Reactivated and latent varicella-zoster virus in human dorsal root ganglia

(latency/reactivation/in situ hybridization/PCR-ELISA)

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ABSTRACT Ganglia obtained at autopsy were examined by in situ hybridization from one patient with zoster (also called herpes zoster or shingles), two varicella-zoster virus (VZV)-seropositive patients without clinical evidence of zoster, one VZV-seronegative child, and one fetus. Ganglia positive for VZV had a hybridization signal in both neuronal and nonneuronal satellite cells. Ganglia obtained from the fetus and from the seronegative infant were consistently negative for VZV. Two striking observations were evident regarding the presence of VZV DNA in ganglia obtained from the individual with zoster at the time of death. First, ganglia innervating the sites of reactivation and ganglia innervating adjacent sites yielded strongly positive signals in neurons and satellite cells, whereas ganglia from distant sites were rarely positive. Second, VZV DNA was found in both the nuclei and the cytoplasm of neurons innervating areas of zoster. However, in neurons innervating zoster-free areas, VZV DNA was found only in the nucleus of neurons and their supporting satellite cells. Immunohistochemistry with a fluorescent monoclonal antibody to the VZV glycoprotein gpl, a late virus protein, revealed a positive signal in the cytoplasm of ganglia with clinical evidence of reactivation. These results illustrate that both neuronal and satellite cells become latently infected following primary VZV infection. The presence of VZV DNA and gpl in the cytoplasm of neurons demonstrates productive infection following reactivation at the site of latency.

Varicella-zoster virus (VZV) is an alphaherpes virus that commonly infects young individuals living in temperate climates (1-3). Following primary infection, clinically presented as chicken pox, latent virus persists in the host and may reactivate at a later date to cause zoster (also called herpes zoster or shingles). Clinical reactivation occurs most frequently in immunocompromised and older individuals and places the affected patient at risk for developing postherpetic neuralgia. This debilitating pain syndrome is associated with significant morbidity that may not be relieved by antiviral and analgesic therapy (4). It is therefore desirable to prevent zoster and postherpetic neuralgia in individuals harboring latent virus. Understanding the cellular and viral factors governing VZV latency and reactivation is essential to achieve this goal. Determining the location of persistent and reactivated virus is essential for defining the molecular basis of VZV pathogenesis.

Electron microscopy has demonstrated VZV in sensory ganglia of patients with zoster (5, 6), and polymerase chain reaction (PCR) and Southern blot hybridization have detected VZV DNA in latently infected dorsal root ganglia (7, 8). Studies utilizing in situ hybridization methods have identified several different VZV transcripts in affected ganglia (7, 9-13).

These studies have led to controversy as to the nature of the cell harboring the latent VZV genome. VZV transcripts have been localized exclusively in neurons (10, 13, 14) or in non-neuronal satellite cells (9, 12) depending on the in situ hybridization technique used.

In the current study, the cellular and intracellular location of the VZV genome in latently infected human dorsal root ganglia and in ganglia with clinical evidence of reactivated virus was determined by in situ hybridization. Evidence for virus replication in affected ganglia was provided by polymerase chain reaction (PCR) and immunohistochemistry. These results demonstrate that latent VZV resides in both neurons and their supporting satellite cells and that latent virus can reactivate in neurons.

MATERIALS AND METHODS

Tissue Specimens. Dorsal root ganglia from two VZV-seropositive patients without clinical evidence of zoster, one VZV-seronegative child and one fetus without a maternal history of varicella, were obtained at autopsy. Ganglia from an 85-year-old man with malignant glioma of the thoracic spinal cord who had a zosteriform rash in the distribution of the right T11, T12, and L1 sensory nerves at the time of death were also obtained. Prior to developing zoster, the patient had radiation therapy to the spinal cord at the levels of T4 through L1. At autopsy, he had a vesicular eruption in the right T11 and L1 distribution, separated by an area of recently healed epidermis in the right T12 distribution.

Ganglia were fixed in 10% formalin and embedded in paraffin. Six-micrometer-thick sections were mounted on Silanated slides (Digene Diagnostics, Beltsville, MD) for in situ hybridization and immunohistochemistry studies or were placed in sterile Eppendorf tubes for solution PCR. Tissue sections were heat-fixed at 60°C. The microtome blade was cleaned with 70% ethanol between cutting each tissue block.

In Situ Hybridization. The sections were deparaffinized with xylene, rinsed twice with ethyl alcohol, and digested with proteinase K (PK) diluted to 10 μg/ml in PK buffer (100 mM Tris, pH 7.4/150 mM NaCl/12.5 mM EDTA) for 10 min at room temperature. After rinsing with in situ hybridization buffer (50 mM KCl/10 mM Tris, pH 7.4/3 mM MgCl2), the slides were placed in this buffer containing 40 pmol of the fluorescein-labeled VZV oligonucleotide probe (Table 1) per ml and denatured at 95°C for 10 min. The hybridization reaction was performed at 60°C for 1-2 hr. The slides were rinsed twice, and the hybridized probe was detected by incubating the sections with a 1:200 dilution of alkaline phosphatase (AP)-conjugated anti-fluorescein antibody (Boehringer Mannheim) in 5× SSC.

Abbreviations: AP, alkaline phosphatase; ORF, open reading frame; DAPI, 4',6-diamidino-2'-phenylindole dihydrochloride; VZV, varicella-zoster virus.

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with a VZV-specific fluorescein-labeled oligonucleotide probe (Table 1).

ELISA detection of the PCR products was performed as described (16). Briefly, the 222-bp amplification product with a 5' biotin moiety was captured on streptavidin-coated polystyrene plates (Pierce) and hybridized with 10 pmol of the complementary fluorescein-labeled oligonucleotide probe (Table 1). PCR product–probe hybrids were detected with AP-conjugated anti-fluorescein antibody (Boehringer Mannheim). One hundred microliters of AP substrate solution (1 mg/ml dilution of p-nitrophenyl phosphate; Sigma) was prepared and added to each well as recommended by the manufacturer. The absorbance at 405 nm was determined after a 1-hr incubation. An absorbance greater than 0.4 indicated the presence of amplified product. This PCR–ELISA method detects 1–5 fg of DNA.

RESULTS

Oligonucleotide in situ hybridization probes may detect both DNA and RNA species. The fluorescein-labeled VZV probe (Table 1) is complementary to 27 bp from ORF 54, a gene contained within the BamHI D fragment of VZV, which is not known to be transcribed during latency (9, 14). In situ hybridization of ganglia obtained from two patients without evidence of acute VZV infection or reactivation revealed that both the fluorescent and antibody-mediated AP hybridization signals were detected in the nucleus of affected cells. However, greater resolution of detail and less background staining were obtained when the signal was amplified with anti-fluorescein antibody conjugated to AP. Positive sections exhibited hybridization signal in both neurons and nonneuronal satellite cells (Fig. 1 A). The proportion of positive cells differed among the

![Image](https://example.com/image1.png)

Table 1. Oligonucleotide primers and probes used for in situ hybridization and PCR–ELISA

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLA</td>
<td>GGAACCGCTGCACCATAGAA</td>
</tr>
<tr>
<td>FOK</td>
<td>Bio-TCCCTTATGCACGGTCTCAT</td>
</tr>
<tr>
<td>Probe</td>
<td>CCGGTGATGCAACGGGTGGGCGCCAGAGGAGGCGG</td>
</tr>
</tbody>
</table>

Bio denotes conjugated biotin and Flu denotes conjugated fluorescein. The probe is complementary to 27 bp within the VZV open reading frame (ORF) 54 region amplified by the NLA/FOK primers.

![Image](https://example.com/image2.png)

Fig. 1. In situ hybridization of VZV-positive and -negative ganglia. (A) A ganglion harboring VZV illustrates in situ hybridization AP signal in the nuclei of neurons and satellite cells. (B) Ganglion from a VZV-seronegative infant lacks AP in situ hybridization signal.
Table 2. In situ hybridization results of ganglia obtained from four patients without evidence of acute VZV infection

<table>
<thead>
<tr>
<th>Patients</th>
<th>Specimen</th>
<th>Neurons</th>
<th>Satellite cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive, %</td>
<td>positive, %</td>
</tr>
<tr>
<td>Adult 1</td>
<td>1 ganglion</td>
<td>20–30</td>
<td>20–30</td>
</tr>
<tr>
<td></td>
<td>2 ganglia</td>
<td>5–10</td>
<td>5–10</td>
</tr>
<tr>
<td></td>
<td>4 ganglia</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Adult 2</td>
<td>1 ganglion</td>
<td>15–20</td>
<td>20–30</td>
</tr>
<tr>
<td></td>
<td>1 ganglion</td>
<td>3–8</td>
<td>5–10</td>
</tr>
<tr>
<td>Infant</td>
<td>1 ganglion</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Fetus</td>
<td>1 ganglion</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

At least four sections were examined from each ganglion.

ganglia examined; some had many positive cells and others had only a few positive cells (Table 2). Five percent to 30% of neurons and/or satellite cells in ganglia from patients with zoster or a history of varicella were positive for VZV DNA by in situ hybridization.

To determine the specificity of the hybridization probe, two ganglia obtained from a stillborn fetus with no maternal history of recent varicella or herpes zoster infection and three ganglia from an infant with no history of varicella infection were examined. In situ hybridization studies of these specimens were consistently negative (Fig. 1B and Table 2). Studies with other control fluorescent-labeled oligonucleotide probes for several human papillomaviruses and Mycobacterium tuberculosis were negative. Thus, the probe is specific for VZV DNA, and the fluorescence moiety does not nonspecifically label ganglionic tissue.

The dorsal root ganglia of an 85-year-old man with malignant glioma of the thoracic spinal cord who had a zosteriform rash in the distribution of the right T11, T12, and L1 sensory nerves at the time of death were studied. On histologic examination, the right T12 ganglion showed severe necrosis and inflammation, while the right T11 and L1 ganglia were mildly inflamed. Histology of ganglia not associated with clinical zoster was unremarkable. In situ hybridization and PCR results of individual ganglia from this case are summarized in Table 3. Ganglia innervating the sites of reactivation and adjacent ganglia yielded strongly positive signals. Distant ganglia were rarely positive. The right T11 ganglion had many neurons and satellite cells that were positive for VZV DNA by in situ hybridization. The right T12 ganglion was diffusely necrotic, and cellular definition was obscured in all but a few neurons that were mostly positive for VZV DNA. The right T8, T9, T10, L1, and left L4 ganglia had few positive cells. AP signal representing VZV DNA was found in both the nuclei and the cytoplasm of some affected T11 neurons and most affected T12 neurons (Fig. 2A). In contrast, VZV-positive ganglia from dermatomes without corresponding evidence of reactivation, such as the left L4 ganglion, had detectable viral DNA in the nuclei but not in the cytoplasm of affected neurons (Fig. 2D). Although the patient’s rash also involved the right L1 dermatome, this ganglia had in situ hybridization signals only in nuclei of VZV-positive cells.

The presence of VZV DNA in the cytoplasm of neurons from the right T11 and T12 ganglia indicates productive infection. During VZV replication, late genes such as those encoding structural glycoproteins are expressed. To demonstrate that late gene products were being produced in ganglia with cytoplastic VZV signals, these ganglia were examined for expression of gp1. gp1 was detected in the cytoplasm of neurons from the right T11 and T12 ganglia (Fig. 2B). The left L4 ganglion did not have detectable gp1 (Fig. 2E). When these ganglia were stained with DAPI, a fluorochrome that interacts with double-stranded DNA, the neuronal cytoplasm of the right T11 and T12 ganglia exhibited punctate fluorescence in a pattern indistinguishable from the pattern seen by in situ hybridization (Fig. 2C). No cytoplasmic DAPI fluorescence was detected in the left L4 ganglion (Fig. 2F).

The results from PCR amplification confirmed the in situ hybridization findings. These results correlated, with the exception that five ganglia positive by in situ hybridization were negative by PCR. Four of the five ganglia had only a few positive cells by in situ hybridization, and it is likely that the amount of VZV DNA present in these specimens was below the level of detection of the PCR-ELISA assay. The right T10 ganglion had many positive cells by in situ hybridization but was negative by PCR-ELISA. The VZV PCR-negative tissue sections contained amplifiable β-globin DNA.

**DISCUSSION**

The establishment of latent VZV infection is thought to involve either axonal transport of virus from skin lesions or hematogenous seeding of virus to the dorsal root ganglia during primary infection. As the result of interactions between the virus, host immune responses, and the cellular environment, VZV establishes and maintains a dormant state in sensory ganglia. Reactivation is thought to occur when alterations in the balance between virus and host factors allow local

Table 3. Comparison of results of in situ hybridization (ISH) and PCR–ELISA analyses for the presence of VZV DNA in left and right ganglia obtained from an individual with zoster involving the T11, T12, and L1 dermatomes

<table>
<thead>
<tr>
<th>Ganglion</th>
<th>Neurons positive, %</th>
<th>Satellite cells positive, %</th>
<th>PCR</th>
<th>Neurons positive, %</th>
<th>Satellite cells positive, %</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>T8</td>
<td>0</td>
<td>0</td>
<td></td>
<td>5–10</td>
<td>5–10</td>
<td></td>
</tr>
<tr>
<td>T9</td>
<td>0</td>
<td>0</td>
<td></td>
<td>10–15</td>
<td>10–15</td>
<td></td>
</tr>
<tr>
<td>T10</td>
<td>0</td>
<td>0</td>
<td></td>
<td>30–40</td>
<td>25–30</td>
<td></td>
</tr>
<tr>
<td>T11</td>
<td>3–6</td>
<td>3–6</td>
<td></td>
<td>60–70†</td>
<td>50–70†</td>
<td></td>
</tr>
<tr>
<td>T12</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>70–80</td>
<td>Necrotic</td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>0</td>
<td>0</td>
<td></td>
<td>5–10</td>
<td>10–15</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>4–8</td>
<td>5–10‡</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>L5</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NA, not available for study.

*gp1*.
†Neuronal cytoplasm was positive by ISH.
‡gp1*. 

replication of virus in the ganglion and axonal transport to the skin, resulting in zoster (reviewed in ref. 2).

Determining the cellular location of persistent virus is essential to understanding the regulation of latency and reactivation. In situ hybridization methods have detected VZV nucleic acids exclusively in the satellite cells of latently infected trigeminal ganglia (9, 12) or in the neurons (10, 14). These differences in latency sites may occur because the probes used in these studies hybridized to different VZV transcripts and genes. It is possible that unique regions of the viral genome are transcribed in different cell types.

In this study, ganglia from adults that were positive for VZV exhibited signal in both neurons and satellite cells. The question arises whether virus underwent reactivation prior to obtaining the tissue. This seems unlikely because no in situ hybridization signal was found in the cytoplasm of positive neurons from patients without active zoster.

These data are consistent with those obtained from animal models and in vitro studies. The latent virus is not entirely quiescent; several VZV genes have been shown to be expressed during latency including ORFs 4, 21, 29, 62, and 63 (9, 10, 12, 14, 17). Ganglia harboring latent VZV obtained from a rat model contained the product of ORF 63, a VZV-transactivating protein, in neurons and satellite cells (18). Additionally, investigators were able to recover VZV from an in vitro model of latency and reactivation only when both neurons and satellite cells were included in the system (19). These results and those presented here lead to the conclusion that latent VZV is maintained in neurons and satellite cells of dorsal root ganglia and that both cell types are essential to the processes of VZV latency and reactivation.

Ganglia obtained from a patient with zoster at the time of death had intense in situ hybridization signals in the nuclei and cytoplasm of many neurons and in the nuclei of satellite cells. VZV DNA was present in the neurons and satellite cells predominantly in the ganglia corresponding to the affected dermatomes and to a lesser extent in the adjacent ipsilateral ganglia without corresponding dermatologic signs of reactivation. Ganglia innervating asymptomatic dermatomes had fewer VZV-positive neurons and satellite cells.

The presence of cytoplasmic VZV DNA and gpl illustrates replication of VZV in neurons during reactivation. These data are consistent with electron microscopic studies (5, 6). The location of virus in the satellite cells could not be determined because of their large nucleus-to-cytoplasm ratio. However, the absence of gpl in these cells suggests that during reactivation, replication of VZV occurs predominantly in neurons.

Previous in situ hybridization studies found only 0.01–0.3% of ganglion cells harboring VZV nucleic acids (9, 10). Our results illustrate that VZV infects a larger population of ganglion cells than previously recognized. VZV DNA was detected by in situ hybridization in 5% to 30% of neurons and satellite cells in affected ganglia obtained from individuals without signs of active VZV infection. In the individual with
zoster, the majority of neurons and satellite cells in ganglia with corresponding clinical evidence of reactivation were positive for VZV DNA. Results of in situ hybridization of the remaining ganglia studied from this individual were similar to the results of ganglia obtained from individuals with latent virus. It appears that initially only a few cells within a ganglion harbor latent VZV. Upon reactivation the virus replicates and interneuronal spread occurs, leading to clinical disease. Determining the role each ganglion cell type plays in maintaining nonproductive VZV infection and its involvement in the process of reactivation may suggest novel strategies to prevent the clinical manifestations of zoster and its sequelae.

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