Journal of NeuroVirology, 8(suppl. 2): 80–84, 2002
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DOI: 10.1080/13550280290101058

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Key issues in varicella-zoster virus latency

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The molecular mechanisms by which varicella-zoster virus (VZV) causes a latent infection in human trigeminal and spinal ganglia are not well understood. It is known that VZV establishes latency in ganglia following the primary infection causing varicella (chickenpox), and that the virus may reactivate after years of dormancy to produce herpes zoster (shingles). Two key issues have been the cell-type localization of latent VZV in human ganglia, and the nature and extent of VZV gene expression during latency. Although the cell specificity of latent VZV has been controversial for almost a decade, it is now widely accepted that the virus is mainly latent in neuronal cells, with only a small proportion of non-neuronal cells infected. All of the studies carried out so far have indicated that VZV gene expression is highly restricted during ganglionic latency. Although at least four VZV genes have been identified as being expressed, the possibility that latent gene expression is significantly greater than this cannot yet be excluded. There is also evidence for VZV gene–encoded proteins being expressed during latency, although the precise extent of this is unclear. Advances in this difficult field may be expected to arise from both newly developed molecular technology and more refined animal models of VZV latency. *Journal of NeuroVirology* (2002) **8(suppl. 2),** 80–84.

Keywords: latency; trigeminal ganglion; varicella-zoster virus (VZV)

Introduction

Varicella-zoster virus (VZV) is a human herpesvirus, which is the causative agent of varicella (also known as chickenpox). Following this primary infection, which generally, but not always, occurs in children, VZV is transported by sensory nerve fibers to the trigeminal ganglia (TG) and the dorsal root ganglia (DRG) where the virus establishes a latent infection (Gilden *et al*, 2000b). After a variable interval, which may be decades, the virus may reactivate, either spontaneously or following one or a number of triggering factors, to produce herpes zoster (also known as shingles), which is a painful skin eruption that usually affects an entire dermatomal region (Gilden *et al*, 2000b). Factors that increase the possibility of VZV reactivations include increasing age and the coexistence of a state of immunosuppression. VZV reactivations may be followed by a number of neurological complications, including postherpetic neuralgia, which is a major cause of morbidity, and a variety of other central nervous system (CNS) conditions, including small and large vessel vasculopathy, segmental weakness, and cranial nerve palsies (Gilden *et al*, 2000a).

The burden of VZV-associated disease is considerable and much greater than was previously thought. The importance of CNS VZV infections was recently highlighted by a study from Finland in which VZV was identified as being the causal agent in no less than 29% of all probable or confirmed etiological agents of encephalitis, meningitis, and myelitis in a total of 3231 patients with suspected CNS viral disease (Koskiniemi *et al*, 2001). Most hospital admissions of VZV infections in a recent study in Scotland were

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This article is based on both an oral presentation given at the 4th International Symposium of NeuroVirology in Düsseldorf, Germany, on June 20, 2002, and an article in *Reviews in Medical Virology* (2002, Vol. 12: 1–8). I wish to thank Drs. Donald Gilden and Randall Cohrs for manuscript review. The author's VZV research described here was supported by sequential grants from the Wellcome Trust.

Received 31 July 2002; revised 6 August 2002; accepted 19 August 2002.

for trigeminal zoster, with a length of stay ranging from 1 to 70 days (median 11 days), and it was predicted that the incidence of zoster would increase as the population ages (Torrens *et al*, 1998). In the USA, a previous estimate of about 300,000 cases of zoster annually (Weller, 1992) is now almost certainly an underestimate, especially because VZV reactivates in about 15% of individuals (Cohen *et al*, 1999).

Details of the VZV genome will not be given here and have been described elsewhere (Ostrove, 1990). Essentially, the VZV genome contains double-stranded DNA, with a size just under 125 kb, with 71 predicted open reading frames (ORFs), of which about 65 have known homologs in herpes simplex virus type 1 (HSV-1) (Cohen *et al*, 1999; Ostrove, 1990). The genome has been shown to comprise a unique long (U_L) segment and a unique short (U_S) segment, which are covalently linked, and these segments are both flanked by terminal and internal repeat regions (Cohen *et al*, 1999; Ostrove, 1990). Our knowledge of VZV infections has tended to lag behind that of HSV-1, due primarily to the considerable difficulty in working with VZV because of its very cell-associated nature, making growth of the virus to high titer difficult, and the fact that it only grows in human or primate cells. The VZV genome contains putative immediate-early, early, and late genes, and viral gene expression during acute lytic infection appears to be sequentially regulated, as is the case with HSV-1 (Cohen *et al*, 1999).

VZV ganglionic latency

The ultimate aim of VZV latency studies is to understand the molecular mechanisms of the establishment and maintenance of ganglionic latency, and of viral reactivation, with the long-term goal of devising more effective measures to treat, if not prevent, VZV-associated neurological disease. Unfortunately, the only method of investigating VZV latency in humans at present is to study postmortem TG and DRG, in which mechanistic studies are not feasible (Gilden *et al*, 2000b). Moreover, it is not possible to exclude the possibility that a degree of viral reactivation may have already occurred at the time of death or removal of tissues at autopsy. This restriction is compounded by the absence of very good animal models of VZV latency and reactivation, although there have been some recent promising advances in this area.

It is known from careful and detailed polymerase chain reaction (PCR)-based studies that almost 90% of normal individuals harbor latent VZV DNA in their TG (Mahalingam *et al*, 1990). Correct identification of the ganglionic cell type in which latent VZV resides is important in that it should enhance our understanding of the latency process. Although one might strongly suspect intuitively that VZV may mirror the neuronal specificity of latent HSV-1 (Steiner and Kennedy, 1993), a compelling case has also been made for the conceptual advantage in pathogenetic terms of VZV residing in non-neuronal satellite cells (Meier and Straus, 1992). Thus Meier and Straus (1992) argued that non-neuronally located VZV, when reactivated, may proliferate extensively, with spread to both neuronal and non-neuronal cells, and that this might help explain the extensive dermatomal distribution of herpes zoster, in contrast to the limited cutaneous lesions of reactivated HSV-1. However, early studies in human TG using in situ hybridization (ISH) reported that latent VZV was located in neurons (Hyman *et al*, 1983), findings that were later confirmed by Gilden and colleagues (1987). On the other hand, two subsequent studies from Straus and colleagues using ISH strongly suggested that latent VZV resided in non-neuronal satellite cells and not neurons (Croen *et al*, 1988; Meier *et al*, 1993). In the 'third phase' of the investigation of this issue, Lungu *et al* (1995), using ISH, found that VZV was located in a high percentage of both neurons and satellite cells. In the most extensive study to date of this issue, Kennedy *et al* (1998) used ISH and *in situ* PCR amplification on a large number of TG (including coded samples obtained from both the Straus and Gilden laboratories) to show unambiguously that latent VZV was located predominantly in neurons, with only occasional non-neuronal satellite cells infected (Figure 1). A subsequent study confirmed these findings in human DRG, in which it was also shown that more than one region

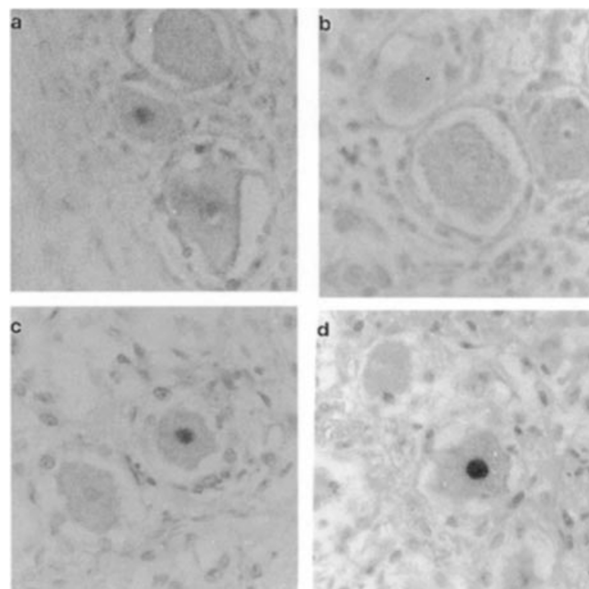

Figure 1 *In situ* hybridization of human trigeminal ganglia (TG) with DIG-labeled probes. (**a**) Normal TG hybridized with VZV gene 29 probe. (**b**) Control, TG hybridized with non-VZV plasmid DNA probe. (**c** and **d**) HIV-positive TG hybridized with VZV gene 63 probe and with VZV gene 29 probe, respectively. Positive nucleic acid signals in neurons can be seen in **a**, **c**, and **d**, but not in **b** (×450). Reproduced, with permission, from *Proc Natl Acad Sci USA* (1998) **95:** 4658–4662. Copyright (1998), National Academy of Sciences, USA.

of the viral genome was present in neurons during latency (Kennedy *et al*, 1999). A neuronal localization of latent VZV was also demonstrated by LaGuardia *et al* (1999) by analysis of dissociated TG neuronal and non-neuronal cells, thus providing an elegant confirmation of the neuronal site of viral latency (Mahalingam *et al*, 1999).

The second key issue in VZV latency is the extent of viral gene expression during ganglionic latency (Kennedy, 2002). This is an important question to address for two reasons. First, this should theoretically enhance our understanding of the latency process because the functions of so many of the VZV genes are already known or inferred through homology with HSV-1. However, there are two caveats that should be added here, namely, that the inferred homologies may not all be completely correct as has been demonstrated for gene 21 (Cohrs *et al*, 2002), and also that it is very difficult to prove the biological function of a known expressed VZV gene during latency because mechanistic studies are not possible in human ganglia. Second, viral genes that are expressed during latency may be targeted for antiviral strategies. But caveats to be added in this context include the problem of selection of particular gene products out of possibly multiple expressed genes, and also the mode of delivery of potential therapies, e.g., antisense oligonucleotides. In addition, there is the problem of deciding whether to use such therapies to prevent the viral reactivation process, e.g., immunization protocols against expressed viral proteins, or to attempt to limit reactivation once it has started.

There have been several published studies of latent VZV gene expression using a variety of different techniques on pooled and individual human ganglia, including Northern blot analysis, ISH, and cDNA library construction (Kennedy, 2002). Most studies have reported the presence of RNA for VZV genes 21, 29, 62, and 63 (Cohrs *et al*, 1994, 1995, 1996; Kennedy *et al*, 2000; Meier *et al*, 1993), with conflicting results for the presence of RNA for gene 4, which has been reported by some (Croen *et al*, 1988; Kennedy *et al*, 2000) but not other (Meier *et al*, 1993) groups. In our own studies using ISH, RNA for VZV genes 21, 29, 62, and 63 were the most frequently detected in TG from both normal and human immunodeficiency virus (HIV)-infected individuals, and RNA for both genes 4 and 18 were also detected, but at

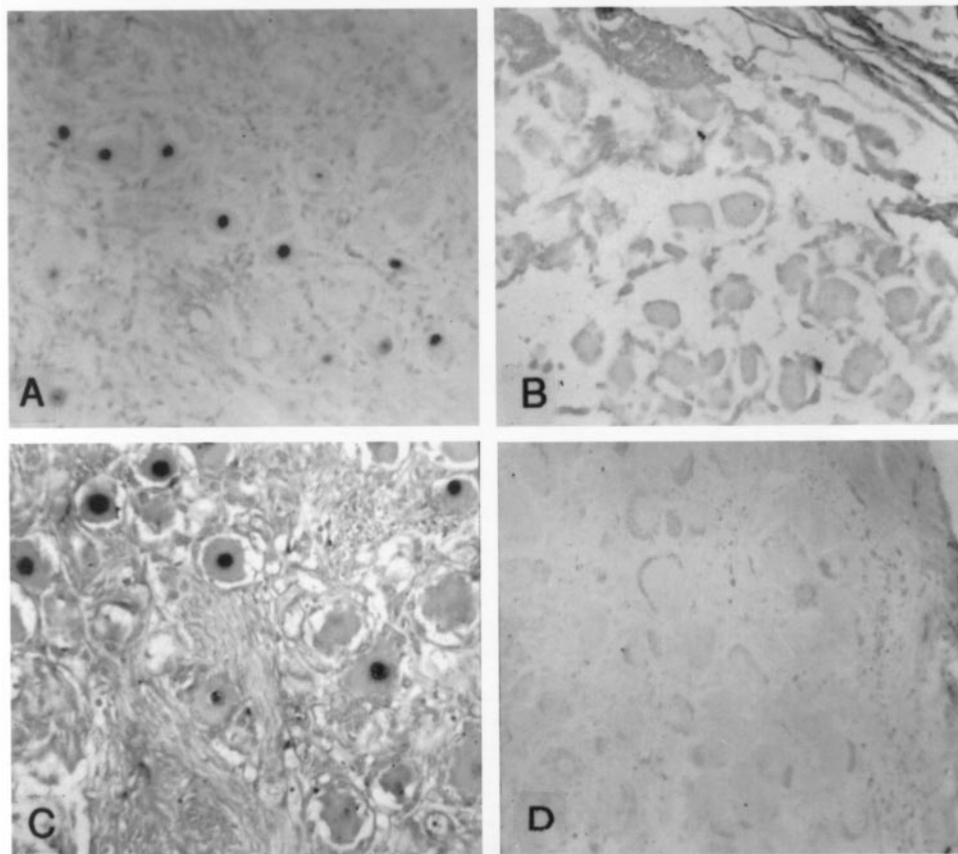

Figure 2 *In situ* hybridization for VZV RNA of human TG with DIG-labeled probes. (**A**) Normal TG hybridized with VZV gene 62 probe. Several neurons are labeled. (**B**) Control, infant TG hybridized with VZV gene 62 probe. No cells are labeled. (**C**) HIV-positive TG hybridized with VZV gene 29 probe. Several neurons are labeled. (**D**) Normal TG hybridized with VZV gene 4 probe. No cells are labeled. (×450.) Reproduced, with permission, from *J Virol* (2000) **74:** 11893–11898.

a lower frequency (Kennedy *et al*, 2000) (Figure 2). The most frequently detected VZV transcript in this study, and also a recent detailed study using real-time PCR (Cohrs *et al*, 2000), was that corresponding to VZV gene 63. The VZV RNA signals were again localized predominantly in neurons, with only occasional satellite cells being positive. There is now a consensus that VZV gene 63–encoded protein is present in a significant proportion of TG (Kennedy, 2002; Mahalingam *et al*, 1996), up to 25% in a recent study (Kennedy *et al*, 2000). There has also been a report by Lungu *et al* (1998) that demonstrated the presence of proteins encoded by VZV genes 4, 21, 29, 62, and 63 in the cytoplasm of neurons and non-neuronal cells in latently infected TG. These studies await confirmation but are very important as they indicate possible immunization targets for preventing VZV-associated neurological disease. Recent studies on a rat model of VZV latency have also strongly suggested that VZV gene expression is limited to these small number of genes in latently infected DRG (Kennedy *et al*, 2001). Whether latent ganglionic VZV gene expression in humans is significantly more extensive than has been demonstrated hitherto has yet to be determined.

Conclusions

The continued hard work of many laboratories over the last 2 decades has shown that answers to some of the problems of VZV latency in ganglia are possible, but others are likely to take many more years to unravel. Whether VZV gene expression is more extensive than is currently thought, and whether such studies will ultimately lead to new and effective therapies for VZV-associated neurological disease, are certainly possible but as yet unknown. These studies should be enhanced by both new molecular technologies and the development of more refined animal models of VZV latency.

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