promoters regulate their differing biological properties such as the enhanced neuroinvasiveness, more frequent reactivation from latency, and the ability to infect individuals previously infected with HSV-1 displayed by HSV-2. Testing of a mutant and genomically restored pair in which the VP16p1 in strain 17syn+ was replaced with VP16p2 of HSV-2 revealed increased entry into lytic infection in trigeminal ganglion (TG) neurons very early pi, prolonged viral protein expression in multiple neurons at day 16 pi (persistence and/or spontaneous reactivation), and increased virulence. These findings suggest the VP16 promoter has a major impact on the pathobiology of HSV and differs significantly between the two serotypes. All animal studies were performed following guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Cincinnati Children's Hospital Medical Center IACUC #2013-0162. No human studies were performed.

Abel Viejo-Borbolla, *Hannover Medical School*, reviewed the attempts to establish latency models for VZV using human peripheral neurons. VZV is a highly prevalent human pathogen that establishes latency in peripheral neurons. The long-term objective of this project is to discover the virus and host

factors responsible for VZV latency and reactivation. VZV is highly host-specific and therefore the use of human neurons is required to study these processes. Thus, this lab derived human neurons from inducible pluripotent stem cells. Human neurons are highly heterogeneous in vivo differing in expression pattern and activity and this may influence VZV biology. Therefore, the lab performed a thorough characterization of the derived neurons by RNA-Seq, RT-qPCR, immunofluorescence, HPLC, calcium imaging, and patch clamp to determine the neuronal subtype. These neurons have characteristics of low threshold mechanoreceptors found in human dorsal root ganglia. They were infected with two VZV strains in the presence of acyclovir, type I interferon (IFN), or a combination of both and measured viral gene expression and spread. A phenotype reminiscent of latency was achieved when infecting with a bacterial artificial chromosome-derived POka strain in the presence of acyclovir or IFN. However, the low-passage clinical isolate EMC-1 replicated upon withdrawal of acyclovir or IFN, indicating that these compounds did not repress this virus. However, EMC-1 infection in combination of both acyclovir and IFN resulted in very low viral gene expression, maintenance of viral genome and lack of spread during more than 40 days after removal of the compounds. Reactivation was not achieved by classical methods such as inhibition of phosphoinositide 3 kinase or removal of growth factors. However, EMC-1 infection was not abortive since spontaneous reactivation occurred at about 50 days post-infection. Results indicate that VZV quiescent infection and reactivation can be achieved through different pathways, permitting the investigation of efficient mechanisms to block VZV reactivation and spread. No animal or human studies were performed.

Anna Wald, *University of Washington*, presented data from a study led by Dr. Christine Johnston, describing genital and oral mucosal shedding and cellular immune response after first-episode genital HSV-1 infection. HSV-1 has emerged as the predominant pathogen in first-episode genital herpes, with subsequent recurrences and viral shedding less frequent than in genital HSV-2 infection. This lab hypothesized that understanding the relationship between mucosal shedding patterns and the development of the cellular immune response following genital HSV-1 acquisition may clarify whether the reduced shedding reflects virus-specific latency attributes versus a potent immune response that successfully prevents mucosal replication of HSV-1. Following laboratory-documented first-episode genital HSV-1 infection, participants obtained self-collected genital and oral swabs for two 30-day sessions at 2 months and 11 months post-infection. HSV was detected using quantitative HSV PCR. CD4 and CD8 T cell responses were measured by IFN-γ ELISpot. Forty-three persons with documented first-episode genital HSV-1 acquisition completed the follow-up. Genital HSV-1 shedding was detected in 173 (15.1%) of 1149 swabs from the first shedding session and declined to 62 (5.7%) of 1087 swabs in the second session. The frequency of genital shedding in the second session was 62% lower than the first session (RR = 0.38, 95% CI = 0.17–0.84, $p = 0.018$). Genital lesions were detected on 2.6% of days in the first session and 2.5% of days in the second session ($p = 0.74$). HSV-1-specific CD4 and CD8 T cell responses were detected as early as 2 weeks post first-episode HSV-1. Polyfunctional HSV-1-specific CD4 cells were detected throughout the first year of infection at stable levels. A median of one HSV-1-specific CD8 epitope was detected per person using a limited set of curated CD8 epitopes. The conclusions presented was that genital HSV-1 shedding frequency declines substantially in the first year after genital HSV-1 acquisition. HSV-1-specific T cells are detectable up to 1 year post-infection, despite low recurrence rates. Further analyses from this lab will address the correlation of viral shedding and the immune response. This protocol was approved by the Institutional Review Board at University of Washington (protocol number is STUDY00001465). No animal studies were performed.

Benjamin E. Warner, *University of Pittsburgh*, discussed the essential gene mutations in VZV without the need for complementation. VZV causes herpes zoster (shingles) upon reactivation, which is often followed by considerable pain that may become chronic and debilitating (post-herpetic neuralgia, PHN). This lab has been exploiting a rat model of pain where VZV inoculation in the foot or whisker pad induces behaviors that indicate mechanical hypersensitivity. Their results suggest pain behaviors develop without complete VZV replication. Rather, an abortive infection with limited gene expression may be sufficient for a hypersensitive state in the rat. This may reflect events in VZV reactivated human neurons that survive lytic reactivation, but continue to signal pain. This group reasons that VZV mutants lacking certain essential genes will reveal the requirements for VZV-induced pain. VZV with essential gene deletions have been difficult to create due to obstacles in developing complementing cell lines. Here, they have developed conditionally replicating VZV in which the essential DNA replication protein ORF29 or tegument protein ORF9 are fused to a degron motif taken from *E. coli* dihydrofolate reductase (DHFR). This motif allows for targeted, conditional protein degradation. In the presence of cell-permeable antibiotic trimethoprim (TMP), fused proteins are stable and functional. In the absence of TMP, proteins are rapidly degraded through the ubiquitin-proteasome pathway. The efficiency of this system is gene dependent, but has enabled us to block VZV infections completely at the DNA replication or assembly stage of VZV infection. This confirms that both proteins are critical for VZV and provides a method to examine the role of complete VZV replication in the rat pain model. This group is now using the system to genetically analyze ORF9 by ectopic expression of a second copy of the gene with otherwise detrimental mutations. All animal studies were performed following guidelines and protocols approved

by the Institutional Animal Care and Use Committee of the University of Pittsburgh (protocol number: 18022168). No studies using human subjects or human tissue samples were performed.

Angus C. Wilson, *New York University School of Medicine*, summarized progress towards a general model of HSV-1 reactivation. Latency in peripheral ganglia is defined by (a) restricted viral gene expression and (b) the absence of viral DNA replication. These are faithfully recapitulated in an ex vivo infection model using sympathetic neurons from rat superior cervical ganglia. Using this and similar models, seemingly unrelated reactivation cues have been shown to act through major signal integrators Akt and mTORC1 to regulate cap-dependent mRNA translation and maintain latency. Altering these signals using inhibitors or RNAi results in reactivation and is best explained by a two-step model that begins with a generalized burst of viral transcription (phase I) initiated by the JNK stress-activated kinases. Viral effector proteins produced in phase I, including ICP0 and VP16 help to overcome host epigenetic suppression, antagonize innate host defenses, and drive an ordered transcriptional cascade (phase II) leading to infectious virus production. By investigating viral and host transcription during phase I at both the population and single cell level, this lab found that the majority of viral genes are transcribed, consistent with activation of most or all viral promoters. Measuring reactivation in individual neurons using single cell RNA-seq and cycloaddition chemistry, while challenging, has potential to uncover physiological states or neuronal subtypes that influence the decision to trigger phase I or progression into phase II. Although genes encoding viral replication proteins are transcribed in phase I, viral DNA amplification is restricted to phase II. Little is known about HSV-1 genome replication in post-mitotic neurons or during reactivation. His group has begun quantifying the expression and sub-neuronal localization of viral replication factors and are developing methods to characterize neurons that support viral DNA replication. It is hoped these multidisciplinary studies will allow them to build a detailed, mechanistic model for how different physiological stresses reanimate latent HSV-1 genomes, culminating in the production of new infectious virus. 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 160713-03). No human studies were performed.

Qiaojuan Zhang, *University of Maryland Eastern Shore*, discussed the regulation of T-type Ca^{2+} channel expression by interleukin-6 in sensory-like ND7/23 neurons post HSV-1 infection. HSV-1 infection of sensory neurons results in a significant reduction in the expression of voltage-activated Na^{+} and Ca^{2+} channels, which may disrupt the transmission of pain information following viral infection and latency. Viral infection or reactivation from latency may also results in the secretion of various pro-inflammatory cytokines including IL-6. The role of IL-6 in regulating the electrical activity and ion channel expression post HSV-1 infection has not been investigated. This lab hypothesized that IL-6 regulates the expression of Na^{+} and Ca^{2+} channels post HSV-1 infection in ND7/23 sensory-like neurons. Their results demonstrate that HSV-1 acute infection causes a significant reduced protein expression of the Cav3.2 T-type Ca^{2+} channel subunit but the Cav3.2 mRNA expression was increased by HSV-1 infection. Neither Cav3.2 mRNA nor protein expression was affected by IL-6 treatment post HSV-1 infection. In ND7/23 cells, HSV-1 infection caused a significant reduction in the expression of Na^{+} and T-type Ca^{2+} channels within 48 h. Similarly, in HEK cells stable transected with the T-type Ca^{2+} channel subunit Cav3.2, HSV-1 infection caused a significant reduction in the expression of T-type Ca^{2+} currents. Exposure of ND7/23 cells to IL-6 for 24 h post-infection reverses the effect of HSV-1, resulting in a significant increase in T-type Ca^{2+} current density. However, the reduction of Na^{+} currents by HSV-1 infection of ND7/23 cells was not reversed by 24 h treatment with IL-6. The ability of IL-6 to increase the functional expression of T-type Ca^{2+} channels on the membrane was blocked by inhibition of protein trafficking with brefelding-A. These results indicate that IL-6 release following HSV infection regulates the electrical properties of sensory neurons, which may alter the transmission of pain information. No animal or human studies were performed.

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Compliance with ethical standards

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