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Viral Isolates Derived from Simian Varicella Epizootics Are Genetically Related

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Epizootics of a natural varicella-like disease occur in populations of nonhuman primates. Several primate herpesviruses have been isolated from these epizootics, but the relatedness of these isolates to each other is not well-defined. In this study, we demonstrated that the restriction endonuclease (REn) profiles of four epidemiologically distinct isolates were similar, although not identical, indicating that simian varicella epizootics are caused by various strains of simian varicella virus (SVV). The genetic variation among the isolates did not map to a specific region of the SVV genome and REn differences were detected within the SVV DNA long component and the inverted repeat region. Southern blot hybridization demonstrated that SVV is more closely related to varicella-zoster virus than to other primate herpesviruses. The study indicates that the current herpesvirus classification scheme should be changed to include SVV as a single taxonomic group within the *Varicellovirus* genus of alphaherpesviruses. In addition, REn profiles of SVV isolates, derived from primary and secondary episodes of simian varicella in the same monkey, were identical, providing evidence for SVV reactivation in a latently infected monkey.

INTRODUCTION

Simian varicella is a naturally occurring disease of Old World monkeys (Superfamily Cercopithecoidea, Oakes and d'Offay, 1988). The disease is characterized by varicella-like symptoms including fever, lethargy, and vesicular rash on the face, abdomen, and extremities. Disseminated infection often results in life-threatening pneumonia and hepatitis. Clarkson *et al.* (1967) initially described a simian varicella epizootic involving recently acquired African green (vervet) monkeys (*Cercopithecus aethiops*) at the Liverpool School of Tropical Medicine. Since then, several epizootics have occurred in captive primate populations of African green, patas (*Erythrocebus patas*), and several species of macaque monkeys (*Macaca* sp., Table 1; Soike, 1992).

The causative agents isolated from several simian varicella epizootics were identified as primate herpesviruses (Table 1). Felsenfeld and Schmidt (1977) demonstrated that the Delta herpesvirus (DHV), Liverpool vervet virus (LVV), and Medical Lake Macaque virus (MLMV) are antigenically related as determined by cross-neutralization and complement fixation assays. A later study suggested that serological assays can differentiate DHV from LVV, MLMV, and the Patas herpesvirus (PHV) (Harbour and Caunt, 1979).

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The current herpesvirus classification scheme differentiates the simian varicella-like herpesviruses into three distinct alphaherpesvirus groups (Roizman *et al.,* 1992; Roizman, 1996). LVV is designated as Cercopithecus herpesvirus 6, DHV and PHV as Cercopithecus herpesvirus 7, and MLMV as Cercopithecus herpesvirus 9. The Cercopithecus herpesvirus 9 group alone includes the common name, simian varicella herpesvirus, although the virus is most commonly referred to as simian varicella virus (SVV). This classification scheme for these primate herpesviruses causes confusion in the scientific literature and in DNA and protein sequence databases.

DHV, LVV, MLMV, and PHV are antigenically related to varicella-zoster virus (VZV), which causes varicella (chickenpox) and herpes zoster (shingles) in humans (Felsenfeld and Schmidt, 1975, 1977; Fletcher and Gray, 1992). Prior immunization with VZV protects monkeys from simian varicella following challenge with DHV (Felsenfeld and Schmidt, 1979). Recent studies confirm that DHV is genetically related to VZV. The DHV and VZV genomes are similar in size, structure, and genetic organization and share 70–75% DNA homology (Fig. 1; Gray and Oakes, 1984; Gray *et al.,* 1992; Clarke *et al.,* 1992; Pumphrey and Gray, 1992).

Due to the genetic relatedness of DHV and VZV and the clinical similarities of human and simian varicella, DHV infection of nonhuman primates serves as an experimental model for VZV pathogenesis and development of antiviral strategies (Soike, 1992). DHV, like VZV, establishes latent infection in sensory ganglia of infected monkeys (Mahalingam *et al.,* 1991). Reactivation of virus from

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TABLE 1

Simian Varicella Epizootics and Viral Isolates

Date and location of epizootic	Species	Clinical infections	Deaths	Viral isolate
1966 Liverpool, UK	*Cercopithecus aethiops*	17	9	Liverpool vervet virus (LVV)
1967 Glaxo Labs, UK	*Erythrocebus patas*		36	Patas herpesvirus (PHV)
1968 Delta Primate Center, USA	*Erythrocebus patas*	24	12	Delta herpesvirus (DHV1)
1969–1971 Washington Primate Center, USA	*Macaca nemestrina*	20	9	Medical Lake Macaque virus (MLMV)
	Macaca fasicularis	50	4	
1974 Delta Primate Center, USA	*Erythrocebus patas*	24	14	Delta herpesvirus (DHV2)
1981 Litton Labs, USA	*Erythrocebus patas*	101	44	Litton herpesvirus
1983 Bowman Gray University, USA	*Cercopithecus aethiops*	9	5	
1984 Hazelton Labs, USA	*Cercopithecus aethiops*		8	Hazelton herpesvirus (HAZV)
	Macaca fasicularis		5	
	Macaca mulatta		2	
1989 Tsukuba Primate Center, Japan	*Macaca fasicularis*	111	43[a]	Tsukuba herpesvirus

[a] Twenty-eight monkeys with simian varicella were sacrificed in an effort to end the epizootic. Reference: Soike, 1992.

latently infected monkeys and transmission of the highly contagious agent to susceptible monkeys are presumably important causes of simian varicella epizootics. However, documentation of simian varicella reactivation disease is limited (Soike *et al.,* 1984).

In this study, we determined the genetic relatedness of isolates derived from distinct simian varicella epizootics to each other and to other primate herpesviruses. The results support the reclassification of the simian varicella-like herpesviruses into a single taxonomic group. In addition, the data provide evidence for SVV reactivation from a latently infected monkey.

METHODS

Viruses and cell culture

DHV (derived from the 1974 epizootic at the Delta Regional Primate Center — now the Tulane Regional Primate Center), DHV-Reactivation (DHV-RE), and the Hazelton virus (HAZV) were provided by Dr. Ken Soike (Tulane Regional Primate Center, Covington, LA). DHV-RE is an isolate derived from an African green monkey with secondary simian varicella, presumably caused by reactivation of latent endogenous virus. The LVV and MLMV were provided by Dr. Bagher Forghani (California State Department of Health Services). The SVV isolates, herpes simplex virus type 1 (HSV-1) strain MacIntyre, and HSV-2 strain 333 were propagated in African green monkey kidney (Vero) cells cultured in Eagle's minimal essential medium supplemented with gentamicin (50 μg/ml) and 5% newborn calf serum. VZV strain Ellen was propagated in MRC-5 cells.

Viral DNA isolation

DHV, DHV-RE, HAZV, LVV, MLMV, VZV, HSV-1, and HSV-2 DNAs were isolated from purified nucleocapsids of infected cells as previously described (Gray *et al.,* 1992). Purified DNA samples of herpes B virus (HBV), SA8, herpesvirus saimiri (HVS), and herpesvirus ateles (HVA) were provided by Dr. Richard Eberle (Oklahoma State University).

Restriction endonuclease (REn) and Southern blot hybridization analyses

These procedures were conducted as previously described (Pumphrey and Gray, 1992). DNA samples were digested with *Bam*HI, *Bgl*II, or *Eco*RI. DNA fragments were fractionated by agarose gel electrophoresis and photographed under ultraviolet illumination. The DNA was alkali denatured and transferred to Gene Screen filters for Southern blot hybridization. DHV DNA probes were 32P-labeled and hybridized to the immobilized DNA fragments. Standard hybridizations were performed at 42° in buffer containing 2×10^6 cpm of labeled DNA probe, 100 μg/ml salmon sperm DNA, 1% SDS, 10% dextran sulfate, 1 *M* NaCl, and 50% formamide. Reduced stringency hybridizations were conducted at 37° in the same buffer, but with 40% formamide.

RESULTS

Genetic relatedness of simian varicella-like herpesvirus isolates

REn analysis of DHV, DHV-RE, HAZV, LVV, and MLMV DNAs revealed that these primate herpesviruses have similar *Bam*HI, *Bgl*II, and *Eco*RI profiles (Fig. 2). This result demonstrates the genetic relatedness of these viral isolates. The *Bam*HI, *Bgl*II, and *Eco*RI DNA profiles of the DHV and DHV-RE isolates, derived from primary and reactivated simian varicella, respectively, of the same

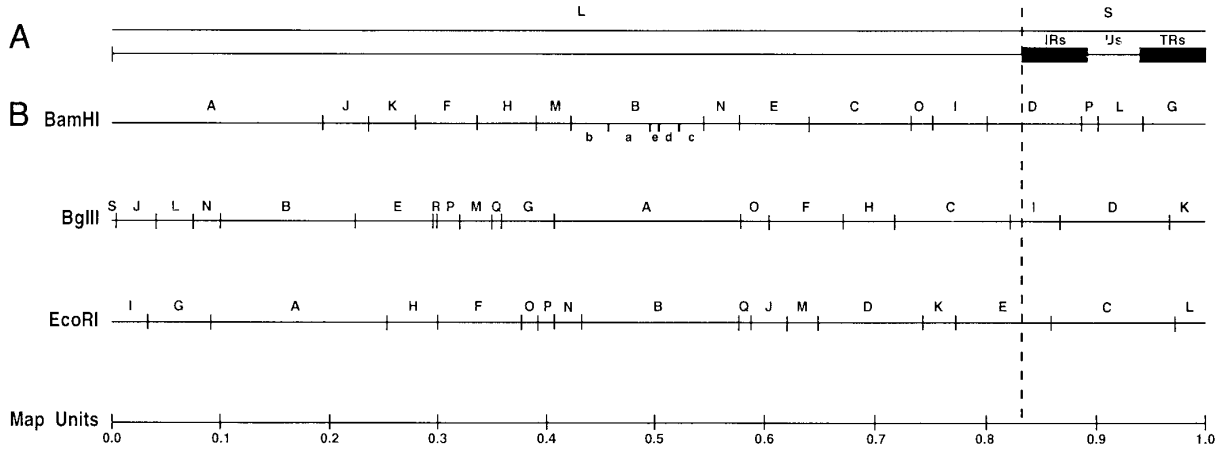

FIG. 1. Structure and REn maps of the DHV genome (Gray *et al.,* 1992; Clarke *et al.,* 1992). (A) The double-stranded viral genome consists of a long (L, ≅100 kbp) component covalently linked to a short (S) component. The S component includes a unique short (Us, 4.9 kbp) sequence bracketed by internal and terminal inverted repeats (IRs and TRs, 7.5 kbp). (B) *Bam*HI, *Bgl*II, and *Eco*RI maps of the DHV genome. *Sma*I subclones of the *Bam*HI B are indicated by the lowercase letters.

monkey, were identical. However, distinct differences were observed between the REn profiles of the other isolates. The DHV and LVV REn profiles were nearly identical, but with a slight difference in size of the *Bgl*II M bands. The HAZV REn profiles differed from the DHV profiles in several respects. The HAZV *Bam*HI DNA bands corresponding in size to the DHV *Bam*HI I and *Bam*HI O were absent and reduced in size, respectively. In addition, the HAZV *Bgl*II profile lacked a band corresponding in size to the DHV *Bgl*II M (3.2 kbp), but included a slightly larger band (3.4 kbp). Based upon the established REn maps of the DHV genome (Fig. 1), each of the differences between the DHV, LVV, and HAZV REn profiles occurs within the viral genome long (L) components, although the nature of these differences is not yet precisely defined.

The MLMV DNA *Bam*HI, *Bgl*II, and *Eco*RI profiles included distinct differences from the corresponding DHV, HAZV, and LVV DNA REn profiles. MLMV REn fragments corresponding in size to the DHV *Bam*HI D and G, *Bgl*II D, and *Eco*RI C were either absent or reduced in intensity (Fig. 2). In each case, these bands were replaced with a number of less intense, submolar bands. Based upon the DHV REn maps, the differences map to the short (S) component of the viral genomes. The remaining MLMV fragments correspond in size to DHV bands, indicating similarity in the REn patterns of the MLMV and DHV DNA long (L) components.

To confirm the genomic location of the MLMV genetic variability, ^{32}P-labeled DHV S component DNA probes were hybridized to the DHV, LVV, HAZV, and MLMV DNA REn fragments by Southern blot hybridization. Each of the DHV probes hybridized to DHV DNA as well as DHV-RE, HAZV, LVV, and MLMV DNAs, confirming the genetic relatedness of these viral isolates (Fig. 3 and other data not shown). The DHV *Bam*HI D probe hybridized to the DHV *Bam*HI D [map units (m.u.) 0.80–0.89] and G (m.u. 0.95–1.0), as expected, and also to corresponding DHV-RE, HAZV, and LVV *Bam*HI bands (Figs. 1 and 3A). However, the DHV *Bam*HI D probe hybridized to a heterogeneous series of MLMV *Bam*HI bands. The DHV *Bam*HI P probe hybridized to the DHV *Bam*HI P (m.u. 0.89–0.91) and *Bam*HI L (m.u. 0.91–0.94) fragments, derived from the DHV S component, as well as to homologous MLMV bands of similar size (Fig. 3B). The DHV *Bam*HI P probe also hybridized to the DHV *Bgl*II D (m.u. 0.87–0.96, Fig. 3C). In contrast, the DHV *Bam*HI P probe hybridized to a series of heterogeneous-sized MLMV *Bgl*II bands. Therefore, MLMV DNA heterogeneity was detected within the *Bam*HI D and *Bgl*II D, but not within the *Bam*HI P or L. The results indicate a region of heterogeneity which maps within the viral inverted repeat components of the MLMV genome (m.u. 0.87–0.89 and also 0.94–0.96, Fig. 1).

The REn differences observed in the DNAs of the SVV isolates were not likely induced during *in vitro* propagation. DNA was derived from each of the virus isolates at an early passage (<5) level. In addition, the REn profiles of DHV do not change after multiple passages (>15) in Vero cells (data not shown). Similarly, VZV DNA REn profiles are stable after prolonged passage in culture (Hondo *et al.,* 1987).

Relatedness of DHV to other primate herpesviruses

The genetic relatedness of DHV to other primate herpesviruses was evaluated by Southern blot hybridization. The analysis determined homology between DHV DNA and DNA derived from three human alphaherpesviruses (VZV, HSV-1, and HSV-2), two Old World monkey alphaherpesviruses [SA8 and herpes B virus (HBV)], and two New World monkey (Superfamily Ceboidea) herpesviruses [herpesvirus saimiri (HVS) and herpesvirus ateles (HVA)]). Each of the primate herpesvirus DNAs was di-

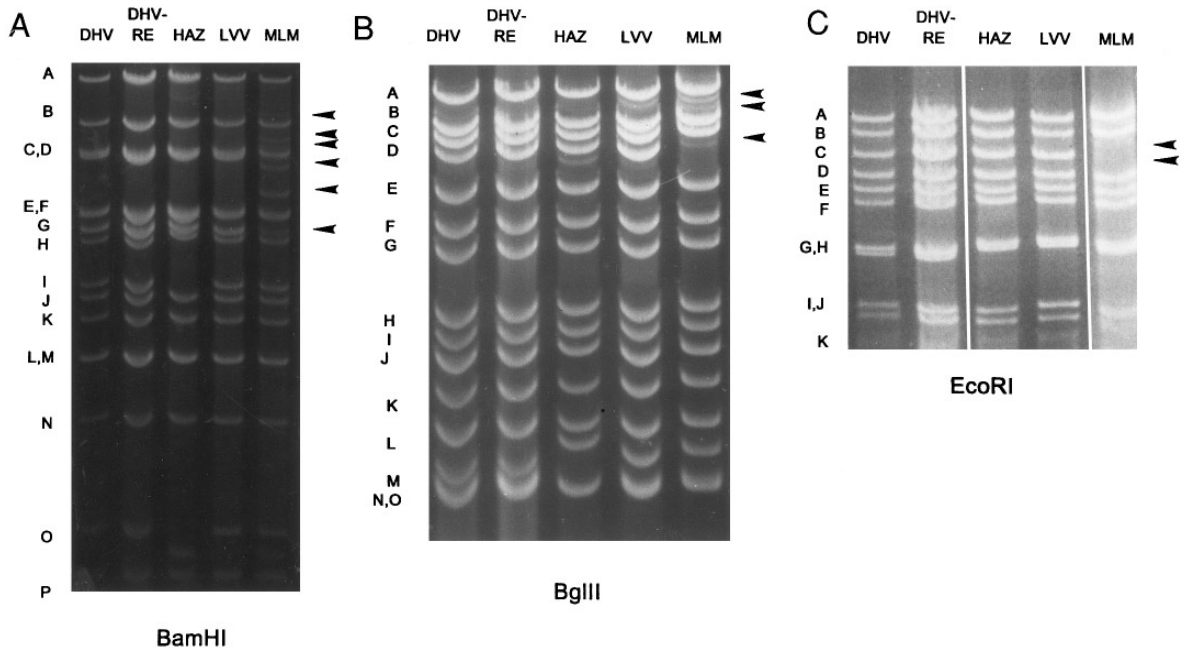

FIG. 2. REn profiles of SVV isolates. *Bam*HI (A), *Bgl*II (B), and *Eco*RI (C) REn profiles of DHV, DHV-RE, HAZV, LVV, and MLMV DNAs are shown. Arrowheads indicate submolar MLMV REn fragments.

gested with *Bam*HI, immobilized on a hybridization membrane, and hybridized to a ^{32}P-labeled DHV *Bam*HI B DNA probe (m.u. 0.42–0.54, Fig. 1). Under standard stringent hybridization conditions ($T_m \cong -25°$), the DHV probe hybridized only to DHV DNA (data not shown). Under less stringent hybridization conditions ($T_m \cong -36°$), the DHV probe hybridized to VZV DNA REn fragments (*Bam*HI A, H, and P, m.u. 0.35–0.55; Straus *et al.,* 1983), but not to any of the other primate DNAs (Fig. 4). The results indicate that DHV and, by analogy, other SVV isolates are genetically more closely related to VZV than to the other primate herpesviruses.

DISCUSSION

This study confirms that simian varicella epizootics are caused by various strains of the same primate herpesvirus, rather than distinct primate herpesviruses. The cur-

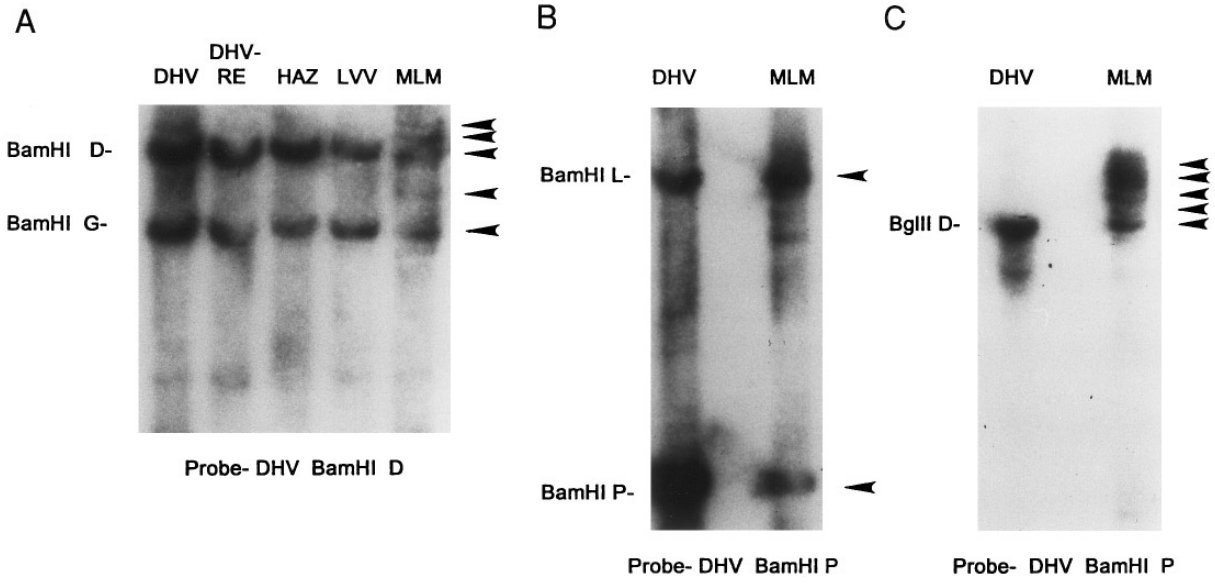

FIG. 3. Hybridization of DHV S region probes to immobilized, REn-digested, DNA of SVV isolates. (A) DHV *Bam*HI D probe hybridized to *Bam*HI-digested DHV, DHV-RE, HAZV, LVV, and MLMV DNA. (B) DHV *Bam*HI P probe hybridized to *Bam*HI-digested DHV and MLMV DNA. (C) DHV *Bam*HI P probe hybridized to *Bgl*II-digested DHV and MLMV DNA. Arrowheads indicate novel MLMV DNA bands.

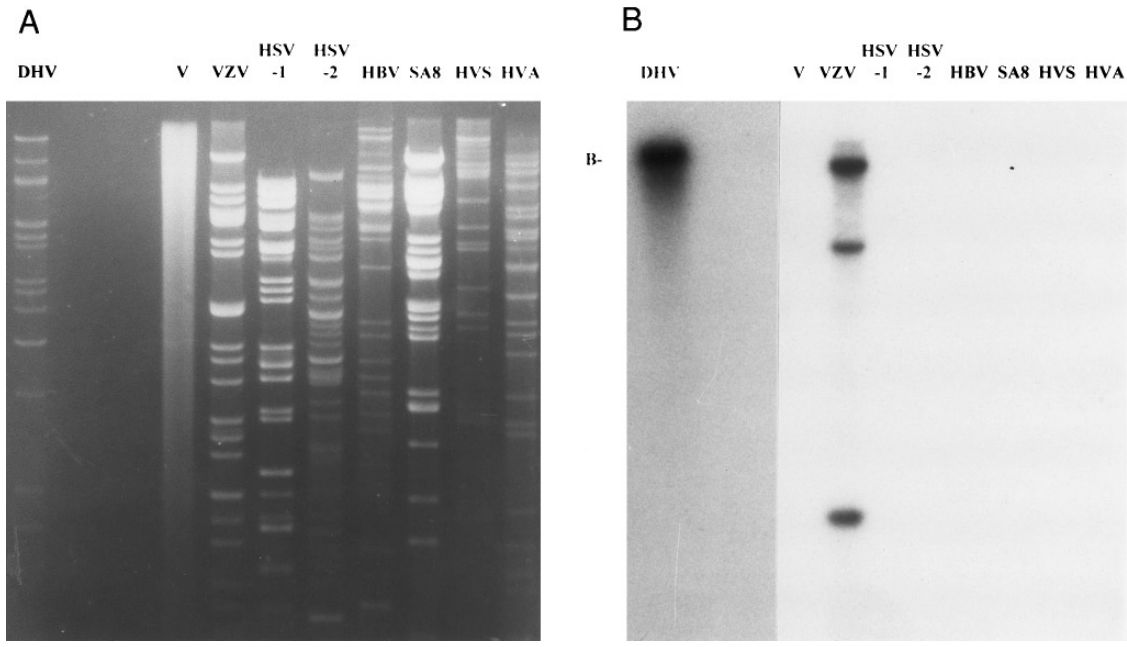

FIG. 4. Relatedness between DHV and other primate herpesviruses. (A) *Bam*HI REn profiles of DHV, Vero (V), VZV, HSV-1, HSV-2, herpes B virus (HBV), SA8, herpesvirus saimeri (HVS), and herpesvirus ateles (HVA). (B) Hybridization of a DHV *Bam*HI B probe to immobilized primate herpesvirus DNAs under reduced stringency hybridization conditions. The hybridized VZV bands correspond to *Bam*HI A, H, and P fragments (Straus *et al.,* 1983).

rent herpesvirus taxonomy places the simian varicella-like herpesviruses into three groups, Cercopithecus herpesviruses 6, 7, and 9 (Roizman *et al.,* 1992; Roizman, 1996). Based upon the results of this study, the classification should be changed to incorporate SVV as a single taxonomic group. The results also confirm that SVV is more closely related to VZV than to other primate herpesviruses. Therefore, SVV should be included within the *Varicellovirus* genus of alphaherpesviruses, along with VZV, equine herpesvirus type 1 (EHV-1), bovine herpesvirus type 1 (BHV-1), and pseudorabies virus (PRV) (Roizman *et al.,* 1992; Roizman, 1996). SVV has the same genomic structure as VZV, EHV-1, BHV-1, and PRV and shares partial DNA homology with these varicelloviruses (Fletcher and Gray, 1993; Gray *et al.,* 1995a).

The diversity in the REn profiles of SVV strains offers an opportunity to employ REn analysis for tracing the epidemiology of SVV epizootics. In this study, four epidemiologically unrelated SVV isolates were distinguished by REn analysis. Genetic variability between herpesvirus isolates is not unusual, and REn profile differences also occur among isolates of VZV, HSV-1, and other herpesviruses (Straus *et al.,* 1983; Rojas *et al.,* 1993). REn analysis of VZV isolates indicates that variations occur predominantly within the inverted repeats and three regions of the L component (m.u. 0.1–0.2, 0.3–0.4, and 0.78–0.82) (Straus *et al.,* 1983; Hondo *et al.,* 1987). In this limited analysis of five SVV isolates, variations were also detected within the inverted repeats and L component of the SVV genome.

The REn profiles of the MLMV strain of SVV were the most divergent from the patterns of the prototype DHV strain. REn and Southern blot hybridization analyses of the MLMV DNA suggest a region of heterogeneity which maps to the MLMV DNA internal (m.u. 0.87–0.89) and terminal inverted repeat (0.94–0.96) sequences. This region of the SVV inverted repeat contains the viral reiterated sequence (Rs, Gray *et al.,* 1995a). DNA sequence analysis demonstrates that the DHV Rs includes a 16-base sequence which is repeated seven times plus a partial repeat. An analogous 27-base pair sequence is repeated a variable number of times in different VZV strains (Casey *et al.,* 1985; Davison and Scott, 1986). In addition, tandem duplication of a 2760-base pair sequence has been reported in the same region of the inverted repeat of VZV DNA isolated from persistently infected Mewo cells (Dohner *et al.,* 1988). Further studies of MLMV DNA are needed to determine the molecular basis of the heterogeneous region.

VZV isolates derived from varicella and zoster in the same individual have identical REn profiles, indicating that herpes zoster is caused by reactivation of latent VZV (Straus *et al.,* 1984). The REn analysis of SVV isolates also provides evidence for SVV reactivation from latently infected monkeys. Since primary varicella confers protective immunity in monkeys (Gray *et al.,* 1995b), subsequent SVV-induced clinical disease in monkeys is presumed to be caused by reactivation of endogenous latent virus. SVV reactivation disease in monkeys is usually limited to a mild vesicular rash, rather than the severe, dissemin-

ated disease that often occurs during primary simian varicella (K. Soike, personal communication). In addition, reactivation disease appears as a generalized whole-body rash, rather than a unilateral, segmental rash as occurs in herpes zoster (Soike *et al.,* 1984). DHV-RE was isolated from a vesicular skin lesion of an African green monkey which had been experimentally infected with DHV 2 years previously (K. Soike, personal communication). This monkey had no exposure to other monkeys with simian varicella during the months prior to the secondary disease. Based upon REn analysis, the DHV and DHV-RE isolates are identical to each other and distinct from other SVV isolates. This result is consistent with the hypothesis that the secondary SVV disease was caused by reactivation of endogenous latent virus, rather than infection by exogenous SVV.

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