

VARICELLA ZOSTER VIRUS LATENCY, NEUROLOGICAL DISEASE AND EXPERIMENTAL MODELS: AN UPDATE

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1. ABSTRACT

Varicella zoster virus (VZV), a ubiquitous neurotropic human herpesvirus, causes chickenpox (varicella) and then remains latent for decades in cranial nerve, dorsal root and autonomic nervous system ganglia along the entire neuraxis. Virus reactivation, most often after age 60, produces shingles (zoster), characterized by pain and rash usually restricted to 1-3 dermatomes. In elderly individuals, zoster is frequently complicated by postherpetic neuralgia (PHN), pain that persists for months to years after the resolution of rash. Virus may also spread beyond ganglia to the spinal cord to cause myelitis, as well as to blood vessels of the brain, producing a unifocal or multifocal vasculopathy. The increased incidence of zoster in the elderly and immunocompromised individuals appears to be due to a VZV-specific host immunodeficiency. Recent studies indicate that PHN may be due to a chronic active VZV ganglionitis, and that VZV vasculopathy is caused by a productive virus infection in cerebral arteries. Since neurological disease produced by VZV is due to reactivation from ganglia, the physical state of viral nucleic acid and expression during latency as well as the possible mechanisms by which VZV latency is maintained and reactivates are discussed. Finally, VZV is an exclusively

human herpesvirus, and experimental infection of animals with VZV does not produce disease nor does VZV reactivate from ganglia. Two varicella models in primates have proven useful: one that mimics varicella latency in humans, and one that can be used to study the efficacy of antiviral agent in driving varicella virus back to a latent state.

2. INTRODUCTION

Varicella zoster virus (VZV) is an exclusively human neurotropic alphaherpesvirus. Primary infection produces ~4 million cases of chickenpox annually. After chickenpox, VZV becomes latent in cranial nerve, dorsal root and autonomic nervous system ganglia along the entire neuraxis (1-3). Virus reactivation results in shingles (zoster). Zoster is characterized by severe, sharp, lancinating radicular pain and rash restricted to 1-3 dermatomes. In more than 40% of zoster patients over age 60, pain persists for months and sometimes years, so-called postherpetic neuralgia (PHN). Although neither the pain of zoster nor the more chronic pain of PHN is life-threatening, it is difficult to manage. Less often, VZV reactivates from

VZV latency; neurologic disease; varicella models

ganglia and virus spreads to arteries of the brain and spinal cord, providing a vasculopathy that affects large and small cerebral vessels. Compared to zoster and PHN, VZV vasculopathy is more serious, causing considerable neurologic deficit and sometimes death.

3. VARICELLA ZOSTER VIRUS (VZV) LATENCY

3.1. Location and burden of viral nucleic acid

Initially, *in situ* hybridization (ISH) revealed that VZV was latent exclusively in neurons (4,5). These findings were later disputed by the apparent detection of VZV in non-neuronal satellite cells (6,7) and by a single report that found VZV in many neurons and non-neuronal cells (8). However, ISH results of autopsy samples containing low amounts of latent virus DNA are capricious and prone to misinterpretation (9). Improvements in ISH that incorporated polymerase chain reaction (PCR) and amplified low-copy number latent virus DNA revealed VZV DNA predominately, if not exclusively, in neurons (10-12).

The question of latently infected cell type has since been addressed by an ISH-independent approach using the procedure of contextual cell analysis. Initially developed by Sawtell (13) to analyze HSV-1 latency in mice, cells in ganglia are dissociated by mild collagenase treatment followed by isolation of neurons using rate zonal centrifugation. Contextual analysis was later modified for application to human autopsy samples, wherein fractions enriched in neurons were prepared from collagenase-treated trigeminal ganglia (TG) cell suspensions by differential filtration. VZV DNA was detected by PCR only in neurons (14). More recently, human TG neurons enriched from mechanically dissociated TG by differential sedimentation were analyzed by PCR (15). A total of 2226 neurons and 20,736 satellite cells from 12 autopsy cases were studied. Cell DNA was found in all samples; VZV DNA was found in 34 neurons and never in non-neuronal cells. Thus, like all neurotropic alphaherpesviruses analyzed to date, latent VZV resides in neurons.

The burden of latent VZV in ganglia has also received recent attention. Assuming 15.6 pg total DNA per TG cell (16) and one neuron for each 100 non-neuronal cells (17), and assuming that approximately 2% of neurons contain latent VZV DNA (15), semi-quantitative PCR revealed 0.3-1.6 (18) and later <1 to 84 (15) copies of VZV DNA copies per latently infected neuron. Linear transformation of real-time PCR data with the above frequency assumptions indicated 2-50 (16) and 14-1390 (19) copies of VZV DNA per latently infected neuron. Analysis of individual TG from any single subject indicated no significant difference in the virus DNA burden, although the latent virus DNA content among individuals appeared to vary widely. This variation could be due to limited virus reactivation after death, although the time between death and autopsy did not correlate with VZV DNA copy number. More likely, this range in the amount of latent VZV DNA reflects the severity of primary infection, the time at which latency is established. During acute varicella, the virus load in blood ranges from 200-5000

copies per 150,000 peripheral blood mononuclear cells (MNCs), 100-1000 copies per ml whole blood, and 100 to >10,000 copies per ml serum (20,21). Overall, just as the amount of VZV available to seed TG varies greatly within humans, so does the latent VZV DNA burden.

3.2. Configuration of latent VZV DNA

The VZV genome is a linear double-stranded DNA molecule containing long and short segments covalently connected through inverted-repeated DNA sequences (22). To determine the configuration of latent VZV DNA, Clarke *et al.* (23) developed a PCR-based strategy that differentiates linear and circular molecules. Primers were designed and used for DNA synthesis outward from the genome termini. End primers would amplify only if DNA was circular or concatameric (end-to-end) or when the VZV unique long region was inverted as it is in 5% of virions; internal primers were selected to amplify DNA in all configurations. Amplification of DNA from latently infected TG revealed a 1:1 ratio of internal-to-terminal sequences compared to 15:1 in virion DNA, indicating that the VZV genome termini are adjacent. This finding is consistent with an extrachromosomal circular configuration, as shown for latent HSV-1 DNA in humans and mice (24,25).

3.3. VZV gene expression during latency

The VZV genome has been sequenced (26). Several regions, especially those encoding surface proteins, show mutations that have allowed geographic phylogenetic analysis (27). However, the VZV genome is a stable molecule overall (28,29). This stability extends to multiple clinical isolates (30,31) and has allowed the application of PCR to the analysis of latent VZV gene transcription. Previously, VZV genes 29, 62 and occasionally gene 4 were detected by RNA ISH (6), a method fraught with interpretation problems. Northern analysis also revealed VZV gene 29 and 62 transcripts, but TGs from 200 individuals were needed to obtain sufficient polyadenylated RNA (7). In a series of experiments, PCR was used to detect, clone, and quantitate latently transcribed VZV genes (19,32-34). The 3'-polyadenylated termini of VZV genes 21, 29, 62 and 63 were sequenced from a cDNA library constructed from latently infected TG RNA enriched for virus transcripts by hybridization selection. The relative abundance of VZV gene 21, 29 and 63 transcripts was determined by real-time RT-PCR applied to RNA extracted from individual TGs. VZV gene 63 transcripts were detected in 19 of 22 (86%) of these TGs, and the abundance varied by a factor of ~2000. VZV gene 21 and 29 transcription was detected in 5 and 3 of 22 individual TG, respectively. While VZV gene 29 transcripts were more abundant than those of VZV gene 21, the levels of both VZV genes 21 and 29 transcripts were far below that of VZV gene 63. Overall, VZV gene 63 transcription appears to be a consistent hallmark of VZV latency.

To demonstrate translation of the latently transcribed VZV genes, antibodies directed against VZV genes 21, 29, 62 and 63 have been used to stain TG sections. Initially, immunohistochemistry (IHC) detected VZV gene 63 protein in the cytoplasm of neurons (35).

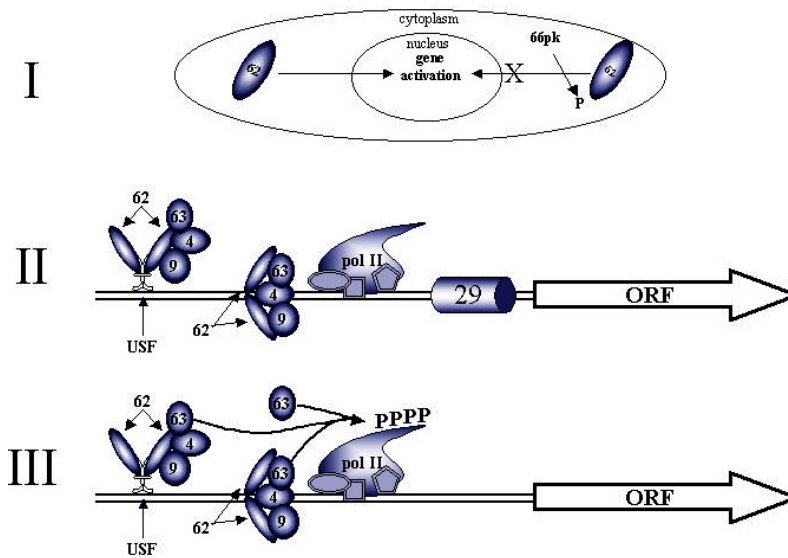


Figure 1. Regulation of IE62 function. From the available data, three non-mutually exclusive models might describe the regulation of IE62 function in latently infected human ganglia. **Model I: regulation by location.** Cytoplasmic VZV IE62 is translocated into the nucleus where gene activation in trans is initiated. Phosphorylation of IE62 by VZV gene 66 protein kinase halts IE62 nuclear import, IE62 is restricted to the cytoplasm and gene activation does not occur. **Model II: regulation at the promoter.** Nuclear IE62 is associated with at least 3 virus proteins encoded by open reading frames 4, 9, and 63. The IE62 complex binds to VZV gene promoters either through cellular transcription factors, in this case the previously identified upstream stimulating factor (USF), or directly to the promoter itself. The IE62 complex further associates with cellular RNA pol II and its associated transcription factors. However, RNA transcription is blocked by downstream binding of VZV gene 29 protein. **Model III: regulation by transcription factor modification.** As in Model II, nuclear IE62 and the associated VZV and cellular proteins bind to gene promoters. However, transcription is blocked by modification of the cellular RNA pol II complex. In this model, either free or bound VZV gene 63 protein alters the phosphorylation pattern of the C-terminal domain of cellular RNA pol II, resulting in transcription control.

Subsequently, IHC detected gene 63 protein in multiple human TG neurons (36,37), as well as VZV gene 21, 29 and 62 proteins in the cytoplasm of human TG neurons during latency (36). However, Lungu *et al.* (36) reported detection of VZV gene 63 protein in 6% neurons and VZV gene 29 protein in 21% of neurons, frequencies greater than the reported frequency of VZV latency (~5% of TG neurons harbor latent virus). Thus, those data require confirmation.

Recently, the VZV gene 66 protein kinase, 66pk, was detected in latently infected human TG (38). In tissue culture, the phosphorylation of IE62 (the immediate-early protein encoded by VZV gene 62) by 66pk restricts IE62 to the cytoplasm of infected cells (39). Analysis of human TG by IHC with rabbit anti-IE62 antibody confirmed the cytoplasmic location of IE62 during latency (Kennedy, unpublished data). To determine if VZV 66pk phosphorylation might account for the cytoplasmic restriction of IE62, a search was made for VZV 66pk transcription and protein in latently infected human TG. Using reverse transcriptase-linked PCR, Cohrs *et al.* (38) sequenced the 3'-terminus of latent VZV gene 66 transcripts, revealing polyadenylation typical of VZV transcripts. ISH revealed VZV gene 66 transcripts exclusively in the nucleus of TG neurons, and IHC located VZV 66pk protein in the cytoplasm of TG neurons.

3.4. Mechanism of latency maintenance and reactivation

The central paradigm in herpesvirus research is that infectious virus is not released during latency.

Accordingly, expression of VZV proteins leading to the assembly and release of infectious VZV is blocked in latently infected neurons. Understanding the mechanism involved in the establishment and maintenance of latent infection is the goal of current research and is propelled by the severity of disease associated with virus reactivation (40). The promoters for VZV genes 21, 29, 62, and 63 have been identified (41-44). Transient transfection of neuroblastoma cells in culture with reporter plasmids in which luciferase gene expression is driven by VZV gene 21, 29 or 63 promoters demonstrated that these promoters are silent (Cohrs, unpublished data). While continuously dividing neuroblastoma cells in culture are not the same as terminally differentiated sensory neurons, all available information suggests that the VZV gene 21, 29 and 63 transcripts detected during latent infection *in vivo* are not the result of promoter activation by unmodified cellular transcription factors, but instead are the result of a signal derived from latent VZV. Thus, these proteins may function to maintain repression of virus replication.

The presence of IE62 in latently infected neurons is intriguing, since this essential 1310-amino acid phosphoprotein is a promiscuous activator of virus and cellular genes, and enhances the infectivity of transfected VZV DNA (45-49). To maintain latency, the effect of IE62 on subsequent virus gene expression must be controlled. From the available data, three models of the control of IE62 function seem possible (figure 1). One model of

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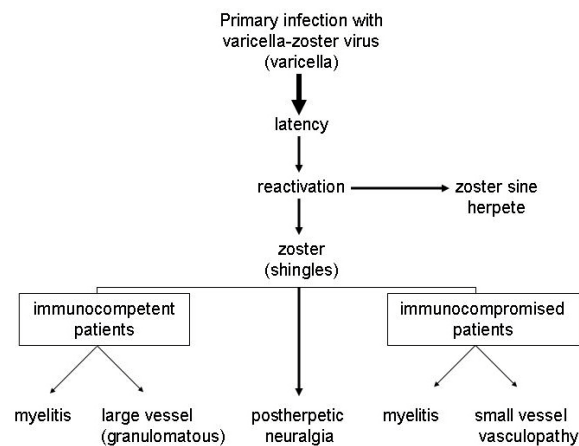


Figure 2. Neurologic disease produced by reactivation of varicella zoster virus.

modulating IE62 activity is based on cellular location. In a series of studies, Kinchington *et al.* (39,50,51) demonstrated that the distribution of IE62 is dependent on 66pk phosphorylation. Late in productive virus infection, 66pk phosphorylation of IE62 restricts IE62 to the cytoplasm. While IE62 phosphorylation may be a mechanism of incorporating IE62 into the developing virus, nuclear exclusion would also efficiently control the function of this potent gene transactivator. As noted above, polyadenylated VZV 66pk transcripts as well as 66pk protein have been detected in latently infected human TG (38).

A second mechanism of modulating IE62 activity is through control at the promoter level. Consistent with this hypothesis is the finding that the protein encoded by VZV gene 29 binds to a 40-bp region on the glycoprotein I promoter (52,53). Gene 29 protein binding results in increased IE62 induced gl expression in permissive cells and reduced IE62 transactivation in nonpermissive cells as determined by transient plasmid transfections or site-specific mutated virus *in vivo* (54). Thus, the major DNA binding protein encoded by gene 29 may function to direct IE62 transactivation (figure 2). Interestingly, the HSV-1 homologues of VZV gene 29 and gene 21 form a DNA binding complex in the nucleus of virus-infected cells (55). The HSV-1 homologue of VZV gene 29 also affects transcription of late HSV-1 genes (56). Based on the functions of these HSV-1 homologues and the modulation of IE62 function by VZV gene 29 protein, we tested whether VZV gene 29 and 21 proteins might synergistically modulate IE62-induced gene transactivation; in tissue culture, VZV gene 29 and 21 proteins had no significant effect on IE62-induced activation of gene 20, 21, 28 or 29 promoters (44). Whether these proteins affect IE62 function in TG neurons remains to be determined.

A third mechanism of modulating IE62 activity is through transcriptional control. The HSV-1 homologue of the VZV gene 63 protein (ICP22) has been shown to alter phosphorylation of cellular RNA pol II, thereby reprogramming the cellular transcription apparatus to more

readily transcribe viral genes (57,58). By extension, VZV gene 63 protein might modify gene expression by interacting with transcription factors. Conclusive evidence that gene 63 protein alters RNA pol II phosphorylation or that gene 63 protein alone affects gene expression is lacking (42,59). However, VZV gene 63 protein can complex with IE62 (60) and phosphorylated forms of VZV gene 63 protein reduce IE62-induced promoter activation (61). Since the effect of ICP22 on RNA pol II is dependent on its phosphorylation (62), the possibility exists that phosphorylated forms of VZV gene 63 protein alter cellular transcription to reduce the effect of IE62 on virus gene activation.

Overall, considerable advances have been made in the field of VZV latency. Current evidence indicates that: (1) neurons are the site of latency; (2) VZV is latent in 2-5% of TG neurons; (3) the virus DNA copy number ranges from 14-1390 molecules per neuron, most likely reflecting the severity of primary infection; (4) the VZV genome termini are adjacent, consistent with an extrachromosomal circular configuration; and (5) at least 5 VZV genes are transcribed. Models for the action of the latently expressed VZV genes have been suggested and are now being tested. Unfortunately, the only tissue available for studying VZV latency is human ganglia removed after death, unlike HSV-1 where animal models exist in which virus reactivates spontaneously or after experimental induction (63).

4. CLINICAL FEATURES

4.1. Relationship of reactivation to the host anti-VZV cell-mediated immune response

Although zoster can develop at any age, most VZV reactivation occurs in elderly and immunocompromised individuals. Thus, zoster and its attendant neurological complications are likely to continue increasing. As humans age, they undergo a natural decline in cell-mediated immunity (CMI) to VZV (64,65). Furthermore, increasing numbers of cancer patients and transplant recipients are being treated with cytotoxic drugs that impair CMI to VZV. The most extreme example of host immunodeficiency to VZV is encountered in patients with AIDS, who not only have the highest incidence of zoster, but who also develop multiple episodes of zoster. The development of zoster may be viewed in the context of a continuum in immunodeficient individuals, ranging from a natural decline in VZV-specific immunity with age, to more serious immune deficits seen in cancer patients and transplant recipients, and ultimately to patients with AIDS.

4.2. Postherpetic neuralgia (PHN)

The most common complication of zoster is PHN. Zoster anywhere on the body in individuals >60 years old is associated with a 40-44% risk of PHN (66). The cause of PHN is unknown. However, clinical-virological correlations suggest that virus persistence in ganglia, which produces a chronic ganglionitis, is the cause. VZV-specific DNA (67,68) and VZV-specific late glycoproteins (69) have been found in blood MNCs of PHN patients 1-8 years after zoster. In zoster patients who

did not develop PHN, VZV DNA was only found in MNCs up to 38 days, or not at all, after disappearance of zoster pain (70). A more extensive study of zoster patients with and without PHN revealed that VZV DNA could be detected in MNCs up to 8 years after zoster in 11/51 patients with PHN, but not in MNCs of 19 zoster patients without PHN who were analyzed 1-31 years after zoster, or in any of 11 elderly age- and gender-matched subjects with no history of zoster (68). Further evidence that the longstanding radicular pain of PHN reflects a chronic ganglionitis has come from the detection of VZV DNA in blood MNCs and cerebrospinal fluid (CSF) of two patients with zoster sine herpete [pain without rash (71,72)], including the fact that pain disappeared after both patients were treated with intravenous acyclovir (71).

We also conducted a detailed analysis of an elderly immunocompetent elderly woman with PHN monitored over an 11-year period (73). Blood MNCs contained VZV DNA initially on two occasions, but not after she was treated with famciclovir. However, she voluntarily stopped treatment five times, and pain always recurred within one week. On all five occasions when she discontinued famciclovir, VZV DNA was found in her blood MNCs. Overall, the repeated detection of VZV DNA in this patient's MNCs whenever famciclovir was discontinued, as well as the gratifying clinical response to famciclovir, is best explained by a chronic VZV ganglionitis-induced PHN. Of interest is that several, but not all, regions of the VZV genome were detected at different times in MNCs, in contrast with the detection of every region of the VZV genome in latently infected human ganglia (68,73). The cumulative data from our (68,69,71,74) and other laboratories (72) suggest that the detection of VZV DNA in MNCs of PHN patients probably reflects incomplete digestion of VZV DNA by DNases in blood MNCs, particularly antigen-presenting cells, which acquire VZV when they traffic through productively infected ganglia where viral persistence accounts for the continuous pain. Whereas neither Mainka *et al.* (20) nor Schunemann *et al.* (75) detected VZV DNA or RNA in MNCs of 16 PHN patients, we have studied a larger number of patients over a longer period of time, including the patient reported above in whom VZV DNA was detected in MNCs on multiple occasions.

4.3. VZV vasculopathies

CNS disease is the most alarming complication of herpes zoster. Two conditions predominate: small vessel vasculopathy primarily in immunocompromised individuals and large vessel granulomatous arteritis in immunocompetent patients (figure 2).

4.3.1. Multifocal vasculopathy

Multifocal vasculopathy is the most common form of CNS involvement caused by VZV. Disease develops on a background of cancer, immunosuppression and AIDS (76). Neurologic disease is subacute and death is common. Herpes zoster small vessel vasculopathy presents as headache, fever, vomiting, mental changes, seizures and focal deficit. Brain imaging reveals large and small ischemic or hemorrhagic infarcts—often both—of

cortex and subcortical gray and white matter. Deep-seated white-matter lesions often predominate and are ischemic or demyelinative. The demyelinative lesions are smaller and less coalescent than those seen in progressive multifocal leukoencephalopathy. The CSF shows a mild to moderate pleocytosis (predominantly mononuclear), normal or mildly elevated concentrations of CSF protein, and a normal CSF glucose content—findings that do not differ significantly from herpes zoster without vasculopathy. Treatment of herpes zoster vasculopathy includes intravenous acyclovir at a dosage of 15 to 30 mg/kg/day for 10 days. Longer treatment may be necessary in severely immunocompromised patients.

4.3.2. Unifocal vasculopathy

The salient feature of this form of VZV-induced CNS disease is acute focal deficit that develops weeks or months after contralateral trigeminal distribution herpes zoster. Stroke results from a necrotizing arteritis, primarily of large cerebral arteries. One comprehensive review showed that most patients with large vessel vasculopathy were older than 60 years and that there was no sex bias (77). The mean onset of neurologic disease was seven weeks, and the longest interval between the onset of herpes zoster and the onset of neurologic disease was six months. Transient ischemic attacks and mental symptoms were common, and 25% of patients died. The majority of patients had a CSF pleocytosis, usually fewer than 100 cells (predominantly mononuclear), oligoclonal bands and increased CSF IgG. Besides contralateral hemiplegia, ipsilateral central retinal artery occlusion and posterior circulation involvement have been described. Angiographic examination reveals focal constriction and segmental narrowing, primarily in the large internal carotid, anterior, middle and posterior cerebral arteries (78). Microscopic examination has revealed a necrotizing arteritis (primarily involving the intima and adventitia), inflammation with multinucleated giant cells, VZV antigen, Cowdry type A inclusions, and herpesvirus particles. Most herpes zoster-associated granulomatous angiitis infarcts are pale (79), but hemorrhagic infarction also occurs. Afferent trigeminal ganglionic fibers to both intracranial and extracranial blood vessels provide an anatomic pathway for the spread of virus.

Clinicians do not have a definitive treatment for large vessel herpes zoster vasculopathy. Nevertheless, because productive virus infection is found in arteries, patients should receive intravenous acyclovir (to kill persistent replicating virus) and steroids (to exert anti-inflammatory effects).

4.3.3. VZV disease in the absence of rash

In addition to vasculopathy, VZV may cause disease at multiple levels of the neuraxis involving the central and peripheral nervous system in the absence of rash. This includes cases of encephalomyeloradiculoneuropathy; mixed small and large vessel vasculopathy; acute, chronic and recurrent neuropathy (80); and myelopathy (81,82). Magnetic resonance imaging scanning, cerebral angiography and examination of CSF with virological analysis are needed to confirm the diagnosis. Proof of VZV etiology of disease is based on detection of VZV DNA and VZV antibody in

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CSF, along with reduced serum/CSF ratios of VZV IgG compared to total IgG or albumin, or both. Some of these patients respond well to treatment with intravenous acyclovir, underscoring the value of aggressive testing for VZV in unusual cases of CNS disease or neuropathy.

5. EXPERIMENTAL ANIMAL MODELS OF VARICELLA VIRUS INFECTION

Because VZV causes disease exclusively in humans, it has not been possible to develop an animal model to study VZV latency, pathogenesis and reactivation. Experimental inoculation of rodents and primates leads only to seroconversion without clinical signs (83-88). In adult rats inoculated subcutaneously with VZV along the spine, ganglionic infection was established, and analysis of dissociated dorsal root ganglia at multiple intervals for 9 months after inoculation detected VZV nucleic acid and protein in neurons (89). Later, VZV DNA was detected by ISH in ganglionic neurons and non-neuronal cells 1-3 months after inoculation (90). Another study detected VZV gene 63 protein only in ganglionic neurons 1-18 months after inoculation (91,92). VZV did not reactivate in any of the rats, and none of the above studies analyzed non-ganglionic tissue for virus. Thus, while multiple investigators have demonstrated that ganglionic infection can be established after peripheral inoculation of VZV, the rat model cannot be considered to be one of latency because: (1) VZV does not reactivate; (2) non-ganglionic tissues have not been shown to be free of VZV; and (3) VZV in rats is not restricted to ganglionic neurons. The same holds true for guinea pigs in which ocular (93) and subcutaneous (94) inoculation of VZV resulted in ganglionic infection. Unfortunately, non-ganglionic tissues were not analyzed for VZV, and virus did not reactivate.

Subcutaneous inoculation of the Oka VZV (vaccine strain) into the breast of a chimpanzee produced viremia and mild rash restricted to the inoculation site; however, ganglia and other tissues were not studied (95). In another study, human fetal thymus and liver were implanted under the kidney capsule or subcutaneously in the skin of severe combined immunodeficient (SCID-hu) mice and VZV was injected into the implants 3-5 weeks later (96). Human lymphocytes circulating in these mice were shown to harbor infectious VZV up to 21 days after infection and VZV proteins were detected in CD4+ and CD8+ T cells obtained from the infected implants. The disadvantage of this model is that the implants were externally infected rather than by viremia.

5.1. Simian varicella virus (SVV)

Simian varicella virus (SVV) is a herpesvirus that causes varicella in primates. The clinical (97,98), pathological (97,99,100) and immunological (101,102) features of SVV resemble those of human varicella. Like human chickenpox, varicella in primates is preceded by an incubation period of one-to-two weeks, followed by fever and a papulovesicular rash of skin and mucous membranes (103). Primary SVV infection produces viremia, and infectious virus can be recovered from blood MNCs (104-107). Disseminated SVV infection is frequent in

monkeys after intratracheal inoculation. Lung and liver are the most severely affected organs, much like disseminated varicella in immunosuppressed patients (103,108). Histological examination of skin and viscera reveals foci of hemorrhagic necrosis, inflammation and eosinophilic intranuclear inclusions (104,105). Like VZV after recovery from acute disease, SVV becomes latent in sensory ganglia at all levels of the neuraxis (109). SVV reactivates in virus-infected monkeys exposed to social and environmental stress, and virus can be isolated from skin vesicles of these monkeys (106,107).

5.2. SVV and VZV

Complement fixation, viral neutralization, immunofluorescence and immunoprecipitation assays have shown that VZV and SVV are antigenically related (101,102,110,111). Like VZV, SVV is an enveloped, double-stranded DNA virus. The SVV genome is colinear with that of VZV (112,113), similar in size, and shares 70-75% DNA homology with VZV (26,114-116). Based on the complete sequence of the SVV genome, the virus DNA has been found to be 124,139 bp in size, 745 bp shorter than VZV DNA, with a G+C content of 40.4% compared to 46% for VZV (117). Comparison of the SVV DNA sequence to the previously published VZV sequence (118) reveals one major difference at the leftward end of the SVV genome (119), which is currently under study with regard to species specificity.

5.3. SVV pathogenesis

Two models of varicella infection have been established, one by intratracheal inoculation of SVV, and the other by natural exposure of SVV-seronegative monkeys to SVV-infected monkeys. In the first model, intratracheal inoculation of monkeys with 10^3 - 10^4 plaque-forming units of SVV produces varicella. Infectious virus can be recovered from blood MNCs at 2-11 days after inoculation (10,100,106,107,120). Monkey ganglia become infected before rash appears (121). Histopathological analysis reveals necrosis and intranuclear inclusions in lung, liver and spleen (122). Immunohistochemical analysis reveals SVV-specific antigens in liver, lung, spleen, adrenal gland, kidney, lymph node, bone marrow and in ganglia at all levels of the neuraxis (99,122). In addition, SVV-specific immediate early, early and late transcripts can be detected in skin, lung, liver and ganglia from acutely infected (11-12 days post-infection) monkeys (122).

Months to years after intratracheal inoculation, SVV DNA can still be detected in several tissues, including ganglia, liver and blood MNCs, primarily in CD4+ and CD8+ cells but not in CD14+ or CD20+ cells (123). The significance of persistent MNC infection is unclear. This persistence model of SVV infection can be used to study the ability of antiviral agents to drive virus into a latent state, of particular importance for patients taking immunosuppressive drugs.

In the second model of SVV infection, adult SVV-seronegative monkeys are exposed to monkeys previously inoculated intratracheally with SVV. After 10-

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14 days, a mild varicella rash develops in monkeys caged with the intratracheally inoculated monkeys, and PCR amplification of SVV DNA in skin scrapings of the naturally infected monkeys confirms that SVV causes the disease. SVV DNA is detected only occasionally in blood MNCs of naturally infected monkeys at the time of varicella. At 6-8 weeks after the resolution of rash, SVV DNA is present in multiple ganglia along the neuraxis, but not in lung or liver (109), indicating latent SVV infection. Thus, SVV infection of primates fulfills the above-mentioned criteria of latency. One particular advantage of this model is that latently infected monkey ganglia can be removed from anaesthetized monkeys before death to determine the physical state of viral nucleic acid and gene expression, thus obviating concerns about virus reactivation after death. This not only allows a comparison of SVV transcription during latency and the cell types harboring latent SVV with that found for VZV, but also serves in dissecting the molecular events in the cascade of cellular and immune factors involved in virus reactivation, studies not possible in humans.

6. PERSPECTIVES

6.1. Possible mechanisms for maintenance and reactivation of latent herpesvirus

VZV might have developed a mechanism to maintain latency involving virus genes 4, 18, 21, 29, 62, 63 and 66 through restriction of typically nuclear proteins to the cytoplasm. Alternatively, latent VZV might simply reactivate sporadically from a small number of neurons. Feldman *et al.* (124) recently documented rare occurrences of spontaneous subclinical HSV-1 reactivation from a small subset of neurons in latently infected mouse trigeminal ganglia. If VZV reactivates from a small population of latently infected neurons, IE62 is likely to be among the first virus genes expressed and translated, setting the stage for production of infectious virions. IE62-induced transcripts of VZV genes 4, 18, 21, 29, 63 and 66 would follow, all of which have been detected in latently infected human ganglia. In the case of gene 66, the protein kinase product might function as a reactivation-agonist, since its phosphorylation of IE62 would restrict the latter to the cytoplasm and thereby abort further virus reactivation. Although this hypothesis is difficult to test in humans, it is testable in the developing simian model of varicella latency (125). Overall, VZV latency is characterized by the transcription of multiple virus genes, whereas latent virus DNA transcription in other alphaherpesviruses is limited. The interplay of the latency-related VZV proteins may control the production of infectious virus to maintain latency.

6.2. Clinical implications

One of the most important interpretations of clinical-virological studies is that PHN might be caused by persistent VZV infection in ganglia. Unfortunately, ganglia of PHN patients cannot be studied during life; furthermore, even after death, ganglia corresponding to the site of pain during the life of PHN patients have not been studied pathologically or virologically. Thus, the causal relationship between PHN and chronic active VZV

infection in ganglia remains hypothetical. Nevertheless, the details provided in 4.2 have considerable implications for the treatment of patients with PHN. Adequate treatment of patients with VZV infection in the nervous system (myelitis, unifocal or multifocal vasculopathy) requires intravenous acyclovir. Further studies are needed to determine which of the antiviral agents (acyclovir, valacyclovir or famciclovir) used to treat VZV infection provides the greatest drug levels in ganglia, and whether any of these drugs given orally achieve levels comparable to those after intravenous injection. A comparison of ganglionic levels of these drugs after oral dosing can be conducted in primates, allowing subsequent comparison with ganglionic drug levels after intravenous acyclovir administration. Finally, determination of the drug and route of administration that provides the highest levels in monkey ganglia will allow treatment efforts in these persistently SVV-infected animals to drive virus in to the latent state.

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