2015 Colorado Alphaherpesvirus Latency Society Symposium

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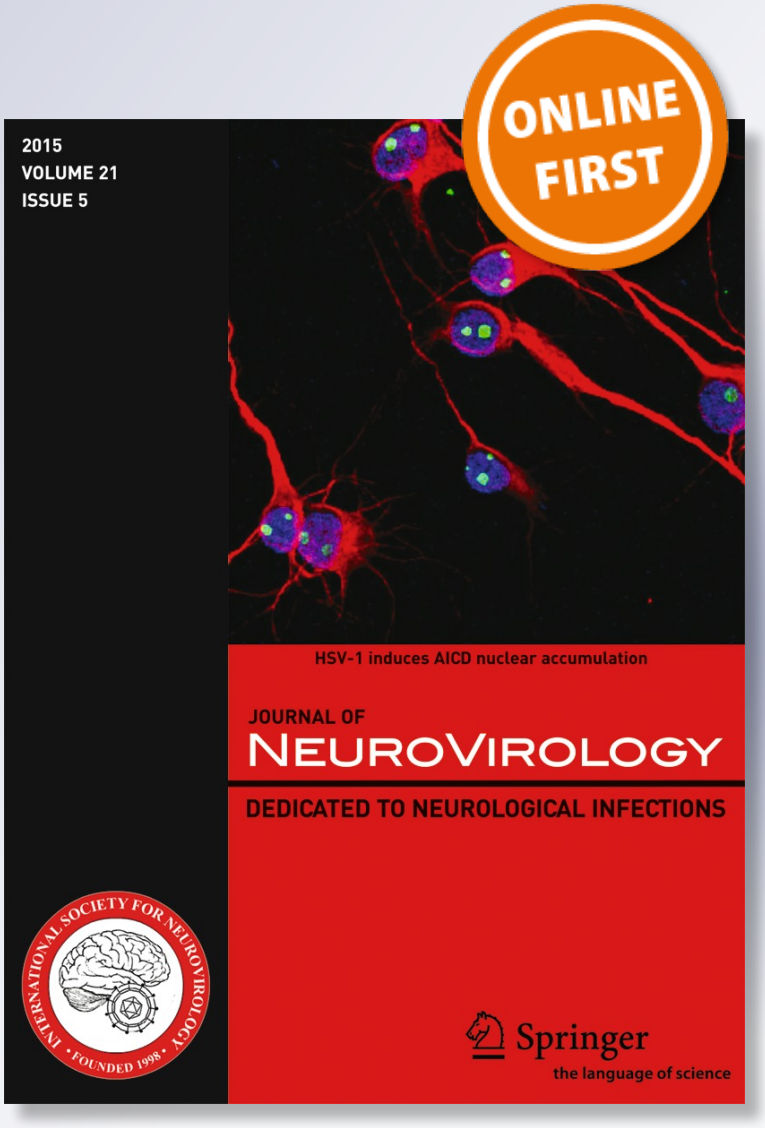
Journal of NeuroVirology

ISSN 1355-0284

J. Neurovirol.
DOI 10.1007/s13365-015-0396-1



Springer

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DOI 10.1007/s13365-015-0396-1

MEETING REPORT

2015 Colorado Alphaherpesvirus Latency Society Symposium

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Received: 7 October 2015 / Accepted: 12 October 2015

Introduction

The fifth Colorado Alphaherpesvirus Latency Society (CALS) Symposium from May 14–16, 2015 brought together 63 investigators (Fig. 1), who have authored over 2945 PubMed-listed publications and who traveled 118,225 miles to discuss current advances in the field of alphaherpesvirus latency in the relaxed setting of The Christiania Lodge in Vail, Colorado. The 2-day symposium consisted of 29 oral and 9 poster presentations concerning latency of herpes simplex virus 1 and 2, varicella zoster virus, bovine herpesvirus, and simian varicella and herpes B virus. In keeping with the theme of inviting speakers outside the field of virology to help view latency from a different perspective, George Smith from the Temple University provided the plenary talk describing neurotrophin-mediated regeneration of sensory afferents after dorsal root rhizotomy. Back by popular demand was an evening fireside open forum to discuss topics not addressed in formal presentations. CALS remains a joint venture wherein individuals from around the world unite with the common goal of eradicating disease produced by alphaherpesvirus reactivation through understanding the molecular mechanisms underlying establishment, maintenance, and reactivation from latency. A brief summary of each presentation follows.

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Leonardo D'Aiuto, *University of Pittsburgh*, described the antiviral effects of Amaryllidacea alkaloid analogs. Type 1 herpes simplex virus (HSV-1) and varicella zoster virus (VZV) are ubiquitous herpes DNA viruses that cause substantial human morbidity and mortality due to uncontrollable cycles of lytic and latent intracellular infections. Common treatments include nucleoside analogs, of which acyclovir is the current standard. However, acyclovir resistance in immunocompromised patients and acyclovir-induced neurotoxicity in patients with renal failure have been reported, pointing to the need for better anti-herpetic drugs. Dr. D'Aiuto described the production of alkaloid trans-dihydrolycoricidine 7 and 4 other C3-analogs 8–10 via total asymmetric synthesis, as well as the effect of these compounds on HSV-1 infection in Vero cells and human induced pluripotent stem cell (iPSC)-derived neurons. A configurationally defined secondary alcohol at C3 was found to be crucial for inhibition of lytic HSV-1 infection. Additionally, compound 7 significantly reduced HSV-1 reactivation from latency and inhibited VZV infection, features superior to those of acyclovir. Overall, the studies demonstrated a relationship between the structure of lycorane-type alkaloids and anti-herpes virus activity. The novel synthetic method and the iPSC neuron-based platform can be adapted to screen drugs for other neurotropic virus infections.

Daniel Depledge, *University College London*, presented a molecular analysis of virus and host factors involved in VZV latency and reactivation in human ganglia. The host and viral mechanisms that maintain latency and/or trigger reactivation of VZV remain unknown, mostly due to the lack of an effective model. While the use of tissue explants and neuronal cultures has shown some promise in studying VZV latency, samples of human trigeminal ganglia (TG) removed shortly after death and split for ChIP assays and nucleic acid extraction can provide snapshots of the viral and host transcriptome and enable characterization of the epigenetic modifications

Published online: 27 October 2015

Fig. 1 Participants of the 2015 Colorado Alphaherpesvirus Latency Symposium. (*left to right*, *row 1*) Paul Kinchington, Thomas J. Goodwin, Randy Cohrs; (*row 2*) Todd Margolis, David Koelle, Lynn Enquist, Ken Jones, Jason Chen, Maria Nagel, Andrea Bertke, Michael Gershon, Nancy Sawtell, Anne Gershon, Martine Aubert, Esteban Engel, Georges Verjans, George Smith, Hua Zhu, Vicki Traina-Dorge; (*row 3*) Dan Carr, Ilhem Messaoudi, Ravi Mahalingam, David Bloom, Angus Wilson, Adit Dhummakupt, Seth Frietze, Julia LeCher, Julia Hilliard, Leigh Zerboni, Richard Thompson, Keith Jerome, Marielle Lebrun, Moriah Szpara; (*row 4*) Chandra Kroll, Patrick Stuart, Pamela Roehm, Charles Grose, Daniel Depledge, Peter Kennedy, Judith Breuer, Donna Neumann, Victor Hsia, Edouard Cantin, Patrick Lomonte; (*row 5*) William Halford, Deepak Shukla, Scott Schmid, Klaus Osterrieder, John Blaho, Werner Ouwendijk, Thomas Heineman, Susanne Himmelein, Leonardo D'Aiuto, Vishwajit Nimgaonkar, Clinton Jones, Catherine Sadzot, David Davido, Aldo Pourchet; (*row 6*) Nicholas Baird, Homayon Ghiasi, Ron Goldstein

present on histones bound to the viral genome. Profile changes between short (<9 h) and longer (>9 h) post-mortem intervals (PMI) promise to provide insights into how changes in transcriptional and epigenetic control relate to virus reactivation. Such analyses require the use of next-generation sequencing to enable accurate detection and mapping of all RNA transcripts and histone binding sites within the viral genome. The main barrier to success, however, is the minimal amount of viral DNA and RNA present in a TG, underscoring the need to first optimize methods of target enrichment to maximize capture of the viral genome and transcriptome. Data were presented on the capture of viral DNA bound to H3K9ac histones in late PMI samples and the identification of multiple sites bound by H3K9ac across the viral genome. In addition, studies were conducted using a stranded RNA-seq approach, with enrichment for viral RNA, to examine viral transcriptomes in early and late PMI samples.

Adit Dhummakupt, *University of Florida*, discussed ChIP-seq analysis of H3K27me3 and SUZ12 on latent genomes of HSV-1 wild-type and latency associated transcript (LAT) promoter deletion viruses. HSV-1 establishes latency in sensory neurons where lytic genes are silenced. Tri-methylation of lysine 27 on histone 3 (H3K27me3) is abundant on latent genomes and likely plays an important role in the heterochromatic repression of lytic gene expression. Moreover, previous studies suggest a role for LAT in controlling the amount of heterochromatin associated with the viral genome. ChIP-seq analyses to generate a high-resolution profile of H3K27me3 on the latent genomes of HSV-1 strain 17+ and a LAT promoter deletion mutant (17ΔPST) revealed not only global enrichment of H3K27me3 over much of the latent genome but also more robust enrichment of H3K27me3 in specific genomic regions of both viruses. Furthermore, 17ΔPST was enriched with higher levels of H3K27me3 in several locations within the repeat region. These data confirm differential enrichment of H3K27me3 between 17+ and 17ΔPST in specific regions, consistent with the previous finding in this latency model that LAT interferes with H3K27me3 enrichment in a targeted manner. Finally, ChIP-seq analyses for SUZ12, a protein in the polycomb repressive complex 2 (PRC2) responsible for deposition of H3K27me3, showed a generally good correlation between enrichment of SUZ12 on the viral genome and regions of high H3K27me3 enrichment, although computer analysis using CisGenome identified several regions where SUZ12 may be recruited more specifically and robustly. Overall, these data suggest that particular regions of the HSV-1 genome play a critical role in the degree of heterochromatic repression of lytic genes during latency.

Esteban Engel, *Princeton University*, discussed findings on the mechanism of anterograde transport and spread of HSV-1 in neurons. Studies were done using a naturally occurring HSV-1 mutant called MacIntyre, which was isolated from

the brain of a patient who died of HSV encephalitis. MacIntyre can spread exclusively in the retrograde direction, as verified in vivo in the CNS of infected primates and rodents, and in vitro in infected neurons cultured in modified Campenot tri-chambers. The spread defect of MacIntyre was found to be due to a block in axonal sorting of viral particles. Sequencing the entire MacIntyre genome with the goal of identifying the viral proteins responsible for the mutant spread phenotype showed missense mutations in genes gI and gE and one nonsense mutation in the Us9 gene. When neuronal cell bodies were infected with MacIntyre and complemented in cis or trans with wild-type Us9, anterograde spread was not restored; however, MacIntyre-infected cell bodies complemented in cis or trans with both wild-type Us9 and gI showed restored anterograde spread, suggesting a role for Us9 and gI in recruiting host motor proteins to facilitate efficient axonal sorting and anterograde transport in infected neurons. In other studies, the microtubule-dependent kinesin-3 motor protein KIF1A was found to be essential for efficient axonal transport of HSV-1 capsids. Live cell microscopy of neurons infected with fluorescently labeled HSV-1 showed that KIF1A is co-transported with HSV-1 capsids in axons, while transcomplementation of infected cell bodies with dominant-negative KIF1A dramatically reduced anterograde spread. Together, these studies have helped to dissect the molecular mechanism of HSV-1 anterograde spread in neurons, identifying viral and host proteins essential for this process.

Chandra Krol, *University of Oklahoma Health Science Center*, showed that the consequences of HSV-1 infection and latency vary depending on the type of tissue infected within the nervous system. HSV-1 infection can result in life-threatening encephalitis (HSE), but the mechanism(s) underlying long-term HSE-mediated pathology is unknown. Studies were carried out using HSV-1-inoculated scarified corneas of C57BL/6 or reporter-inducible Rosa mice to characterize viral dissemination pathways in the peripheral and central nervous system (CNS), as determined by RT-PCR and confocal microscopy. In addition, leukocyte infiltration, T cell function, and the resident microglia activation signature were analyzed by flow cytometry to examine the local immune responses during acute and latent infection. After ocular infection, red fluorescent protein, representing HSV-1-infected cells from Rosa mice, and HSV-1 lytic genes were detected in the olfactory bulb (OB) before these genes were detected in other areas of the CNS (~2 days post-infection). During acute encephalitis, viral lytic genes were identified in all regions of the brain. During latency (day 30 and day 60), latency associated transcripts were found in the ependyma (CNS tissue lining the ventricles), the midbrain, as well as in TG. Unexpectedly, HSV-1 lytic genes were also observed in the ependyma during latency but not in the OB, TG, or brainstem. Unlike the TG and the brainstem, the ependyma and OB maintained elevated levels of effector T lymphocytes and MHC class II-positive resident microglial cells during latency up to day 60 post-infection. $CD4^+$ and $CD8^+$ T cell populations from each CNS site (OB, ependyma, and midbrain) responded to PMA/ionomycin with production of IFN-γ. This seminal study evaluating the OB as a site of viral dissemination after ocular infection suggests the importance of ependymal cells in maintaining effector T lymphocyte populations during latency.

Marielle Lebrun, *University of Liege*, described the interplay of "VZV assemblons" with promyelocytic leukemia (PML) bodies. VZV assemblons have been defined as the dynamic capsid aggregrates in the nuclei of infected cells, based on the use of a virus expressing the small capsid protein fused to enhanced green fluorescent protein (eGFP-ORF23 VZV). Studies were carried out to test for a possible link between such assemblons and recently described similar structures containing capsids entrapped in PML "cages" in nuclei of wild-type VZV-infected cells. VZV-infected MeWo cells in which expression of each PML subunit was downregulated by shRNA still revealed VZV assemblons. While immunostaining of eGFP-ORF23 VZV-infected MeWo cells with antibody against PML proteins showed only partial co-localization of VZV assemblons with PML bodies, infection of HEK293 cells overexpressing PML proteins fused to eGFP with VZV containing ORF23 fused to red fluorescent protein (RFP-ORF23) showed complete co-localization, suggesting that the partial co-localization findings reflect expression levels of PML proteins. It is also possible that PML proteins were recruited to sites where VZV assemblons develop. However, although PML bodies decreased during infection, all cell lines tested were permissive for VZV. Given the results in productive infection, analysis of the relationship between VZV assemblons and PML bodies in latent or non-permissive VZV infections should prove informative.

Julia LeCher, *Georgia State University*, presented data on the immunological response of mixed cell cultures derived from non-dissociated macaque dorsal root ganglia (DRG) to herpes B virus infection. B virus is endemic in macaque monkeys, which are widely used in biomedical research. Since B virus can cause deadly human zoonoses, the virus poses an extreme occupational hazard. The lack of a suitable animal model and restrictions on handling potentially infected macaque nervous tissue has previously limited studies on events surrounding infection of and establishment of latency in macaque ganglia. To address these issues, macaque non-dissociated DRG were cultured resulting in outgrowth of mixed cell culture. Large neuronal clusters (300 μm) formed into both multipolar and bipolar bodies with projections of 500 and >1000 μm, respectively. During growth, axonal projections formed tracks with glial-like cells that formed connections with other neighboring neuronal clusters. Use of this mixed culture model to test whether acute B virus infection of macaque ganglia might trigger cellular defense networks for

lymphocyte recruitment revealed upregulated intracellular pattern recognition receptors of proteins they regulate (interferon, MAPK, and NF-κB). Further, TNF-α and IL-6 were upregulated with no change in IL-1β. Chemokines were also upregulated and infected cell medium was sufficient to induce lymphocyte chemotaxis, as evidenced by transmigration assays. B virus-infected cultures showed upregulated IRF-1 mRNA and MHC class I protein expression, indicating both T_H-1- and T_H-2-mediated immune responses, with the microenvironment supporting $CD8^+$ T cell recruitment and recognition of infected cells. Together, these data support the notion that acute B virus infection of macaque ganglia triggers cellular defense networks to recruit lymphocytes and provide an ex vivo model to study virus-cell interactions in macaque DRG.

Werner Ouwendijk, *Erasmus Medical Center*, presented analyses of the immune response in ganglia after primary simian varicella virus (SVV) infection. Primary SVV infection in non-human primates causes varicella, after which virus becomes latent in ganglionic neurons and reactivates to cause zoster. The host immune response in ganglia during establishment of latency is ill-defined. Ganglia from five African green monkeys (AGM) obtained at 9, 13, and 20 days post (dpi)-intratracheal SVV inoculation were analyzed by ex vivo flow cytometry, immunohistochemistry, and in situ hybridization. Mild inflammation was seen in ganglia at 13 and 20 dpi. Immune infiltrates consisted mostly of $CD8^{dim}$ and $CD8^{bright}$ memory T cells, some of which expressed granzyme B, and fewer $CD11c^+$ myeloid cells. Chemoattractant CXCL10 transcripts were expressed by neurons and infiltrating inflammatory cells, but did not co-localize with SVV ORF63 RNA in neurons. Satellite glial cells expressed increased levels of activation markers CD68 and MHC class II at 13 and 20 dpi compared to 9 dpi. Overall, primary SVV infection of ganglia is associated with recruitment of T cells, possibly mediated by CXCL10, that likely control virus replication.

Aldo Pourchet, *New York University*, presented models for HSV-1 latency using human stem cell-derived neurons. New models of HSV-1 latency/reactivation using neurons generated from human embryonic stem cell (hESC) lines are being developed to enable analyses of HSV-1 in a truly species-homologous and accessible context. The schemes for generating neuron cultures from two different hESC cell lines and how these two models differ from each other and from the existing rat SCG-derived neuron model were described.

Edouard Cantin, *City of Hope*, showed that T cells control the inflammatory monocytes that drive HSV-1 reactivation. Analysis to test whether maintenance of latency is an active process using wild-type C57BL/6 (B6) mice infected with a high dose of HSV1 17+ and, at 30 dpi, subjected to lethal total body irradiation (TBI) to deplete all leukocytes unexpectedly revealed no virus in the TG, brainstem, or brain, and all mice survived, contrary to the accepted idea that immunosuppression promotes reactivation. Remarkably, heat stress of TBI B6 mice induced transient reactivation in vivo (IVR), with infectious HSV detected in ~65 % of TGs 24 h after IVR but not later. Thus, "animation" of latent HSV genomes is not under active T cell control. A new model of latency was established in immunodeficient B6. Rag mice using human immunoglobulin and acyclovir treatment to suppress viral replication and promote survival of latently infected Rag mice inoculated at high dose (HD) or low dose (LD). In this model, heat stress resulted in efficient IVR, culminating in fatal herpes encephalitis in all HD but not in LD mice, which showed only transient reactivation despite the only 3-fold difference in inoculum dose. The major difference between latent LD-Rag and HD-Rag mice was a significant accumulation of $Ly6C^{high}$ inflammatory monocytes only in the brainstem of HD mice. Notably, transfer of wild-type memory T cells to HD-Rag mice inhibited accumulation of inflammatory monocytes and blocked IVR, suggesting that in the absence of resident inflammatory monocytes (LD-Rags and B6 TBI mice), intrinsic neuronal restriction mechanisms and T cell suppression of inflammatory monocytes efficiently inhibit progression from "animation" to lytic replication and full reactivation.

Dan Carr, *University of Oklahoma Health Sciences Center*, discussed evidence that leukocytes do not directly contribute to HSV-1-induced corneal angiogenesis. HSV-1 elicits robust neovascularization of the mouse cornea primarily during establishment of latency, when no infectious virus or virus-encoded antigen is detected. The mechanism that drives neovascularization during this time is unknown, but has been thought to include neutrophils (PMNs). Analysis of the role of infiltrating leukocytes in cornea neovascularization showed that a single bolus of dexamethasone (DEX) at 10 dpi resulted in a significant reduction in blood vessel intrusion but not lymphatic vessel genesis into the central cornea at 14 dpi, with no apparent differences in the number of infiltrating leukocytes, including myeloid-derived cells and T cells. By 30 dpi, DEX-treated mice displayed no corneal neovascularization, despite the presence of leukocytes including PMNs. Moreover, mice treated with anti-Gr-1 antibody, resulting in a complete loss of neutrophils ($Gr1^+F4/80^-$), T cells, and inflammatory monocytes ($Gr1^+F4/80^+$) in the cornea, showed no difference in blood or lymphatic vessel density/area compared to isotype control-treated mouse corneas. However, similar to the DEX-treated mice, levels of some pro-angiogenic factors differed. Overall, no direct contribution of leukocytes to the development of corneal angiogenesis after HSV-1 infection was found.

Michael Gershon, *Columbia University*, described T cells and neurons as the "odd couple" of VZV. Most VZV-infected cells in vitro do not release infectious virions; spread of VZV is cell-to-cell, both in vitro and within hosts. Except in the suprabasal epidermis, cells degrade VZV in late endosomes

before secreting inactivated virions. Retrograde transport from skin provides VZV with a route to neuronal perikarya in dorsal root ganglia (DRG), where VZV establishes latency. Viremia during varicella is an alternative route to neuronal perikarya, such as those of the enteric nervous system, which lack cutaneous projections. Although most VZV-infected cells transmit lytic infection to co-cultured enteric neurons, VZV-infected T cells are exceptional because the type of infection they transmit is always latent. VZV establishes lytic infection in T cells and, as in other lytically infected cells, newly enveloped VZV traffics to late endosomes where it is degraded. T cells do not secrete filterable cell-free infectious virions and their ability to transmit latent infection to co-cultured neurons is not prevented by agents, such as mannose 6-phosphate (40 mM), which prevent infection of neurons by cell-free VZV. In microfluidic chambers, where cell bodies and axon terminals of neurons are separated, VZV-infected T cells transmit latent infection independently of whether they are added to terminals or cell bodies. When injected intravenously, VZV-infected T cells establish latent infection in virtually every neuron of the enteric nervous system and DRG; thus, VZV-infected T cells are as efficient in establishing latent VZV infection in neurons in vivo as they are in vitro. Cells infected with HSV-1 have recently been shown to export stimulator of interferon genes (STING) in exosomes, which deliver these genes to non-infected cells. VZV-infected T cells upregulate STING and concentrate transcripts encoding STING in exosomes. STING is also highly expressed in latently infected enteric neurons. It is possible that T cells transiently fuse with neurons to transmit VZV, but that a STING-mediated type 1 interferon response inhibits viral proliferation, thus facilitating latent infection.

Homayon Ghiasi, *Cedars-Sinai Medical Center*, showed that $CD8^+$ T cells play a bystander role in HSV-1 latently infected mice. In an explant reactivation model, it has been proposed that $CD8^+$ T cells maintain latency in trigeminal ganglia of HSV-1 latently infected mice. This iconoclastic notion was examined using a combination of knockout mice, adoptive transfers, depletion studies, and blocking CD8 antibody both in vitro and in vivo. Analysis of C57BL/6 mice ocularly infected with the avirulent HSV-1 strain RE after corneal scarification or with the virulent HSV-1 strain McKrae without corneal scarification showed that $CD8\alpha^+$ dendritic cells contribute more than $CD8^+$ T cells to HSV-1 latency and reactivation in trigeminal ganglia of ocularly infected mice. Overall, the data suggest that $CD8^+$ T cells do not account for the increase or maintenance of latency in ocularly infected mice in the presence of the latency associated transcript.

Ron Goldstein, *Bar-Ilan University*, showed that experimental reactivation of quiescent VZV in human embryonic stem cell-derived neurons results in virus production and spread of infection to neighboring cells. Most adults worldwide harbor latent VZV in their ganglia and reactivation can cause herpes zoster. VZV latency in sensory and autonomic neurons has remained enigmatic and difficult to study, and experimental reactivation has not yet been achieved. Human embryonic stem cell-derived neurons have proven permissive for productive and spreading VZV infections. Analyses also show that such neurons can host a persistent non-productive infection lasting for weeks, which can subsequently be reactivated by multiple experimental stimuli. qPCR of quiescent infections established by exposing neurons to low-titer, cell-free VZV and using acyclovir or by infection of axons in compartmented microfluidic chambers without acyclovir revealed VZV DNA and low levels of viral transcription for up to 7 weeks. Moreover, quiescently infected human neuronal cultures induced to undergo renewed viral gene and protein expression by growth factor removal or by inhibition of PI3 kinase activity showed enhanced VZV reactivation resulting in productive infections when reactivated at a lower temperature (34 °C). Comparison of VZV transcription in quiescently infected versus productively infected neurons using RNA-seq revealed preferential transcription from specific genome regions, especially the duplicated regions. These experiments establish a powerful new system for modeling the VZV latent state and reveal a potential role for temperature in VZV reactivation and disease.

Charles Grose, *University of Iowa*, discussed reactivation of VZV infection in a human brain with no evidence of arterial involvement. Analyses of a brain biopsy obtained from an immunocompetent young adult who recovered from focal VZV encephalitis were conducted to investigate VZV infection in the brain of a living person. VZV genome copy numbers were, on average, 5 copies/cell, far fewer than found in cultured cells, while transcript analysis by qPCR indicated levels of VZV regulatory and structural genes similar to those in cell culture. Both 2D and 3D confocal microscopy to examine the distribution of VZV infection in neurons, astrocytes, microglia, and oligodendrocytes documented the presence of gE and gH, although in a restricted pattern in the cytoplasm, while gC expression was even more limited. Co-localization of VZV immunoreactivity with individual brain cell types revealed VZV mostly in astrocytes, based on expression of glial fibrillary acidic protein (GFAP), with some areas of GFAP-reactivity representing a reactive gliosis containing numerous VZV-positive astrocytes. Neither confocal nor electron microscopy revealed large infected blood vessels. These results indicate VZV infection in the brain without rash, i.e., zoster sine herpe, presumably after reactivation from cranial nerve ganglia and suggest that astrocytes can halt spread of VZV infection in the brain and thus protect the host from a fatal outcome.

William P. Halford, *Southern Illinois University School of Medicine*, showed that virus-specific antibodies are needed for complete vaccine-induced protection against HSV-2. HSV-2

0ΔNLS is a live-attenuated HSV-2*ICP0*$^-$ vaccine strain that normally elicits complete protection against HSV-2. Mice immunized with an ultraviolet-inactivated form of the HSV-2 0ΔNLS vaccine exhibited a profound defect in their capacity to restrict the replication of wild-type HSV-2 during the first 48 h post-challenge, whereas mice immunized with the live HSV-2 0ΔNLS vaccine shed ~200-fold less virus at 24 and 48 h post-challenge. To test whether antibodies mediate this early protection because only antibodies (not T cells) are present in the lymphatics that bathe epithelial cells before inflammation, live HSV-2 0ΔNLS vaccine-induced protection was compared in C57BL/10 and B cell-deficient μMT mice. ELISpot and CD8$^+$ T cell activation marker analyses indicated that the live-0ΔNLS vaccine elicited an equivalent T cell response in C57BL/10 and μMT mice. Nonetheless, live 0ΔNLS-immunized μMT mice shed ~40-fold higher levels of HSV-2 at 24 h post-challenge compared to B cell$^+$ (C57BL/10) recipients of the same vaccine. Moreover, >60 % of live 0ΔNLS-immunized μMT mice succumbed to a slowly progressing HSV-2 challenge that was lethal between 10 and 21 days post-challenge. Passive transfer of HSV-2 antiserum restored complete protection to live 0ΔNLS-immunized μMT mice. These results suggest that virus-specific antibodies play a critical role in promoting rapid and efficient T cell recruitment to sites of HSV-2 infection.

Thomas Heineman, *GSK Vaccines*, described HZ/su, a novel adjuvanted subunit candidate vaccine for the prevention of herpes zoster. HZ/su, which contains VZV glycoprotein E (gE) and the AS01$_B$ adjuvant system, is being developed to prevent herpes zoster in older adults and immunocompromised individuals. Phases 1 and 2 clinical trials demonstrated that 2 doses of HZ/su elicit robust and durable cellular and humoral immune responses in all populations studied. In people ≥60 years, 2 doses of HZ/su induced a 15.2-fold increase in the frequency of gE-specific CD4$^+$ T cells, which remained 4.0-fold above baseline 6 years after vaccination. Studies in older adults stratified by age (50–59, 60–69, and ≥70 years) demonstrated minimal age-related declines in immune responses to HZ/su. Similarly, 2 doses of HZ/su induced strong CD4$^+$ T cell responses in immunocompromised subjects—15.1- and 20.7-fold increases compared to placebo, respectively, in HIV-infected adults and autologous stem cell transplant recipients. HZ/su efficacy was evaluated in an observer-blinded, placebo-controlled trial (ZOE-50; NCT01165177) in adults ≥50 years. Subjects were randomized 1:1 to receive 2 doses 2 months apart of HZ/su or saline placebo with efficacy follow-up beginning 1 month after the second dose (N=14,759 in the efficacy cohort). In this study, 6 cases of herpes zoster occurred in the HZ/su-treated group compared to 210 cases in the placebo group during a mean follow-up of 3.2 years. HZ/su efficacy in reducing the risk of herpes zoster was 97.2 % in subjects ≥50 years and ranged from 96.6 % in subjects 50–59 years of age to 97.9 % in subjects ≥70 years. During the 7 days after vaccination, injection site and systemic reactions were more frequent in HZ/su compared to placebo recipients. No apparent differences were observed in the incidence of serious adverse events, deaths, or potential immune-mediated diseases between the HZ/su and placebo groups. These results indicate that HZ/su is highly efficacious in reducing the risk of herpes zoster in older adults.

Susanne Himmelein, *Ludwig Maximilians University Munich*, showed that latent HSV-1 does not induce apoptosis in human trigeminal ganglia. Ganglia latently infected with HSV-1 contain CD8$^+$ T cells, which secrete granzyme B and are thus able to induce neuronal apoptosis. Use of immunohistochemistry and single-cell RT-qPCR revealed a greater frequency and greater transcript levels of caspase-3 in HSV-1-negative ganglia compared to HSV-1-positive ganglia and neurons, respectively. No neurons positive for terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end-labeling were detected. Thus, infiltrating T cells do not induce apoptosis in latently infected neurons.

Victor Hsia, *University of Maryland Eastern Shore*, described the effects of thyroid hormone (TH) on alphaherpesvirus latency and reactivation. Several mechanisms have been suggested to underlie the establishment of alphaherpesvirus latency and reactivation, such as an altered immune response, LAT-mediated anti-apoptosis, microRNA-induced gene silencing, differential neuronal suppression, hormonal regulation, and repressive chromatin. Studies aimed at identifying new transcription factor binding sites in alphaherpesviruses revealed TH response elements located adjacent to important promoter regions. Analyses to test whether TH fluctuations might participate in controlling viral latency and reactivation via interaction of TH to its nuclear receptor showed that TH decreased viral replication in the differentiated neuroendocrine cell line LNCaP and that the removal of TH reversed the repression. Negative TH response elements were identified in thymidine kinase (TK) promoters of HSV-1 and VZV. Introduction of a point mutation in the HSV-1 TK TH response element disrupted TH-mediated regulation. This described mode of action was observed only in differentiated cells. Recent studies also suggest that TH can modulate PI3K signaling to regulate HSV-1 replication and gene expression. Preliminary results showed that casein kinase 2 (CK2), a key component of the PI3k pathway, was upregulated by TH and that a CK2 inhibitor was sufficient to reverse TH-mediated suppression. Use of a mouse latency model to investigate the effects of TH on HSV-1 latency/reactivation revealed that TH not only reduced viral reactivation but also delayed release of infectious virus by 2 days. The clinical relevance of these findings, as addressed in a retrospective case-controlled analysis using hospital diagnostic codes for treatment of thyroid disorders and alphaherpesvirus outbreak as proxies for pathological outcomes, was suggested by data analysis showing that patients with TH dysfunction

had an increased risk of HSV-1 or VZV reactivation. Together, these results point to the role of TH dysfunction in herpesvirus pathogenesis and may have implications in the control of reactivation from viral latency.

Keith Jerome, *Fred Hutchinson Cancer Research Center*, discussed inactivation of latent HSV-1 by homing endonuclease (HE)-directed mutagenesis in primary neurons. Studies indicate a potentially curative approach tor HSV infection based on the introduction of DNA double-strand breaks in latent HSV genomes by highly specific HEs, leading to mutagenesis of essential viral genes and thus disabling the virus. Susceptibility of HSV genomes to HE-mediated mutagenesis was demonstrated in a model for latent HSV-1 infection involving in situ-targeted mutagenesis of HSV genomes in fibroblasts quiescently infected with HSV and subsequent exposure to an HSV-specific HE (HSV-HE) along with Trex2, a 3-′ to 5-′ DNA exonuclease shown to increase the efficiency of mutagenesis by HEs. The frequency of mutagenesis increased 2- to 5-fold when cells were exposed to HE/Trex2 in the presence of HDAC inhibitors (HDACi), suggesting that chromatin structure of quiescent HSV genomes can affect the efficiency of HE-mediated mutagenesis. Initial experiments in neurons, the ganglionic cell type in which HSV establishes latency in vivo, showed that HSV replication in vitro was decreased up to one log by exposure to HSV-HE and Trex2. Amplicon analysis of HE target sites obtained from these treated cells revealed the expected mutations in HSV genomes. In other experiments, HSV-HE/Trex2 exposure of cultured neurons obtained from HSV-infected mice resulted in mutations at the site targeted by HSV-HE. Experiments are ongoing to test whether HDACi treatment improves the mutagenesis efficiency in HSV-infected neurons and whether HSV-HE/Trex2 exposure limits viral reactivation from HSV-infected neurons. Finally, an animal model has been developed to evaluate approaches for in vivo enzyme delivery to trigeminal ganglia following ocular HSV infection. These studies provide the groundwork for further in vivo research aimed at developing a cure for HSV infection by HE-mediated targeted mutagenesis of virus DNA in latently infected neurons.

Clinton Jones, *University of Nebraska*, showed that the bovine herpesvirus 1 (BHV-1) genome contains multiple glucocorticoid response elements, two of which are important for transactivation by the glucocorticoid receptor (GR). After acute infection, BHV-1 becomes latent in sensory neurons, wherein viral gene expression is restricted. Stress, including the synthetic corticosteroid dexamethasone, increases reactivation from latency, in part by inducing lytic cycle viral gene expression. The immediate early transcription unit 1 (IEtu1) promoter drives expression of two important viral transcriptional regulatory proteins, ICP0 and ICP4, suggesting that stress-induced factors activate the IEtu1 promoter during reactivation. Bioinformatic analysis revealed numerous potential GR binding sites (GREs) in the BHV-1 genome, with enrichment for these sites in the repeat sequences containing the IEtu1 and IEtu2 promoter, origin of replication, and bICP4 coding sequences. Transient transfection studies of neuroblastoma cells treated with dexamethasone demonstrated that the GR stimulated IEtu1 promoter activity, but not IEtu2 or VP16 promoter sequences. A 416-bp IEtu1 promoter fragment containing two putative GREs conferred GR responsiveness to a heterologous promoter. Mutagenesis of each GRE or both GREs demonstrated that the 5′ GRE (GRE#1) was more important than GRE#2. Electrophoretic mobility shift assays showed that cellular factors bind to an oligonucleotide containing GRE#1 if GRE is intact. Although stimulation of IEtu1 promoter activity by activated GR appears to be an important event during reactivation, stress is predicted to have pleiotropic effects on the latently infected neuron and viral genome necessary for reactivation.

Paul Kinchington, *University of Pittsburgh*, presented an update on the VZV-induced pain model in the rat. A common complication of herpes zoster after reactivation of VZV from sensory and autonomic ganglia is chronic pain, i.e., postherpetic neuralgia (PHN), which causes depression, withdrawal from society, reduced quality of life, and even suicide. Many PHN patients do not respond to current therapies. A rat model of PHN, in which prolonged mechanical hypersensitivity develops in rat footpads after inoculation with live VZV that mirrors the crippling allodynia seen in PHN patients, has been obtained. Three new aspects of this model are now being addressed: (1) studies regarding why VZV lacking ORF47 kinase, which is required to correctly assemble virions and may underlie a reduced ability to initiate infection of rats, does not induce pain in rats; (2) expansion of the rat platform to model trigeminal ganglia (TG)-distribution PHN, one of the most common sites of zoster and PHN in humans, by inoculating rat whisker pads and inducing prolonged nocifensive and defensive behaviors suggestive of pain; and (3) studies to determine whether pain-inducing events occur at the periphery or the ganglia. Analyses together with Phillip Kramer at the Baylor College of Dentistry showed that direct stereotactic injection of the rat TG with VZV results in mechanical and thermal sensitivity indicative of pain, compared to rats directly inoculated in the TG with uninfected cells. Given that VZV reactivation from the TG can cause blinding ocular zoster and chronic facial pain, this model might shed light on human facial pain as well as TG-associated neurological disease in rats.

David Koelle, *University of Washington*, presented a genome-wide look at the CD4 T cell response to live attenuated VZV vaccine. This licensed zoster prevention vaccine boosts antibody and the CD4 T cell response to the whole virus. Recently, a subunit vaccine containing VZV gE has shown promising results in phase III trials. Further analysis in a longitudinal open-label study in 12 persons, mostly age 50–60 years, using the licensed vaccine to examine the

immune response in ex vivo CD4 T cell and gpELISA IgG assays revealed a relatively modest boost, consistent with literature findings. VZV-reactive CD4 T cell lines examined before and 28 and 182 days after vaccination were expanded from blood and tested for responses to every known VZV ORF, scored as "yes/no" for CD4 T cell response to each VZV protein using an objective cut-off based on irrelevant microbial proteins. The breadth of the CD4 response approximately quadrupled after vaccination and then returned to baseline. While CD4 T cell responses to gE were observed in most individuals at each time point, several other VZV ORFs were similarly strong CD4 immunogens. Numerous specific CD4 T cell epitopes in ORFs 9, 12, and 18 were determined, and several new examples of T cell cross-reactivity between VZV and HSV-1 and/or HSV-2 were uncovered. Future studies include use of VZV antigen panels to examine younger and older vaccine recipients and study of local T cell responses in skin.

Patrick Lomonte, *National Center for Scientific Research*, described prerequisites for formation of HSV-1 latency associated, viral DNA-containing promyelocytic leukemia nuclear bodies (PML-NBs). PML-NBs are nuclear relays of the intrinsic antiviral pathway that control infections by both RNA and DNA viruses. Latent HSV-1 genomes can be trapped in PML-NBs, forming nuclear structures called viral DNA-containing PML-NBs (vDCP-NBs), which are hallmarks of latency established during acute infection in mouse TG neurons and which persist during early latency (~28–30 dpi). Fluorescence in situ hybridization combined with immunofluorescence to study viral genome patterns were used to examine viral and/or cellular features that favor formation/disassembly of vDCP-NBs in wild-type or mutant HSV-1-infected mouse primary TG neurons. Infection with wild-type virus in the presence of specific inhibitors or after boosting the type I interferon response never led to vDCP-NBs, but instead to replication compartment-like patterns also observed in some neurons during acute infection. Prevention of PML-NBs destabilization by infection with an ICP0-negative virus or slowing of viral replication in neurons by infection with a thymidine kinase-negative virus did not enable formation of vDCP-NBs. Previous in vivo studies showed that during acute infection, latency might be favored over the lytic cycle in the absence of (1) the lytic program/viral replication (e.g., in the absence of functional ICP4), (2) VP16 expression, and (3) ICP0 synthesis. Analyses of neurons infected with temperature-sensitive mutant viruses *in1374* (tsICP4, VP16 mutated on its transactivation domain, ICP0 non-functional) or *in1330* (tsICP4, VP16 functional, ICP0 non-functional), which are unable to replicate at the restrictive temperature of 38.5 °C due to synthesis of a non-functional ICP4, showed that vDCP-NBs were formed at restrictive but not permissive (32 °C) temperatures with both viruses. These data demonstrate that defects in the onset of the lytic program due to the absence of functional ICP4, combined with the absence of ICP0, are viral features that lead to formation of vDCP-NBs.

Ilhem Messaoudi, *University California Riverside*, showed that acute SVV infection results in robust and sustained gene expression changes in the ganglia. Intrabronchial inoculation of rhesus macaques with SVV, a homolog of VZV, recapitulates the hallmarks of acute and latent VZV infection in humans, in which the mechanisms of viral transport to the ganglia, as well as the host response in the ganglia and its relationship to latency are not well understood. Analyses in the simian model showed that SVV is present in the ganglia as early as 3 dpi, supporting a hematogenous route of dissemination and that the SVV transcriptome replicates robustly at 3 dpi followed by latency at 7 dpi. RNA-seq analysis of the host response to SVV infection in uninfected and acutely infected ganglia collected at 3, 4, 7, 10, and 14 dpi revealed 393 differentially expressed genes at day 3, 1118 at day 7, 809 at day 10, and 576 at day 14. Enrichment analysis using Gene Ontology showed changes in genes associated with muscle system processes, defense response to virus, apoptotic processes, endocytosis, and neurogenesis. Further analysis after acute infection revealed neuronal damage and a type 1 interferon response in the ganglia. Changes in expression of immune-related genes were detected at day 3, peaking at 7 dpi before subsiding and consistent with the establishment of latency, whereas expression of genes associated with neurogenesis remained downregulated throughout acute infection.

Maria Nagel, *University of Colorado School of Medicine*, described "burning mouth syndrome" due to HSV-1. Burning mouth syndrome is characterized by chronic orofacial burning pain. No dental or medical cause has been found. In a case of burning mouth syndrome of 6-month duration in a healthy 65-year-old woman, high copy numbers of HSV-1 DNA were found in the saliva at the height of pain. After antiviral treatment, her pain resolved completely with a corresponding absence of salivary HSV-1 DNA 4 weeks and 6 months later. This case expands the spectrum of disease caused by HSV with and without rash.

Donna Neumann, *Louisiana State University Health Sciences Center*, showed that the protein CTCF nucleates the formation of chromatin loops during HSV-1 latency. The cellular protein CTCF occupies 7 conserved CTCF binding domains in the HSV-1 genome during latency. At least 3 of these sites, i.e., the CTRL2 site downstream from the LAT enhancer, the CTa'$_m$ site upstream of the ICP0 promoter and the CTRS3 site upstream of the ICP4 promoter, undergo CTCF eviction at early times (2 h) post-reactivation in mice latently infected with 17Syn+. Loss of CTCF binding required ongoing transcription, supporting the possibility that each CTCF domain in HSV-1 is regulated independently. Analyses to test whether each motif might contribute to latency by quantifying CTCF occupation of the remaining 4 CTCF

binding motifs in HSV-1 during latency revealed one particular site that differed significantly with respect to CTCF binding and function, i.e., CTRL1 upstream of the LAT promoter was significantly enriched (>3-fold, $p<0.05$) in CTCF protein binding during latency. Further assays using reporter constructs containing the CTRL1 binding domain showed that the CTRL1 domain was not an enhancer-blocker, suggesting a unique and complex role for the CTRL1 domain in regulating gene expression. Because CTCF insulators are often key functional components in the formation of chromatin loop protein-mediated complexes that regulate gene expression through long-distance interactions, conventional chromosome conformation capture assays (3C) were combined with next-generation sequencing to identify potential chromatin loops in latently infected mouse trigeminal ganglia to identify spatial interactions in the HSV-1 genome during latency. These 3C-seq experiments revealed 3 independent chromatin loops, 2 of which involve the CTRL1 domain of HSV-1. This is the first evidence that the latent HSV-1 genome may be organized into district chromatin domains maintained by spatial interactions via chromatin loops that regulate latent HSV-1 gene expression.

Vishwajit Nimgaonkar, *University of Pittsburgh*, described persistent, non-encephalitic HSV-1 infection as a risk factor for cognitive impairment. Extensive research indicates that inherited factors and environmental toxins can impair human cognition, but the risk conferred by latent virus infections has received less attention. Ongoing studies indicate such a role for HSV-1, a virus that causes highly prevalent, lifelong latent infection in human sensory ganglia. Although HSV-1 DNA has been detected in ~1/3 of non-encephalitic post-mortem brain tissues, its presence has been considered benign for the brain. Yet eight recent studies (with only two other discrepant reports) revealed a significant cognitive dysfunction in individuals with elevated HSV-1 antibody titers in the serum but no prior history of encephalitis. The associations were detected initially in persons with schizophrenia (SZ), but have also been observed in otherwise healthy persons. In several studies, the cognitive dysfunction affected attention and working memory domains in a manner more focal and less severe than the widespread deficits from HSV-1 encephalitis. They were not traceable to other herpes infections or to socio-economic factors. Latent HSV-1 infection is also associated with volume reductions in the prefrontal cortex among SZ patients; such deficits appear to be progressive. In an initial randomized controlled trial, HSV-1-seropositive SZ patients who received adjunctive valacylovir (1.5 g twice daily) plus anti-psychotic drugs, for 16 weeks, showed a significant improvement in working, verbal, and visual memory compared with the placebo plus anti-psychotics group. A second similar trial is ongoing in India, where a progressive cognitive decline was earlier noted over 1–2 years in controls and SZ patients, leading to the hypothesis that latent HSV-1 infection causes cognitive impairment across the lifespan.

Pamela Roehm, *Temple University School of Medicine*, described the use of an RNA-guided DNA editing system to modify the HSV-1 genome. HSV-I infects 80–90 % of adults, becoming latent in ganglionic neurons and reactivating in response to multiple stimuli to manifest as herpes labialis and rarely encephalitis. While medical treatment for productive HSV-1 infection is effective, antiviral treatment does not eliminate latent infection or affect further reactivation. Recently, an RNA-guided, DNA-editing system based on the CRISPR/Cas9 editing system in bacteria has been modified for use in mammalian cells. Use of this system to cut specifically targeted DNA in the ICP0 gene of HSV-1, followed by stable transfection of Vero cells with guide RNAs against ICP0 and the Cas9 gene, revealed a decreased cell infectability similar to that seen with ICP0-null HSV-1 virus. This study demonstrates that, once optimized, this technology will be useful to prevent HSV-1 reactivation by inactivating latent virus DNA.

Nancy Sawtell, *Cincinnati Children's Hospital Medical Center*, described acute and long-term outcomes of simulated deep-space radiation exposure on latent viral CNS infection and pathology. There is mounting evidence that in combination with genetic risk factors, HSV infection in the CNS further increases the probability of neurodegenerative disease. While the pathology and disease outcomes associated with acute HSV infection in the CNS are well appreciated, much less is known about the effects of long-term presence of virus DNA in the brain. In certain mouse genetic backgrounds, mice latently infected with HSV, but not uninfected mice, develop CNS lesions associated with long-term exposure to periodic reactivation stress. In an ongoing project with NASA, this model is being used to determine acute and long-term effects of HSV, with and without repeated reactivation triggers, on pathology in the nervous system.

Deepak Shukla, *University of Illinois at Chicago*, described the potential use of HSV-1 entry receptor expression to identify latently infected neurons. Little is known about the expression of HSV-1 entry receptors and co-receptors such as nectin-1, herpes virus entry mediator (HVEM), and heparan sulfate after the start of lytic infection in epithelial cells and during latency in neurons. Use of an ocular infection model in mice to study post-entry receptor expression during primary and latent infections showed that HVEM and nectin-1 are widely expressed in normal corneal epithelium and endothelium, but are almost undetectable in corneal stroma. Similarly, both HVEM and nectin-1 are expressed on neurons and non-neuronal cells in the TG. Acute HSV-1 keratitis and ganglionitis resulted in an increased HVEM expression in corneal epithelium and stroma and in TG neurons and non-neuronal cells, and many inflammatory cells in these tissues also expressed HVEM but without significant changes in nectin-1 expression. TG obtained from mice 7 months after

virus inoculation revealed an association between latent HSV-1 infection and increased HVEM expression in neurons and non-neuronal cells, whereas nectin-1 expression did not change significantly. Latently infected TG also contained focal infiltrates of mononuclear inflammatory cells, many of which expressed increased HVEM. Corneas derived from latently infected mice demonstrated chronic keratitis, with no evidence of virus replication or altered HVEM/nectin-1 expression in the corneal epithelium, and inflammatory cells present showed only weak HVEM expression. Yet heparin sulfate was removed from the cell surface via heparanase enzymatic activity and remained at low levels at 24 h post-infection of murine corneal epithelium. Overall, the findings raise the possibility that changes in HVEM expression after ocular HSV-1 infection modulate HSV latency and infection-induced inflammation in the cornea and TG. Higher expression of HVEM is a potential marker of latently infected neurons.

George Smith, *Temple University School of Medicine*, described neurotrophin-mediated regeneration of sensory afferents after dorsal root (DR) rhizotomy. DR injuries, which include those in the brachial plexus, lumbosacral plexus, and cauda equina, result in permanent loss of primary afferent terminals in the spinal cord. Upon these injuries, axons regenerate within the peripheral nerve component of the DR, but fail to regenerate through the DR entry zone and into the spinal cord. Analyses during the past 15 years to examine the role of neurotrophins in mediating regeneration of sensory afferents back into the spinal cord have shown that nerve growth factor (NGF) or artemin, a glial cell line-derived neurotrophic factor (GDNF), induces regeneration of calcitonin gene-related peptide-positive axons when expressed in the spinal cord. Accordingly, artemin or NGF enhanced recovery of only nociceptive behavior and showed a distribution of the cFos proto-oncogene similar to the topography of regenerating axons. GDNF and, to a lesser extent, artemin induced regeneration of non-peptidergic (isolectin B4-positive) nociceptive axons back into the spinal cord, again showing topographic cFos labeling and a return to correct nociceptive function. The combination of artemin and GDNF led to near complete regeneration of nociceptive axons and restoration of function. Of these treatments, only GDNF and neurotrophic factor-3 (NT-3) led to some regeneration of large-diameter axons from tactile or proprioceptive axons. Ongoing studies to examine mechanisms that augment regeneration of large-diameter axons by co-expression of NT-3 with either constitutively active Rheb (Ras homolog enriched in the brain), a direct activator of mTor, or BRSK2 (brain-restricted serine kinase), an axonal polarity factor important in development of proprioceptive axons, thus far indicate better regeneration and functional recovery of tactile and proprioceptive responses than that seen with NT-3 alone in the spinal cord.

Patrick Stuart, *Saint Louis University*, described the role of CD28 in establishment of HSV-1 latency. Mice are resistant to herpetic reactivation, which results in peripheral virus shedding; however, mice deficient in CD28 demonstrated "spontaneous" reactivation ranging from 1020 %. Because virus sheds for longer time periods from the cornea after reactivation in CD28KO mice (12 vs. 6 days for wild-type mice), raising the possibility that trigeminal ganglia (TG) infection is greater, total virus genomes in infected TGs were measured by real-time PCR analysis; indeed, TGs harvested during the first 2 weeks of infection had more total HSV-1 genomes in CD28KO mice than in wild-type B6 mice. Over time, both strains of mice show reduced genome loads and while more remained in CD28KO mice, these differences were not significant by day 35. In contrast, FISH analysis for LAT gene message revealed a greater number of neurons with a latent viral genome in wild-type than in CD28KO mice, a difference that persisted throughout the analysis; however, as with genome copy numbers, the difference was not significant 32 days post-infection. Analyses to test whether the generation of antigen-specific CD8 T cell responses was impaired in CD28KO mice, leading to less robust control of latency than in wild-type mice, revealed fewer total numbers of CD8 tells isolated from CD28KO mice than from wild-type mice and a significantly lower percent of CD8 T cells expressing the immunodominant T cell receptor in CD28 KO mice (30 vs. 50 %) at day 30. Data thus far suggest that the primary mechanism for "spontaneous" reactivation in CD28KO mice reflects an inefficient expansion and migration of antigen-specific CD8 cells to the TG.

Moriah Szpara, *The Pennsylvania State University*, presented on genome-wide surveillance of HSV-1 from natural virus shedding. Both HSV-1 and HSV-2 cause recurrent epithelial and genital infections, with the latent viral reservoir in ganglionic neurons providing a source of lifelong reactivation and shedding. The epidemiology of genital herpes has undergone a significant transformation over the past 2 decades, including the emergence of HSV-1 as a leading cause of first-episode genital herpes in certain populations. This shift in disease presentation provides a unique opportunity to study viral adaptation in action. Because viral genome sequencing can reveal genetic features that underlie HSV tropism and virulence in an unbiased way, total DNA from oral and genital swabs was sequenced using Illumina high-throughput sequencing technology. Taxonomic diversity in these samples was addressed by computational enrichment for HSV-specific sequences. HSV-1 genomes were assembled from 4 of 6 samples, correlating with the amount of HSV-specific sequence data obtained for each sample. This underscores the need to develop enrichment methods for naturally shed HSV DNA, to enable viral genome sequencing from low copy-number samples. This will be critical for genome-wide surveillance during asymptomatic shedding or at early time points after

reactivation. Phylogenetic comparison of these uncultured HSV-1 genomes revealed a significant diversity. Sequence analysis and comparative genomics are underway to elucidate unique features of these first uncultured HSV-1 genome isolates. The ability to sequence genomes directly from naturally shed virus will enable observation of viral adaptation to new hosts and new body niches and provide insights on viral flexibility in the face of selective pressures.

Richard Thompson, *University of Cincinnati College of Medicine*, showed that long-term periodic reactivation stress results in recurrent and progressive eye disease in mice. HSV-induced stromal keratitis (HSK) is a leading cause of infectious blindness in the USA. Both virus and host genetics contribute to the risk of loss of visual acuity. HSK develops during primary infection of mouse eyes in susceptible strains. While this also can occur in humans, human HSK is more common after one or more recurrent ocular disease episodes. There is some evidence that the mechanisms resulting in HSK differ between primary and recurrent disease. In a collaborative study with NASA, male C57B6 mice were infected with HSV-1 strain 17syn+ or mock-infected and at 30 dpi, infected and mock-infected groups were exposed to reactivation stress twice per month or left unstressed. At 120 dpi, mice with healthy eyes (more than half of those initially infected) were selected for further study. All groups were examined for signs of eye disease before and for several days after reactivation stresses. Corneal clouding was observed in some mice at 1 2 days after stress, with patterns of appearance and resolution as well as progression to severe outcomes observed over the next 120 days. Disease developed in two eyes in the infected unstressed group (N=30), a number significantly lower than the 12/29 observed in the stressed group. No disease developed at any time pi in mice infected with virus mutants selectively defective for replication in TG, even in highly susceptible mouse strains. Thus, a viral "round-trip" (eye to TG to eye) is required for all HSK development. This study demonstrates that like humans, mice can develop eye disease months after primary infection and that the frequency of disease development is linked to the frequency of reactivation stress exposure.

Angus Wilson, *New York University School of Medicine*, described the establishment of an interferon (IFN)-resistant state preceding the onset of HSV-1 productive replication during reactivation from latency. HSV-1 becomes latent in ganglionic neurons and reactivates in response to stress. Recent work using a cultured neuron model of HSV latency has shown that accumulation of viral mRNA during reactivation occurs in two discrete phases. Phase I involves widespread derepression of viral lytic genes, independent of protein synthesis and viral DNA replication, whereas phase II involves sequential transcription of lytic genes that is dependent upon viral proteins and results in production of infectious virus. Although there is strong evidence that innate immune defenses such as type I and II IFNs limit HSV-1 reactivation, exactly how and when they act remains poorly understood. Analysis using a primary neuronal culture model of latency in which reactivation can be induced by inhibiting mTORC1 signaling showed that both IFNβ and IFNγ selectively prevented phase I transcription in a neuron-autonomous manner. IFN treatment did not detectably activate mTORC1 to override the reactivation-inducing signal, but instead prevented initiation of transcription from previously silent lytic genes. Interestingly, addition of IFN after phase I had begun did not prevent phase II from proceeding, suggesting that after a certain time during reactivation, viral gene products can counteract the actions of IFN. Indeed, advanced delivery of ICP0 allowed HSV-1 to reactivate effectively in the presence of IFNγ, but could not neutralize IFNβ. These findings show that in latently infected neuron cultures, type I and II IFNs block HSV-1 reactivation at a very early stage by preventing the first wave of transcription from latent viral episomes and that a key function of phase I rests in providing viral factors that antagonize intrinsic defenses to allow reactivation.

Acknowledgments Funding for this conference was provided, in part, by Public Health Service grant AG04651 (R.C.) from the National Institute on Aging, National Institutes of Health. We are grateful for financial support from Biomedical Solutions, Inc., Cellular Dynamics International, Fisher Scientific, Genewiz, Inc., Integrated DNA Technology, Light Labs, Miltenyi Biotech, Inc., PerkinElmer, Phoenix Biosystems, Rocky Mountain SIMS and Tivoli Distributing Company, The VZV Foundation, and from Don Gilden. We especially thank the owners of The Christiania Lodge at Vail. We thank Marina Hoffman for the editorial assistance.

Compliance with ethical standards

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