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Simian varicella virus DNA in dorsal root ganglia

(herpesvirus/monkey ganglia/polymerase chain reaction)

RAVI MAHALINGAM*†, DIANA SMITH*, MARY WELLISH*, WILLIAM WOLF*, AUD N. DUELAND*, RANDALL COHRS*, KENNETH SOIKE‡, AND DONALD GILDEN*§

Departments of *Neurology and of §Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262; and ‡Delta Regional Primate Research Center, Tulane University, Covington, LA 70433

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ABSTRACT **Clinical, pathological, immunological, and virological evidence suggests that simian varicella virus (SVV) infection of primates is the counterpart of varicella–zoster virus infection of humans. To determine whether these two viruses share similarities in their properties during latency, we analyzed ganglia and brain of an African green monkey experimentally infected with SVV for the presence of viral nucleic acid using the polymerase chain reaction technique. We detected SVV DNA in dorsal root ganglia but not in brain of this monkey, which demonstrated no apparent clinical signs of SVV infection. Our results suggest that SVV becomes latent in monkey ganglia and that latency can develop in the absence of clinical varicella (chickenpox). These studies provide an animal model system to study varicella virus latency.**

Varicella–zoster virus (VZV) causes chickenpox (varicella) in childhood, becomes latent in dorsal root ganglia, and reactivates decades later to produce shingles (zoster) in adults. The host range of VZV is restricted exclusively to humans, so that no animal model has been developed to study VZV pathogenesis and latency. However, there have been various reports of spontaneous epizootic outbreaks of varicella in housed monkeys. Like human varicella, simian varicella frequently occurs in epidemics, has an incubation period of 1 or more weeks, and histologic examination of skin lesions reveals necrosis and hemorrhagic foci of epidermal cells containing intranuclear inclusions (1). The causative agent of simian varicella is a member of the herpesvirus family, simian varicella virus (SVV), which is antigenically crossreactive with VZV (2, 3). SVV also reactivates in monkeys to produce disseminated varicella (3). If the site of SVV latency is ganglionic as has been shown for human VZV (4, 5), then studies of SVV pathogenesis and latency in primates would be relevant to human VZV infection. In the present study, we demonstrate the presence of latent SVV DNA in ganglia and the establishment of latent infection in a monkey which seroconverted after experimental infection with SVV but failed to develop clinical varicella.

METHODS

SVV. The deltaherpesvirus (DHV) strain of SVV was originally isolated from a naturally infected patas monkey (*Erythocebus patas*) as described (6) and propagated in Vero cells, and a virus stock was prepared as described (7, 8).

Virus Inoculation. A 6-year-old female African green monkey (*Cercopithecus aethiops*) was inoculated with SVV as described (8). Briefly, 10^5 plaque-forming units of SVV in 1.5 ml was administered by intratracheal catheter into the upper bronchi, and 1.5 ml was injected subcutaneously over the abdomen. Two days after inoculation, the monkey was treated intramuscularly with a purine nucleoside, B722U (Burroughs Wellcome), at 150 mg/kg of body weight per day for 10 days. The monkey was examined daily for a varicelliform eruption. Blood was collected every few days for detection of viremia and on days 14 and 21 after inoculation for determination of antibody titer to SVV (9).

DNA Isolation. Total DNA was extracted from brain and from ganglia—two trigeminal, six thoracic, and four cervical ganglia—of the monkey. Tissues were thawed and homogenized (Dounce homogenizer) in 2 ml of 0.010 M Tris·HCl, pH 7.5/0.1 M NaCl/50 mM EDTA (TNE buffer) per 0.5 cm^2 of tissue at 4°C and were digested with sodium dodecyl sulfate (0.5% final concentration) and proteinase K (200 μg/ml) for 1 hr at 37°C or overnight at room temperature. DNA was extracted three times with phenol, twice with phenol/chloroform, 1:1 (vol/vol), and three times with chloroform; was precipitated with ethanol; and was redissolved in 0.010 M Tris·HCl, pH 8.0/0.001 M EDTA (TE buffer) (100 μl per 0.5 cm^2 of tissue). DNA was also extracted from BSC-1 cells, VZV-infected BSC-1 cells, and SVV-infected BSC-1 cells as described (10). DNA concentrations were estimated by optical density at 260 nm, and final concentrations were adjusted to 1 μg/μl.

Cloning and Sequencing of SVV *Bam*HI Fragment P Recombinant Clone. DNA isolated from purified SVV was digested with *Bam*HI and ligated to a transcription vector (pGEM-4; Promega) that had been linearized with *Bam*HI. The ligation mixture was used to transform *Escherichia coli* strain HB101. A recombinant clone (p53) containing the 1-kilobase-pair (kbp) SVV *Bam*HI P fragment was selected for sequencing by using [α-^{32}P]dATP at a specific activity of >600 Ci/mmol (1 Ci = 37 GBq; ICN), SP6 promoter/primer, and the T7 DNA polymerase system (Promega) as described (11). The products were separated on a 6% polyacrylamide sequencing gel and exposed for 16 hr to a Kodak X-Omat AR film.

Polymerase Chain Reaction (PCR). Oligonucleotide primers were obtained from Research Genetics, Huntsville, AL. Stock solutions containing 1 μg of the oligonucleotide per ml in sterile water were diluted to 20 μM for use in PCR reactions. PCR was performed with the commercially available GeneAmp Kit (Perkin–Elmer/Cetus). In a typical reaction, 1 μg of tissue DNA or 1 ng of uninfected or virus-infected cell DNA or an equivalent volume of sterile water was used for template in a final 100-μl reaction mixture containing 0.010 M Tris·HCl (pH 8.3); 0.050 M KCl; 0.015 M $MgCl_2$; 0.01% gelatin; dNTPs, each at a final concentration of 200 μM; 1 μM of each primer; and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase. Samples were heated to 95°C for 5 min before adding *Taq* DNA polymerase. PCR data were obtained with an automated DNA thermal cycler (Perkin–Elmer/Cetus) set for a 1-min denaturation step at 94°C,

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Abbreviations: SVV, simian varicella virus; VZV, varicella–zoster virus; PCR, polymerase chain reaction.

†To whom reprint requests should be addressed.

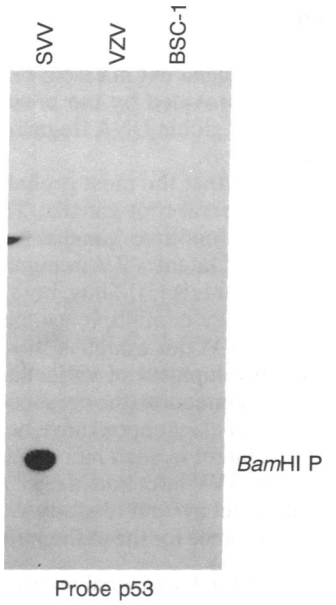

FIG. 1. Specific hybridization of the cloned SVV *Bam*HI P fragment to a *Bam*HI digest of SVV DNA. DNA extracted from SVV-infected BSC-1 cells, VZV-infected BSC-1 cells, and uninfected BSC-1 cells (1, 3, and 10 μg, respectively) was digested with *Bam*HI and separated by electrophoresis (16 hr of 35V) on a 0.6% agarose gel in a buffer of 40 mM Tris acetate/2 mM EDTA, pH 8.0. The DNA fragments were transferred to nitrocellulose filters and hybridized to ^{32}P-labeled riboprobes synthesized by using SP6 RNA polymerase and *Hin*dIII-linearized recombinant clone p53 containing the SVV *Bam*HI P (1000 bp) fragment in pGEM-4. Filters were washed and exposed to Kodak X-Omat film for 16 hr.

a 2-min annealing step at 45°C, and an elongation step of 3 min at 72°C with an automatic extension of the elongation step by 15 sec per cycle. The total number of cycles was 35. As a control, no DNA was included in one of the reaction tubes.

Detection of Virus-Specific Sequences in PCR-Amplified Products. Synthetic oligonucleotides representing a region internal to the amplified fragment were dissolved in sterile water to a final concentration of 0.1 μg/μl, and an aliquot (20 μl) was used for end-labeling with [γ-^{32}P]ATP (7000 Ci/mmol; ICN) as described (11). Three microliters of the 100-μl PCR mixture containing SVV-infected cell DNA and 12 μl from the others were used for analysis. Amplified DNA fragments were separated on a 2% agarose gel in 0.04 M Tris acetate/0.002 M EDTA, pH 8.0. DNA fragments in the agarose gel were transferred to Zeta-Probe (Bio-Rad) membranes, hybridized to ^{32}P-end-labeled oligonucleotide probes, and detected by autoradiography per the manufacturer's instructions. The size of the amplified product was determined by comparison with standard molecular weight markers (123-bp ladder, Bethesda Research Laboratories).

RESULTS AND DISCUSSION

A 6-year-old female African green monkey was inoculated with 10^5 plaque-forming units of SVV by the intratracheal and subcutaneous routes (12). As part of a study designed to determine the efficacy of a new antiviral agent, the monkey was treated 2 days after inoculation with a purine nucleoside for 10 days. SVV was isolated from blood 3–4 days later. The monkey failed to develop a localized or disseminated varicelliform eruption. A neutralizing antibody titer of 1:20 (the serum dilution resulting in a reduction of 80% of viral plaques) was detected 21 days after inoculation. In a study investigating immune responsiveness, the monkey was treated 4 months after inoculation with interleukin 4 (5 μg/kg per day) for 17 days and then was rechallenged with 2.5×10^5

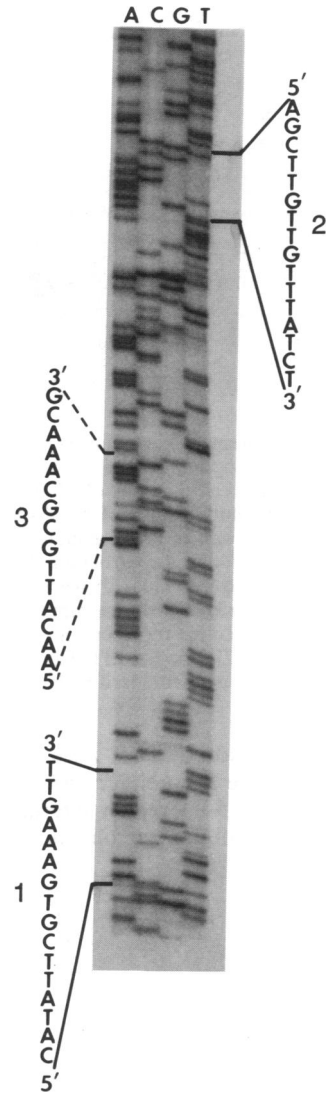

FIG. 2. Sequencing of SVV *Bam*HI P recombinant clone. The recombinant clone (p53) containing the SVV *Bam*HI P fragment (1000 bp) was sequenced by using [α-^{32}P]dATP. The products were separated on a 6% polyacrylamide sequencing gel and exposed for 16 hr to Kodak X-Omat AR film. A portion of the gel containing the 123-bp region used for amplification by PCR is presented. The sequence of the 16-mer oligonucleotide primers 1 and 2 on the two opposite DNA strands chosen for PCR and the 15-mer internal region 3 chosen for detection of the amplified material are indicated.

plaque-forming units of SVV. Ten days after reinoculation with SVV, the anti-SVV antibody titer was 1:2560. Again, no rash or sign of clinical disease was observed. Five months after primary infection, the monkey was sacrificed, and DNA was extracted from two trigeminal, four cervical, and six thoracic ganglia and from brain for PCR analysis.

A recombinant clone (p53) containing the 1.0-kilobase (kb) SVV DNA *Bam*HI P fragment in pGEM-4 was used to synthesize ^{32}P-labeled riboprobe which was hybridized to *Bam*HI-digested DNA from SVV-infected, VZV-infected, and uninfected BSC-1 cells. The specificity of the SVV riboprobe was demonstrated by its hybridization to only DNA from SVV-infected BSC-1 cells, corresponding to the 1.0-kbp SVV DNA *Bam*HI P fragment but not to DNA from VZV-infected or uninfected BSC-1 cells (Fig. 1).

The sequence of a 123-bp region of the SVV insert in clone p53 was determined by using the T7 DNA polymerase sequencing system (Promega) in the presence of SP6 promotor/primer and [α-^{32}P]dATP (Fig. 2). Oligonucleotide primers

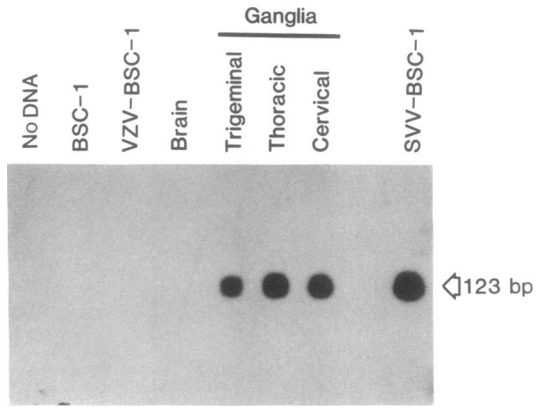

FIG. 3. Detection of latent SVV sequences in DNA from monkey ganglia by PCR. One nanogram of DNA from uninfected, VZV-infected, and SVV-infected cells and 1 μg of DNA from the remaining samples were used for amplification of SVV-specific sequences. Primers used for amplification of SVV sequences are described in Fig. 2. No DNA was included as a control (lane No DNA). Aliquots (12 μl) of the reaction mixtures (100 μl) were separated on a 2% agarose gel, transferred to a Zeta-Probe membrane and hybridized to a ^{32}P-end-labeled oligonucleotide representing sequences internal to the amplified product (Fig. 2). The size of the amplified products was determined by comparison with standard molecular weight markers (123-bp ladder).

(16-mers) for amplification by PCR were obtained based on the nucleotide sequence from either end of the 123-bp segment. Sequence of a 15-bp region internal to the 123-bp segment was used to obtain an oligonucleotide probe to detect the amplification products. The 123-bp SVV DNA fragment was amplified in preparations from trigeminal, cervical, and thoracic ganglia but not from brain (Fig. 3). An additional control was provided by the presence of an amplifiable 83-bp monkey β-globin DNA fragment in DNA from all monkey tissues (Fig. 4).

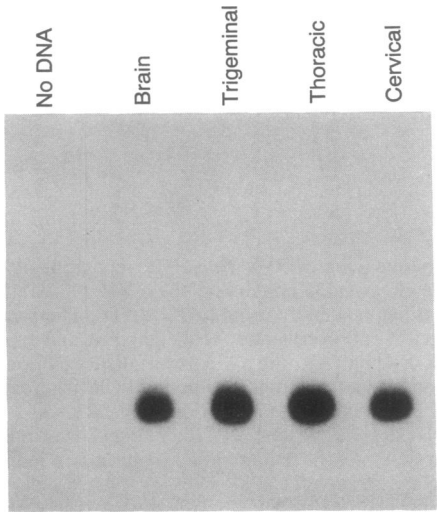

FIG. 4. Detection of monkey β-globin sequences in DNA from monkey brain and trigeminal, thoracic, and cervical ganglia. The monkey β-globin sequences (83 bp) were amplified and detected as described in Fig. 3. The sequences of the monkey β-globin primers used for amplification were 5'-AGCTCGCTTTCTTGCTGTCC-3' and 5'-GCCCTTCATAATATCCCCCA-3', and that used for detection was 5'-TTCCCAAAGTCCAAC-3' (13). No DNA was included as a control (lane No DNA).

These results indicate that the most probable site of SVV latency in primates is dorsal root ganglia. The detection of SVV DNA by PCR in multiple ganglia but not in brain parallels the detection of latent VZV in human ganglia from multiple levels of the neuraxis (5). Thus, both SVV and VZV appear to establish latency in multiple ganglia after primary infection. Moreover, SVV can establish latency in multiple ganglia without the development of varicella. Cases of subclinical human varicella infection (the presence of antibody to VZV without a history of chickenpox) have been documented (14); whether VZV is latent in such individuals has not been studied. In conclusion, SVV infection in primates provides an appropriate animal model system to study varicella latency, an issue of major importance for the entire human population.

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