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Simian Varicella Virus Expresses a Latency-Associated Transcript That Is Antisense to Open Reading Frame 61 (ICP0) mRNA in Neural Ganglia of Latently Infected Monkeys▽

Yang Ou,[1] Kara A. Davis,[1] Vicki Traina-Dorge,[2] and Wayne L. Gray[1]*

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205,[1] and Tulane National Primate Research Center, Covington, Louisiana 70433[2]

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Simian varicella virus (SVV) and varicella-zoster virus (VZV) are closely related alphaherpesviruses that cause varicella (chickenpox) in nonhuman primates and humans, respectively. After resolution of the primary disease, SVV and VZV establish latent infection of neural ganglia and may later reactivate to cause a secondary disease (herpes zoster). This study investigated SVV gene expression in neural ganglia derived from latently infected vervet monkeys. SVV transcripts were detected in neural ganglia, but not in liver or lung tissues, of latently infected animals. A transcript mapping to open reading frame (ORF) 61 (herpes simplex virus type 1 [HSV-1] ICP0 homolog) was consistently detected in latently infected trigeminal, cervical, and lumbar ganglia by reverse transcriptase PCR. Further analysis confirmed that this SVV latency-associated transcript (LAT) was oriented antisense to the gene 61 mRNA. SVV ORF 21 transcripts were also detected in 42% of neural ganglia during latency. In contrast, SVV ORF 28, 29, 31, 62, and 63 transcripts were not detected in ganglia, liver, or lung tissues of latently infected animals. The results demonstrate that viral gene expression is limited during SVV latency and that a LAT antisense to an ICP0 homolog is expressed. In this regard, SVV gene expression during latency is similar to that of HSV-1 and other neurotropic animal alphaherpesviruses but differs from that reported for VZV.

A hallmark of herpesviruses is the ability to persist in the host by establishing latency following resolution of the primary infection and to later reactivate, possibly causing a secondary disease. Human alphaherpesviruses, including herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus (VZV), establish latent infections in trigeminal and dorsal root neural ganglia (37). Likewise, neurotropic animal alphaherpesviruses, including simian varicella virus (SVV), equine herpesvirus type 1 (EHV-1), EHV-4, pseudorabies virus of pigs (PRV), bovine herpesvirus type 1 (BHV-1), and feline herpesvirus (FHV), persist in a latent state in neural ganglia (2–4, 25, 30, 39). However, the molecular basis of herpesvirus latency and reactivation is not understood.

SVV infection of nonhuman primates is a useful model for investigation of the molecular aspects of herpesvirus pathogenesis and latency (14). SVV, which causes a natural varicella-like disease of Old World monkeys, is closely related to VZV, the causative agent of human varicella (chickenpox) and herpes zoster (shingles). While the viruses are species specific, SVV and VZV share extensive antigenic cross-reactivity and DNA homology (12, 17–19). The SVV and VZV genomes are similar in size and structure and are colinear in gene arrangement (17–19). The pathogenesis and clinical features of simian and human varicella are comparable (13, 20). Following resolution of primary disease, SVV and VZV establish latent infections within neurons of the neural ganglia and may subsequently reactivate to cause a secondary disease, such as herpes zoster (13, 22, 26, 30).

Little is known concerning SVV gene expression during latent infection. Herpesvirus gene expression during lytic or acute herpesvirus infection is coordinated into immediate-early (IE), early, and late phases, involving all or most of the viral genes located throughout the viral genome. In contrast, herpesvirus gene expression during latent infection is restricted to transcription of one or a few specific viral genes within cells of a particular tissue. For example, HSV-1 gene expression is limited to the latency-associated transcripts (LATs), which partially overlap and are antisense to the viral ICP0 gene (37). Neurotropic animal alphaherpesviruses, including EHV-1, EHV-4, PRV, and BHV-1, also express a LAT that is antisense to a ICP0 homolog gene in latently infected ganglia (1, 2, 4, 25). VZV gene expression in latently infected neural ganglia is restricted to transcription and possibly translation of specific open reading frames (ORFs), including ORFs 21, 29, 62, 63, and 66 (7, 27, 37).

In this study, we have investigated SVV transcription in neural ganglia derived from acutely and latently infected nonhuman primates. SVV ORF 61, which is located at nucleotides (nt) 102,915 to 104,426 of the SVV genome (Fig. 1), is a homolog of the HSV-1 ICP0 gene and VZV gene 61 and encodes a viral protein that transactivates viral IE, early, and late genes (15). This study demonstrates that SVV expresses a LAT that is antisense to the ORF 61 mRNA in the neural ganglia of latently infected vervet monkeys.

MATERIALS AND METHODS

SVV infection of nonhuman primates. Six St. Kitts vervet monkeys (*Chlorocebus aethiops*) (3 to 4 years old), designated ET66, ET68, ET69, ET70, FV91, and FV93, were confirmed to be seronegative for SVV by serum neutralization

* Corresponding author. Mailing address: Dept. of Microbiology and Immunology, Slot 511, University of Arkansas for Medical Sciences, 4301 W. Markham St., Little Rock, AR 72205. Phone: (501) 686-5187. Fax: (501) 686-5359. E-mail: graywaynel@uams.edu.

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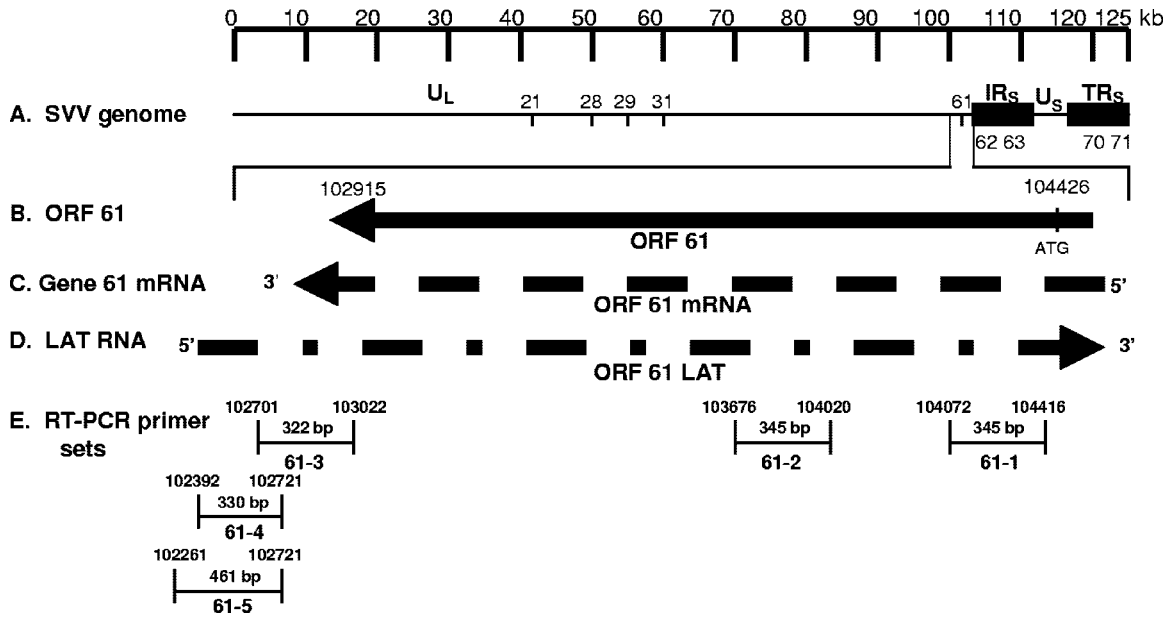

FIG. 1. SVV genome and locations of ORF 61, gene 61 mRNA, and LAT. (A) The 124.7-kb SVV genome consists of a long component, including a unique long (U_L) sequence bracketed by 64-bp inverted repeats, and a short component, including a unique short (U_S) sequence bracketed by internal and terminal inverted repeats (IR_S and TR_S). Map locations of SVV ORFs 21, 28, 29, 31, 61, 62, 63, 70, and 71 are indicated. The expanded ORF 61 region illustrates the map locations of gene 61 (B), gene 61 mRNA (C), and the LAT (D). (E) ORF 61 primers used to map the LAT are indicated. Nucleotide numbers are based on the DNA sequence of the SVV genome (GenBank accession no. AF275348).

assay prior to experimental infection. The animals were infected with 5×10^4 PFU of SVV strain Delta by intratracheal inoculation. One animal (FV91) was part of a study to investigate the immunogenicity of recombinant SVV expressing SIV Env and Gag antigens (40). The clinical and virological parameters of simian varicella were evaluated as previously described (20). Viremia was monitored by harvesting peripheral blood mononuclear cells (PBMCs) from Ficoll-Hypaque gradients and determining infectious SVV titers by coculture of PBMCs on Vero cells. Skin rash was scored on an established scale of 0 to 4, with 0 for no rash and 4 for severe rash. Hepatitis was assessed by aspartate transaminase assays of blood specimens. Serum neutralization assays were used to assess antibody responses to SVV, with the neutralization titer determined to be the serum dilution that reduced the viral plaque count by 50%. One animal (FV93) developed severe simian varicella, and tissues were collected at necropsy on day 10 postinfection (p.i.). Monkeys ET66, ET68, ET69, and ET70 were given booster immunizations on days 28 and 56 p.i. by intratracheal inoculation with 5×10^4 PFU of SVV and were sacrificed by ketamine anesthesia on day 119 p.i. Monkey FV91 was given a booster immunization on day 42 by intratracheal and subcutaneous inoculation with 5×10^4 PFU of SVV and was sacrificed on day 108 p.i. Liver, lung, and neural ganglia (trigeminal, cervical, and lumbar) tissues were harvested at necropsy and quick-frozen for subsequent analysis. Studies involving animals were conducted in compliance with the Public Health Service policy on humane care and use of laboratory animals.

RNA isolation and detection of transcripts in tissues. Total cellular RNA was isolated from liver, lung, and ganglion tissues by the TRI Reagent protocol (Molecular Research Center Inc., Cincinnati, OH). Briefly, 1 ml of TRI Reagent was added to 50 to 100 mg of tissue and immediately homogenized in a Polytron homogenizer. After addition of a phase separation reagent and a 15-min incubation, the mixture was centrifuged at 10,000 rpm for 15 min at 4°C. RNA in the upper aqueous phase was precipitated by the addition of isopropanol and centrifugation at 10,000 rpm for 8 min at 4°C. The RNA pellet was washed with 75% ethanol, dissolved in FORMAzol (300 to 500 μl/50 to 100 mg tissue), and stored at −20°C. RNA was precipitated by centrifugation at 14,000 rpm for 5 min, resuspended in H_2O, and DNase I treated with a commercial kit (Ambion Inc., Austin, TX).

Standard one-step reverse transcriptase PCR (RT-PCR) was conducted with the Access RT-PCR system (Promega Corp.). The RT reaction mixture, including RNA (0.5 μg) and gene-specific primers (1 μM), was incubated at 45°C for 45 min to generate cDNA and was followed by PCR amplification conducted under the following conditions: initial denaturation, 94°C for 2 min; 31 cycles of 94°C for 30 s, 60°C for 30 s, and 68°C for 1 min; and a final 5-min extension at 68°C. Control reactions without RT were conducted to confirm the absence of contaminating SVV DNA. Amplified cDNA products were fractionated by 1.0% agarose gel electrophoresis and visualized by ethidium bromide staining and UV illumination. The oligonucleotide primers used for RT-PCR are indicated in Table 1.

The specificity of RT-PCR products was confirmed by Southern blot hybridization. Briefly, the amplified cDNA was fractionated by agarose gel (1.0%) electrophoresis and then transferred to a nylon membrane by capillary transfer and UV cross-linking (Roche Applied Science). SVV DNA probes were labeled with digoxigenin (DIG)-dUTP by random priming, denatured, and then hybridized to the immobilized cDNA on the filters at 42°C for 16 h in Easy Hyb hybridization buffer (Roche Applied Science). After extensive washing, hybridized products were incubated with anti-DIG antibody conjugated with alkaline phosphatase, followed by washing and incubation with chemiluminescent substrate for alkaline phosphatase. The membranes were exposed to X-ray film, and the hybridized DNA products were detected by chemiluminescence.

In some cases, RT-PCR products were confirmed by nested PCR. Briefly, 2 μl of cDNA amplified in the initial PCR was used as the template for a second PCR using nested primers internal to the original PCR primers. Amplified DNA was analyzed by 1.0% agarose gel electrophoresis, ethidium bromide staining, and UV illumination.

Determination of SVV transcript orientation. A two-step RT-PCR procedure to determine the orientation of the SVV LAT was performed with the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen Corp.). Briefly, individual RT reaction mixtures, including either sense or antisense primers (0.1 μM) and DNase I-treated total cellular RNA template (0.5 μg), were incubated for 15 min at 50°C and then terminated at 85°C, after which the RNA template was degraded with RNase H. The cDNA product was amplified by PCR using gene-specific primers and the PCR conditions described above. The identity of the amplified products was confirmed by agarose gel electrophoresis and Southern blot hybridization analysis using DIG-labeled SVV ORF 61 DNA probes, as described above.

RESULTS

SVV infection of nonhuman primates. Five vervet monkeys (ET66, ET68, ET69, ET70, and FV93) were infected with wild-type SVV by intratracheal inoculation. An additional

TABLE 1. Oligonucleotide primers used for RT-PCR

Primer[a]	Sequence	Location[b]
ORF 21-2 sense	5′ GTG GAT ATT ATG GAA ACC GC 3′	31,924
ORF 21-2 sense-nested	5′ GGA AGC TGG AAT CGC ATT GC 3′	32,195
ORF21-2 antisense-nested	5′ TCC AAG TTT AGC CGA AGT GC 3′	32,331
ORF 21-2 antisense	5′ AGT CCA TCC TGA ACG ATA GG 3′	32,519
ORF 21-1 sense	5′ CTA CCT GCG ATA GTA TAA ACC 3′	34,427
ORF 21-1 antisense	5′ TTC GTC TAT CAT AAG CCA TC 3′	34,965
ORF 28 antisense	5′ TTG CGA CAT ACC AAG TAA CG 3′	51,473
ORF 28 antisense-nested	5′ GTT CCA TGG CGA GAC GAA TCG CAT ATA TCA GC 3′	51,522
ORF 28 sense-nested	5′ TTC ATG GCT AAC AGA ACA G 3′	51,916
ORF 28 sense	5′ TTC ATG GCT AAC AGA ACA G 3′	51,916
ORF 29 sense	5′ AAG AGA TGG AAG CTA CAC AG 3′	52,094
ORF 29 sense-nested	5′ AAG AGA TGG AAG CTA CAC AG 3′	52,094
ORF 29 antisense-nested	5′ AAT GGA TGG CGT TCA CGT GCA GTG TTA CAT G 3′	52,463
ORF 29 antisense	5′ GCA TAT GTC AGC TCC AGT AC 3′	52,524
ORF 31 sense	5′ GGA TCG AAA GCG TGG CAT ACA 3′	58,640
ORF 31 sense-nested	5′ GTA GAA GAA GTC GAT GCT CGA TC 3′	58,730
ORF 31 antisense-nested	5′ GTA GAA CAT CTT CGA CCT CTC G 3′	59,039
ORF 31 antisense	5′ ACT GTT TGC ATA TTC GTC GC 3′	59,059
ORF 61-5 antisense	5′ CCT AAT GAC AAG GTG CTT ACC 3′	102,261
ORF 61-4 antisense	5′ GCT GCA TTA TCT TCC ATT GCC 3′	102,392
ORF 61-3 antisense	5′ CGA GAA ACG GAG ATT TAA CGG 3′	102,701
ORF 61-5 sense	5′ CCG TTA AAT CTC CGT TTC TCG 3′	102,721
ORF 61-4 sense	5′ CCG TTA AAT CTC CGT TTC TCG 3′	102,721
ORF 61-3 antisense-nested	5′ GCT ACC CAA TCC TCG TAA TAG 3′	102,725
ORF 61-3 sense-nested	5′ GGA AAC AGG GTA TTG CTC GC 3′	102,970
ORF 61-3 sense	5′ GAA CAG CCA AGT ACA TCA GG 3′	103,022
ORF 61-2 antisense	5′ CTT GTG CAG TTA GAA CAG CG 3′	103,676
ORF 61-2 antisense-nested	5′ GGA TAT GAC CCA TGG AGA GC 3′	103,696
ORF 61-2 sense-nested	5′ ATG CTT CCT TCT CAT CTG GC 3′	103,993
ORF 61-2 sense	5′ GCG TCT TTC AGC CTT CAA CG 3′	104,020
ORF 61-1 antisense	5′ CTG TGT TAG ATG CGC TAT TCG 3′	104,072
ORF 61-1 antisense-nested	5′ TCT GTA ATG GTG CAT CTT CG 3′	104,147
ORF 61-1 sense-nested	5′ CGG CGT ATA CCA GTA CTA GC 3′	104,416
ORF 61-1 sense	5′ CGG CGT ATA CCA GTA CTA GC 3′	104,416
ORF 62 antisense	5′ GGA TCT TTG TGT GAC CTT GG 3′	108,509
ORF 62 antisense-nested	5′ ATC GGT AGG TTT CTG GGA AGG 3′	108,568
ORF 62 sense-nested	5′ GCG AGC GAA CAG TTT GAA CG 3′	108,939
ORF 62 sense	5′ CCG CAG AAC GAT CTA CTT GT 3′	109,010
ORF 63 sense	5′ GCA GGA TCC CCG AGA TGA AAT GAC G 3′	110,492
ORF 63 sense-nested	5′ GAA TGT TTC TCT GTA TCG CTC 3′	110,683
ORF 63 antisense-nested	5′ GTT GAT TGG TCG TCA TCG CT 3′	110,992
ORF 63 antisense	5′ CCG TAC AAC ATT CAT CAT CCG 3′	111,042

[a] Sense and antisense are primer orientations relative to the specific ORF (mRNA).
[b] Nucleotide positions are based on the SVV genome sequence (GenBank accession no. AF274348 [January 2007 version]).

monkey (FV91) was infected with a recombinant SVV expressing the SIV Env and Gag antigens (40). The clinical and virological parameters of infection for each animal are given in Table 2. A transient viremia occurred between days 3 and 6 p.i., as indicated by detection of infectious SVV in PBMCs. Each animal exhibited clinical signs of simian varicella, including vesicular skin rash and hepatitis during the first 2 weeks of infection. One monkey (FV93) developed severe disease, and acutely infected tissues were harvested from this animal on day 10 p.i. The remaining five animals generated immune responses to SVV, including neutralizing antibody, and resolved the acute disease by 20 days p.i. Monkeys ET66, ET68, ET69, and ET70 were administered booster immunizations with SVV on days 28 and 56 p.i., and monkey FV91 was given a booster immunization with the recombinant SVV on day 42. Neither viremia nor clinical symptoms (rash, hepatitis) were detected following these booster immunizations. The animals were sacrificed 9 weeks after the final immunization, and liver, lung, and neural ganglion tissues were collected and analyzed as indicated below. These animals were initially determined to be latently infected, based on the lack of clinical symptoms of simian varicella at the time of sacrifice, an inability to detect infectious SVV in tissues by coculture on CV-1 cell monolayers, and the ability to detect SVV DNA in the neural ganglia (data not shown).

Detection of SVV transcripts in tissues of acutely and latently infected monkeys. SVV transcripts in acutely and latently infected tissues were detected by RT-PCR and confirmed by nested PCR and/or Southern blot hybridization. Table 3 summarizes the analysis leading to the detection of SVV transcripts in liver, lung, and various neural ganglion tissues in each of the infected monkeys. Figure 2 shows representative RT-PCR/DNA hybridization results for detection of SVV transcripts in tissues derived on day 10 p.i. from an acutely infected monkey (FV93) (Fig. 2A) and in tissues derived on day 119 p.i. from a latently infected monkey (ET68) (Fig. 2B).

TABLE 2. Clinical, virological, and immunological parameters of SVV infection

Monkey	Viremia[a] on day:						Rash[b] on day:					Hepatitis[c] on day:			SVV NAT[d] on day:			
	0	3	6	10	12	14	6	10	12	14	20	0	10	30	0	14	21	Final[e]
ET66	0	38	110	0	0	0	0	3	2	1	0	56	87	42	<20	320	1,280	10,240
ET68	0	20	215	0	0	0	0	3	2	1	0	36	87	36	<20	1,280	2,560	5,120
ET69	0	89	170	0	0	0	0	3	2	1	0	43	126	39	<20	640	2,560	5,120
ET70	0	59	102	0	0	0	0	3	2	1	0	74	115	43	<20	640	640	1,280
FV91	0	29	0	0	0	0	0	4	3	1	0	30	100	40	<20	160	640	2,560
FV93[f]	0	397	443	ND			0	4	4			33	480		<20			

[a] Mean SVV PFU in PBMCs per milliliter of blood. ND, not determined.
[b] Skin rash assessed on a scale of 0 (no rash) to 4 (severe vesicular rash).
[c] Assessment of viral hepatitis based on elevated mean aspartate transaminase levels in blood.
[d] Neutralizing antibody titer (NAT) expressed as the reciprocal of the serum dilution that reduced SVV plaques by at least 50%.
[e] Day 119 for ET66, ET68, ET69, and ET70; day 108 for FV91.
[f] Animal necropsy on day 10 p.i.

SVV IE (ORFs 61, 62, and 63), early (ORF 29), and late (ORF 31) genes were expressed in acutely infected liver and lung tissues and trigeminal, cervical, and lumbar ganglia derived from monkey FV93 on day 10 p.i., as indicated by detection of viral transcripts by RT-PCR and Southern blot hybridization (Fig. 2A; Table 3). The results confirm active viral replication in these tissues during the acute stage of simian varicella.

TABLE 3. Detection of SVV transcripts in tissues derived from latently and acutely infected monkeys

Monkey (infection) and tissue[a]	Detection of SVV transcript with indicated primer set[b]								
	61-1	61-2	61-3	21	28	29	31	62	63
ET66 (latent)*									
Liver	−				−	−	−		−
Lung	−	−	−		−	−	−		−
Trigeminal	+	+	+	+	−	−	−	−	−
Cervical	+		+	−	−	−	−		−
Lumbar	+		+	+	−	−	−		−
ET68 (latent)*									
Liver	−		−		−	−	−		
Lung	−		−	−	−	−	−	−	−
Trigeminal	+	+	+	+	−	−	−	−	−
Cervical	+		+	−	−	−	−	−	−
Lumbar	+		+	+	−	−	−	−	−
ET69 (latent)*									
Lung	−	−	−		−	−	−		
Trigeminal	+	+	+	−	−	−	−	−	−
Cervical	+		+	−	−	−	−		
Lumbar	+	+	+		−	−	−	−	−
ET70 (latent)*									
Liver	−		−		−	−	−		−
Lung	−		−		−	−	−		−
Trigeminal	+	+	+	−	−	−	−	−	−
Cervical	+		+	+	−	−	−	−	−
Lumbar	+		+		−	−	−	−	−
FV91 (latent)*†									
Trigeminal		+	+	−	−		−	−	−
Cervical		+	+	+	−		−	−	−
FV93 (acute)‡									
Liver	+	+	+	+		+	+	+	+
Lung	+	+	+	+		+	+	+	+
Trigeminal	+	+	+	+		+	+	+	+
Cervical	+	+	+	+		+	+	+	−
Lumbar	+	−	+			−	+		−

[a] Symbols: *, tissues collected on day 119 p.i. (ET66, ET68, ET69, and ET70) or day 108 p.i. (FV91); †, SIV *env* and *gag* transcripts not detected in tissues of monkey FV91, infected with recombinant SVV expressing SIV *env* and *gag* (40); ‡, tissues collected from acutely infected monkey FV93 on day 10 p.i.
[b] Symbols: +, detection of SVV transcript by RT-PCR and confirmation by Southern blot hybridization or nested PCR; −, no transcript detected by the same methods. A blank space indicates that the tissue was not tested with the indicated primer set.

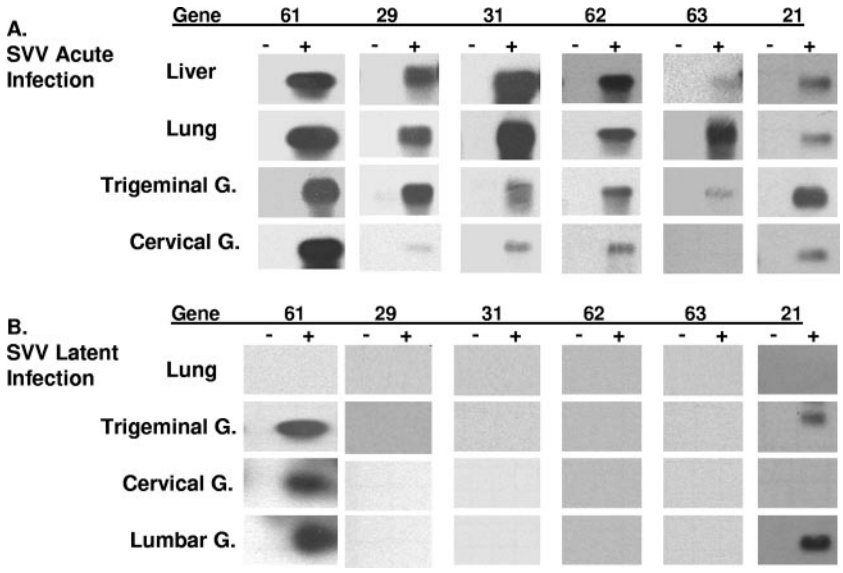

FIG. 2. Detection of viral transcripts in tissues of an acutely infected monkey (FV93) (A) and a latently infected monkey (ET68) (B). SVV transcripts were detected by RT-PCR using RT (+) and gene-specific primer sets, followed by Southern blot hybridization with DIG-labeled gene-specific DNA probes. Control reactions without RT (−) were conducted to confirm the absence of contaminating DNA. Based on molecular size standards (100-bp ladder), the sizes of the RT-PCR products corresponded to the expected size for each primer set: 345 bp for ORF 61, 431 bp for ORF 29, 420 bp for ORF 31, 502 bp for ORF 62, 551 for ORF 63, and 539 bp for ORF 21.

SVV transcripts mapping to SVV 61 ORF were consistently detected in neural ganglia (trigeminal, cervical, and lumbar) of each of the five animals (ET66, ET68, ET69, ET70, and FV91) that were latently infected with SVV (Fig. 2B; Table 3). Transcripts mapping to SVV ORF 21 were detected in 5 of 12 of the ganglia analyzed and in four of the five latently infected monkeys, confirming a previous report that ORF 21 is expressed in SVV latently infected ganglia (6). The ORF 61 and ORF 21 transcripts were detected exclusively in the neural ganglia; they were not detected in liver or lung tissues. In contrast, SVV ORF 28, 29, 31, 62, and 63 transcripts were not detected in the neural ganglia or liver or lung tissues, indicating a lack of viral replication in these tissues and confirming latent infection.

In order to confirm ORF 61 transcription in latent neural ganglia, five different ORF 61 primer sets (61-1, 61-2, 61-3, 61-4, and 61-5 [Fig. 1]) were used to detect transcripts in latent ganglia by RT-PCR. Figure 3 demonstrates that RT-PCR primer sets 61-1, 61-2, 61-3, and 61-4 each amplified cDNA from RNA template isolated from the trigeminal and lumbar ganglia, but not lung tissue, of a latently infected monkey (ET69). Primer set 61-5 did not detect ORF 61 RNA, indicating that the left-most terminus of the ORF 61 transcript maps within a 132-bp region between SVV DNA nt 102,392 and 102,261. Neither SVV ORF 61 transcripts nor cellular transcripts homologous to ORF 61 were detected in neural ganglia derived from an uninfected monkey, as confirmed in a previous study (15).

Orientation of the SVV ORF 61 LAT. In order to determine the orientation of the SVV ORF 61 transcript, oligonucleotide sense or antisense primers, relative to the ORF 61 mRNA, were used in RT reactions, and the cDNA products were amplified by PCR using ORF 61-specific primer sets. The size and specificity of the amplified products were confirmed by Southern blot hybridization using ORF 61 DIG-labeled DNA probes. Figure 4A demonstrates that RT reactions including the ORF 61 sense primers generated a cDNA product of the expected size from an RNA template derived from the neural ganglia of latently infected monkeys (ET66, ET70, and FV91), but RT reactions including antisense primers were less efficient at generating such a cDNA product. Because the sense primer has the same orientation as gene 61 mRNA, this primer must bind to a transcript that is antisense to ORF 61 mRNA. In contrast, when RNA template derived from liver tissue or neural ganglia of an SVV acutely infected monkey (FV93) was used, RT reactions including ORF 61 antisense primers, which bind to gene 61 mRNA, efficiently generated the expected cDNA product (Fig. 4B). Although the ORF 61 sense primers

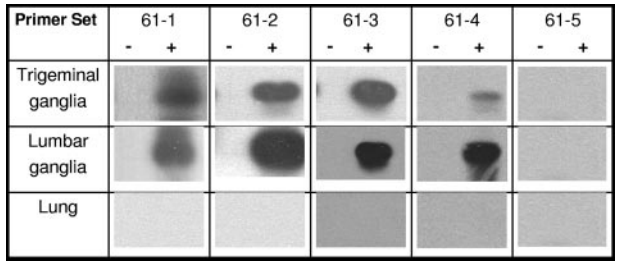

FIG. 3. Detection of the SVV ORF 61 LAT in the trigeminal and lumbar ganglia of a latently infected monkey (ET69) by RT-PCR employing multiple ORF 61 primer sets. Total cellular RNA isolated from ganglia or lung tissue was used as a template for RT-PCRs with RT (+) and without RT (−) and five different primer sets. Amplified LAT was detected by Southern blot hybridization using an ORF 61-specific DIG-labeled DNA probe. The map location of each RT-PCR primer set is shown in Fig. 1. Based on molecular size standards (100-bp ladder), the sizes of the RT-PCR products corresponded to the expected size for each primer set: 345 bp for 61-1, 345 for 61-2, 322 for 61-3, and 330 for 61-4.

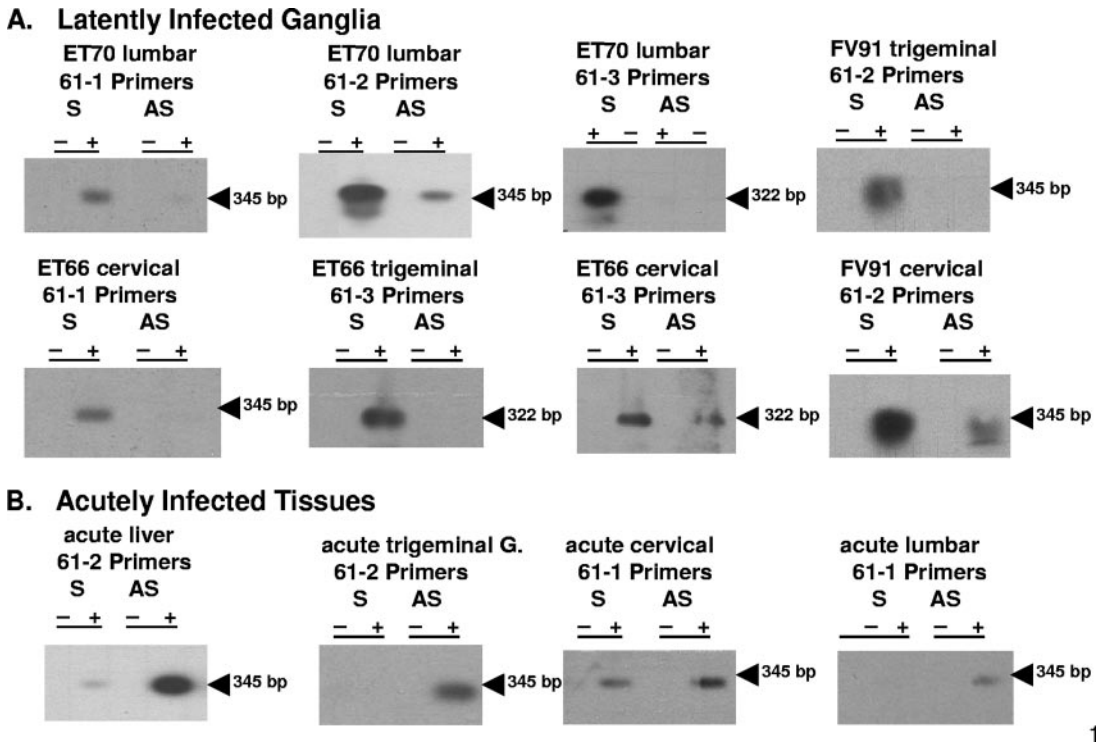

FIG. 4. Determination of SVV ORF 61 LAT orientation by strand-specific RT-PCR. Total cellular RNA isolated from ganglia derived from the latently infected monkeys ET66, ET70, and FV91 and the acutely infected monkey FV93 was used as a template for RT reactions conducted with ORF 61 sense (S) and antisense (AS) primers. Reactions were conducted with (+) and without (−) RT. The cDNA product was amplified by PCR and detected by Southern blot hybridization using an ORF 61-specific DIG-labeled DNA probe. The map location of each RT-PCR primer set is shown in Fig. 1. Based on molecular size standards (100-bp ladder), the sizes of the amplified products corresponded to the expected size for each primer set, as indicated.

did detect antisense transcripts in acutely infected liver and cervical ganglia tissues, the sense primers were not as efficient as the antisense primers for generating a cDNA product from RNA template derived from acutely infected tissues. These results indicate that SVV gene 61 mRNA is predominantly expressed in acutely infected neural ganglia, while an antisense LAT is primarily expressed in latently infected ganglia (Fig. 1). Both ORF 61 mRNA and the LAT could be detected in some ganglia, but gene 61 mRNA was detected most efficiently in acutely infected ganglia and the LAT was detected most efficiently in latently infected ganglia.

DISCUSSION

This study demonstrates that a viral transcript antisense to SVV gene 61 mRNA is expressed in the neural ganglia of SVV latently infected monkeys. This LAT was consistently detected in multiple ganglia (trigeminal, cervical, and lumbar) derived from each of five latently infected monkeys by RT-PCR employing multiple ORF 61 RT-PCR primer sets (Tables 3 and 4). The transcript is present in low copy numbers in latently infected ganglia, as RT-PCR followed by nested PCR or Southern blot hybridization was usually required for detection. Further characterization is needed to determine whether multiple transcripts are present, or whether the transcript is spliced or polyadenylated, and to determine the 5′ and 3′ ends of the transcript(s). Transcripts mapping to SVV ORF 21 were also detected in 42% of ganglia and four of the five latently infected monkeys, confirming a previous report that ORF 21 is expressed in SVV latently infected ganglia (6). SVV ORFs 28, 29, 31, 62, and 63 were not expressed in latently infected ganglia but were expressed in tissues of an acutely infected animal. Recent data have also demonstrated that SVV ORFs 14 (dUTPase), 19 (ribonucleotide reductase), and 59 (uracil DNA glycosylase) are expressed in acutely infected, but not latently infected, ganglia (T. M. Ward and W. L. Gray, unpublished data). The results suggest that viral gene expression

TABLE 4. Summary of detection of SVV transcripts in latently infected ganglia

SVV ORF	Function	Kinetic class	No. positive/total	
			Monkeys[a]	Ganglia[b]
ORF 61	Transactivator	IE (?)	5/5	14/14[c]
ORF 62/71	Transactivator	IE	0/5	0/11
ORF 63/70	Transactivator	IE	0/5	0/13
ORF 28	DNA polymerase	Early	0/5	0/14
ORF 29	DNA binding protein	Early	0/4	0/12
ORF 21	Unknown	Early	4/5	5/12
ORF 31	Glycoprotein B	Late	0/5	0/14

[a] Number of monkeys with ganglia expressing transcript/total number of monkeys.
[b] Number of ganglia expressing transcript/total number of ganglia examined.
[c] Detection of ORF 61 transcripts (LAT) in ganglia was confirmed with multiple primer sets.

during latency is limited to at least two specific regions, ORF 61 and ORF 21, of the SVV genome, although further studies are needed to determine whether additional SVV genes are expressed during viral latency.

SVV IE, early, and late transcripts were detected in liver, lung, and ganglia at day 10 p.i. of an acutely infected monkey, indicating active virus replication in these tissues and confirming a previous study of SVV gene expression during acute disease (16). In contrast, IE (ORFs 62 and 63), early (ORFs 28 and 29), and late (ORF 31, glycoprotein B) transcripts were not detected at 15 to 17 weeks p.i. in liver or lung tissues derived from animals that had resolved the acute disease. This result, combined with the finding of SVV DNA and restricted viral gene expression in the neural ganglia, confirmed latent infection in these animals. This finding is not consistent with a previous study which indicated that SVV IE, early, and late genes were transcribed in liver, lung, and ganglia for as long as 12 months p.i. following intratracheal inoculation of African green monkeys, suggesting persistent infection (44). The basis for this discrepancy is unclear. It is possible that the booster SVV immunizations used in this study played a role in promoting SVV latent infection. Other reports have supported SVV latency following resolution of simian varicella as indicated by an inability to detect SVV nucleic acids in liver and lung tissues of SVV intratracheally infected monkeys at 2 months to 4 years p.i. (6, 22, 30, 34).

The finding that latent SVV infection of monkey neural ganglia is characterized by limited gene expression, including a transcript that overlaps and is antisense to a viral regulatory gene (ORF 61, ICP0), is consistent with viral latency of other neurotropic alphaherpesviruses, including HSV-1, HSV-2, EHV-1, EHV-4, BHV-1, PRV-1, and FHV-1. During latent infection of neural ganglia, HSV-1 and HSV-2 express primary transcripts that partially overlap and are antisense to the ICP0 gene and are spliced into stable 1.5- to 2.0-kb LATs (10, 11). EHV-1 and BHV-1 express LATs that are antisense to ICP0 homolog genes in neurons of trigeminal ganglia derived from latently infected horses and cows, respectively (1, 25). LATs antisense to ICP0 and ICP4 (IE) homolog genes are expressed by EHV-4 and PRV in latently infected ganglia of horses and pigs, respectively (2, 4). FHV-1 LATs antisense to the ICP4 IE gene are detected in the trigeminal ganglia of latently infected cats (43). Even the lymphotropic Marek's disease virus expresses a LAT that is antisense to the ICP4 IE gene in T lymphocytes of latently infected chickens (38).

In contrast, studies of VZV latency indicate a pattern of gene expression in neural ganglia that is different from that typical during latent infection of other alphaherpesviruses, including SVV. Transcripts of VZV ORFs 21, 29, 62, and 63 have been most consistently detected in latent human neural ganglia, with ORFs 4, 14, 18, and 66 RNA more variably identified (37). In addition, VZV ORF 4, 21, 29, 63, and 66 proteins are reported to be expressed in latently infected human ganglia (9, 29, 33). Only a few of these studies have attempted to detect VZV gene 61 expression, and these studies used methods (in situ and Northern hybridization) that may not have been sensitive enough to detect low-copy-number transcripts or tried to detect LATs in poly(A)-enriched RNA, even though a VZV ORF 61 LAT, like the HSV-1 LAT, may not be polyadenylated (7, 27, 36). The basis for the novel transcription pattern in VZV latency is unknown. It is possible that aberrant gene expression and/or partial reactivation occurs following death, as human ganglia are not usually available for analysis until 12 to 48 h postmortem, although a recent study employing a rat model of VZV latency argues against this hypothesis (21). The simian varicella latency model avoids this problem, as ganglia are harvested from animals immediately after sacrifice.

The role of the SVV ORF 61 and ORF 21 transcripts in viral latency and reactivation remains to be elucidated. The finding that SVV gene 61 mRNA predominates in acutely infected ganglia while the ORF 61 antisense LAT predominates in latently infected ganglia suggests that the balance of these transcripts may play a role in the molecular basis of SVV latency and reactivation. Possibly, viral latency is maintained by LAT binding to the complementary gene 61 mRNA, thus inhibiting translation of the ORF 61 protein, a transactivator of SVV IE, early, and late gene expression (15). Conversely, viral reactivation could be induced by changes in the neuronal cell environment, resulting in a shift in the gene 61 mRNA/LAT ratio such that the ORF 61 transactivator protein is expressed. However, evidence indicates that HSV-1 LAT antisense regulation of the ICP0 gene is not a mechanism involved in HSV-1 reactivation in mice (41). An alternative hypothesis is that the SVV LAT may serve as an inhibitor of neuronal apoptosis, thus promoting neuronal cell survival and enhancing latency and reactivation, as has been proposed for the HSV-1 LAT and the BHV-1 latency-related transcript (5, 42). In addition, VZV induces apoptosis in human foreskin cells and lymphocytes but not in neuronal cells (24, 28). Thus, inhibition of apoptosis may also play a role in VZV latency and reactivation by promoting neuronal cell survival. Indeed, VZV ORF 63 has been shown to inhibit apoptosis in neurons (23). Sequence analysis of the SVV LAT does not identify an obvious ORF, so a LAT protein may not be involved in viral latency and reactivation. The SVV LAT/ORF 61 has 53% sequence homology to corresponding sequences in VZV but little homology to the HSV-1 LAT/ICP0. Comparison of homologous SVV and VZV ORF 61 sequences does not provide an obvious explanation for the differences between SVV and VZV gene expression during latency.

The detection of SVV and VZV ORF 21 transcripts in latently infected monkey and human ganglia, respectively, suggests that expression of this viral gene may play a role in varicella latency. VZV gene 21 encodes a 115-kDa nucleocapsid protein that does not transactivate VZV IE, early, or late genes and whose function is presently unknown (32, 46). Studies of VZV ORF 21 deletion mutants indicate that gene 21 expression is essential for viral replication in vitro but is not required for the establishment of latent infection in a cotton rat model (45). The SVV and VZV ORF 21 promoters are stimulated in infected, but not uninfected, monkey kidney cells (BSC-1) and are transactivated by the ORF 62 IE protein (8, 35). This IE62-mediated transactivation of SVV ORF 21 is regulated by the SVV ORF 63 protein in a cell-dependent manner (31). Studies are in progress to characterize the SVV ORF 21 transcript detected in latently infected monkey ganglia.

SVV and VZV are closely related herpesviruses that induce clinically similar diseases sharing aspects of viral pathogenesis,

including establishment of latent infection in neural ganglia and viral reactivation. Despite these similarities, this study indicates differences between SVV and VZV gene expression in the neural ganglia during viral latency. Further studies using the simian varicella model may provide information to help elucidate the molecular mechanisms of herpesvirus latency and reactivation.

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