Varicella-Zoster Virus (VZV) Transcription during Latency in Human Ganglia: Prevalence of VZV Gene 21 Transcripts in Latently Infected Human Ganglia

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Received 17 November 1994/Accepted 13 January 1995

Reverse transcriptase-linked PCR was used to determine the prevalence of varicella-zoster virus (VZV) gene 21 transcription in latently infected human ganglia. Under conditions wherein reverse transcriptase-linked PCR detected $\geq 1,000$ transcripts, VZV gene 21 RNA, but not VZV gene 40 RNA, was found in ganglia but not other tissues from five of seven humans.

The hallmark of the alphaherpesvirus family is the ability to become latent in the nervous system. After primary infection, herpes simplex virus (HSV), varicella-zoster virus (VZV), bovine herpesvirus, pseudorabies virus, and simian varicella virus all become latent in the ganglia of their natural hosts (10–13, 20, 24, 25). Although the mechanisms by which latency is maintained and virus reactivates is unknown, it is likely that both depend upon the extent of virus gene transcription.

In a cDNA library constructed from latently infected human trigeminal ganglionic mRNA, we identified a polyadenylated transcript mapping to VZV gene 21 (5). The present study was designed to determine the prevalence of VZV gene 21 transcription in tissues from multiple humans. Table 1 lists the

oligonucleotide primer used to amplify from the 3' poly(A)⁺ tail of mRNA (Table 2).

To determine the sensitivity of reverse transcriptase (RT)-linked PCR, we constructed recombinant bacterial plasmids containing the 3' ends of VZV genes 21 and 40 for use in *in vitro* transcription (Fig. 1). Reconstruction experiments using RT and nested PCR detected as few as 1,000 molecules of either VZV gene 21 or 40 *in vitro*-synthesized transcripts in a background of 5 μ g of total BSC-1 cell RNA (Fig. 2), indicating equivalent sensitivity for either VZV gene 21 or 40 transcripts. No PCR product (VZV or β -actin) was obtained when RT was omitted from the reaction, indicating that the RT-linked PCR assay was RNA dependent.

Analysis of VZV transcription in tissues of subject 1. Nucleic acids were extracted from both trigeminal ganglia, six thoracic ganglia, and the brain, heart, liver, and kidney tissues of subject 1. DNA from each tissue was PCR amplified by

TABLE 1. Subject history

Subject ^a no.	Age (yr)	VZV antibody ^b	Cause of death
1	53	+	Respiratory arrest
2	85	+	Pneumonia
3	65	+	Lung cancer
4	70	+	Pneumonia
5	78	+	Cardiac arrest
6	55	+	Ruptured aorta
7	59	+	Myocardial infarction

^a All seven were men.

^b Antibody to VZV was detected by enzyme-linked immunoassay (8).

clinical features of the seven persons whose tissues were studied. None was immunocompromised before death, and there were no skin lesions characteristic of recent varicella or zoster at autopsy. DNA and RNA were extracted from tissues, RNA was treated with RNase-free DNase, and cDNA was synthesized as previously described (3, 5). PCR amplification was performed as previously described (5), with synthetic oligonucleotide primers (Operon, Inc., Alameda, Calif.) specific for VZV genes 21 and 40 (6) and human β -actin (17) and an

TABLE 2. Oligonucleotide primers

Specificity and name	Sequence	Location
VZV gene 21		
F0	TCACTCACTCCTCTAACACG	31,434 ^a
F1	AGTAATGTAGCAGAACACACCC	31,803
F2	AGCGTTGTAGCAGACGAGCAT	31,968
R1	CGGGTAAAAGGTTCTA	32,743
R2	ATGACAGCAACCTTCCCTGT	32,473
R3	ACCCACTAAAGCGAGACATCC	32,033
VZV gene 40		
f1	TCACACACAATCGGATGTTG	75,279
f2	ATACGGTGACAGGCTATACAACGG	75,315
r1	ATCGCTTGAGCATAGTGGTG	75,613
r2	GATCCTGTGTCATTTCCGGTAGA	75,566
r3	ATACGGTGACAGGCTATACAACGGAA	75,348
β -Actin		
Act1	GCACTCTTCCAGCCTTCCTT	818 ^b
Act2	GGAGTACTTGCCTCAGGAG	1,055
ActP	GCAAAGACCTGTACGCCAACACA	909
Poly(A), d(T)		
	GAGAGAGAGAACTAGTAGTCTCGAGC GC(T ₁₅)	

^a Location of first 5' base on the DNA sequence of the VZV genome (6).

^b Location of first 5' base on the sequence of human β -actin cDNA (17).

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FIG. 1. Cloning and in vitro transcription of the 3' ends of VZV genes 21 and 40. The 3' ends of VZV genes 21 and 40 were subcloned from a lambda-based cDNA library constructed from VZV-infected BSC-1 cell mRNA. The PCR primer sets used included one VZV gene-specific primer and a second primer complementary to the 3' poly(A)⁺ mRNA tail (shaded region). The amplified DNA was inserted into pCRII by T/A ligation. Insert size was determined by *Eco*RI digestion and agarose gel electrophoresis (top right panel). Digestion of the plasmid containing the 3' end of VZV gene 21 with *Eco*RI (lane 1) yielded a 3.9-kbp vector fragment and two VZV gene 21-specific fragments (2.2 and 0.3 kbp). Digestion of the plasmid containing the 3' end of VZV gene 40 with *Eco*RI (lane 2) yielded a 3.9-kbp vector fragment and a 0.55-kbp VZV gene 40-specific fragment. DNA size standards included lambda digested with *Hind*III (lane m) and a 123-bp ladder (Gibco-BRL) (lane m). Each plasmid was linearized by *Xho*I digestion before SP6 transcription. Template DNA was digested, and the RNA product (lower right panel) was sized on denaturing agarose containing methylmercuric hydroxide (4). The size of the VZV gene 21-specific RNA was 2.5 kb, and that of the VZV gene 40-specific RNA was 0.6 kb. RNA size markers included a 0.16- to 1.77-kb RNA ladder (lane m) and a 0.24- to 9.5-kb RNA ladder (Gibco-BRL) (lane m).

using primers specific for VZV genes 40 and 21 and β -actin (Fig. 3). VZV gene 40 and 21 DNAs were detected in trigeminal ganglionic DNA but not in DNA from thoracic ganglia, the brain, or the viscera; β -actin DNA was found in all tissues (Fig. 3A). RNA from each tissue was treated with DNase until no DNA remained which could be amplified by using primers specific for VZV gene 40 or 21 or human β -actin (Fig. 3B). cDNAs prepared from tissue RNAs revealed VZV gene 21, but not VZV gene 40, transcripts in trigeminal ganglia; all cDNAs contained β -actin transcripts (Fig. 3C).

Prevalence of latent VZV gene 21 transcripts. We then analyzed the trigeminal and thoracic ganglia, brains, hearts, livers, and kidneys of six additional humans and repeated the analysis of all tissues from subject 1 (Fig. 4). VZV gene 40-specific DNA was detected in the trigeminal ganglionic DNAs of six of the seven subjects, and VZV gene 21-specific DNA was detected in all of the subjects. VZV gene 40 DNA was found in the thoracic ganglia of three of the seven subjects and VZV gene 21 DNA was found in four of the seven subjects; β -actin DNA was found in the ganglia of all seven subjects (Fig. 4A). RNA was extracted from all ganglia and treated with DNase until it was free of amplifiable VZV DNA or β -actin DNA (Fig. 4B). To increase PCR sensitivity, nested PCR was

FIG. 2. Sensitivity of detection of VZV gene 21 and 40 transcripts by RT-linked PCR. Reverse transcription followed by nested PCR was conducted with various dilutions of in vitro-synthesized VZV gene 21 and 40 transcripts. Both VZV genes were detected with equal sensitivity ($\sim 1,000$ copies). The detection assay is RNA dependent, since no cDNA amplification was detected when RT was omitted (lanes marked with a minus sign). Because the VZV-specific transcripts were added to $5 \mu\text{g}$ of control (BSC-1) RNA, a β -actin amplification product was detected in all samples subjected to reverse transcription (lanes marked with a plus sign).

FIG. 3. PCR analysis of tissue nucleic acids from subject 1. DNA was extracted from a portion of pooled trigeminal ganglia (TG), pooled thoracic ganglia (TH), brain (BR), heart (HT), liver (LIV), and kidney (KID). VZV gene 40 and 21 DNA sequences were detected by PCR amplification in DNA from trigeminal ganglia but not in DNAs from other tissues; amplification of a 237-bp DNA fragment within the human β -actin gene indicated the lack of PCR inhibitors in the DNA samples (A). After DNase treatment of the RNA, no PCR-amplifiable VZV gene 40 or 21 or β -actin DNA was detected (B). VZV gene 21, but not gene 40, transcripts were detected in cDNA prepared from trigeminal ganglionic RNA (C); neither VZV gene 40 nor VZV gene 21 transcripts were detected in cDNAs prepared from RNAs of any other tissues; β -actin transcripts were detected in cDNAs from RNAs of all tissues. Controls for PCR included no DNA (NO DNA) and DNAs extracted from uninfected BSC-1 cells (BSC-1) and VZV-infected BSC-1 cells (VZV-BSC-1).

used to detect VZV gene 21- and 40-specific DNA sequences. At the increased level of sensitivity afforded by nested PCR, no RNA sample contained detectable amounts of VZV DNA. Analysis of single-stranded cDNA synthesized from each RNA sample by nested PCR revealed VZV gene 21 transcripts in cDNAs prepared from trigeminal ganglionic RNAs from three of seven humans and in cDNAs from thoracic ganglionic RNAs from three of seven humans; no VZV gene 40 transcripts were found by nested PCR in cDNAs prepared from any human trigeminal or thoracic ganglionic RNAs (Fig. 4C).

Nested PCR analysis of cDNAs prepared from RNAs of non-ganglionic tissues from the seven subjects did not reveal VZV gene 21-specific cDNA in brain, heart, liver, and kidney tissues (data not shown). Table 3 summarizes the results from the seven subjects.

The detection of latent VZV gene 21 transcripts in ganglia, but not in brain, heart, liver, or kidney tissue, parallels the detection of latent VZV DNA in ganglia (13). In the only study to identify latent VZV transcripts by Northern (RNA) blotting, hundreds of human ganglia were pooled and VZV gene 29 and

TABLE 3. Detection of VZV DNA in ganglionic DNAs, RNAs, and cDNAs of seven human subjects

Subject	Ganglia	DNA			RNA			cDNA		
		Gene 40	Gene 21	β -Actin	Gene 40	Gene 21	β -Actin	Gene 40	Gene 21	β -Actin
1	Trigeminal	+	+	+	-	-	-	-	+	+
1	Thoracic	-	-	+	-	-	-	-	-	+
2	Trigeminal	+	+	+	-	-	-	-	-	+
2	Thoracic	+	+	+	-	-	-	-	+	+
3	Trigeminal	+	+	+	-	-	-	-	-	+
3	Thoracic	-	-	+	-	-	-	-	-	+
4	Trigeminal	-	+	+	-	-	-	-	-	+
4	Thoracic	-	+	+	-	-	-	-	+	+
5	Trigeminal	+	+	+	-	-	-	-	-	+
5	Thoracic	+	+	+	-	-	-	-	-	+
6	Trigeminal	+	+	+	-	-	-	-	+	+
6	Thoracic	+	+	+	-	-	-	-	+	+
7	Trigeminal	+	+	+	-	-	-	-	+	+
7	Thoracic	-	-	+	-	-	-	-	-	+

FIG. 4. Prevalence of VZV gene 21 transcripts in latently infected human trigeminal and thoracic ganglia from seven subjects. VZV gene 40 DNA was found in trigeminal ganglionic DNAs extracted from subjects 1, 2, 3, 5, 6, and 7, and VZV gene 21 DNA was detected in all seven subjects; VZV gene 40 DNA was found in thoracic ganglionic DNAs from subjects 2, 5, and 6, and VZV gene 21 DNA was found in subjects 2, 4, 5, and 6; β -actin DNA was found in trigeminal and thoracic ganglionic DNAs of all seven subjects (A). RNAs extracted from trigeminal and thoracic ganglia of all seven subjects were treated with DNase until no PCR-amplifiable VZV gene 40 or 21 or β -actin DNA was detected (B). VZV gene 40 transcripts were not detected by nested PCR in cDNA prepared from ganglionic RNA of any subject; VZV gene 21 transcripts were found by nested PCR in cDNAs prepared from trigeminal ganglionic RNAs of subjects 1, 6, and 7 and in cDNAs prepared from thoracic ganglionic RNAs of subjects 2, 4, and 6. β -Actin transcripts were detected in cDNAs prepared from the ganglionic RNAs of all seven subjects (C). Controls for all PCRs included no DNA (NO DNA) and DNAs extracted from uninfected BSC-1 cells (BSC-1) and VZV-infected BSC-1 cells (VZV-BSC-1).

open reading frame is present in latently infected human trigeminal and thoracic ganglia. VZV gene 29 and 62 transcripts were found in the poly(A)⁺ fraction of ganglionic RNA (15), and VZV gene 21 cDNA contains a poly(A)⁺ tail (5); thus, all three latent VZV transcripts presumably encode functional proteins. Whether the translation products of VZV genes 21, 29, and 62 can be detected during latency remains to be determined.

The product of VZV gene 21 has not been identified. However, the HSV type 1 (HSV-1) homolog, *UL37*, encodes a late (γ 1) 120-kDa phosphoprotein which associates with HSV-1 ICP8 (the major DNA-binding protein) in infected cells (1, 14, 22, 23). VZV gene 29 encodes the major DNA-binding protein in VZV-infected cells which, by analogy to HSV-1 ICP8, is an early protein (6, 16). VZV gene 62 encodes an essential, virion-associated, phosphorylated, immediate-early, 140-kDa transactivator homologous to HSV-1 ICP4 (2, 9, 18, 19, 26). VZV gene 40 encodes the major 156-kDa capsid protein synthesized late in infection (6).

Overall, expression of human herpesvirus family genes during latency appears to vary for HSV-1, VZV, and Epstein-Barr virus. In contrast to the apparently limited expression of HSV-1 during latency (7), multiple Epstein-Barr virus genes are expressed (21). To date, the expression of a few VZV genes is greater than that of HSV-1 genes but less than that of Epstein-Barr virus genes.

This work was supported in part by Public Health Service grants AG 06127, NS 32623, and NS 07321 from the National Institutes of Health.

We are grateful to the Departments of Pathology at University Hospital, the Veterans Administration Hospital, and the Presbyterian-St. Luke's Medical Center in Denver for allowing access to autopsy materials. We thank B. Forghani for performing the enzyme immunoassays, Mary Devlin and Marina Hoffman for editorial review, and Cathy Allen for preparation of the manuscript.

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62 transcripts were identified (15). Thus, there appear to be no fewer than three different VZV genes transcribed during latency in human ganglia. Our detection of VZV gene 21 was based on amplification of a primary PCR product (940 bp) and a nested PCR product (505 bp) within the 3,113-bp open reading frame. Thus, a major portion of the predicted VZV gene 21

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