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Latent Varicella-Zoster Virus in Human Dorsal Root Ganglia

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To understand further the molecular events underlying the process of Varicella-zoster virus (VZV) latency in human ganglionic tissues, *in situ* hybridisation (ISH) for VZV RNA and DNA, and PCR *in situ* amplification for VZV DNA were used in human dorsal root ganglia from 12 individuals (3 normal and 9 who had died with AIDS). The results showed that (a) two separate regions of the VZV genome, represented by genes 4 and 40, were detected in neurons in two normal and three AIDS ganglia, (b) evidence of transcription of VZV genes 4, 21, 29, and 63 was found in normal and AIDS cases, and (c) VZV DNA and RNA for the same gene (gene 29) was detected in neurons in serial tissue sections in three cases. Thus more than one region of the VZV genome is present in neurons during VZV ganglionic latency, and the presence of both a VZV gene and its corresponding RNA transcript can be shown to occur in the same localised region of DRG tissue.

Key Words: varicella-zoster virus; neuron; dorsal root ganglion.

INTRODUCTION

The human herpesvirus Varicella-zoster virus (VZV) causes varicella (chickenpox) as a primary infection, usually in children, following which it establishes a latent infection in human trigeminal and dorsal root ganglia (DRG) (Kennedy 1987; Johnson 1998). Following a variety of possible triggering factors, or spontaneously, VZV may then reactivate to cause herpes zoster (shingles), which may be followed by postherpetic neuralgia. The latter is an important cause of human morbidity, and both types of VZV infection may lead to a variety of serious neurological complications, particularly in immunocompromised individuals, including those with Human Immunodeficiency Virus (HIV) infection (Kennedy 1987). An important pathogenetic question is the cell specificity of latent VZV because knowledge of this will help the understanding of how viral reactivation and the various complications occur. The cellular location of latent VZV in human ganglia had for several years remained a controversial issue (Hyman *et al.,* 1983; Gilden *et al.,* 1987; Croen *et al.,* 1988; Meier *et al.,* 1993), but it has recently been established that the neuron is the predominant site of latent viral infection (Kennedy *et al.,* 1998), although there is not currently a consensus as to the exact frequency of neuronal or nonneuronal satellite cells, which support latent VZV (Lungu *et al.,* 1995; Kennedy *et al.,* 1998). We have consistently detected latent VZV in only a small percentage of satellite cells (Kennedy *et al.,* 1998).

Three of the important questions that remain to be clarified and confirmed following previous studies are (a) whether the predominant neuronal localisation of latent VZV in trigeminal ganglia also exists in the DRG—the other main site of human VZV latency, (b) whether separate regions of the VZV genome can be detected in neurons in areas of the same human ganglion indicating the presence of the entire genome rather than fragments, and (c) whether it is possible to detect the presence of both a particular VZV gene and evidence of its transcription in the same ganglionic region and/or cell. We therefore used previously employed molecular techniques to address these issues.

RESULTS AND DISCUSSION

Using the PCR *in situ* technique, DNA for more than one VZV gene was detected in the same areas in neurons in serial sections of several of the DRG tissues. Thus two separate regions of the VZV genome, represented by genes 4 and 40, were detected in five of the cases (2 of the 3 normal and 3 of the AIDS cases) (Fig 1). Approximately 2–5% of the neurons in the DRG sections were positive for viral DNA, and only occasional nonneuronal satellite cells were labeled. The relatively small sample size did not allow for statistical analysis. As expected, the results using ISH showed a very similar pattern of VZV DNA labelling. A limited screen for VZV RNA using ISH with probes to VZV genes 4, 21, 29, and 63 showed that all of these genes were transcribed in varying numbers of cases. Thus RNA for gene 21 was detected in one case, for gene 29 in nine cases, for gene 63 in eight cases, and for gene 4 in two cases. Furthermore in three cases, both VZV DNA and RNA for the same gene (gene 29) were detected in neurons in serial tissue

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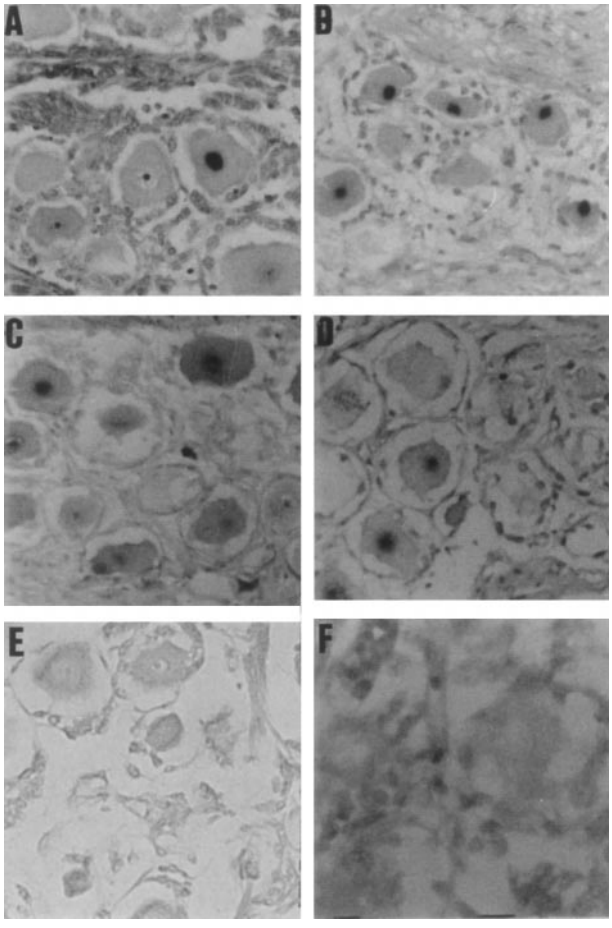

FIG. 1. (A) and (B) *In situ* PCR for VZV DNA of serial sections of human dorsal root ganglion from an AIDS case for genes 4 and 40, respectively. (C) and (D) *In situ* hybridisation (ISH) of serial sections from human dorsal root ganglion (DRG) from a normal case for VZV gene 29 DNA and RNA, respectively. Positive signals in neuronal nuclei can be seen in all fields. (E) and (F), Examples of negative controls. (E) shows ISH for VZV gene 29 RNA on a 12-day-old infant trigeminal ganglion. (F) shows *in situ* PCR using enterovirus primers on a human DRG from an AIDS case. (x450).

sections (Fig. 1). It was not possible to determine unequivocally whether the exact same neuron containing VZV DNA also contained RNA of the same gene, but our data suggest that this is probably the case, at least in some instances. VZV DNA or RNA was always detected in the neuronal nucleus, and not the cytoplasm, in the positive cases. No DNA or RNA signals were detected in any of the human or rodent control tissues.

These results both confirm and extend in three ways our previous observations of VZV latency in human ganglia. First, the assumption that the predominant neuronal localisation of latent VZV seen in trigeminal ganglia would also extend to human DRG has been proved to be correct. The results that were obtained here show no difference from those obtained in trigeminal ganglia, either in the normal or HIV-positive cases in which alterations of cellular tropism might be expected because of the immunosuppression (Kennedy *et al.,* 1998). Second, it is clear that more than one region of the VZV genome is present in neurons during the latency process as evidenced by the detection of two separate regions of VZV DNA (represented by genes 4 and 40) in neurons within the same tissue area. Our previous studies had also identified DNA of VZV genes 18, 21, 29, and 63 in different trigeminal ganglia using a combination of techniques (Kennedy *et al.,* 1998), but here we specifically identified in five DRG multiple genes in neurons in the same tissue area, thereby confirming what was previously assumed. Third, evidence was obtained to demonstrate the presence of both a particular VZV gene (gene 29) and its transcription in the same area of the DRG tissue. Whether this was actually the case in the same cell can only be established unequivocally by the development of double-gene-labelling techniques in these tissue sections. Nevertheless the current data, using serial sections, lead us to believe that this is the case in VZV latency. The results for the other VZV genes that we found to be transcribed in neurons in this limited RNA screen are consistent with a number of previous studies (Croen *et al.,* 1988; Cohrs *et al.,* 1992, 1994–1996; Meier *et al.,* 1993). We are currently employing these techniques to provide a detailed analysis of the transcription of a large number of VZV genes in specific cell types in both normal and immunocompromised individuals.

MATERIALS AND METHODS

Tissues and probes

All of the experimental protocols, including extensive negative and positive control procedures, used in the current study have been previously described in detail elsewhere (Kennedy *et al.,* 1998) and therefore will only be given in outline here. Formalin-fixed wax-embedded human lumbar DRG tissue samples from 12 individuals were used (kindly donated by J. Bell, Edinburgh; Table 1). Three of these were from normal individuals and nine

TABLE 1

Human Dorsal Root Ganglia (DRG) Studied

Case	Age (Yr)	Sex	Region	Diagnosis
1	33	Male	Lumbar	AIDS
2	37	Male	Lumbar	AIDS
3	28	Male	Lumbar	AIDS
4	50	Male	Lumbar	AIDS
5	30	Male	Lumbar	AIDS
6	35	Male	Lumbar	pre-AIDS
7	27	Male	Lumbar	Normal (overdose)
8	26	Male	Lumbar	Normal (overdose)
9	26	Male	Lumbar	Normal (overdose)
10	46	Male	Lumbar	AIDS
11	26	Male	Lumbar	AIDS
12	29	Male	Lumbar	pre-AIDS

TABLE 2

Oligonucleotide Sequences

VZV gene	Oligonucleotide sequence
4	5′-TGCAACCTCGAAGTCACTT-3′*
	5′-CCACGGACAACTATATTAG-3′
21	5′-GGTCACTCCCACTTGTATTCC-3′*
29	5′-TCATCTAGAATCTTTACTGCTTCTAGAGCGCCTTCTACGGTCCAGGGCGTTTCCAGGGTTTGGATAATC-3′*
40	5′-ATGACAACGGTTTCATGTCCCGTC-3′
	5′-TCTAGAAAACGCACAAAGTTTAAT-3′*
63	5′-CGCGCTTAAGCTACACGCCATGGGGGGGCGG-3′*
Enterovirus	Z_1 5′-CAAGCACTTCTGTTTCCCCGG-3′
	Z_3 5′-ATTGTCACCATAAGCAGCCA-3′
β-Globin (180 bp)	5′-CTGTGGGGCAAGGTGAACG-3′
	5′-CAAAGGACTCAAAGAACCTC-3′

Note. All VZV sequences obtained from Davison and Scott (1986). *, those sequences end-labelled with digoxygenin and used as probes.

from patients with acquired immunodeficiency syndrome (AIDS). Control tissues included human and rodent neural and nonneural tissues as well as a trigeminal ganglion from a 12-day-old infant (Kennedy *et al.,* 1998). The oligonucleotide probes and PCR primers used in the study are shown in Table 2. They included oligonucleotide probes and primer sequences for VZV genes 4, 21, 29, 40, and 63, enterovirus (as a negative control) (Zoll *et al.,* 1992) and also human β-globin. All oligonucleotides were synthesized by Genosys UK.

In situ hybridisation

For most experiments, viral RNA and DNA were detected using *in situ* hybridisation (ISH), which was carried out as previously described in detail (Kennedy *et al.,* 1998). In brief, five-μm tissue sections on APES-treated glass slides were incubated overnight at 37°C, dewaxed in xylene, and rehydrated through graded ethanol. After permeabilisation with HCl and proteinase K and then inactivation of proteinase K by glycine/PBS followed by acetylation, the sections were dehydrated and prehybridized in buffer containing 50% formamide, 2× SSC, Denhardt's solution, and denatured salmon sperm DNA. The sections were hybridised overnight with an appropriate digoxigenin (DIG)-labeled probe in dextran sulfate prehybridisation buffer, and standard DIG agents then used to detect any resulting hybrids. The slides were dipped in appropriate buffer and blocking agent for 30 min and treated for 60 min with antibody conjugated to alkaline phosphatase (AP). Following equilibration in buffer, slides were treated with the detection agent (Kennedy *et al.,* 1998) and 2.4 mg/ml levamisole (in the dark) for a period of 15 min to 2 h until the development of the purple colour. All slides were read blind. To colocalize VZV DNA and RNA in the same region, serial sections were processed and analysed. Double-labelling the sections with two different VZV probes was not possible, and not all of the probes were used in all cases.

PCR *in situ* amplification

PCR *in situ* amplification to detect viral DNA was carried out as previously described in detail (Kennedy *et al.,* 1998). In brief, 5-μm wax sections on APES-coated glass slides were incubated at 60°C for 24–48 h and then dewaxed in xylene and rehydrated in graded ethanol prior to treatment with 0.02 M HCl. After treatment with Triton-X and then proteinase K, the tissue samples were boiled in citric acid for 3 min, fixed in 20% acetic acid, washed in PBS, and then dehydrated through graded ethanols. For PCR *in situ,* we used the Perkin–Elmer Gene Amp system 1000. The composition of the PCR mastermixes was as previously described (Kennedy *et al.,* 1998). Slides were air-dried and 50 μl of the PCR mix placed onto each section prior to amplification. The cycling parameters for VZV amplifications were as previously detailed (Kennedy *et al.,* 1998), following which the slides were cooled to 4°C, washed in 2× SSC, and processed for colour development as above. The maximum duration of slide immersion in the detection reagent was 30 min.

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