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MEETING REPORT

Colorado alphaherpesvirus latency symposium

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The inaugural symposium of the Colorado Alphaherpesvirus Latency Society (CALS) convened May 11-13, 2011 at the Christiania Lodge in Vail Colorado. Human alphaherpesviruses include herpes simplex virus (HSV) types 1 and 2 and varicella zoster virus (VZV) and are characterized by their ability to establish latency in cranial nerve ganglia (HSV-1 and VZV), dorsal root ganglia (VZV), sacral ganglia (VZV and HSV-2) and autonomic ganglia (VZV). Along with these neurotropic human pathogens, alphaherpesviruses are significant pathogens of nonhuman primates (simian varicella virus) and domestic animals (bovine herpesvirus and Marek's disease virus). The CALS mission is to convene researchers active in alphaherpesvirus latency to discuss current advances in a relaxed venue. The mission was a success on both fronts: 47 senior researchers traveled over 79,000 miles to discuss their ongoing work concerning all facets of alphaherpesvirus latency in a quiet town in the heart of Colorado's Rocky Mountains. The CALS symposium was the first meeting specifically dedicated to understanding alphaherpesvirus latency. The participants (Fig. 1) account for more than 2600 PubMed cited publications in the field. The success of this first symposium promises that futures meetings will be of the same high caliber. A brief summary of each presentation follows.

Reports

Don Gilden at the University of Colorado School of Medicine emphasized the importance of conducting basic molecular virological studies of alphaherpesvirus latency. A more complete understanding of the physical state of viral nucleic acid and virus gene expression in latently infected ganglia will lead to functional hypotheses designed to prevent reactivation, which, in turn may lead to serious neurological disease. Reactivation of HSV-1 produces encephalitis, while recurrent meningitis is the most common neurological disorder resulting from HSV-2 reactivation. Reactivation of VZV from latently infected ganglia produces multiple neurological diseases (zoster, postherpetic neuralgia, vasculopathy, meningoencephalitis, cerebellitis, and myelitis) as well as various ocular disorders, the most common of which is retinal necrosis.

Charles Grose at the University of Iowa School of Medicine provided a historical introduction about the evolution of herpesviruses. Primordial herpesviruses may have arisen 400 mya. The beta and gamma herpesviruses arose 300 mya. These geologic times indicate that many herpesviruses evolved when our planet consisted of one supercontinent called Pangaea. Alphaherpesviruses are the most recent, originating around 100 mya. Thus, it is likely that VZV originated in primates in Africa. Dr. Grose also discussed the velocity of VZV based on calculations in a boy with sciatic nerve distribution zoster. He first complained of severe left low back pain 6 days before rash appeared on the ipsilateral great toe. Based on an estimated 80-cm length of his sciatic nerve, VZV traveled just over 13 cm per day, an anterograde velocity of 1.5 μm/s. Prior laboratory studies in an animal model demonstrated that the anterograde velocity of the closely related porcine pseudorabies virus is 1.9 μm/s. The two highly correlative studies reveal that the velocity of alphaherpesvirus along a sensory axon is 1.5 to 1.9 μm/s.

Greg Smith at Northwestern University School of Medicine summarized previous findings of HSV-1 and PRV retrograde transport in axons of cultured neurons. The implication of HSV-1 sending down a small proportion of VP11/12 and VP16 to neural somas was raised. The presentation next focused on the mechanism of retrograde

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Fig. 1 1, Barbara Hill; 2, Linda van Dyk; 3, David Koelle; 4, Benedikt Kaufer; 5, John Blaho; 6, Scott Schmid; 7, David Bloom; 8, Tobias Derfuss; 9, Nigel Fraser; 10, Dale Carpenter; 11, Marius Birlea; 12, Jim Hill; 13, Georges Verjans; 14, Werner Ouwendijk; 15, Clinton Jones; 16, Bernard Roizman; 17, Monique van Velzen; 18, Kip Kinchington; 19, Joel Rovnak; 20, Caroline Kulesza; 21, Nicole Giordani; 22, Stephanie James; 23, Jason Chen; 24, Christopher Abraham; 25, Charles Grose; 26, Robert Hendricks; 27, Andrea Bertke; 28, Ravi Mahalingam; 29, Stacey Efstathiou; 30, Peter Kennedy; 31, Vicki Traina-Dorge; 32, Randy Cohrs; 33, Marieke Verweij; 34, Sandy Weiss; 35, Leigh Zerboni; 36, Ann Arvin; 37, Igor Jurak; 38, Richard Thompson; 39, Nancy Sawtell; 40, Don Gilden; 41, Maria Nagel; 42, Subbiah Pugazhenthi; 43, Michael Gershon; 44, Anne Gershon; 45, Greg Smith; 46, Chandrasen Soans; (Ilhem Messaoudi, Christine Meyer; not pictured)

transport. The large tegument protein, VP1/2, was shown to be sufficient to engage microtubule transport in cells after transient transfection. A PRV mutant encoding an in-frame deletion in most of the proline-rich region had decreased transport kinetics, as measured by single-capsid tracking by time-lapse fluorescence microscopy. Using a new assay in which mutant and wild-type viruses are allowed to race to neural soma during co-infection, the VP1/2 mutant virus consistently arrived at the soma and nuclear rim with delayed kinetics relative to PRV encoding wild-type VP1/2 protein. Together, these results document the first mutant, perhaps of any virus, that has a measurable defect in retrograde transport as a result of weakened dynein recruitment.

Igor Jurak at Harvard Medical School presented data on the role of HSV microRNAs in latency. Several HSV microRNAs are expressed more abundantly during latent infection than during lytic infection. Using both wild-type and latency-associated transcript (LAT) mutant viruses, expression of HSV-1 miR-HI–miR-H6 during lytic infection, as well as in acute and latent ganglionic infection, was quantified. In lytically infected cells and in acutely infected ganglia, expression of miR-H2–H6 was weak and was not significantly affected by a 1.8-kbp deletion that included the LAT promoter. In contrast, in latently infected mouse ganglia, the microRNAs accumulated to $>10^2$ molecules/viral genome, and deletions of the LAT promoter markedly reduced expression of LAT-encoded microRNAs and also miR-H6, which is encoded upstream of LAT and can repress expression of ICP4. The primary RNA transcript from which miR-H6 is processed is unknown. It is possible that: (1) its expression is driven by the LAT promoter, in which case that promoter would be bi-directional; (2) it is also possible that the promoter is different than that for LAT, but the LAT deletion somehow affects expression of miR-H6, or (3) that products of the LAT transcriptional unit are necessary for miR-H6 expression. Because LAT mutants establish and reactivate from latency, our results argue that miR-H2–H6 are not essential for latency, at least in mouse trigeminal ganglia. Studies of HSV-1 mutants in which miRNAs are inactivated are in progress.

Tobias Derfuss at the Unversity of Basel in Switzerland pointed out that HSV-1 latency is accompanied by a local immune cell infiltrate, whereas VZV latency seems to be controlled by lymphocytes in the periphery since inflammatory infiltrates are not seen in ganglia latently infected with VZV. Furthermore, HSV-1 encoded miRNAs, which are expressed in latently infected human neurons, can block transcription of IE genes. Thus, both the immune system and viral miRNAs may cooperate to maintain HSV-1 latency.

Linda van Dyk at the University of Colorado School of Medicine noted that all herpesviruses share conserved coding genes that are highly organized in the genome as well as unique genes which differ among subfamilies and specific viruses. She proposed that this paradigm of both shared and unique functions among herpesviruses extends to herpesvirus-encoded miRNAs. She showed that viral miRNAs of gammaherpesvirus-68 are unique in their biogenesis, in that they are polymerase III transcripts that are processed in a drosha-independent manner. Yet, these uniquely generated miRNAs confer miRNA-mediated translation inhibition during virus infection. Additionally, characterization of target genes revealed that gammaherpesvirus-68 miRNAs regulate both viral and cellular genes in a

sequence-specific manner during virus infection. Finally, despite any lack of sequence conservation among herpesvirus miRNAs, significant conservation of cellular target genes and pathways unique to gammaherpesviruses could be distinguished from those that are shared by other herpesvirus subfamilies.

Richard Thompson at the University of Cincinnati School of Medicine presented findings that demonstrate HSV-1 first enters the latent transcriptional program in neurons infected from the body surface. He and his collaborator, Nancy Sawtell, employed a series of viral promoter/LacZ reporter viruses combined with whole ganglion histochemistry and immunohistochemical staining for viral proteins to examine viral promoter activity at the earliest stages of infection. The latency-associated transcript promoter was expressed first in neurons at 18–20 h post infection, and lytic phase promoters from the ICP0 and VP16 genes were first expressed about 14 h later (32–36 h pi). Early transcription from the LAT locus was biologically important. In the absence of LAT locus transcription, a higher proportion of neurons exited the latent transcriptional program at early times pi.

Nigel Fraser at the University of Pennsylvania School of Medicine presented work on genome-wide nucleosome mapping for HSV-1 and its role in viral gene expression. The HSV virion does not contain histones. Yet, the viral genome acquires some histone within 1 h after entering the infected cell nucleus. Nucleosomes accumulate on the viral genome peaking around 6 h, after which the ratio of nucleosomes to viral DNA diminishes. Partial micrococcal nuclease (MNase) digestion does not yield a classic ladder band pattern, although complete digestion does reveal a mononucleosome band. This strongly suggests that some nucleosomes are present on the viral genome during acute infection, but are insufficient to generate a ladder on partial Mnase digestion. Where, then, are the nucleosomes? Using a microarray designed with 50 mers overlapping by 25 bp that cover the entire 152-kb viral genome, Dr. Fraser showed the first snapshot of HSV-1 genome-wide nucleosome mapping at a time when viral replication was well-established (6 h after infection). After Cy 3 labeling, the arrays were probed with fragments of DNA protected from MNase digestion by nucleosomes. The data which was normalized for hybridization efficiency showed that the density of nucleosome signal was located at preferred positions on the genome; some positions were never occupied or were occupied at a frequency below the level of detection, revealing that histone deposition is not random. Occupancy of only a fraction of the available sites may explain the lack of a MNase partial digestion band ladder for HSV DNA during acute infection. DNA encoding early and late genes had a higher density of nucleosomes than immediate early genes. This difference in nucleosomal occupancy may be related to the tightly regulated and sequentially ordered cascade of viral gene expression.

David Bloom at the University of Florida School of Medicine presented studies on the regulation of heterochromatin formation on the HSV-1 genome by the HSV-1 LAT and its role in establishment and maintenance of latency. He showed that epigenetic modifications of chromatin associated with the latent HSV genome play a key role in the transcriptional control of lytic genes during latency. First, facultative heterochromatin marked by trimethyl H3K27 and histone variant macroH2A is present on the latent HSV genome. In addition, the polycomb-group protein Bmi1, a member of the PRC1 maintenance complex, associates with specific sites in the latent genome; thus, recruitment of PRC to the HSV-1 genome and subsequent deposition of repressive trimethyl H3K27 likely dampens lytic viral gene repression during latency. Dr. Bloom also compared 17ΔPst (an HSV-1 recombinant with a deletion in the LAT core promoter) with the reconstituted virus (LAT core promoter reinserted in 17ΔPst) as well as with wild-type HSV-1. He showed that reduced reactivation of 17ΔPst was associated with a dramatic increase in trimethyl H3K27 and reduced transcription of lytic viral genes. These data reveal that a less repressed heterochromatic state results in more efficient reactivation. Overall, LAT expression during latency modulates the physical state of the latent HSV-1 genome.

Clinton Jones at the University of Nebraska presented findings on cellular factors that are induced by a single intravenous injection of dexamethasone (DEX) in ganglia latently infected with bovine herpesvirus 1 (BHV-1). A commercially available Bovine Gene Chip was used to compare cellular gene expression in trigeminal ganglia of calves latently infected with BHV-1 with and without DEX treatment. Three hours after DEX treatment, 11 cellular genes were induced more than ten-fold. Two transcription factors, promyelocytic leukemia zinc finger (PLZF) and Slug, were induced more than 15-fold. PLZF or Slug stimulated productive infection 20-fold and 5-fold, respectively, and Slug stimulated the late BHV-1 glycoprotein C promoter more than ten-fold. Additional DEX-induced transcription factors also stimulated productive infection and some viral promoters. Importantly, because cellular transcription factors preceded lytic cycle viral gene expression by 3 h, it is likely that DEX-induced cellular transcription factors stimulated lytic cycle viral gene expression in a subset of latently infected neurons.

Nancy Sawtell at the University of Cincinnati School of Medicine stated that HSV-1 reactivates from ~0.03% of latently infected neurons. The HSV-1 tegument and transactivating protein, VP16, is critical for the earliest stage of reactivation in vivo. Viral mutants in which the interaction of VP16 with Oct-1 is disrupted, fail to exit latency,

indicating that this interaction plays a critical role early in reactivation. Importantly, expressing either VP16 or ICP0 behind the latency-associated promoter revealed that VP16, but not ICP0 is sufficient to initiate the viral lytic cycle in ganglia, suggesting that VP16 is unique in this role.

Bernard Roizman at the University of Chicago School of Medicine posed a fundamental question regarding HSV latency. Why during latency are only latency-associated transcripts (LAT) and miRNAs expressed and the neuron survives, whereas in non-neuronal cells, HSV replicates and kills the infected cells. Current evidence indicates that in productive infection, there are two checkpoints. The first is at activation of α genes and requires a viral protein (VP16) that recruits HCF-1, Oct1, and LSD1 to demethylate histones and initiate transcription. The second checkpoint involves activation of β and γ genes. An α protein, ICP0, activates transcription by displacing HDAC1 or 2 from the HDAC/CoREST/LSD1/REST repressor complex from its DNA binding sites. Current data suggest that in ganglia, VP16 and HCF-1 are not translocated to the neuronal nucleus, and the HDAC/CoREST/LSD1/REST complex is not suppressed—a first step in silencing the viral genome and establishment of heterochromatin. The viral genome remains in a state of equilibrium with respect to viral gene expression. The function of both LAT and the micro RNAs is to silence low level expression of viral genes that could reactivate the latent genome.

Stacey Efstathiou at the University of Cambridge in the UK described a reporter mouse model system to examine HSV promoter activation in vivo. This system utilizes recombinant viruses expressing Cre-recombinase under the control of viral promoters of distinct kinetic class and the reporter mouse strain Rosa26R. The viral promoter driven Cre-recombinase mediates a permanent genetic change of the host resulting in reporter gene (LacZ) activation and permanent marking of latently infected cells. Using this approach, a stable population (10–30%) of latently infected neurons was identified that experience and survive transient activation of immediate early promoters. Thus, failure to activate IE ICP0 and ICP4 promoters after neuronal infection is not a pre-requisite to establish latency. Of potential significance is the identification of a small but stable reservoir of latently infected neurons that have experienced and survived late viral promoter activation.

Andrea Bertke at the University of California at San Francisco presented data on HSV LAT regulation of productive infection in specific types of neurons in mouse trigeminal ganglia (TG). The neuronal subtype that supports latent infection of HSV-1 (A5+ neurons) or HSV-2 (KH10+ neurons) in vivo is non-permissive for productive infection in vitro in dissociated adult TG neurons. Non-permissive infection is regulated in part by LAT. To determine the mechanisms regulating productive infection in vitro, cultured adult TG neurons were infected with HSV-1 LAT deletion viruses, all of which productively infected KH10+ but not A5+ neurons, similar to wild-type HSV1. In contrast, deletions in the HSV-2 LAT region altered the ability of HSV-2 to productively infect A5+ and KH10+ neurons, indicating that HSV-2 contains LAT regulatory elements necessary for productive infection in A5+ neurons and non-permissive infection in KH10+ neurons. Chimeric viruses in which portions of the LAT region were swapped between HSV-1 and HSV-2 showed that the HSV-2 LAT element that promotes productive infection in A5+ neurons is a cis-acting sequence, and that the inhibitory regulatory element for KH10+ neurons requires additional trans-acting factor(s) from outside the HSV-2 LAT region. Overall, HSV-2 infection of neurons in vitro is regulated by both stimulatory and inhibitory sequences in the LAT region.

Christine Meyer at the Oregon Health and Science University presented data on the simian varicella virus (SVV) transcriptome during acute infection in bronchial alveolar lavage (BAL) cells and in latently infected ganglia of rhesus macaques. During acute infection, transcripts from all SVV open reading frames (ORFs) were detected; those involved in virus replication and assembly such as transactivators (ORF 63) and structural proteins (ORFs 23, 41) were the most abundant. In contrast, latent SVV gene expression was highly restricted; ORF 61, a viral transactivator and latency-associated transcript, was the most prevalent. Age and immune status impacted viral gene expression in latently-infected ganglia. Aged rhesus macaques displayed a “leaky” profile with the detection of 14 additional transcripts, mainly in the core region of the genome, not found in adult rhesus macaques. CD4 depletion of young rhesus macaques resulted in a viral transcription profile that paralleled acute replication in BALs with the detection of 47 out of 69 ORFs, although at lower copy numbers. A detailed understanding of the molecular biology of SVV will aid the studies of VZV pathogenesis, antiviral therapy, and vaccines.

Michael Gershon at Columbia University discussed the association of VZV with gastrointestinal disorders, including chronic intestinal pseudoobstruction (Ogilvie's syndrome). VZV DNA was detected in 77% of archived paraffin sections of human intestine from 30 patients with multiple gastrointestinal diseases, and VZV-specific transcripts were found in small and large intestines removed at surgery from 21/24 (88%) humans. VZV-specific DNA and RNA were also detected in gut resected from six out of six children with a history of varicella and in six out of seven of vaccinated children, but not in any of three control children less than 1 year old with no history of varicella or who had not been vaccinated. These data suggest that exposure to VZV by natural infection or vaccination leads to VZV latency in the enteric nervous system (ENS). Evidence

from tracing studies and after intravenous inoculation of VZV-infected human or guinea pig lymphocytes into guinea pigs suggested that cutaneous VZV can reach the ENS via dorsal root ganglionic neurons that project to skin and the gut; alternatively, the ENS may become infected by hematogenous spread of virus. After delivery via either route, VZV becomes latent in enteric neurons, but not in glia. The causes and consequences of reactivation of VZV in the ENS ("enteric zoster") require further study.

Subbiah Pughazenthi at the University of Colorado School of Medicine developed an in vitro model to study the VZV–host relationship in neurons. Human neural stem cells were induced to differentiate in the presence of neurotrophic growth factors and retinoic acid. After differentiation, neurons constituted approximately 90% of the cell population as shown by immunofluorescent staining for markers of neurons (MAP2a and β-tubulin), astrocytes (GFAP) and oligodendrocytes (CNPase). These neurons were maintained in culture for up to 8 weeks. No cytopathic effect (CPE) developed in neurons infected with cell-free VZV (Zostavax vaccine) compared to human fetal lung fibroblasts infected with VZV. VZV DNA, virus-specific transcripts (ORFs 21, 29, 62, and 63) were detected in infected neurons. Dual immunofluorescence staining revealed the presence of VZV IE63 and gE exclusively in healthy-appearing neurons, but not in astrocytes. Neither the tissue culture medium nor a homogenate prepared from VZV-infected neurons produced a CPE in fibroblasts. VZV induced apoptosis in fibroblasts, as shown by activation of caspase-3 and by TUNEL staining, but not in neurons. This model provides a unique in vitro system to study the VZV-neuronal relationship.

Paul Kinchington at the University of Pittsburgh School of Medicine is studying VZV-induced allodynia (pain elicited by a light touch to the skin in zoster patients) in a rat model. Swiss Webster rats inoculated in the footpad with 10^5 VZV-infected cells developed a prolonged (8–12 weeks) hypersensitivity to mechanical stimuli as measured by Von Frey filament hair stimulation. Both allodynia and immune infiltrates in ganglia peaked at 4–6 weeks. Mutant VZV lacking two viral protein kinases did not induce allodynia. Real-time PCR analysis revealed approximately 1–2,000 copies of VZV DNA per ganglion after infection with wild-type VZV in contrast to the absence of amplifiable VZV DNA in ganglia after infection with mutant VZV lacking viral kinase genes. These early results suggest that kinases are required for delivery of VZV from the periphery to ganglia and efficient neuronal infection.

Benedikt Kaufer Institut für Virologie, Freie Universität, Berlin presented data on the ability of two lymphotropic herpesviruses, human herpesvirus 6 (HHV-6), and Marek's disease virus (MDV), to integrate their genomes into host telomeres. Intriguingly, HHV-6, MDV, and other herpesviruses harbor telomeric repeats identical to host telomere sequences $(TTAGGG)_n$ at either end of the linear viral genomes. To investigate the role of the herpesvirus telomeric repeats, MDV, an alphaherpesvirus that infects chickens and serves as a natural virus-host model for herpesvirus-induced lymphomagenesis, repeat sequences were mutated or the hexameric repeats were deleted. Compared to parental and revertant viruses, absence of the viral telomeric repeats significantly decreased mortality and tumor incidence in infected chickens. Tumor cells derived from animals infected with telomere mutant viruses contained only a single integration site that was not located in host telomeres. Furthermore, reactivation of the mutant viruses from tumor cells was significantly less efficient when compared to parental and revertant viruses from corresponding tumor cells. This is the first evidence that MDV telomeric repeats, and possibly telomeric repeats of other herpesviruses (e.g., HHV-6 and HHV-7), mediate integration into host telomeres that allows maintenance of the virus genome and crucially contributes to transformation, tumor formation, and reactivation.

Dale Carpenter at the University of California, Irvine summarized that during latency, the HSV genome is generally silent. The only RNA transcripts detectable at appreciable levels are the LATs. During lytic infection, viral gene expression occurs in a temporal fashion with immediate early (IE) genes controlling the expression of the early (E) and late (L) genes. Additionally, during the lytic cycle, the IE protein ICP4 binds directly to the transcriptional start site of LAT and tightly downregulates LAT expression. In addition, LAT− mutants reactivate inefficiently. Thus, a long-speculated hypothesis is that LAT suppresses HSV IE gene expression which favors latency. To test this hypothesis, ICP4 binding sites were removed from the LAT promoter. This should impair the ability of ICP4 to suppress LAT during lytic infection, which, in turn, should hamper lytic viral growth. However, allowing LAT expression during early acute infection did not affect viral growth in tissue culture and resulted in a virus with an enhanced virulence and reactivation phenotype in an animal model. This preliminary data leads Dr. Carpenter to hypothesize that LAT is a positive factor that enhances viral growth in the nervous system resulting in "enhanced" infection/establishment of latency in the TG and promotion of a more robust reactivation from latency. It follows then that LAT expression is not constitutive during latency and may be induced by stress to facilitate reactivation of the virus from the latent neuron. There are also implications that a viruses' ability to downregulate LAT expression during acute infection may be a factor in encephalitis.

Leigh Zerboni and Ann Arvin at Stanford University School of Medicine presented data on HSV-1 replication in human dorsal root ganglion (DRG) xenografts maintained in immunodeficient mice. Live bioluminescent imaging was performed every 3–5 days on HSV-infected DRG xeno-

grafts in SCID mice for 60 days. Despite absence of adaptive immune responses, SCID mice exhibited little HSV-related morbidity, and viral replication was localized to the xenograft. The intensity of the bioluminescent signal was significantly less than previously observed in DRG infected with VZV expressing luciferase. Histological analysis revealed that in contrast to the rapid cytolytic spread of VZV in DRG, HSV-1 spread more slowly, with little cytopathic effect. Satellite cell tropism and fusion of neuronal and satellite cell membranes characteristic of VZV neuronal infection were not observed. Initially, many HSV-1 infected neurons expressed either LAT or lytic viral proteins, but as infection progressed, dual LAT/antigen positive neurons were seen. These results demonstrate that (1) initial HSV gene silencing in human neurons does not require an adaptive immune response, (2) HSV-1 replication and spread within the human ganglion is less robust than for VZV, and (3) the capacity for fusogenic spread and satellite cell tropism are pathogenic mechanisms that are unique to VZV and not shared among the other human alphaherpesviruses.

John Blaho at the City University of New York presented data on the ability of the HSV tegument protein to prevent human dendritic cell (DC) activation. Initially, the kinetics of HSV replication in human DCs was measured by qPCR analyses of representative HSV genes. Release of pro-inflammatory cytokines by conventional DC (cDC) during HSV infection was triggered by both virus replication-dependent and replication-independent pathways. Removal of the HSV virion host shut-off (vhs) protein revealed a block to DC activation during wild-type HSV infection. Using cDCs isolated from mice deficient in both the toll-like receptor (TLR) adaptor proteins MyD88 and TLR3, wild-type HSV, and vhs-deleted virus activated cDCs independent of TLR signaling, showing for the first time that a viral protein contained with the HSV-1 tegument can block induction of DC activation by TLR-independent pathways.

David Koelle at the University of Washington School of Medicine, in collaboration with Georges Verjans' group at Erasmus University, used novel cell enrichment and T-cell query tools based on cross-presentation, activation marker-based cell sorting, and panels of artificial antigen presenting cells to conduct an ORFome-wide screen of CD8 and CD4 T-cell responses to HSV-1. Dr. Koelle presented the blood-based studies, and the Verjans laboratory presented work based on ocular and neural samples. In blood, the average person has CD8 responses to 17 HSV-1 ORFs and CD4 responses to 23 HSV-1 ORFs. The responses cluster by HLA alleles and some were detected in unmanipulated peripheral blood cells. Many novel HSV-1 T-cell epitopes were discovered which are useful for making fluorescent tetramers for blood- and tissue-based studies. Novel ORFs that may be rational for next-generation HSV subunit vaccines were discussed.

Robert Hendricks at the University of Pittsburgh School of Medicine presented data that identified HSV-1 specific epitopes recognized by $CD8^+$ T cells in trigeminal ganglia (TG) of latently infected C57BL/6 mice. In TGs, 50% of $CD8^+$ T cells recognized a single dominant linear epitope on glycoprotein B (gB), while the other 50% recognized one previously defined and 17 newly identified epitopes. Approximately 80% of HSV-specific $CD8^+$ T cells were directed against epitopes on viral proteins that are expressed before HSV-1 DNA synthesis. In HSV-1 infected TG, $CD8^+$ T-cells specific for the dominant gB epitope exhibit increased functionality during latency, whereas those specific for less prevalent epitopes showed a dramatic loss of function indicative of T-cell exhaustion. Depletion of $CD8^+$ T cells from latently infected ganglia that recognized the immunodominant gB epitope lead to reactivation as indicated by a significantly increased viral genome copy number. The findings suggest that targeting $CD8^+$ T cells specific for gB might be a good vaccine strategy to prevent HSV-1 reactivation.

lhem Messaoudi at the Oregon Health and Science University presented data on the role of CD4, CD8, and B cell (CD20) responses after primary SVV infection. CD20 cell depletion led to a significant decrease in the antibody response, but did not affect the severity of varicella or the T-cell response. Loss of CD8 T cells was associated with slightly higher peak viral loads. In contrast, CD4 depletion resulted in higher viral loads and disseminated varicella. Overall, CD4 T cells play a critical role in the control of primary SVV infection that is distinct from their helper function.

Georges Verjans, Werner Ouwendijk and Monique van Velzen at the Erasmus Medical Center in Rotterdam, Netherlands presented new data on their studies of HSV-1 and VZV latency in human trigeminal ganglia (TG) obtained within 6 h after death. In situ analysis revealed that IE expression in neurons was rare and dependent on co-expression of IE62. VZV proteins accumulated in large cytoplasmatic vacuoles interconnected via small channel-like structures. CD8 T cells infiltrating human TG recognized a limited number of HSV-1 proteins and localized close to infected neurons. Sequence analyses of the viral gene encoding the acyclovir (ACV)-converting enzyme thymidine kinase (TK) of latent HSV-1 isolates showed that human TG contain genetically related HSV-1 strains encompassing ACV-sensitive and ACV-resistant viruses.

Ravi Mahalingam at the University of Colorado School of Medicine presented findings on reactivation of SVV in latently infected immunosuppressed monkeys. SVV infection of primates closely parallels human varicella zoster virus infection. After becoming latent in ganglia, SVV reactivates upon social and environmental stress and after experimental immunosuppression. Two models of SVV latency have been

established: the first is natural SVV infection of seronegative monkeys by exposure to monkeys that had been inoculated intratracheally; the second is by intrabronchial inoculation of rhesus macaques. Using the first model, latently infected monkeys were immunosuppressed with various combinations of X-irradiation and treatment with tacrolimus and prednisone. In four irradiated monkeys treated with tacrolimus and prednisone, one developed zoster 2 weeks later. In four monkeys treated with tacrolimus and prednisone alone, none developed zoster. In four monkeys treated with tacrolimus alone, three developed zoster 1–2 weeks later. In four irradiated monkeys, one developed zoster 6 weeks later. In four irradiated monkeys treated with tacrolimus, all four developed zoster 2 weeks later. Overall, the combination of irradiation and tacrolimus was optimal for SVV reactivation. $CD3^+$ T cells were not found in latently infected ganglia, but were seen around neurons in ganglia of monkeys after zoster or subclinical reactivation. CD3+ T cells did not always co-localize with SVV antigens. The phenotype and antigen specificity of infiltrating T cells in ganglia undergoing virus reactivation is being further investigated.

Jim Hill at the LSU Eye Center in New Orleans discussed the possible association of HSV-1 reactivation and Alzheimer's disease (AD). Multiple studies have documented the presence of HSV-1 DNA in human brain and in the brain of latently infected animals. The abundance of HSV-1 DNA is greatest in the hippocampus and entorhinal cortex. Brains from humans with AD exhibit oxidative damage, chronic focal inflammation, apoptosis, necrosis, and immunocytotoxicity; the same is found in animals that harbor latent HSV-1. Most humans (~95%) have antibodies against HSV-1 and are latently infected with HSV-1. Humans frequently shed HSV-1 DNA in tears and saliva. Dr. Hill hypothesized that high phenotypic reactivators of HSV-1 in brain could initiate AD. In a mouse model, infectious HSV-1 was recovered by explantation of brain tissues. In contrast, HSV-1 has not been recovered from normal brain of immunocompetent humans.