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SYMPOSIUM: Herpesvirus Infections of the Nervous System

Human Herpesvirus Latency

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Herpesviruses are among the most successful human pathogens. In healthy individuals, primary infection is most often inapparent. After primary infection, the virus becomes latent in ganglia or blood mononuclear cells. Three major subfamilies of herpesviruses have been identified based on similar growth characteristics, genomic structure, and tissue predilection. Each herpesvirus has evolved its own unique ecological niche within the host that allows the maintenance of latency over the life of the individual (*e.g.* the adaptation to specific cell types in establishing latent infection and the mechanisms, including expression of different sets of genes, by which the virus remains latent). Neurotropic alphaherpesviruses become latent in dorsal root ganglia and reactivate to produce epidermal ulceration, either localized (herpes simplex types 1 and 2) or spread over several dermatomes (varicalla-zoster virus). Human cytomegalovirus, the prototype betaherpesvirus, establishes latency in bone marrow-derived myeloid progenitor cells. Reactivation of latent virus is especially serious in transplant recipients and AIDS patients. Lymphotropic gammaherpesviruses (Epstein-Barr virus) reside latent in resting B cells and reactivate to produce various neurologic complications. This review highlights the alphaherpesvirus, specifically herpes simplex virus type 1 and varicella-zoster virus, and describes the characteristics of latent infection.

Introduction

Herpesviruses are among the largest viruses in nature and have adapted very specific mechanisms not only to evade host defenses and usurp the cellular machinery in the production of progeny virions, but also to shut down their own lytic replication in the establishment of latency. Thus latency is a means by which the virus can exist for the life of the host. Reactivation from latency with concomitant virus production is an efficient means of spreading within the host population.

Based on host range, duration of replication cycle, cytopathology and characteristics of latent infection, herpesviruses have been classified into three groups (42, 71). The alphaherpesviruses have a variable host range both *in vivo* and *in vitro*, with a short replication cycle that results in a rapidly spreading infection and cell lysis. Due to the cytopathology of the virus, establishment of latently infected cell cultures with wild-type virus can be difficult. However, *in vivo* latency is readily established in ganglia. The prototype alphaherpesvirus is herpes simplex virus type-1 (HSV-1). Primary HSV-1 infection is an inapparent asymptomatic infection of the mouth and lips, after which the virus becomes latent in trigeminal and other cranial nerve ganglia. Reactivation of latent HSV-1 typically results in localized epithelial eruptions (cold sores) that usually resolve in days with few, if any, consequences (90). Other human herpesviruses of the alpha-subfamily include HSV-2 and varicella-zoster virus (VZV). Primary HSV-2 infection is either subclinical or associated with transient vesicular and ulcerative genital lesions. Reactivation of latent HSV-2 from sacral ganglia is either asymptomatic or associated with recurrent genital lesions (2). Primary VZV infection results in childhood chickenpox, after which the virus becomes latent in cranial nerve ganglia, dorsal root ganglia, and autonomic nervous system ganglia (29). Reactivation of latent VZV typically results in zoster (shingles) and may be further complicated by postherpetic neuralgia (persistent pain), myelitis, and large vessel granulomatous arteritis or small vessel encephalitis (reviewed in ref. 30).

Betaherpesviruses have a narrow host range limited to a single species or genus. *in vitro* virus can be propagated in fibroblasts originating from the natural host. The replication cycle is relatively long, showing a slowly developing focus of infection in which the cells frequently become enlarged before lysis. Establishment of latently infected cultures is not difficult. *in vivo*, virus is usually latent in secretory glands and the lymphoreticular system. Human cytomegalovirus (CMV) is the pro-

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totype betaherpesvirus. In healthy immunocompetent individuals, primary CMV infection is subclinical; however, infection can be life-threatening in the very young, the elderly or the immunocompromised. Virus is latent in peripheral blood and bone marrow-derived myeloid progenitor cells, and reactivation is most frequent after allograft transplantation (6).

The host range for the gammaherpesviruses is also limited, both *in vivo* and *in vitro*. The association of the virus with lymphocytes has been termed "restringent" (47) since the resident virus genome is partially expressed, but virus replication is arrested prior to development of infectious virions. The lytic cycle of the virus is variable, as is its cytopathology, since the predominant outcome of the virus infection is lymphocyte transformation. Primary infection with Epstein-Barr virus (EBV), the prototypical gammaherpesvirus, is typically uneventful, but may result in heterophile-positive infectious mononucleosis, nasopharyngeal carcinoma, and non-Hodgkin's lymphoma (9). Over 90% of humans harbor latent EBV in resting memory B cells. Reactivation of latent EBV has been associated with meningitis, meningoencephalitis, central nervous system (CNS) lymphoma, transverse myelitis, psychiatric disorders, and polyneuropathies (35), including autonomic neuropathy (3).

An in-depth review of each herpesvirus is beyond the scope of this article; therefore, this review focuses on the alphaherpesviruses, especially HSV-1 and VZV.

Alphaherpesvirus latent infection

Analysis of latent human alphaherpesvirus infection in the natural host is difficult since ganglia (the latently infected tissue) are not accessible in living individuals. Furthermore, ganglia most suitable for analysis must be limited to cadavers of immunocompetent individuals, without a recent history of herpesvirus reactivation and without a long delay between death and autopsy. Thus, considerable research has attempted to develop a standard animal model of herpesvirus infection, latency, and reactivation. For HSV-1, both mice and rabbits have been used. A small aliquot of infectious virus is dropped onto either unscarified (rabbit) or scarified (mouse) eyes, or inoculated subcutaneously into either animal. After recovery from transient eye disease (keratitis and retinitis), virus can be isolated from the trigeminal ganglion on the same side (76, 88). In latently infected rabbits, virus reactivates sporadically or can be induced by a single course of iontophoresis of epinephrine (21). Sporadic virus reactivation does not occur in the mouse model, but can be induced by heat shock, UV irradiation (64, 74, 86), or irritation of skin by Scotch tape. While reactivation of latent virus results in shedding of infectious virus in tear film in the rabbit model, virus recovery from latently infected mice is detected best by seeding the dissociated trigeminal ganglion cells onto indicator cells (79).

Unlike HSV-1, which has a wide *in vivo* host range, VZV is an exclusively human pathogen. Nevertheless, VZV DNA and proteins were detected in neurons up to 9 months after bilateral subcutaneous injection of cell-associated virus into rats (20, 73). After unilateral injection of cell-free VZV into rat foot pads or beneath skin around the paraspinal region, virus DNA was detectable in both ipsilateral and contralateral ganglia, suggesting that virus replication had occurred (1). Overall, VZV inoculation into rodents will lead to the presence of viral DNA in ganglia; however, there is no evidence of virus reactivation.

Configuration of latent alphaherpesvirus DNA

An initial step in the replication of herpesvirus DNA is the formation of circular molecules from the infecting linear genome. Both HSV-1 and VZV contain a single unpaired nucleotide at the terminus of the genome that may facilitate circularization before DNA replication via a rolling circle mechanism (19, 63). After DNA replication, intramolecular recombination ensues, resulting in the formation of isomers of the genome. The anatomy of the herpesvirus genome is described in more detail later; here, we note only that the virus DNA is composed of two covalently joined segments bracketed by repeat sequences. Thus, when the long and short segments of the alphaherpesvirus genome invert during synthesis, four isomeric structures are obtained.

Southern blot analysis of DNA extracted from latently infected mouse brainstem and trigeminal ganglia indicated the presence of "endless" HSV-1 DNA molecules (26, 69). No signal was detected associated with the genomic termini; however, a new segment of HSV-1 DNA was seen during latency. The novel DNA corresponded to the ends of the molecule joined together (26, 69). Cesium chloride centrifugation of DNA, extracted from latently infected mice, showed that most HSV-1 DNA is extrachromosomal (59). Overall, latent HSV-1 DNA exists as non-integrated circular molecules.

Clarke *et al.* (7) exploited the fact that the long segment of the VZV genome rarely inverts with respect to the short segment (18, 37, 46, 82) to investigate the structure of latent VZV DNA. DNA was extracted from latently infected human trigeminal and thoracic ganglia and used in quantitative polymerase chain reaction

(PCR) with oligonucleotide primers that amplify only linear or circular VZV molecules. The ratio of internal to terminal viral DNA sequences was used to determine the configuration of the latent virus genome. The average ratio in samples from 11 individuals was 1.0 (± 0.2) compared to 15.0 during productive virus infection, indicating that the ends of the VZV DNA molecule are also covalently joined during latency. The simplest interpretation is that alphaherpesvirus genomes assume a circular, episomal state during latency. Since both HSV-1 and VZV DNA are infectious when transfected into permissive cells, it is not surprising that these potentially lethal molecules are sequestered in nucleosomal structures within the neuron during latency.

Site of alphaherpesvirus DNA during latency

Identification of the cell type harboring latent virus is important, since the pathogenesis of initial infection and reactivation is likely to depend on the number and type of cells initially infected (17, 58). The trigeminal ganglion is composed of neurons and non-neuronal satellite cells, fibroblasts, and endothelial cells. The ratio of neurons to non-neuronal cells in ganglia is approximately 1 to 100 (50). *In situ* hybridization (ISH) has been used to identify the cell type harboring latent HSV-1 in ganglia. After photographic development of the overlaying silver grains, the cell type is revealed by its size and distinctive morphology. In ganglia from mice and rabbits latently infected with HSV-1, silver grains were deposited over neurons (70, 83). Further analysis of HSV-1 latency in human trigeminal ganglia revealed virus located exclusively in 0.2 to 4.3% of neurons (14, 34).

Identifying the ganglionic cell type that harbors latent VZV has been more problematic. While RNA mapping to a restricted region of the HSV-1 genome is abundantly transcribed and accumulates within the nucleus of latently infected cells, thereby offering a convenient target for ISH, this is not the case for VZV. Initially, ISH, with a radioactive DNA probe made by nick-translation of the entire VZV genome that would hybridize to any and all VZV RNA transcripts, revealed VZV exclusively in ganglionic neurons at an abundance of 0 to 0.3% of neurons from the trigeminal ganglia of five subjects (38). ISH, with a radioactive RNA probe (specific activity > 10^8 cpm/μg) transcribed from the *Sal*I-P VZV DNA fragment that would detect VZV DNA or RNA, also identified neurons as the site of latency in an analysis of 4 thoracic ganglia of a single subject (31). However, cytoplasmic ^{32}P signal was also noted in both of these studies instead of the expected predominant nuclear signal seen with HSV-1 DNA and its latency-associated transcript. There was also a significant discrepancy between the two studies in the numbers of positive neurons. Transcripts corresponding to VZV genes 29 and 62 were later detected by ISH, primarily in a small number of non-neuronal satellite cells, but not in neurons (15, 57). However, the subjectivity associated with localization of silver grains over smaller satellite cells makes these studies difficult to interpret. Using ISH to detect VZV DNA mapping to open reading frame (ORF) 54, Lungu *et al.* (53) reported a positive signal in 5-30% of both neurons and non-neuronal cells in latently infected ganglia from two individuals, and in the majority of neurons and non-neuronal cells in ganglia corresponding to the distribution of zoster from an individual with clinical reactivation. *In situ* PCR combined with ISH identified an amplified product from VZV gene 40 exclusively in neurons, albeit with some leakage of signal into the cytoplasm (25). In a well-designed and carefully controlled study in which several investigators submitted latently infected human ganglia for analysis by a single laboratory, latent VZV DNA was detected almost exclusively in the nuclei of neurons, with 2-5% of neurons and less than 0.1% of non-neuronal cells showing a positive signal by ISH alone (genes 29 and 63), by *in situ* PCR (genes 21 and 29), and by ISH PCR followed by probing (gene 29) (43). A second report from the same laboratory found 2-5% of neurons and only occasional non-neuronal cells positive for VZV DNA and RNA (genes 4, 21, 29 and 63) in normal humans (44).

Given the problems of background and interpretation inherent with *in situ* technology, we adopted a strategy using quantitative PCR analysis to study neurons and non-neuronal cells from postmortem ganglion cells sorted by size. In this protocol, human ganglia were removed at autopsy ,and cellular morphology was stabilized by fixation. Ganglia were dissociated by treatment with collagenase, and individual cells were sorted through increasingly smaller filters. This approach revealed VZV DNA primarily, if not exclusively, in neurons (49). Overall, most data indicate that VZV is latent primarily in neurons (25, 31, 38, 43, 49), analogous to other alphaherpesviruses (5, 16, 48, 61, 62, 68, 81).

The ganglionic viral DNA burden

Analysis of HSV-1 in animal models indicates that the likelihood of reactivation depends on the copy number of latent virus (52, 75). The accuracy of virus DNA quantitation depends not only on the technique used, but also on the quality of the sample. When using an animal model, conditions related to virus strain, load of inocu-

lum, duration of infection, sample removal and storage before DNA extraction can be controlled, whereas results obtained with samples of human trigeminal ganglia removed at autopsy are potentially confounded by variables beyond the control of the investigator. Using the mouse model for HSV-1 latency, it was shown that the concentrations of virus DNA (approximately 200 copies) were highest in neuronal nuclei associated with ganglia directly innervating the inoculated skin (77). This result confirmed earlier evidence that virus seeded the ganglia by retrograde movement from the skin (40, 83). However, analysis of latency is an average of all cells in the ganglion. Even in animal models, each neuron is exposed to different amounts of virus. The need for a method by which individual neuronal cells could be separately analyzed was met by contextual analysis in which latently infected murine trigeminal ganglia were dissociated and enriched for neurons or non-neuronal cells by differential centrifugation (74). Analysis of individual neurons for HSV-1 DNA by quantitative PCR showed that latent virus DNA content within individual cells ranged from <10 to >1,000 and that the virus burden directly correlated with the amount of input virus. Further investigations showed that the total number of latently infected neurons, but not the virus DNA content per neuron, is dependent on the amount of virus entering the ganglion, which in turn, depends on virus replication peripherally (85).

The lack of a satisfactory animal model for VZV latency requires analysis of human tissue. Southern blot analysis detected VZV DNA in human ganglia (32), but the amount of virus DNA present was not determined. PCR-based methods have made it possible to quantitate latent VZV DNA in human ganglia. Semi-quantitative PCR uses samples with known copy numbers of viral DNA generate a standard curve to compare with experimental samples. Drawbacks of this technique include the requirement for standard and experimental samples in separate PCR tubes and for the transfer of PCR products to nylon membranes before probing with radioactive oligonucleotides. Potential errors in quantitation rest in variations during PCR, DNA transfer and immobilization, or probing under conditions where the radiolabeled oligonucleotide is not in excess, and the need to detect the signal within a linear response. In competitive quantitative PCR, samples with known copy numbers of a mutated viral sequence are added to aliquots of an experimental sample, and the copy number is estimated from samples in which mutant and wild-type viral sequences compete equally for PCR reagents. This technique has the advantage of known and experimental samples in the same tube, but still requires transfer to membranes and subsequent probing. In addition, the mutated viral DNA fragment is cloned into a vector sequence, and this fragment may not amplify with the same efficiency as native viral DNA. Real-time quantitative PCR is the most recent advance, wherein viral DNA in samples with known and unknown copy number are assessed during the actual PCR cycling by detecting products that emit fluorescence, thus ensuring that copy numbers are quantitated during exponential amplification. Although probing is not required, this technique does require separate tubes for known and unknown samples.

Semi-quantitative PCR revealed that 10^3-10^5 copies of latent VZV DNA were present in 10^5 ganglionic cells (7), similar to the amount of latent HSV-1 found in human ganglia by Southern blot analysis (26). Using competitive quantitative PCR, Mahalingam *et al.* (56) detected 6-31 copies of the VZV genome per 10^5 cells in ganglia at all levels of the neuraxis. Semi-quantitative PCR using neurons separated from a liquid suspension of human trigeminal ganglia cells revealed 2-5 copies of VZV DNA in 4 of 20 lots of 100 neurons each; assuming 100 non-neuronal cells per neuron, these data average to approximately 5.5 copies of VZV DNA per 10^5 cells (49).

Pevenstein *et al.* (67) used real-time quantitative PCR to analyze both the HSV-1 and VZV DNA content in trigeminal ganglia from 15 humans. VZV DNA was found in 13 of 15 individuals, with a copy number of 258 ± 38 per 10^5 ganglionic cells, whereas HSV-1 DNA was detected in eight of 15 individuals at an average of 2902 ± 1082 copies per 10^5 ganglionic cells. This study was the first report of the simultaneous quantitation (by the accepted best technique) of HSV-1 and VZV DNA in latently infected human ganglia. The authors suggested that the higher frequency of HSV-1 reactivation than that of VZV might rest in its higher abundance in the ganglion. However, in the study, only one ganglion from each of the 15 individuals was used to quantitate virus DNA, and it was not possible to correlate the HSV-1 and VZV content in the same sample. Therefore, we used real-time PCR to quantitate HSV-1 and VZV DNA in both the left and right trigeminal ganglia from 17 subjects (12). Our results showed that the left and right trigeminal ganglion from the same individual did not differ significantly in the number of HSV-1 or VZV genomes, and varied per subject from 42.9 to 677.9 and from 37.0 to 3560.5 copies per 100 ng of DNA, respectively. The overall average of HSV-1 DNA and VZV DNA was 195 and 580 copies per 100 ng total DNA,

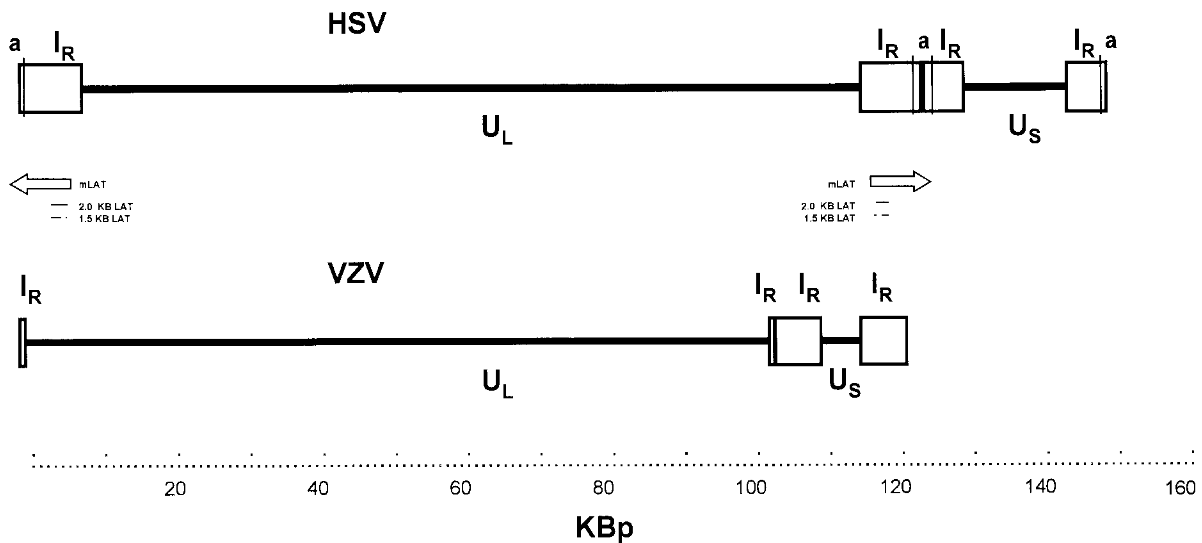

Figure 1. Linear structure of the alphaherpesvirus genome. The genomes of both HSV and VZV consist of unique long and short segments (solid lines) bracketed by inverted repeat DNA sequences (open boxes). However, the repeat region bracketing the unique long segment of HSV-1 is approximately 9 kbp larger than that of VZV. It is within this region that the unstable primary 8.5-kb HSV-1 mRNA maps (mLAT). The 2.0-kb and 1.5-kb LAT introns are spliced from the mLAT and accumulate in the nucleus of latently infected neurons. VZV DNA lacks a large segment of the homologous inverted repeat and does not encode a specific LAT. U_L, unique long; U_S, unique short; I_R, inverted repeat; a, "a" sequence repeat found in HSV-1 DNA.

respectively. Thus, while the virus burden varies widely among individuals, it does not significantly differ between the left and right side in the same person. These results suggest that, as in the mouse model of HSV-1 latency, the latent virus burden is dependent upon the severity of primary disease.

Alphaherpesvirus genes transcribed during latency

Alphaherpesviruses share similar biological features during latency, *i.e.* the cell type harboring latent virus and configuration of latent virus DNA; however, the transcriptional pattern of latent virus DNA differs. This difference could in part arise from the architecture of the HSV-1 and VZV genomes (Figure 1). Both virus DNAs are composed of two covalently joined segments, the unique long and short segments. The long segment is comprised of a unique stretch of DNA bounded at each end by repeat sequences. Similarly, the short segment contains a unique stretch of DNA bounded at the termini by a second set of inverted repeats. While the HSV-1 genome contains an additional segment present at both termini and at the junction of the short and long segments, this "a" sequence is lacking in VZV. The absence of the terminal "a" sequence in VZV might partly explain why VZV DNA has two predominant isomeric forms resulting primarily from inversion of the short segment, while HSV-1 DNA can invert both the long and short segments leading to four isomeric forms.

Both the HSV-1 and VZV DNAs have been sequenced, and computer-assisted analysis of the genome has aided in comparing the anatomy of the genome. The two viruses share a large degree of homology. Only five of the 68 ORFs in VZV do not have counterparts in the HSV-1 genome, and all but 12 of the 80 HSV-1 ORFs have counterparts in the VZV genome. The most obvious difference between the virus DNAs is that the ~9200-bp repeat flanking the unique long segment of HSV-1 DNA is only 88 bp in the VZV genome. It is possible that deletion of this segment within the VZV genome accounts for the difference in the pattern of gene transcription during latency.

A single latent HSV-1 transcriptional unit has been consistently detected in human ganglia and in animal models. The latency-associated transcript (LAT) is a series of differentially spliced, stable, non-polyadenylated RNAs that accumulate in the nuclei of latently infected neurons (62, 81, 87, 91, 92). The unprocessed 8.3-kb LAT message maps to the opposite strand encoding the immediate-early HSV-1 gene, ICPO (28, 62, 80, 89, 92). While mutations that eliminate a large portion of the HSV-1 LAT still establish and reactivate from latency (41), reduced levels of LAT transcripts have been associated with a reduced efficiency of reactivation (51, 65).

Since the LAT is the only transcribed genetic locus consistently detected during HSV-1 latency, an exhaustive search is being made to detect a function for this RNA. LAT RNA is bound to polyribosomes in latently infected murine trigeminal ganglia (33). Furthermore, dissociated neuronal cell cultures infected with HSV-1 produce an 80-kDa protein recognized by antiserum raised against the major LAT ORF (22). Thomas *et al.* (84) demonstrated that deregulation of this major LAT ORF increased the growth potential of HSV-1 ICP0-deficient mutant viruses. While these results all point to a protein encoded by LAT and its importance in virus regulation, Fareed and Spivack (27) showed that virus with defined mutations in the putative LAT ORFs did not differ from the parental wild-type virus in either the rate or frequency of reactivation. To further investigate the possibility of protein involvement in HSV-1 latency, Drolet *et al.* (23) constructed recombinants between high- and low-frequency reactivation HSV-1 and found that the efficiency of reactivation was independent of the LAT ORF. Thus, if the LAT region is involved in HSV-1 latency, the effect is probably not due to a protein encoded within the region examined. Current analysis of the LAT region indicates that a function within this locus promotes survival of the infected neuron by inhibiting apoptosis (39, 66). Overall, despite extensive investigation into the LAT locus, the mechanism by which HSV-1 latency is maintained or virus reactivation takes place is still largely unknown (4, 60).

Since the homologous region encoding HSV-1 LAT is absent in the VZV genome, it would not be surprising if the transcriptional pattern of VZV during latency differs from that of HSV-1. At least 5 VZV genes are transcribed in latently infected human ganglia (11, 44). Reverse Northern analysis revealed a transcript mapping to the *Sal*I fragment C of the VZV genome during latency (10). VZV gene 21, which maps to this region, was then detected in a cDNA library constructed from poly[A]$^+$ RNA extracted from a pool of latently infected human trigeminal ganglia (13). Meier *et al.* (57) detected VZV gene 29 and 62 transcripts in Northern blots of poly[A]$^+$ RNA extracted from hundreds of pooled human ganglia. In a cDNA library constructed from latently infected human trigeminal ganglia enriched for VZV transcripts by hybridization selection, transcripts corresponding to VZV genes 21, 29, 62 and 63 were found (11). Using ISH with and without PCR, Kennedy *et al.* (44) confirmed and extended these data by detecting transcripts from genes 4, 21, 29 and 63 in normal human ganglia. Thus, VZV genes 21, 29, 62, 63, and 4 are actively transcribed during latency; however, the abundance of these transcripts was not determined. Recently, we applied real-time PCR to the analysis of latent HSV-1 and VZV gene transcription (12). Total RNA extracted from the left or right trigeminal ganglion from 11 individuals was reverse-transcribed and cDNA corresponding to HSV-1 LAT, VZV ORF 21, 29 and 63 transcripts was quantitated. HSV-1 LAT transcripts were consistently detected in ganglia containing latent HSV-1 and varied in relative expression by >500-fold. Of the three VZV transcripts analyzed, only transcripts mapping to ORF 63 were consistently detected in latently infected ganglia and varied in relative expression by >2000-fold. Thus, it appears that VZV gene 63 transcription, like LAT transcription in HSV-1 latently infected ganglia, is a hallmark of VZV latency.

Since the VZV transcripts detected during latency are polyadenylated, it is assumed that these genes are also translated. To detect VZV ORF 63 proteins in latently infected human ganglia, rabbit antiserum raised against the purified protein (20) was used to identify the protein in neurons of latently infected human ganglia (55). Another study identified the protein products of VZV genes 4, 21, 29, 62, and 63, but not of genes 10, 14, or 67, primarily in the cytoplasm of neurons from latently infected individuals, and in both the cytoplasm and nucleus of an individual with zoster at the time of death (54). These VZV-specific proteins are normally seen in the nuclei of productively infected cells in tissue culture. The investigators speculated that the aberrant localization might provide a mechanism to maintain VZV in a latent state, namely by restricting regulatory proteins from the nucleus. The latter study awaits confirmation.

Summary

Neurotropic alphaherpesviruses are ubiquitous human pathogens that typically result in subclinical (HSV-1 and HSV-2) or limited disease (VZV). Reactivation of HSV-1 produces a discrete local epithelial eruption while the exanthem of VZV reactivation is spread over 1 to 3 dermatomes. This difference in the size of the area affected after virus reactivation has led to the proposal that HSV-1 and VZV reside in different cell types during latency (45, 78). Nonetheless, most studies, including analysis of ganglion cells sorted by size, indicate that VZV, like all other members of the alphaherpesvirus family, resides in neurons during latency.

During latent infection, both HSV-1 and VZV DNA are present in an episomal structure from which a limited region of the virus DNA is transcribed. The regulation of virus genes is critical to latency. Left unchecked,

the cascade of virus gene expression results in rapid cell death. Thus, much effort has been devoted to identifying the virus genes expressed during latency. For HSV-1, a single transcriptional unit (LAT) has been described, and current evidence suggests that LAT protects the neuron from programmed cell death. VZV lacks a homologous LAT region, but transcribes at least 5 virus genes; nevertheless, quantitative analysis of virus gene expression within individual ganglia suggests that only the ORF 63 gene is highly associated with VZV latency. The mechanism by which the approximately 70 remaining virus genes are silenced during latency is unknown. Islands of CC+GG dinucleotides have been found in the LAT regions of alphaherpesviruses, and it is possible that these structures affect the accessibility of promoter to cellular transcription factors (8).

While it has also been shown that both HSV-1 and VZV can be latent in the same ganglion (12, 67), it is not known whether they can remain latent in the same neuron. If both viruses reside within the same cell, then the question of latency must be expanded to include the interplay between the two viruses. For example, does HSV-1 LAT expression and the genes transcribed by VZV have a reciprocal effect? To recapitulate nature, future research should analyze both alphaherpesviruses within the same experimental model. Current work demonstrates that simian varicella virus in monkeys models resembles VZV infection in humans (24, 36). Since monkeys are also susceptible to HSV-1 infection and virus latency in trigeminal ganglia can be demonstrated (72), the use of SVV and HSV-1 within the same non-human primate may reveal hitherto unknown relationships between these alphaherpesviruses.

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