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Herpes simplex virus-1 and varicella-zoster virus latency in ganglia

Bradley M Mitchell,[1] David C Bloom,[2] Randall J Cohrs,[3] Donald H Gilden,[3,4] and Peter GE Kennedy[5]

[1]Cullen Eye Institute, Department of Ophthalmology and Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA; [2]Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, Florida, USA; [3]Departments of Neurology and [4]Microbiology, University of Colorado Health Sciences Center, Denver, Colorado, USA; and [5]Department of Neurology, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital NHS Trust, Glasgow, Scotland, United Kingdom

Two human alpha-herpesviruses, herpes simplex virus (HSV)-1 and varicella zoster virus (VZV), account for the most frequent and serious neurologic disease caused by any of the eight human herpesviruses. Both HSV-1 and VZV become latent in ganglia. In this review, the authors describe features of latency for these viruses, such as distribution, prevalence, abundance, and configuration of viral DNA in latently infected human ganglia, as well as transcription, translation, and cell type infected. Studies of viral latency in animal models are also discussed. For each virus, remaining questions and future studies to understand the mechanism of latency are discussed with respect to prevention of serious cutaneous, ocular, and neurologic disease produced by virus reactivation. *Journal of NeuroVirology* (2003) **9,** 194–204.

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Herpes simplex virus-1

Introduction

Herpes simplex virus (HSV) is a ubiquitous human pathogen. Primary infection is usually acquired in childhood and is most often asymptomatic, after which virus becomes latent in neurons of cranial nerve ganglia (HSV-1) or sacral ganglia (HSV-2). Here, we focus exclusively on HSV-1. By age 20 to 25 years, approximately 80% of adults in the United States are seropositive, and in many countries in Europe, Africa, and the Far East, the prevalence of antibody to HSV-1 exceeds 95% in adults age 20 to 40. Reactivation from ganglia produces cold sores or fever blisters in the mouth or on the lip, less often infections of the eye (herpes keratitis), and rarely encephalitis. Multiple vaccines have been produced based on wild-type virus, inactivated (killed) or live virus, viral subunit, or genetically engineered virus, but none has met accepted standards for licensure.

Unlike varicella zoster virus (VZV), which produces disease only in humans, HSV causes disease and becomes latent in ganglia of rabbits and mice after experimental inoculation by various routes. Further, virus can be induced to reactivate by various external stimuli. Thus, studies of HSV latency in humans have been paralleled by models of latency in different animals.

The HSV genome

HSV-1 is a double-stranded DNA virus that is 152 kb in size. The entire 152,260-bp genome has been sequenced (accession number X14112) and consists of two covalently linked components, a unique long and a unique short segment, each bracketed by inverted repeat sequences. Thus, there are four possible isomers of the viral DNA molecule. There are about 90 unique transcriptional units, at least 84 of which encode proteins. The genes have been grouped into three general kinetic classes: immediate-early, early, and late. An updated assignment of function to

Address correspondence to Randall J. Cohrs, University of Colorado Health Sciences Center, Department of Neurology, Mail Stop B182, 4200 East 9th Avenue, Denver, CO 80262, USA. E-mail: randall.cohrs@uchsc.edu

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various HSV genes as well as the structure of virions is detailed by Roizman and Knipe (2001).

Distribution and prevalence of HSV DNA in latently infected human ganglia

HSV-1 latency appears to be restricted to cranial nerve ganglia, as manifest by spontaneous, often recurrent, outbreaks of herpetic vesicles on the mouth, or by rescue of HSV-1 from explants of human trigeminal (Bastian *et al*, 1972; Baringer and Swoveland, 1973), nodose and vagal (Warren *et al*, 1978), and ciliary (Bustos and Atherton, 2002) ganglia after death. HSV-1 sequences have also been found in human thoracic ganglia (Mahalingam *et al*, 1992) and brain (Fraser *et al*, 1981; Baringer and Pisani, 1994), but virus has never been recovered by explantation or cocultivation of these tissues with indicator cells. Most humans have latent HSV-1 in their trigeminal ganglia (Mahalingam *et al*, 1992).

Abundance of HSV DNA in latently infected ganglia

Two different techniques have revealed that the viral DNA copy number during latency in mice ranges from less than 10 to more than 1000 copies per cell (Sawtell, 1997; Chen *et al*, 2002). In the mouse, both the strain and dose of virus has been shown to influence the copy number in latently infected neurons (Sawtell *et al*, 1998).

Configuration and physical state of HSV-1 DNA in latently infected ganglia

Initial studies of HSV-1 DNA configuration in latently infected mouse ganglia revealed two copies of the virion DNA joint fragment but no free ends because the termini were joined, indicating that the HSV-1 genome is endless or circular (Rock and Fraser, 1983, 1985). Efstathiou *et al* (1986) confirmed the detection of "endless" HSV-1 DNA in latently infected mouse ganglia and also found the same viral DNA configuration in human trigeminal ganglia. Subsequent analysis of gradient fractions after buoyant density centrifugation in CsCl of latently infected mouse ganglia revealed that most HSV-1–specific DNA is extrachromosomal (Mellerick and Fraser, 1987); there is no evidence that HSV-1 DNA integrates into the host genome.

HSV transcription in latently infected ganglia

The first proof that HSV-1 is transcribed in latently infected ganglia (Stroop *et al*, 1984) was followed by the identification of a latency-associated transcript (LAT) that mapped to repeat sequences flanking the unique long region (Stevens *et al*, 1987; Spivack and Fraser, 1987). The full-length LAT is 8.3 kb and is a low-abundance transcript found exclusively in neurons of latently infected ganglia. Processing of the full-length transcript results in abundant accumulation of 2.0- and 1.5-kb introns, also found in the nuclei of neurons. Another 1.4-kb species appears to result from additional splicing of the 2.0-kb intron. In both mouse and rabbit models, LAT-deficient mutants can become latent but reactivate with decreased efficiency. Recently, some larger LAT-deletion mutants have been shown to be more virulent during acute infection; interestingly, the number of latently infected neurons is reduced two-fold (Perng *et al*, 2000a; Thompson and Sawtell, 2001). One possibility is that the increase in virulence may reflect the ability of LAT transcripts to protect neurons from apoptosis (Perng *et al*, 2000b; Ahmed *et al*, 2002); similarly, a block in LAT function could affect the ability of virus to replicate in and kill cells (Zhu *et al*, 1999; Thompson and Sawtell, 2001). However, LAT mutants with smaller deletions exhibit wild-type virulence or slightly decreased virulence in mice during acute infection (Bloom *et al*, 1994, 1996). Note that these LAT recombinants lacking the core promoter and/or regions within the first 600 bp of the 5′ exon reactivate with decreased efficiency in both mouse and rabbit models. Further studies are needed to determine whether the primary effect of LATs on reactivation is quantitative (i.e., LAT expression results in fewer latently infected neurons) or qualitative (i.e., expression of LAT directly facilitates reactivation).

HSV proteins expressed in latently infected ganglia

Unlike many other herpesviruses, there is no indication that any HSV proteins are made during latency. Although evidence exists for various functional roles of LATs, there is no direct evidence that LATs either encode a protein or that any putative LAT open reading frames (ORFs) are translated during latency. Two ORFs from the LAT region have the potential to be translated during lytic infection (Perng *et al*, 2002; Thomas *et al*, 2002). The first ORF is located within the 2.0-kb intron and when stably integrated into ND7 cells, enhances viral yields after infection *in vitro*. Although this ORF is likely to have some function during acute infection, a viral recombinant with a mutation in this ORF did not affect establishment of, or reactivation from, latency. The second proposed ORF (AL) is encoded on an RNA that is antisense to the 5′ exon of LAT and overlaps with the 5′ transcriptional start site and a portion of the LAT promoter. The AL ORF has been suggested to encode a proapoptotic function, and its deletion slightly decreases virulence (Perng *et al*, 2002). The transcript encoding this ORF can only be detected during productive infection in tissue culture, but not in latently infected rabbit ganglia. Overall, it seems likely that the AL ORF, if it can be shown to be translated, functions during acute infection. Finally, two additional ORFs, ORF O and ORF P, have been shown to be expressed at low levels in tissue culture under conditions where infected cell protein 4 (ICP4) is not expressed (Lagunoff, 1995; Lagunoff and Roizman, 1994). Although these ORFs may be translated during latency, no latent protein

has been detected, and these ORFs are not required for establishment of latency (Randall *et al*, 2000).

Cell type infected in latently infected ganglia

Neurons are the exclusive site of HSV-1 latency, not only in human ganglia, but also in mouse and rabbit models. Studies employing either *in situ* polymerase chain reaction (PCR) or single-cell PCR analysis of latently infected mouse ganglia have revealed that 1% to 30% of neurons contain HSV-1 DNA, a variation that may reflect differences in infection conditions and strain of virus used (Mehta *et al*, 1995; Sawtell, 1997). LAT-positive neurons comprise about one third of the total neurons in latently infected ganglia. Overall, during latency, there are populations of neurons in which LAT expression is abundant, compared to neurons that do not express detectable LAT.

Ganglionic neurons are classified as large, medium, or small. Although the size groups might represent a heterogeneous population comprised of different functional classes of neurons, it remains unknown whether HSV-1 latency favors a particular size neuron. However, immunohistochemical analysis of latently infected mouse trigeminal ganglia has revealed that LAT-positive neurons tend to colocalize with neurons expressing monoclonal markers SSEA3 and A5 (Margolis *et al*, 1992; Yang *et al*, 2000). SSEA3 neurons comprise a heterogeneous population of all sizes that includes up to 40% of neurons in the ophthalmic region of the trigeminal ganglion (Margolis *et al*, 1992; Robertson *et al*, 1991). In contrast, neurons that stain positive for the monoclonal antibody marker KH10 tend to colocalize with neurons that support acute viral replication. Many A5- and SSEA3-positive populations are also positive for the high-affinity nerve growth factor (TrKa), whereas KH10-positive cells are almost always TrKa-negative. This supports a potential link between nerve growth factor responsiveness and the permissiveness of a neuron for establishment of latent infection. Finally, because 30% of latently infected neurons do not express TrKa, multiple cell proteins are likely to play a role in regulation of HSV latency.

Evidence for genetic control of viral transcription

Compared to LAT expression during latency, lytic gene expression is suppressed (Kramer and Coen, 1995), and recent data suggest that the low numbers of lytic transcripts detected in pooled latent ganglia by reverse transcriptase (RT)-PCR may be attributed to an occasional reactivating neuron (Feldman *et al*, 2002). Further, various cellular promoters such as that for phosphoglycerate kinase (PGK) inserted into the HSV genome are silent during latency (Lokensgard *et al*, 1994). One interpretation of the collective data is that a general mechanism suppresses HSV expression during latency. Epigenetic modification of DNA or chromatin is a conservative, but reversible, means of transcriptionally marking genes or large regions of cellular chromosomes for transcriptional repression. One known epigenetic mechanism used by the beta- and gammaherpesviruses (and well as for cellular genes) is DNA methylation. Although early studies suggested that HSV genomes maintained in a quiescent state in lymphocytes were methylated (Youssoufian *et al*, 1982), and that treatment with 5-azacytidine, which promotes hypomethylation, enhanced reactivation in explanted ganglia from guinea pigs latently infected with HSV-2 (Stephanopoulos *et al*, 1988), later studies using higher resolution assays to examine the methylation status of HSV genomes isolated from latently infected nervous tissue have indicated that DNA methylation is not used by HSV (Dressler *et al*, 1987; Tran, McAnany, and Bloom, personal communication). One possible explanation for the earlier suggestion of the involvement of methylation in HSV latency is that the quiescent infection of lymphocytes does not mimic the state of viral latency in neurons. The observation that 5-azacytidine augments reactivation is difficult to interpret, because it is toxic and might enhance reactivation as a result of increased cell stress. Moreover, the hypomethylating activity of this agent is exerted during active replication. Rather than DNA methylation, recent data suggest that HSV may exploit an epigenetic mechanism involving the differential deposition of histones with different tail modifications. Chromatin immunoprecipitation of DNA isolated from latently infected mouse dorsal root ganglia shows that the DNA polymerase gene is associated with H3 K9 methyl histones, a marker of transcriptionally repressed chromatin (heterochromatin), whereas the LAT promoter is associated with H3 lysine 3,14-diacetyl histones, a marker of transcriptionally permissive chromatin (euchromatic regions) (Kubat and Bloom, personal communication). These observations suggest that the HSV genome is ordered into transcriptional domains where the lytic genes are epigenically suppressed by a histone-mediated mechanism, whereas regulatory regions in the repeat regions of the genome are maintained in a state accessible to transcriptional activation. Because the repressive effects of histone modifications can be reversed in a replication-independent manner (unlike DNA methylation), alpha-herpesviruses might be more likely to use histone modification during latent infection in neurons because it does not involve episomal replication. Additional structural studies of chromatin may shed light on the regulatory paradigms that enable silencing of the HSV genome and subsequent reversible reactivation.

Model systems of HSV-1 latency

Mouse models: Within 21 days after infection with HSV-1 on the eye or footpad of mice, infectious virus and lytic transcripts are not detectable in trigeminal or dorsal root ganglia. Nonetheless, these ganglia are latently infected, as evidenced by the development

of a typical HSV cytopathic effect in indicator cells mixed with small pieces of finely minced ganglia and incubated at 37°C for 7 to 10 days. Typically, infectious virus can be recovered from 90% to 100% of ganglia (Stevens and Cook, 1971). Analysis of latently infected mouse ganglia has revealed viral DNA in 1.9% to 24% of neurons without any decrease over time (Sedarati *et al*, 1993; Sawtell, 1997). Similar numbers of neurons have been shown to contain HSV in latently infected human ganglia (Cai *et al*, 2002). The mouse model has also demonstrated that HSV-1 LAT mutants become latent but reactivate with reduced efficiency (Sawtell and Thompson, 1992; Devi-Rao *et al*, 1994).

Rabbit models: Ocular infection of the rabbit with HSV-1 produces latency that mimics the infection of mice and humans. In rabbits, reactivation can be induced and infectious virus can be recovered at the site of primary infection. The two commonly used reactivation models are the spontaneous model (Nesburn *et al*, 1967) and the adrenergic induction model (Hill *et al*, 1986). In the spontaneous model, eye swabs are examined for infectious virus 14 to 28 days after primary infection. The induced reactivation model uses iontophoresis of epinephrine (or another adrenergic agent) applied to the rabbit eye to reactivate virus. After infection with the 17+ or McCrae strain of HSV-1, epinephrine induces reactivation (>28 days post infection [p.i.]) in 70% to 100% of rabbit eyes. Note that although LAT mutants show a reduced ability to become latent in mice, such a restriction has not been observed in rabbits (Bloom *et al*, 1994), although LAT mutants with large deletions are less efficient at establishing latency (Perng *et al*, 2000a). Moreover, although LAT promoter mutants and mutations within the first 600 to 1500 bp of the LAT all demonstrate reduced reactivation in both the rabbit and mouse, a number of smaller deletions within the 5′ exon of LAT exhibit reduced reactivation only in rabbits (Bloom *et al*, 1996; Perng *et al*, 1996; Loutsch *et al*, 1999). Another divergence relates to virulence; when a particular region of the 5′ exon of LAT is deleted, virulence is increased in the rabbit, but decreased in the mouse (Perng *et al*, 2001). The mechanistic bases for these differences are unknown; but if this region of LAT influences the balance between establishment of latency and the potential to reactivate, it is possible that subtle differences between mouse and rabbit neuronal factors determine the extent of virulence. The model that most accurately reflects the human situation remains to be determined.

In vitro *models:* A challenge in studying HSV latency at the molecular level has been the difficulty in developing an *in vitro* model that mimics the *in vivo* state. Two models predominate. The first involves differentiated rat PC-12 pheochromocytoma cells propagated in the presence of nerve growth factor (NGF) and infected with high multiplicities of HSV (50 to 100 plaque-forming unit [pfu]/cell) (Danaher *et al*, 1999; Su *et al*, 1999). Surviving PC-12 cells harbor latent HSV genomes, predominately in circular form, in the absence of detectable lytic gene expression. Although these cultures can be induced to produce infectious virus by cocultivation with other cell lines, concerns exist over whether the apparent "quiescent" infection is truly devoid of a low level of a "smoldering" virus infection. The second system uses rat or mouse dorsal root ganglionic neurons infected with high multiplicities of HSV and maintained in tissue culture in the presence of acyclovir and NGF (Wilcox and Johnson, 1988). After days of such treatment, the cells are propagated in tissue culture medium containing NGF but not acyclovir. A spontaneous HSV cytopathic effect does not develop, and most cells in culture accumulate LAT. Virus reactivates if NGF is removed from the medium, or if the cells are treated with forskolin, an inducer of cAMP (Colgin *et al*, 2001), or with trichostatin A, an inhibitor of histone deacetylases (Arthur *et al*, 2001). Interestingly, withdrawal of NGF or absence of treatment with forskolin seems to result in the disappearance of the LAT intron from the nucleus (Colgin *et al*, 2001), suggesting that LAT may act as a dynamic suppressor of lytic gene expression, and that silencing of LAT expression is required to promote reactivation. However, it remains unclear whether this down-regulation of LAT expression occurs *in vivo*, and if so, how it might affect reactivation in the two thirds of latently infected neurons that do not express LAT.

Future directions

Although many molecular details of HSV latency have been established in the 30 years since the first explant cocultivation of latent HSV was reported, central questions remain about the mechanisms involved. Chief among these are (1) Which aspects of establishment of latency are dictated by the genotype of the virus, the phenotype of the neuron, or the competency of local immunity? (2) What is the biological impact of viral functions that regulate apoptosis, including those that map to the LAT region, on latency and reactivation? (3) Is latency maintained by a passive process resulting from the lack of transcriptional activation, or does it involve a dynamic repression of lytic viral functions? If the latter is the case, then is part of this repression dictated by LAT acting as a chromatin modeling RNA such as *XIST* or *rox*? and (4) What are the molecular events that signal the transition from stress into productive viral reactivation, and what is the first viral factor that responds to this signal—is it VP16, ICP0, or ICP4? Does LAT function in this process as an activator of the lytic cycle, or does repression of LAT during reactivation allow lytic functions to be activated? Answers to these questions await further detailed molecular analyses of the series of events governing reactivation in the complex architecture of sensory nerve ganglia.

Varicella zoster virus

Introduction

VZV is a ubiquitous human pathogen. Primary infection produces varicella (chickenpox), after which virus becomes latent in neurons of cranial nerve, dorsal root, and autonomic ganglia along the entire neuraxis. Reactivation, which may occur decades later, results in zoster (shingles). Unlike HSV-1, in which primary infection is often asymptomatic, varicella is characterized by malaise, fever, and an extensive vesicular rash (Abendroth and Arvin, 2000). A serologic study of 1201 US military basic trainees indicated that 95.8% had been exposed to virus (Jerant *et al*, 1998). Chickenpox is not always mild; in 1994, an epidemic of 292 cases of chickenpox in rural India resulted in 3 deaths (Balraj and John, 1994), and in England, where VZV vaccination is not mandatory, about 25 people die from chickenpox every year (Rawson *et al*, 2001). Although VZV vaccine effectively prevents varicella (Weibel *et al*, 1984; Gershon *et al*, 1992; Arvin and Gershon, 1996), breakthrough varicella (Takayama *et al*, 1997) and virus reactivation still occur (Krause and Klinman, 2000; LaRussa *et al*, 2000).

The study of VZV latency is fraught with obstacles. VZV is exclusively a human pathogen and no animal model exists in which virus latency and reactivation can be studied. VZV is latent only in ganglia, tissue not accessible during life, so that analysis of latent VZV has been restricted to human ganglia obtained at autopsy. Nonetheless, in the past 20 years, thousands of human ganglia have been analyzed by multiple laboratories. Below we review findings on the physical state of VZV nucleic acid and protein in latently infected human ganglia, including studies of latency in a primate model produced by simian varicella virus (SVV). We conclude with a brief discussion of the importance of studying varicella latency to prevent the serious neurological complications of VZV reactivation.

The VZV genome

Analysis of serially propagated VZV (Ecker *et al*, 1984; Hayakawa *et al*, 1986) and multiple clinical isolates (Hawrami *et al*, 1996; Muir *et al*, 2002) has revealed that the virus genome is a stable molecule with little geographic variation. The 124,884-bp VZV genome has been sequenced, and computer-assisted analysis has identified 71 ORFs numbered consecutively from the leftward end of the virus genome (accession numbers X04370, M14891, and M16612; Davison and Scott, 1986). ORFs 42 and 45 are believed to be exons from the same processed transcript and ORFs 62, 63, and 64 are repeated (ORFs 71, 70, and 69); thus there are 68 predicted unique VZV genes. However, recent analysis of the VZV genome has revealed novel transcripts. VZV gene 33.5 is 3′-coterminal with ORF 33 and encodes the virion assembly protein processed by the ORF 33 proteinase (Preston *et al*, 1997; McMillan *et al*, 1997). The novel VZV ORF 9A maps within ORF 8 (Ross *et al*, 1997). Mutation of both ORF 8 and ORF 9A yields virus with impaired syncytia formation and reduced growth in tissue culture. ORF (S/L) is the first demonstrated spliced VZV transcript and maps to the leftward end of the virus genome (Kemble *et al*, 2000). The 21-kDa cytoplasmic ORF (S/L) protein is expressed during lytic virus growth (*in vitro* and *in vivo*), and null mutations of this gene yield virus with altered cell adhesion characteristics. Overall, there are 70 unique VZV genes that have been identified by computer or experimental analysis.

Distribution and prevalence of VZV DNA in latently infected human ganglia

Unlike HSV-1 where latency is mostly restricted to cranial nerve ganglia, VZV is latent in those ganglia (Gilden *et al*, 1983; Hyman *et al*, 1983), as well as in dorsal root (Mahalingam *et al*, 1990) and autonomic nervous system ganglia (Gilden *et al*, 2001). VZV DNA is present in ganglia of more than 90% of normal adults (Mahalingam *et al*, 1992).

Abundance of viral DNA in latently infected human ganglia

Initially, semiquantitative PCR detected 6 to 51 copies of VZV DNA per 100 ng of total ganglionic DNA (Mahalingam *et al*, 1993). Later, real-time, quantitative fluorescent PCR detected 258 copies of VZV DNA per 10^5 ganglionic cells (Pevenstein *et al*, 1999). Assuming the presence of 15.6 pg of DNA per human cell, as well as the presence of 8.1×10^4 neurons, in which latent VZV resides predominantly, and 8×10^6 non-neuronal cells per trigeminal ganglion, then there are 4 to 40 copies (Mahalingam *et al*, 1993) and 20 copies (Pevenstein *et al*, 1999) of VZV DNA in each latently infected trigeminal ganglionic neuron. Recent real-time PCR analysis of left and right trigeminal ganglia from 17 individuals revealed no difference between the left and right trigeminal ganglia of the same individual in VZV DNA copy number, but a wide variation of 19 to 3145 copies of VZV DNA per latently infected neuron (Cohrs *et al*, 2000). Such a large range is likely to reflect the uneven amount of circulating VZV encountered during primary infection. For example, during acute varicella, the virus load in blood varies substantially from 200 to 5000 copies per 150,000 peripheral blood mononuclear cells, 100 to 1000 copies per milliliter whole blood, and 100 to >10,000 copies per milliliter serum (Mainka *et al*, 1998; de Jong *et al*, 2000). The different amount of latent VZV DNA among individuals is not surprising, because humans are outbred, and decades during which uncontrolled stimuli occur separate primary infection from collection and analysis of ganglia after death. Interestingly, the latent

DNA copy number of both VZV and HSV-1 parallels the amount of initial infecting virus.

Configuration of VZV DNA in latently infected ganglia

The configuration of the latent VZV genome has been determined by quantitative PCR analysis of human ganglionic DNA using two different primer sets and exploiting the rare (5%) isomerization of the unique long segment of the virus DNA. The first primer set corresponded to the termini of the virus DNA expected to yield a PCR product if the viral DNA molecule was circular, concatameric (end-to-end), or if the unique long region of the VZV genome was inverted. The second primer set was located internally and expected to amplify VZV DNA of any configuration. After PCR amplification, the latent VZV DNA copy number with both primer sets was the same (ratio 1:1), compared to a 15:1 ratio in DNA extracted from VZV virions (Clarke *et al*, 1995). The simplest interpretation of the data is that latent VZV DNA exists as a circular episome, similar to the structure of latent HSV-1 DNA (Rock and Fraser, 1985; Efstathiou *et al*, 1986).

VZV transcription in latently infected human ganglia

Based on *in situ* hybridization (ISH) studies combined with sequencing, four transcripts corresponding to VZV genes 21, 29, 62, and 63 have been identified in latently infected human ganglia. Initially, transcripts corresponding to VZV genes 29 and 62 were found on Northern blots prepared from the poly[A]-selected RNA extracted from hundreds of human trigeminal ganglia (Meier *et al*, 1993). Later studies used reverse Northern blot analysis in which cDNA probes were synthesized from latently infected human ganglionic RNA and hybridized to restriction endonuclease fragments spanning 95% of the virus genome. These methods provided the first identification of VZV gene 21 transcripts (Cohrs *et al*, 1994, 1995), confirmed the earlier detection of VZV gene 29 and 62 transcripts by Meier *et al* (1993), and identified transcripts corresponding to VZV gene 63 (Cohrs *et al*, 1996). Interestingly, although the earliest studies of VZV transcription during latency did not detect VZV gene 63 transcripts, it appears to be the most abundantly transcribed VZV gene identified to date (Cohrs *et al*, 2000). Finally, ISH studies (Kennedy *et al*, 1999, 2000) have confirmed the presence in human ganglia of transcripts corresponding to VZV genes 21, 29, 62, and 63, and have also suggested that VZV genes 4 and 18 may be transcribed, but the latter findings need to be confirmed by sequencing.

VZV proteins expressed in latently infected human ganglia

Although immunohistochemical staining of normal human ganglia with polyclonal antiserum directed against VZ virions did not reveal any VZV-specific proteins, use of a monospecific polyclonal antiserum directed against VZV ORF 63 protein did identify this protein in the cytoplasm of neurons (Mahalingam *et al*, 1996). Another study also detected proteins in the cytoplasm of neurons corresponding not only to VZV ORF 63, but also to VZV ORFs 4, 21, 29, and 62 (Lungu *et al*, 1998). In the same report, these VZV proteins were shown to be predominantly nuclear during productive infection (zoster), and the investigators speculated that cytoplasmic location might provide a mechanism to maintain VZV in a latent state, namely, by restricting regulatory proteins from the nucleus. This intriguing study, if confirmed, would suggest a mechanism by which latency is maintained in the presence of an efficient transactivator (e.g., IE62) of virus gene transcription.

Cell type infected in latently infected human ganglia

The first ISH studies detected VZV nucleic acid in neurons of human ganglia (Hyman *et al*, 1983; Gilden *et al*, 1987). A later ISH by Croen *et al* (1988) suggested that VZV was latent exclusively in non-neuronal satellite cells, although a consultant neuropathologist at the University of Colorado viewed Croen's ISH figures as indicative of virus presence only in neurons (Gilden and Kennedy, personal communication). Using ISH, Lungu *et al* (1995) detected VZV DNA in neurons and multiple types of non-neuronal cells, a finding that has not been confirmed. The use of PCR combined with ISH to study VZV latency has revealed VZV DNA exclusively in the cytoplasm of neurons, perhaps due to leakage of viral DNA from the nucleus during the rigors of PCR amplification *in situ* (Dueland *et al*, 1995). In two further carefully controlled studies, various groups investigating VZV latency submitted latently infected human ganglia to a single laboratory for analysis by ISH and PCR-ISH; the findings of both studies indicated that VZV was latent predominantly, if not exclusively, in neurons (Kennedy *et al*, 1998, 1999). To circumvent some of the problems associated with ISH (reviewed in Mahalingam *et al*, 1999), we applied a modification of contextual analysis technology (Sawtell, 1997) to the analysis of dissociated human trigeminal ganglia. Nearly all VZV DNA was detected in ganglionic cells >20 μm in diameter (neurons); some VZV DNA was also found in cells <10 μm in diameter (either small neurons or non-neuronal cells), again indicating that VZV is latent primarily, if not exclusively, in neurons (LaGuardia *et al*, 1999). Overall, the neuronal site of latent VZV DNA appears to be the same as for other alphaherpesviruses, including HSV-1 (Stroop *et al*, 1984; Stevens *et al*, 1987), HSV-2 (Mitchell *et al*, 1990; Croen *et al*, 1991), bovine herpesvirus-1 (Bratanich *et al*, 1992; Kutish *et al*, 1990), equine herpesvirus (Borchers *et al*, 1997), and pseudorabies virus (Priola *et al*, 1990).

Models of varicella latency

Unlike HSV-1, VZV does not reactivate from ganglia after experimental infection of primates and multiple species of rodents. Nevertheless, some important information has been acquired from analysis of ganglia after experimental infection of rodents. After footpad inoculation of rats with VZV, abundant VZV gene 63 protein was found in lumbar ganglia harvested 1 month later (Debrus *et al*, 1995). Viral protein was found exclusively in neurons, both in the nuclei and cytoplasm of infected cells. A subsequent ISH study of the same rat model detected VZV gene 63 DNA in 5% to 10% of neurons and VZV RNA in neurons and non-neuronal cells at an approximate ratio of 3:1; immunocytochemistry revealed VZV gene 63 protein exclusively in neurons, both in the nuclei and cytoplasm of latently infected cells (Kennedy *et al*, 2001). The presence of VZV primarily in neurons, with abundant expression of VZV gene 63 nucleic acid and protein, parallels findings in human ganglia.

Finally, we recently simulated natural varicella infection by exposing four SVV-seronegative monkeys to monkeys that had been inoculated intratracheally with SVV. The four naturally exposed monkeys developed mild varicella 10 to 14 days later, and skin scrapings taken at the time of rash contained SVV DNA. Analysis of multiple ganglia, liver, and lung obtained on sacrifice of the four monkeys 6 to 8 weeks after resolution of rash revealed SVV DNA in ganglia at multiple levels of the neuraxis, but not in lung or liver of any of these monkeys (Mahalingam *et al*, 2002). This animal model provides an experimental system to gain information on varicella latency with direct relevance for the human disease.

Neurological disease after reactivation of VZV

The neurological complications after VZV reactivation are serious and often life-threatening. VZV was identified as the causal agent in 29% of 3231 cases of encephalitis, meningitis, and myelitis (Koskiniemi *et al*, 2001), and VZV was the most common cause of encephalitis in 322 patients age 65 or older (Rantalaiho *et al*, 2001). More than 500,000 Americans develop zoster (severe dermatomal distribution pain and rash) every year. Zoster is frequently followed by postherpetic neuralgia (pain that persists for months and often years after rash resolves), myelitis, or unifocal or multifocal vasculopathy. VZV also produces zoster sine herpete (dermatomal distribution pain without rash). These complications underscore the need to understand the molecular biology of latent VZV infection and reactivation (Gilden *et al*, 2000; Kleinschmidt-DeMasters and Gilden, 2001). Finally, it is important to recognize that, like zoster sine herpete, many cases of VZV vasculopathy, myelitis, and polyneuropathy occur in the absence of rash. A high index of suspicion by clinicians who recognize the wide spectrum of neurological disorders potentially caused by VZV should be followed by testing for VZV DNA and antibody in cerebrospinal fluid (CSF). Detection of antibody to VZV in the CSF, even without PCR-amplifiable VZV DNA, can be diagnostic. Proper diagnosis is critical, because antiviral treatment can be curative, even after weeks to months of chronic VZV infection.

Future directions

Important research priorities in this field over the next few years should include (1) the development of an animal model of varicella that incorporates both the establishment of latency as well as viral reactivation; (2) determination of the full range of VZV gene expression during latency; (3) determination of the functional role, if any, of VZV genes that are expressed during latency; and (4) utilization of VZV gene expression data to develop effective therapeutic strategies for VZV infections designed primarily to prevent viral reactivation in susceptible individuals.

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