Varicella zoster virus in human and rat tissue specimens

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Summary. The limited supply of appropriate tissues for study has been an impediment to investigations of varicella zoster virus (VZV) latency. Human dorsal root ganglia (DRG) harboring latent virus are not plentiful and are not amenable to manipulation for studying the events surrounding the establishment, maintenance, and cessation of latency. An alternative to studies in human DRG is the rat model of latency, which appears to provide a reliable method of investigating VZV latency. Other alternatives include studies in other human tissues involved in VZV pathogenesis. In order to improve our understanding of the establishment and cessation of latency, we performed comparative immunohistochemical analysis of chickenpox and zoster skin lesions. This analysis revealed that during primary infection and reactivation productive VZV infection occurs in a variety of cell types and that the major VZV DNA binding protein, ORF29p, is present in peripheral axons early during the course of chickenpox. VZV latency was studied in the rat model by in situ hybridization and compared with similar studies performed in human DRG containing latent virus, confirming that VZV DNA persists in the same sites in DRG of the two species.

Introduction

Primary varicella zoster virus (VZV) infection causes chickenpox and latent infection in host dorsal root ganglia (DRG), placing the host at risk for virus reactivation, which may be clinically recognized as zoster. The events that facilitate the persistence of VZV in the neurons and satellite cells of the DRG remain poorly understood. Although it appears that the entire virus genome is present during latency [9, 14], only a portion of the genome is expressed at this stage [4-7, 15]. Proteins encoded by open reading frames (ORFs) expressed during latency accumulate in neurons but localize to atypical cellular compartments [13]. When VZV reactivates in DRG, the full lytic cycle cascade resumes and the proteins are found in locations that are typical of productive infection [13]. The factors that influence these events have not yet been fully elucidated.
Hindering further investigations of these phenomena is the lack of appropriate tissue that supports latent VZV infection. VZV is highly species-specific and naturally infects only humans and, rarely, some non-human primates [3]. The most reliable small animal model for the study of VZV latency is the rat model [16]. VZV appears to establish a latent infection in DRG of rats following subcutaneous inoculation and virus DNA persists for months at this site [1, 16]. Whether persistence of VZV in rat DRG results from the same events that allow persistence of VZV in human DRG is still to be determined. In the meantime, studies of the establishment, maintenance, and cessation of VZV latency are largely performed in specimens from the natural host.

Here we describe investigations aimed at elucidating the events involved in VZV latency and reactivation. In order to gain better insight into the establishment and cessation of latency, human skin biopsy specimens from patients with chickenpox and zoster were evaluated by immunohistochemistry [2]. VZV latency was studied in the rat model by in situ hybridization [1] and the findings were compared with similar studies performed in human DRG [12].

VZV infection in human skin biopsies

Comparative immunohistochemistry of skin biopsies from patients with chickenpox and zoster were performed using polyclonal antibodies generated against the protein products of immediate early (IE63, ORF29, and ORF14) [2]. These proteins were chosen for this analysis to include a representative of the three kinetic classes of VZV genes. By analogy to the herpes simplex virus homologue, ICP8, ORF29 is an early gene that encodes a DNA binding protein [3]. ORF14, a late gene, encodes the glycoprotein. gC [3]. IE63p and gC are virion components [11]. ORF29p is not a component of the virion [10] and its presence in a cell nucleus implies virus replication at that site.

Six cases of chickenpox and 8 cases of zoster were selected for immunohistochemical analyses (Table 1, Fig. 1) [1]. Five cases of Grover's disease, a

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Tissues from patients with clinical and histopathological diagnoses (DX) of chickenpox (CP) or zoster (Z) were analyzed by immunohistochemistry for immediate early (IE 63p), early (ORF 29p) and late (gC) virus proteins in epithelial (EP), endothelial (EN), inflammatory cells expressing CD43 (WBC), or in dermal nerves (NE). The results are expressed as the absolute number of biopsies with detectable protein. Zero indicates absence of detectable protein.

*The number of cases positive for any VZV protein/the number of cases examined
+2 chickenpox cases and 2 zoster cases with VZV-infected WBC were found to express CD68 and not CD3 or CD20. Reproduced with permission [2]
Fig. 1. Immunohistochemical detection of ORF29p in skin biopsies. Chickenpox (A), zoster (B) and Grover's disease (C) skin lesions were analyzed for ORF29p. The arrows indicate positive epithelial cells. The sections are shown at 100× magnification. Reproduced with permission [2].
non-infectious dermatologic disorder characterized by a vesicular eruption that is distinguishable from herpes virus infections by histopathologic criteria, and 3 cases of herpes simplex infection were included as negative controls. None of the Grover's disease or herpes simplex cases had positive immunostaining (Fig. 1) [2]. Biopsies were obtained as early as 2 days following the onset of rash in both chickenpox and zoster cases. All of the 6 chickenpox cases were positive for ORF29p, 4 were positive for IE63p and 5 were positive for gC. Among the 8 zoster cases, all were positive for ORF29p, 5 were positive for IE63p, and 5 were positive for gC. One zoster patient subsequently developed postherpetic neuralgia. The biopsy specimen was obtained 3 days after the onset of the rash in this case. When compared with the other 7 zoster cases, no differences could be detected in the histopathologic or immunohistochemical features of the case that precipitated postherpetic neuralgia.

VZV was detected in epithelial cells, endothelial cells, nerves, and inflammatory cells of the epidermis and dermis in both chickenpox and zoster cases (Table 1). IE63p, ORF29p, and gC were detected in the expected intracellular compartments. ORF29p was found in cell nuclei (Figs. 1 and 2). gC was found in cell membranes and the cytoplasm (data not shown). IE63p was found in both the cell nucleus and cytoplasm (data not shown). IE63p was detected in epithelial cells and endothelial cells. ORF29p was found in epithelial cells, endothelial cells (Figs. 1 and 2), and inflammatory cells expressing the cell differentiation marker, CD43 (data not shown). ORF29p was also detected in the Schwann cells and axons of nerves in two chickenpox cases but was not detected in these cells in any of the zoster cases (Fig. 2). gC was found in epithelial cells, endothelial cells, and in both axons and Schwann cells of nerves in chickenpox and zoster. Double labeling experiments revealed that the VZV infected inflammatory cells in chickenpox and zoster cases express CD43 and CD68 but not CD20 or CD3, indicating that they are cells of the monocyte macrophage lineage (data not shown).

Finding ORF29p in the peripheral nerve during chickenpox was unexpected because during lytic infection this protein localizes to cell nuclei, where it participates in virus DNA replication. Unlike gC, which as an envelope protein presumably entered peripheral nerves during primary infection as the virus envelope fused with the axonal membrane, ORF29p is not a component of the virion [10]. De novo production of ORF29p in the nucleus of the neuron residing in the DRG, followed by axonal transport to the peripheral axon is not likely because at least one of the biopsies with this finding was obtained two days after the onset of the rash. It is widely thought that the skin is infected by hematogenous spread of VZV during chickenpox and that virus from the skin enters peripheral nerves to infect the neuron. A substantial time period would be necessary for the virus to travel from the skin to the DRG by retrograde axonal transport, a slow process of 200–400 mm/day [17]. Additional hours would lapse before early virus proteins such as ORF29p would be produced in the neuron cell body. A newly synthesized protein could then be detected in peripheral axons only after traveling the considerable distance back to the dermis by anterograde axonal transport.
Fig. 2. Immunohistochemical detection of ORF29p and CD43 in skin biopsies. Skin biopsies from a patient with chickenpox (A, C) or a patient with zoster (B, D) were probed for the presence of ORF29p. The arrows indicate endothelial cells containing ORF29p. C and D show nerves in which ORF29p was present (C) or absent (D). The sections are shown at 600× magnification. These panels were reproduced with permission [2].

[17]. The lack of ORF29p from peripheral axons during zoster is consistent with previous findings that this protein localizes to the nucleus during lytic infection and reactivation [13]. These findings raise the question of whether ORF29p has a previously unrecognized role in the initial infection of neurons during chickenpox.
VZV infection in rat dorsal root ganglia

Observations of VZV in human DRG during the initial stages of infection and establishment of virus latency have not been possible because of the lack of appropriate specimens. Human specimens are of limited supply and are not amenable to experimental manipulation. In addition, their usefulness for the study of latency is constrained by the risk of VZV reactivation at the time of death. Tissue culture systems that support latent infection are not available for VZV. The most promising tool to study VZV latency is the rat model [16]. VZV appears to establish latent infection in rats that have been subcutaneously inoculated with virus [1, 8, 16].

Although the rats do not develop signs or symptoms of acute infection, the virus genome is found in satellite cells and in the nuclei of neurons one to three months following inoculation (Fig. 3) [1]. By in situ hybridization, latent infection in the rat is indistinguishable from latent infection in humans (Fig. 3). In most instances, DRG ipsilateral to the inoculation site contain VZV. Rarely, we have found latent VZV in contralateral DRG [1], raising the possibility that in the rat, DRG may be infected by the hematogenous route. However, we have not detected VZV in rat peripheral blood mononuclear cells by polymerase chain reaction (data not shown) and in the vast majority of cases, VZV is found only in DRG ipsilateral to the inoculation site. Therefore, axonal transport appears to be the predominant mode of spread to DRG in the rat model.

Fig. 3. In situ hybridization detection of VZV DNA in human and rat DRG. DRG obtained from a human without clinical evidence of zoster at the time of death (A) and from a rat inoculated with VZV three months prior to sacrifice (B) underwent in situ hybridization for VZV. VZV DNA is detected in satellite cells and neuronal nuclei in both specimens.

The sections are shown at 600× magnification. B was reproduced with permission [1]
It remains to be determined if VZV latency in the rat is identical to latency in the human. One VZV protein, IE63p, has been found in rat DRG harboring latent virus [8]. Whether other VZV proteins found in human DRG containing latent virus are present in rat DRG containing latent virus has not been determined. VZV has not been found to spontaneously reactivate in the rat, an important distinction from its behavior in humans that may indicate significant differences between the latency state in the two species. However the lack of spontaneous virus reactivation in rat DRG may be advantageous to investigators studying latency.

Conclusion
Specific immediate early and early proteins accumulate in neurons containing latent VZV and localize to atypical cellular compartments [13]. When VZV reactivates in DRG, the full lytic cycle cascade resumes and these proteins are found in their typical locations [13]. The factors that trigger these changes in protein expression and localization remain unknown. Immunohistochemical analysis of chickenpox and zoster skin lesions may provide clues into the initial establishment and cessation of latency. During primary infection, VZV productively infects endothelial cells, epithelial cells, inflammatory cells bearing monocyte/macrophage markers, and peripheral axons. The presence of ORF29p in peripheral axons during primary infection in puzzling and may suggest that ORF29p is involved in the early stages of neuronal infection. The function that ORF29p performs in peripheral axons during chickenpox does not appear to be required for reactivation since this protein was not found in peripheral axons during zoster. When the virus returns to the skin during zoster, productive infection is again established in epithelial cells, endothelial cells, and inflammatory cells.

Systematic investigations of these events are limited by the lack of appropriate tissues that support latent infection. The rat model may provide a reliable means for studying the establishment and maintenance of latency. Initial studies indicate that latent infection in this model is applicable to latent infection in humans.

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References


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