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The simian varicella virus uracil DNA glycosylase and dUTPase genes are expressed *in vivo*, but are non-essential for replication in cell culture

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Abstract

Neurotropic herpesviruses express viral deoxyuridine triphosphate nucleotidohydrolase (dUTPase) and uracil DNA glycosylase (UDG) enzymes which may reduce uracil misincorporation into viral DNA, particularly in neurons of infected ganglia. The simian varicella virus (SVV) dUTPase (ORF 8) and UDG (ORF 59) share 37.7% and 53.9% amino acid identity, respectively, with varicella-zoster virus (VZV) homologs. Infectious SVV mutants defective in either dUTPase (SVV-dUTPase⁻) or UDG (SVV-UDG⁻) activity or both (SVV-dUTPase⁻/UDG⁻) were constructed using recA assisted endonuclease cleavage (RARE) and a cosmid recombination system. Loss of viral dUTPase and UDG enzymatic activity was confirmed in CV-1 cells infected with the SVV mutants. The SVV-dUTPase⁻, SVV-UDG⁻, and SVV-dUTPase⁻/UDG⁻ mutants replicated as efficiently as wild-type SVV in cell culture. SVV dUTPase and UDG expression was detected in tissues derived from acutely infected animals, but not in tissues derived from latently infected animals. Further studies will evaluate the pathogenesis of SVV dUTPase and UDG mutants and their potential as varicella vaccines.

Keywords

simian varicella virus; varicella-zoster virus; dUTPase; uracil DNA glycosylase

1. Introduction

Varicella zoster virus (VZV) causes chickenpox (varicella), generally a mild disease of childhood (Cohen et al., 2006). After resolution of the primary disease, VZV establishes lifelong latency in the neural ganglia of the host. Viral reactivation induced by stress, trauma, immunosuppression, or other means may occur years later, causing herpes zoster (shingles) and postherpetic neuralgia, most commonly in the elderly. VZV does not produce a varicella-like disease in experimental animals, thus hampering study of VZV pathogenesis and antiviral strategies.

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Simian varicella virus (SVV, *Cercopithecine herpesvirus 9*) causes in nonhuman primates a natural varicella disease, and like VZV, establishes latency in neural ganglia, and may reactivate to cause secondary disease (Gray, 2008). SVV and VZV share a high degree of antigenic and genetic similarity (Fletcher, III and Gray, 1992; Gray et al., 2001). Simian varicella provides an experimental model for the study of VZV pathogenesis and latency and for evaluation of antiviral agents and vaccines (Gray, 2004).

Neurotropic alphaherpesviruses, including SVV and VZV, establish latency in terminally differentiated neurons, which do not exhibit the activity of many key enzymes involved in DNA replication (Focher et al., 1990; Chen et al., 2002). Specifically, the viral deoxyuridine triphosphate nucleotidohydrolase (dUTPase), which helps maintain low pools of dUTP during DNA replication, and uracil DNA glycosylase (UDG), which excises misincorporated uracil from DNA, may be important for efficient viral replication in neurons and thus play a key role in viral pathogenesis, latency, and reactivation (Chen et al., 2002; Focher et al., 1992; Pyles and Thompson, 1994a; Jons et al., 1997; Pyles et al., 1992). Therefore, deletion of the SVV and VZV dUTPase and UDG genes may hamper viral replication in neurons and hence prevent or reduce the establishment of viral latency and/or viral reactivation. In this study, SVV mutants defective in dUTPase and/or UDG activity were constructed and characterized as a step toward determining the role of the dUTPase and UDG in varicella virus pathogenesis.

2. Materials and Methods

2.1. Cells and viruses

African green monkey kidney cells (Vero or CV-1) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% newborn calf serum (NCS), penicillin [5,000U/ml], and streptomycin [5,000U/ml]. SVV infected cells were grown in similar medium with 2% NCS and antibiotics.

DNA and amino acid sequence analyses—The DNA sequence of the SVV dUTPase was determined as previously described (Gray et al., 2001) and analyzed using the sequence analysis software package of the Genetics Computer Group (GCG), University of Wisconsin (Devereux et al., 1984). Amino acid sequence homologies and alignment were determined using the Bestfit program, which employs the local homology algorithm of Smith and Waterman, 1981. Sequence analysis of the SVV UDG was reported previously (Ashburn and Gray, 1999). The SVV dUTPase and UDG DNA and amino acid sequences are included in Gene Bank (accession # AF275348).

Construction of SVV-dUTPase⁻ and SVV-UDG⁻ mutants—Manipulation of the SVV genome was performed using the SVV cosmid recombination system (Gray and Mahalingam, 2005). The SVV dUTPase gene (open reading frame [ORF] 8), within SVV cosmid A, and the SVV UDG gene (ORF 59), within cosmid D, were mutated using the recA assisted restriction endonuclease cleavage (RARE) method (Ferrin and Camerini-Otero, 1991). To create a SVV dUTPase mutant (SVV-UTPase⁻), an oligonucleotide 5'-CGGCACAAATCCTGCCTCTGTACCCGGAATCAATCACACCATTG GCTATAAAAACGTCGG-3' was hybridized to a HpaII restriction site (5'-CCGG-3') at nucleotide (nt) 12,326 on the SVV genome (Gray et al., 2001) and its flanking sequence within ORF 8 of cosmid A. The remaining HpaII sites in the cosmid were methylated with HpaII methylase and the DNA was then digested with HpaII to linearize the cosmid. A double-stranded DNA adapter containing stop codons in each reading frame, made by combining oligos 5'-CGGCTAGCTACTAGGGCGCGCCGCTAGGC-3' and 5'-CGGCCTAGCGGCGCGCCCTAGTAGCTAGC-3' was ligated into the HpaII site, and the

resulting dUTPase⁻ cosmid A was packaged into λ -phage (MaxPlax packaging system, Epicentre). Co-transfection of CV-1 cells with the dUTPase⁻ cosmid A and wild-type (wt) SVV cosmids B, C, and D resulted in recombination generating infectious SVV-dUTPase⁻.

To construct a SVV UDG deletion mutant (SVV-UDG⁻), two oligonucleotides 5'-GGACATATTTGCCTGGACTCGCTTTTGTCCACCGGAAAAGGTACGCGTTGTTATT C TTGGGC-3' and 5'-GATGCTAAAAACGCTTTGCTTACAACGTACCGGATTAGTGTTTA TGTTATGGGGTG-3' were hybridized to two HpaII sites (nt 100,596 and 100,908) and flanking sequences within ORF 59 of cosmid D. After methylation of remaining HpaII sites and release of the hybridized oligos, the cosmid DNA was digested with HpaII to delete 313 bp from ORF 59. Following electrophoresis, the UDG⁻ cosmid D DNA was excised from the gel, re-ligated to itself, and packaged. CV-1 cells were co-transfected with UDG⁻ cosmid D and wt cosmids A, B, and C to yield infectious SVV-UDG⁻. In addition, a double mutant was created by co-transfection of CV-1 cells with dUTPase⁻ cosmid A and UDG⁻ cosmid D along with wt cosmids B and C to generate SVV-dUTPase⁻/UDG⁻.

PCR and DNA sequence analysis were used to confirm the insertion of stop codon adapters in SVV-dUTPase⁻ DNA and deletion of sequences in SVV-UDG⁻ DNA harvested from infected cells. PCR conditions were: an initial melting step of 94°C for 2 minutes, followed by 30 cycles of 94°C for 1 minute, 55°C for 3 minutes, 72°C for 2 minutes, and a final extension step of 72°C for 7 minutes. PCR products were cloned into the pGEM-Teasy vector (Promega Corp.) and DNA sequence analysis was performed using the SP6 and T7 primers.

2.3. Viral growth assays

Cell-free SVV virus preparations were generated as previously described (Ward et al., 2008). CV-1 cell monolayers (25 cm²) were infected with 500 PFU cell-free SVV, and virus titers were calculated by plaque assay on CV-1 cells at 0, 8, 24, 48, and 72 hr postinfection (pi). For plaque size calculations, virus plaques were measured at 72 hours pi. At least 20 independent viral plaques were measured with a light microscope, and the average diameter of plaques was calculated. Statistical significance was determined by Student's t-test.

2.4. Detection of SVV transcripts in infected tissues

Neural ganglia, liver, and lung tissues were harvested at necropsy from an SVV infected African green monkey (*Chlorocebus sabeus*) during the acute stage of disease (day 10 pi) as previously described (Gray et al., 2002). Neural ganglia were also collected from a monkey which had resolved clinical disease and was confirmed to be latently infected by the inability to detect infectious virus in the tissues at 105 day pi (Ou et al., 2007). Total RNA was isolated from tissue using the TRI reagent kit (Molecular Research Center, Inc.). For RT-PCR, RNA samples were DNase treated (DNA-free kit, Ambion), and copied into cDNA using the Access-Quick RT-PCR system (Promega Corp.). For detection of SVV ORF 8 (dUTPase) transcripts, primers 5'-CTGGATATGATGTGTGTGCCCC-3' (nt 11,350) and 5'-GTGAACACTTTGGTTGCGGTG-3' (nt 11,064) were employed. For detection of ORF 59 (UDG) transcripts, primers 5'-CGGAACGCCAATTCCTCC-3' (nt 100,825) and 5'-CCAGTTGATAATGGGTTCTCC-3' (nt 100,413) were used. Reactions were incubated at 45°C for 45 minutes for reverse transcription, followed by PCR amplification under the following conditions: 94°C for 2 minutes, 94°C for 30 seconds, 60°C for 30 seconds, 68°C for 1 minute for a total of 31 cycles, followed by a final extension step at 68°C for 5 minutes. Southern blot hybridization analysis was used to confirm detection of SVV dUTPase and UDG transcripts. Five μ l of each cDNA PCR product was fractionated by 1.0% agarose gel electrophoresis, followed by transfer to nylon membranes. SVV UDG and

dUTPase gene specific digoxigenin (DIG) labeled probes were generated using the High Prime DNA labeling system (Roche). The probes were hybridized to immobilized DNA on nylon membranes, and SVV UDG and dUTPase cDNA was detected by autoradiography on Kodak Biomax XAR film.

2.5. UDG assay

UDG activity was determined by modification of a previously described method (Reddy et al., 1998). SVV infected or mock-infected CV-1 cells were scraped from flasks, washed in phosphate-buffered saline (PBS), and pelleted by centrifugation at 1,100 g for 10 minutes. Supernatant was removed and cell pellets were snap frozen at -70°C . Thawed cell pellets were resuspended in 1.5 ml of extraction buffer (10 mM Tris-HCl, pH 7.5, 250 mM NaCl, 1 mM EDTA), sonicated, and centrifuged at 20,000 g for two minutes. Protein concentration of the supernatants was determined by Coomassie blue dye binding assay (BioRad Laboratories). UDG assays were performed at 37°C in 0.2- ml reactions containing 2– 6 μg protein in 50 mM Tris-HCl, pH 7.5, 2 mM DTT, 10 mM EDTA, 0.01% (w/v) bovine serum albumin, and 4 μg of [^3H]-uracil labeled calf thymus DNA (6,000 cpm/ μg), prepared by nick translation with [^3H]-dUTP. Reactions were terminated by adding 25 μl of unlabelled calf thymus DNA (1 mg/ml) and 25 μl of ice-cold 50% trichloroacetic acid. After 20 minutes on ice, samples were centrifuged at 1100 g for 5 minutes, and 0.2 ml of the supernatant was added to 4 ml of scintillation cocktail, and neutralized with 75 μl of 1.2 M KOH. Radioactivity was measured in a Beckman LS-1800 scintillation counter. One unit of UDG activity was defined as the amount of enzyme that released 1 nmol of [^3H]-uracil/minute at 37°C (Winters and Williams, 1990).

2.6. dUTPase assay

dUTPase activity was determined as previously described (Ross et al., 1997). Briefly, SVV infected or mock-infected CV-1 cells were scraped from flasks, washed with PBS, pelleted by centrifugation, resuspended in 1 ml of extraction buffer (10 mM Tris-HCl, pH 8.0, 1 mM MgCl_2 , 20% [vol/vol] glycerol), sonicated, and centrifuged at 20,000 g for two minutes. Supernatants with equal amounts of protein were fractionated by nondenaturing polyacrylamide gel electrophoresis (PAGE) on 5% gels at 200V for 3 hr at 4°C . Following electrophoresis, gels were cut into 3 mm slices each of which was assayed in 150 μl of dUTPase reaction mixture (50 mM Tris-HCl, pH 8.0, 1 mM MgCl_2 , 0.1 mM [^3H]-dUTP (50 $\mu\text{Ci}/\mu\text{mol}$), 2 mM β -mercaptoethanol, 0.1% (w/v) bovine serum albumin, 2 mM p-nitrophenyl phosphate, 5 mM ATP) for 12 hr at 37°C . Reactions were terminated by spotting 50 μl of the reaction mixture onto a DE81 filter disc and immediately washing the disc twice in 4 M formic acid and 1 mM ammonium formate. Discs were washed in ethanol, dried, and the amount of bound radioactivity was determined with one unit of dUTPase activity defined as the amount of enzyme that hydrolyzed 1 nmol of dUTP/minute/ml at 37°C (Williams and Paris, 1987).

3. Results

3.1. Sequence analysis of the SVV dUTPase and UDG

ORF 8 of the SVV genome (nt 11,525 – 12,713) encodes a 396 amino acid peptide (44.9 kDa) which is similar in size and shares 37.7% amino acid identity to the VZV dUTPase homolog (Fig. 1). Alignment of the SVV ORF 8 amino acid sequence with the dUTPase sequences of other alphaherpesvirus homologs demonstrated that the SVV dUTPase includes five highly-conserved motifs (M1–M5) which combine spatially to form the active site of the enzyme as well as a herpesvirus-specific motif (M6) which has an unknown function (McGehean et al., 2001). A previous study demonstrated that SVV ORF 59

encodes a 30 kDa protein that shares 53.9% amino acid identity to the VZV UDG (Ashburn and Gray, 1999).

3.2. Construction and analysis of SVV-dUTPase⁻ and SVV-UDG⁻ mutants

SVV dUTPase and UDG mutants were constructed using the SVV cosmid system (Gray and Mahalingam, 2005) in combination with the RecA assisted restriction endonuclease cleavage (RARE) method (Ferrin and Camerini-Otero, 1991). The SVV-dUTPase⁻ mutant was generated by insertion of a DNA oligo adapter containing translational stop codons into a HpaII site (nt 12,326) of ORF 8, resulting in a truncated protein missing 67% of the amino acid sequence, including all six of the conserved dUTPase motifs (M1–M6, Fig. 1). PCR amplification of the dUTPase gene and DNA sequence analysis of the amplified product confirmed insertion of the stop codon adapter into the dUTPase sequence of SVV-dUTPase⁻ (Fig. 2A).

A SVV-UDG⁻ mutant was constructed by deletion of 313 bp within ORF 59 (34% of the ORF) including the two highly conserved sequences proposed to comprise the UDG catalytic site (Savva et al., 1995; Ashburn and Gray, 1999). Deletion of the 313 bp sequence from the UDG gene of SVV-UDG⁻ (Fig. 2B, lane 3) compared to wt SVV (lane 2) was confirmed by PCR analysis and subsequently by DNA sequence analysis (data not shown).

In addition, a SVV-UDG⁻/dUTPase⁻ double mutant was generated which includes the truncated UDG gene and also the dUTPase gene deletion. Analysis of SVV-dUTPase⁻/UDG⁻ DNA confirmed insertion of the stop codons within the dUTPase gene (Fig. 2A) and the 313 bp deletion in the UDG gene (Fig. 2B, lane 4)

The SVV-dUTPase⁻ and SVV-dUTPase⁻/UDG⁻ mutants were confirmed to be defective for functional dUTPase enzymatic activity. Lysates of infected and mock-infected CV-1 cells were fractionated on nondenaturing PAGE gels, taking advantage of the larger size of herpesvirus dUTPases compared to cellular dUTPase, and gel slices were analyzed for dUTPase activity. Cellular dUTPase activity was detected in gel slices with R_f values ranging from 0.5 to 0.7 (peak 0.6) in lysates of infected (Fig. 3, panels B–E) and mock-infected (panel A) CV-1 cells. Viral dUTPase activity in wt SVV infected cells was detected in gel slices with R_f values ranging from 0.1 to 0.3 (peak 0.23). The viral dUTPase activity was not present in mock-infected cells (panel A), and was inhibited by the specific herpesvirus dUTPase inhibitor mercaptoguanosine[mg]-HgdUTP (panel C) (Studebaker et al., 2001). Viral dUTPase activity was not detected in lysates derived from SVV-dUTPase⁻ (panel D) or SVV-dUTPase⁻/UDG⁻ (panel E) infected CV-1 cells demonstrating that these viral mutants do not express functional viral dUTPases.

Loss of functional viral UDG enzymatic activity in SVV-UDG⁻ and SVV-dUTPase⁻/UDG⁻ infected cells was confirmed by determining the amount of [³H]-uracil removed from labeled DNA by lysates derived from infected and mock-infected CV-1 cells (Table 1). The UDG activity in SVV-UDG⁻ and SVV-dUTPase⁻/UDG⁻ infected CV-1 cells was reduced compared to wt SVV infected cells.

3.3. SVV dUTPase and UDG mutants replicate efficiently in cell culture

To determine if mutation of genes involved in nucleotide metabolism has detrimental effects on the ability of SVV to replicate, the *in vitro* growth kinetics of SVV-UDG⁻ and SVV-dUTPase⁻ were evaluated. The results demonstrated that the SVV-dUTPase⁻, SVV-UDG⁻, and SVV-dUTPase⁻/UDG⁻ mutants all grew with similar kinetics as wt SVV in CV-1 cell culture (Fig. 4A). To confirm this finding, viral plaque sizes on CV-1 cells at 72 hr pi were measured. The results indicated no significant difference in the mean plaque diameters of

the SVV-dUTPase⁻, SVV-UDG⁻, and SVV-dUTPase⁻/UDG⁻ mutants compared to wt SVV (Fig. 4B).

3.4. SVV dUTPase and UDG gene expression in vivo

SVV dUTPase and UDG gene expression was assessed in tissues derived from a monkey during the acute stage of clinical simian varicella (day 10 pi) and also from a monkey that had resolved the acute disease and was confirmed to be latently infected (day 105 pi). Total cell RNA was isolated from liver, lung, and ganglia tissues and SVV dUTPase and UDG transcripts were detected by RT-PCR and Southern blot hybridization analysis using gene specific probes. The results demonstrate expression of the viral UDG in liver, lung, and trigeminal ganglia of an acutely infected monkey (Fig. 5A). SVV dUTPase transcripts were detected in liver and lung tissue, but not trigeminal ganglia, derived from an SVV acutely infected monkey (Fig. 5B). SVV UDG and dUTPase transcripts were not detected in trigeminal or cervical ganglia derived from a latently infected monkey (Fig. 5 A, B).

4. Discussion

This study demonstrates that the SVV dUTPase and UDG genes are not required for viral replication in cell culture, but are expressed in tissues derived from infected monkeys during acute simian varicella disease. SVV dUTPase and UDG transcripts were not detected in neural ganglia harvested from a latently infected animal, confirming that SVV gene expression is restricted during viral latency (Ou et al., 2007). Like SVV, other neurotropic alphaherpesviruses including VZV, HSV-1, equine herpesvirus type 1 (EHV-1), bovine herpesvirus type 1 (BHV-1), and pseudorabies virus (PRV) encode dUTPase and UDG genes that are not required for *in vitro* replication, but express enzymes that are hypothesized to reduce deleterious uracil incorporation into the viral genome, particularly in terminally differentiated neurons which lack these DNA repair enzymes (Ross et al., 1997; Reddy et al., 1998; Pyles et al., 1992; Pyles and Thompson, 1994a; Chung and Hsu, 1996; Liang et al., 1993; Jons et al., 1997; Dean and Cheung, 1993). The generation of the SVV-dUTPase⁻/UDG⁻ double mutant in this study is the first report that a herpesvirus defective for both dUTPase and UDG can replicate efficiently in cell culture. The conservation of these “non-essential” genes in SVV and other neurotropic alphaherpesviruses suggests that the dUTPase and UDG may play an important role in viral pathogenesis, latency, and/or reactivation.

The UDG enzyme functions by excising uracil from DNA, leaving an abasic site that is then filled with the appropriate base by other proofreading enzymes (Krusong et al., 2005). Uracil may exist in DNA as a result of spontaneous deamination of cytosine, or by misincorporation of dUTP by DNA polymerase during DNA replication. Cytosine deamination may cause accumulation of uracil within the genomes of neurotropic herpesviruses during viral latency in neural ganglia. The viral UDG may rid the viral genome of these mutagenic bases before progeny genomes are copied upon reactivation. While HSV-1 mutants defective for UDG expression replicate efficiently in cell-culture, they have a higher mutation rate upon serial passage (Pyles and Thompson, 1994a, 1994b). *In vivo*, HSV-1 UDG⁻ mutants are ten-fold less neurovirulent upon intracranial inoculation and 100,000-fold less neuroinvasive following footpad inoculation compared to wt HSV-1 using a murine infection model (Pyles and Thompson, 1994a). While the HSV-1 UDG⁻ mutants were able to establish latent infection of neural ganglia, the viral genome burden in ganglia was reduced ten-fold, and the frequency of *in vivo* reactivation was significantly reduced.

The cellular dUTPase enzyme hydrolyzes dUTP to dUMP, an important reaction that provides dUMP as a precursor for *de novo* synthesis of the dTTP utilized for DNA synthesis

(Chen et al., 2002). In addition, dUTPase reduces the intracellular dUTP/dTTP ratio and minimizes the mutagenic misincorporation of uracil into DNA. Like HSV-1 UDG⁻ mutants, HSV-1 dUTPase⁻ mutants have an increased mutation frequency upon serial passage in cell culture compared to wt virus (Pyles and Thompson, 1994b). HSV-1 dUTPase⁻ mutants were attenuated in a mouse model being ten-fold less neurovirulent than wt HSV-1 following intracranial inoculation and 1,000-fold less virulent following peripheral inoculation (Pyles et al., 1992). While HSV-1 dUTPase⁻ mutants were able to establish latent infection in mouse neural ganglia, they exhibited a reduced incidence of *in vivo* reactivation.

Alphaherpesvirus UDG⁻ and dUTPase⁻ mutants may be useful as live attenuated vaccines. While infection with a BHV-1 dUTPase⁻ mutant did not induce clinical disease in cattle, intramuscular inoculation induced viral neutralizing antibody titers and immune protection against disease following BHV-1 respiratory challenge (Liang et al., 1997). Similarly, a PRV dUTPase⁻ mutant was attenuated following intranasal infection of pigs and induced immunity against disease following challenge with a potentially lethal inoculation of wt PRV (Jons et al., 1997).

A future study will utilize the simian varicella model to evaluate the pathogenesis of SVV dUTPase⁻ and UDG⁻ mutants, including their ability to establish latency in neural ganglia and to reactivate, and to assess their potential as varicella vaccines.

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Abbreviations

(SVV)	simian varicella virus
(VZV)	varicella-zoster virus
(dUTPase)	deoxyuridine triphosphate nucleotidohydrolase
(UDG)	uracil DNA glycosylase

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Figure 1.

The amino acid sequence of the SVV dUTPase (ORF 8). Best-fit alignment of the predicted SVV dUTPase protein sequence with dUTPase homologs from VZV, EHV-1, PRV, and HSV-1. Conserved amino acid residues between the SVV and VZV dUTPase sequences are indicated with vertical lines (|). The five conserved motifs involved in dUTPase catalysis (M1–M5) and herpes-specific motif 6 (M6) are indicated with asterisks (*). The arrowhead (▼) indicates the insertion site for the stop codon adapter in the SVV-dUTPase[−] mutant. Consensus is defined as identical amino acids at the same position in at least 3 of the 5 homologs. Amino acid numbers are indicated.

Figure 2.

Confirmation of SVV dUTPase and UDG mutants. (A) Sequence analysis of PCR fragments amplified from wt SVV, SVV-dUTPase⁻, and SVV-dUTPase⁻/UDG⁻ DNA confirms inclusion of the stop codon adapter at the HpaII site within ORF 8. Sequence of wt SVV DNA begins at the ORF 8 ATG start site (nt 12,713), resumes at the region including the HpaII site, and ends with the natural TAA stop codon (#). Sequence of the SVV mutant DNAs begins at the ORF 8 ATG start codon and continues with the region containing the HpaII insertion site (bold) and the DNA sequence of the adapter (underlined) including the initial stop codon (#). (B) Confirmation of the deletion within ORF 59 in SVV-UDG⁻ (lane 3) and SVV-dUTPase⁻/UDG⁻ (lane 4) compared to ORF 59 from wt SVV DNA (lane 2) by agarose gel electrophoresis of PCR amplified products using ORF 59 primers to amplify a 498 bp region bracketing the 313 bp deletion. Lane 1– 100 bp molecular size DNA ladder.

Figure 3.

SVV dUTPase mutants have defective dUTPase activity. Total protein lysates from infected or mock-infected CV-1 cells were fractionated by PAGE and individual gel slices were analyzed for dUTPase activity. Panel A- mock-infected. Panel B- wt SVV infected. Panel C- wt SVV infected with mg-HgdUTP inhibitor. Panel D- SVV-dUTPase⁻ infected. Panel E- SVV-dUTPase⁻/UDG⁻ infected. V- viral dUTPase activity, C- cellular dUTPase activity.

Figure 4.

In vitro growth properties of SVV dUTPase and UDG mutants. (A). CV-1 monolayers were infected with cell-free virus (500 pfu) and incubated for 0, 8, 24, 48, or 72 hr. Viral titer for wt SVV, SVV-dUTPase⁻, SVV-UDG⁻, and SVV-dUTPase⁻/UDG⁻ was determined at each time point by plaque assay. (B) Viral plaque diameters on CV-1 cell monolayers were measured by light microscopy at 72 hr pi. At least 20 plaques for each virus were measured.

Figure 5. Detection of UDg and dUTPase transcripts in tissues of infected animals. Total cell RNA was isolated from liver, lung, and trigeminal ganglia harvested from acutely or latently SVV infected monkeys. SVV UDg and dUTPase RT-PCR cDNA products (413 bp and 287 bp, respectively) were detected by Southern blot hybridization using gene specific probes. (A). SVV UDg expression in acutely and latently infected tissues. (B). SVV dUTPase expression in acutely and latently infected tissues.

Table 1

UDG activity in SVV infected CV-1 cells

Virus	UDG Activity ^a (nmol/ min/ mg)
Mock-infected	0.069
wt SVV	0.110
SVV-UDG ⁻	0.038
SVV-dUTPase ⁻ /UDG ⁻	0.053

^aUDG activity was measured as described in Materials and Methods and defined as the amount of uracil liberated from ³H-dUTP labeled calf thymus DNA per minute per milligram of cell lysate.