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EDITORIAL

Alphaherpesvirus latency

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Three human alphaherpesviruses, i.e., herpes simplex virus (HSV) types 1 and 2 and varicella zoster virus (VZV), cause serious diseases of the skin and nervous system. Alphaherpesviruses also cause disease in cattle (bovine herpesvirus and pseudorabies virus), and simian varicella virus (SVV) infects primates. The human alphaherpesviruses are acquired early in life, establishing a lifelong latent infection in ganglionic neurons from which virus reactivates. HSV-1 becomes latent in the cranial nerve ganglia, HSV-2 in the sacral ganglia, and VZV in the cranial nerve ganglia, dorsal root ganglia, and autonomic ganglia along the entire neuraxis. Disease produced when virus reactivates is usually more serious than that encountered during primary infection.

The alphaherpesviruses are readily propagated in multiple cell lines in tissue culture, and there are well-established animal models to study virus pathogenesis and latency: mice and rabbits for HSV-1 and -2, and SVV infection of primates which parallels the biology of human VZV infection. However, the molecular events that lead to virus reactivation remain to be identified. The Colorado Alphaherpesvirus Latency Society (CALS) was formed to offer a venue for clinicians and scientists actively studying alphaherpesvirus latency to present and discuss current and future work in the field. We are grateful to the authors who submitted current research articles or concise reviews that were generated from the inaugural meeting of the CALS on May 11–13, 2011. We are particularly grateful to Dr. Kamel Khalili for his support and guidance throughout this endeavor.

For nearly 100 years, it has been known that sunlight and trauma to the mucosa and skin on the face trigger HSV-1 reactivation. The molecular events that underlie HSV-1 reactivation are under intense study by numerous laboratories. In this issue, Roizman and colleagues pose a fundamental question in alphaherpesvirus latency: why does HSV-1 rapidly destroy nonneuronal cells while becoming latent in ganglionic neurons? Current work indicates two checkpoints that determine the outcome after virus infection. The first is formation of a protein complex consisting of HSV-1 VP16 and cellular proteins HCF-1, Oct1, LSD1, and CLOCK, a histone acetyl transferase. In the nucleus, the VP16 protein complex demethylates histones, resulting in transcription of HSV-1 α genes [infected cell proteins (ICP) 0, 4, 22, 27, and 47]. In neurons, however, the checkpoint is blocked because VP16 and HCF-1 are not translocated to the neuronal nucleus. Consequently, the resident nuclear protein complex consisting of HDAC/CoREST/LSD1/

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REST remains active, which facilitates posttranslational modification of histones bound to incoming nuclear virus DNA that result in gene silencing. The second checkpoint is transcription of HSV-1β and γ genes. In permissive cells, HSV-1 ICP0 is synthesized and displaces HDAC1 or 2 from the nuclear HDAC(1/2)/CoREST/LSD1/REST protein complex, resulting in transcriptional activation of HSV-1 β and γ genes and ultimately in virus replication and cell death. In neurons, however, ICP0 is not synthesized because VP16 and HCF-1 are not translocated to the nucleus, and the HDAC/CoREST/LSD1/REST complex remains active with no transcription of HSV-1 β and γ genes. Furthermore, Sawtell and colleagues examined the novel idea that HSV-1 VP16 is not only a checkpoint for productive infection, but also for virus reactivation. In this issue, the authors extend their earlier work which showed that the exit of HSV-1 from latency requires de novo VP16 synthesis. Herein, they describe an infectious HSV-1 mutant containing a serine-to-alanine substitution in VP16 at position 375, which establishes latency in mouse trigeminal ganglia and is severely impaired in its ability to exit latency after hypothermic stress.

The role of other factors, including HSV-1 LAT, HSV-1-specific microRNA (miRNA) and CD8+ T cell responses to HSV-1 epitopes, in maintaining HSV-1 latency has been studied in latently infected trigeminal ganglia of mice and rabbits. Held and Derfuss review studies that detected the HSV-1 LAT, HSV-1 miRNAs, and CD8+ T cells in latently infected human and mouse ganglia. St. Leger and Hendricks provide a highly focused review of CD8+ T cells in human and mouse ganglia latently infected with HSV-1. Most CD8+ T cells in latently infected ganglia recognize a single seven-amino acid linear epitope in HSV-1 glycoprotein B, with the remaining T cells directed against HSV-1 proteins and able to inhibit virus reactivation in the mouse model. Since HSV-1-specific T cells in ganglia can inhibit virus reactivation, the authors suggest that the design of HSV-1-specific vaccines should consider stimulation of an HSV-1-specific CD8+ T cell population resident in latently infected ganglia.

Thompson and Sawtell showed that HSV LAT modulates the frequency of virus reactivation. In testing their hypothesis that the LAT locus quells virus reactivation, they found that repeated heat shock over a 42-week period to reactivate HSV-1 in mice latently infected with either wild-type HSV-1 or a LAT-null virus reduced the abundance of LAT-null virus DNA, but not of the latent wild-type virus. The mechanism by which LAT controls virus reactivation was further explored by Jones and colleagues in the bovine model of neurotropic alphaherpesvirus latency. Bovine herpesvirus 1 (BHV-1) is a commercially important alphaherpesvirus, which like HSV-1, becomes latent in ganglionic neurons. In latently infected trigeminal ganglia, a single BHV-1 latency-related (LR) gene transcript is readily detected. This transcript is processed into two miRNAs and a third small transcript located within the LR promoter. Mutational analysis of the LR gene identified an open reading frame, ORF2, which is unique in its lack of an initiating ATG. When the LR gene is deleted, apoptosis is increased in ganglia, and BHV-1 fails to reactivate after dexamethasone treatment. Mutational analysis localized the anti-apoptotic function of BHV-1 LR to the novel protein encoded by ORF2. ORF2 regulates transcription of virus promoters, in part, through an interaction with cellular transcription factors C/EBP-alpha, Notch1, and Notch3.

Critical to HSV-1 reactivation is the ability of virus to replicate in nondividing, terminally differentiated neurons. ICP22 is an HSV-1 immediate early regulatory protein essential for virus growth in neurons, and ICP22 deletion mutants are impaired in their ability to replicate in nondividing cells. Thus, ICP22 can be viewed as a HSV-1 neurovirulence factor. Bowles and Blaho constructed and characterized a new ICP22 truncation mutation and found that HSV-1 containing this truncated ICP22 is impaired for growth in rabbit skin cells and is located in the nucleus of infected Vero cells. This novel virus will prove useful in understanding the multiple functions of ICP22.

Unlike HSV-1, VZV reactivation is closely linked to a specific decline in cell-mediated immunity to VZV with advancing age and in immunosuppressed individuals. Zerboni and Arvin review their model of VZV neurotropism and neurovirulence in human dorsal root ganglia maintained as xenografts in SCID mice. VZV replication is rapid and efficient, resulting in syncytia comprised of satellite cell and neurons and release of infectious virus. In surviving neurons, low amounts of VZV DNA and RNA can be detected in the absence of detectable virus proteins. Mutational analysis of the infecting virus shows that VZV glycoproteins I and gE/gI interactions are critical for virus propagation in the xenografts. Chen and colleagues present their investigations of VZV in the enteric nervous system. Because VZV has been linked to gastrointestinal disorders, including severe abdominal pain that preceded fatal varicella as well as acute colonic pseudo-obstruction (Ogilvie's syndrome), the authors used PCR to examine resected bowel samples for the presence of latent VZV; indeed, wild-type VZV was detected in diseased bowel from all of six children with a past history of varicella, and vaccine strain VZV DNA was found in six of seven resected bowel samples from children who had received varicella vaccine. To determine the route(s) by which VZV might gain access to bowel tissue and become latent, guinea pigs were injected with VZV-infected human or guinea pig lymphocytes in which virus was labeled with green fluorescent protein; numerous infected enteric neurons were seen as early as 2 days after injection, indicating a hematopoietic route of infection. Additionally, VZV was detected in

neurons of both guinea pig enteric ganglia and dorsal root ganglia after intradermal inoculation of virus. Use of tracking dyes identified a small number of guinea pig ganglionic neurons that projected both to the skin and viscera. Overall, VZV infection of enteric ganglia can be blood borne or via transaxonal transport from ganglionic neurons.

A major concern of VZV reactivation is the pain known as postherpetic neuralgia (PHN) that often persists for many months to years after zoster. Depending on age, PHN occurs in 20 to more than 40% of patients with zoster. An animal model currently used to study PHN consists in monitoring withdrawal thresholds to mechanical stimuli (allodynia) or increasing temperature in Wistar rats. Intraplantar injection of cell-associated VZV reliably showed increased sensitivity to both mechanical and thermal stimuli that normalized with time. Kinchington and Goins are using this model to identify VZV genes that might be involved in the induction of pain.

Significant advances in the study of VZV pathogenesis and latency have come from analysis of monkeys infected with the simian counterpart SVV. SVV infection of rhesus macaques recapitulates the hallmarks of VZV infection, i.e., primary varicella, latency with limited gene expression, and zoster with virus reactivation. Meyer and colleagues used multiple reverse transcription-linked real-time PCR to show that all predicted SVV genes are transcribed in both bronchial alveolar lavage cells and peripheral blood mononuclear cells during acute infection of rhesus macaques. The most abundant SVV transcripts detected during acute infection corresponded to genes involved in virus DNA replication and assembly (ORFs 63, 41, and 49). Analysis of the complete SVV transcriptome in ganglia of latently infected rhesus macaques showed that ORF 61 is the most prevalent transcript, followed by ORFs A, B, 4, 10, 63, 64, 65, 66, and 68.

Overall, while all alphaherpesviruses become latent in neurons, HSV-1 latency is limited to cranial nerve ganglia, transcription is restricted to a single genetic locus, and no HSV-1 proteins have been detected. In contrast, both VZV and SVV become latent in multiple ganglia along the entire neuraxis, multiple virus genes are transcribed and some virus-specific proteins have been found. Also, in ganglia latently infected with HSV-1, virus-specific sentinel T cells and virus-encoded miRNAs work together to quell HSV reactivation, whereas T cell infiltration and virus-specific miRNA are not detected in ganglia latently infected with VZV. We anticipate that future Colorado Alphaherpesvirus Latency Society symposia will disseminate rapidly accumulating critical data and novel ideas concerning these important differences in neurotropic alphaherpesvirus latency.