Herpesvirus saimiri Strain Variability

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Herpesvirus saimiri was isolated from 22 squirrel monkeys by cocultivation of peripheral lymphocytes with permissive owl monkey kidney monolayer cells. Comparison of virion DNA fragments produced from restriction endonuclease digestion was used as a sensitive measure of strain variability. Although all isolates contained similarities and common features, 19 of the 22 were readily distinguished. Three of the isolates, however, were indistinguishable and possibly were related epidemiologically. Distinct subtypes of *H. saimiri* were not evident by these criteria; Peruvian, Colombian, Guyanan, and Bolivian squirrel monkeys yielded isolates without characteristic features peculiar to the geographic region. Three of three colony-born squirrel monkeys that were tested yielded a strain of virus distinct from that obtained from the mother. In separate experiments, two of three animals chosen at random yielded a strain of virus different from that originally obtained 16 and 22 months previously; only one of the three animals examined yielded the same strain of virus 22 months after the original isolation. The degree of restriction endonuclease fragment variability among *H. saimiri* strains appeared to be greater than previously observed for other herpesviruses.

Herpesvirus saimiri naturally infects most squirrel monkeys (*Saimiri sciureus*) in which it causes no apparent disease. *H. saimiri* infection of other species of New World primates frequently results in lymphoma or leukemia. Squirrel monkeys, the natural hosts, are commonly found in the rain forests of South America, and animals obtained from different South American regions have previously been shown to have distinct physical appearances and karyotypes (15).

Previous analyses of *H. saimiri* virion DNA revealed a high degree of intramolecular heterogeneity in guanine-plus-cytosine (G+C) content (for review see reference 11). Unsheared DNA purified from virions is composed of about 90% infectious DNA that bands at a density of 1.705 g/cm³ (45% G+C) in CsCl and about 10% defective, noninfectious DNA that bands at a density of 1.730 g/cm³ (71% G+C) in CsCl. The defective DNA consists of identical tandem repeat units of 1.3 kilobase pairs (kb). Because of its high G+C content and density in CsCl, repetitive DNA is called H-DNA. Infectious virion DNA that bands at a density of 1.705 g/cm³ is composed of 115 kb of unique DNA with an average G+C content of 36% (density in CsCl, 1.695 g/cm³) to which H-DNA repeat units are covalently attached in the same orientation at each end. Since the number of H-DNA repeat units at each end varies, the size of infectious *H. saimiri* DNA is somewhat variable; the average

size of intact infectious DNA is around 160 kb. Infectious virion DNA is called M-DNA, and the central unique 115 kb is called L-DNA.

Analysis of size and number of herpesvirus DNA fragments generated by restriction endonuclease digestion has been used by others to distinguish different strains of herpes simplex virus (5, 18), Epstein-Barr virus (2, 12, 22), cytomegalovirus (13), varicella-zoster virus (20), and equine herpesvirus (23), to trace the spread of herpes simplex virus (3, 5, 17), and to establish equine herpesvirus subtypes 1 and 4 (23). The results have indicated that epidemiologically unrelated herpesvirus isolates can be readily distinguished by the number and distribution of restriction endonuclease cleavage sites in their DNAs. This report presents some unique aspects of *H. saimiri* strain variability.

All new *H. saimiri* isolates used in this study (numbered 101 to 122 in Table 1) were obtained from squirrel monkeys of the New England Regional Primate Research Center (NERPRC) colony. The origin of imported squirrel monkeys was determined primarily from phenotypic appearance and importation records; the proper subspecies assignment was further verified for four of the animals by karyotype analysis kindly provided by N. Ma of NERPRC (Table 1). Colombian and Peruvian squirrel monkeys were housed in separate group cages containing 15 to 30 members with no intermixing of monkeys from the two geographic regions. It is possible,

TABLE 1. *H. saimiri* isolates used for analysis of strain variability

Virus isolate no.	Animal no. ^a	Origin of animal	Comment or reference
101	458-78	Colony born	Offspring of 385-70
102	197-71	Guyana	Karyotyped
103	119-70	Peru	Karyotyped
104	385-70	Peru	
105	201-71	Guyana	
106	254-71	Peru	
107	256-71	Peru	
108	302-78	Colony born	Offspring of 254-71
109	417-78	Colony born	Offspring of 256-71
110	483-77	Colombia	
111	24-69	Colombia	Karyotyped
112	505-77	Colombia	
113	488-77	Colombia	
114	494-77	Colombia	
115	634-71	Peru	
116	482-77	Colombia	
117	484-77	Colombia	
118	486-77	Colombia	
119	487-77	Colombia	
120	29-76	Bolivia	
121	24-76	Bolivia	
122	487-80	Bolivia	
11	?	?	10
S295C	83-67	?	19
SMHI	139-67	?	6
OMI	Owl monkey isolate	?	14

^a The last two digits of the animal numbers refer to the year the animal was acquired by NERPRC or born into the colony. For example, squirrel monkey 254-71 was imported from Peru in 1971, and squirrel monkey 302-78 was born in 1978.

however, that many of these animals were exposed to squirrel monkeys from other geographic regions, for example, during importation or through contact with adjacent cages. Squirrel monkeys 458-78, 302-78, and 418-78, born to Peruvian parents, were kept in the same group cage with their Peruvian parents and other Peruvian squirrel monkeys.

Peripheral lymphocytes were separated from whole blood collected from apparently healthy squirrel monkeys by banding over Ficoll-Hypaque (density, 1.076 g/cm³). Lymphocytes were cocultivated with monolayers of permissive owl monkey kidney cells in culture until virus-induced cytopathic effect was complete. Virus-induced foci of the cytopathic effect usually became evident 5 to 10 days after cocultivation. More than 90% of virus rescue attempts were successful. The cell lysates were used as virus stock to inoculate owl monkey kidney cells in 150-cm² flasks for purification of virions and extraction of virion DNA according to previously published procedures (1).

Virion M-DNA was analyzed with the following restriction endonucleases: *Sma*I, *Kpn*I, *Bam*HI, *Eco*RI, *Hpa*II, *Sac*I, *Taq*I, *Pst*I, *Hpa*I, and *Fnu*DII. Maps have been published for strains 11, S295C, OMI, and SMHI by using restriction endonucleases *Sma*I, *Kpn*I, *Bam*HI, and *Eco*RI (11). These last four enzymes were sufficient for distinguishing many of the isolates, but the other enzymes which cleave *H. saimiri* DNA more frequently were used to establish more definitively the identity or nonidentity of any two isolates. *Sac*I, *Hpa*II, and *Eco*RI + *Sma*I were chosen to depict the strain variability (Fig. 1). Of 22 isolates, 19 were readily distinguished by these criteria, and when the 4 previously used laboratory strains were included in the analysis, 23 of the 26 isolates were distinguishable from one another. Three new isolates (102, 106, and 109) could not be distinguished and appeared to be identical. Isolate 106 was obtained from an animal born in the Peruvian group cage. It seems likely that these three animals were infected by the same strain of virus through some common source at NERPRC, but the possibility that this virus strain was introduced into the colony independently by more than one imported animal cannot be excluded.

Based on the molarities of fragments and sum molecular weights, the patterns shown in Fig. 1 and others not shown were generally consistent with a single virus strain being present in each isolation. This is in spite of the facts that viruses were not plaque purified and that a crude cell lysate was used for preparation of viral DNA. Only isolate 104 gave several less-than-molar fragments, suggesting that more than one virus strain was present (Fig. 1). Also, isolate 113 yielded an unusual *Sac*I fragment greater than two molar; this probably arose from a population of defective DNA molecules (Fig. 1).

Sufficient similarity existed among these isolates to certify that we were indeed analyzing various strains of *H. saimiri*. For example, *Sma*I characteristically cleaved several times in each repeat unit of H-DNA but did not appear to cleave L-DNA; the supermolar fragments in the lower portion of the *Eco*RI + *Sma*I gel of Fig. 1 were derived from the repetitive H-DNA. Nevertheless, a single discriminating enzyme such as *Hpa*II or *Sac*I was usually sufficient for distinguishing any two nonidentical isolates. Differences in the degree of variability observed with enzymes that cleaved with similar frequency were consistently noted. For example, most differences in *Eco*RI fragments among strains could be explained by a simple gain or loss of one to three cleavage sites. With *Bam*HI, however, two isolates were frequently found to differ at 50% or more of the cleavage sites (data not shown and see reference 11). The greatest vari-

FIG. 1. Agarose gel electrophoresis of *H. saimiri* DNA cleaved with restriction endonucleases. Virion DNA (approximately 0.6 μ g) was cleaved with each restriction endonuclease according to the recommendations of the manufacturer, and fragments were separated on a 0.6% agarose gel by electrophoresis at 60 V (18 mA) for 1.5 to 20 h (8). The gel was soaked in 1 μ g of ethidium bromide per ml, and the DNA fragments were visualized over UV light. The virus isolate number is indicated at the top of each slot. The last slot in each gel contained molecular weight markers (*Hind*III digest of lambda DNA).

ability among isolates was observed with the restriction endonuclease *HpaII*. As an extreme example, strain S295C yielded 16 *HpaII* fragments greater than 1 kb, and strain 11 yielded 17 *HpaII* fragments greater than 1 kb, with total sizes of around 115 kb; yet only two fragments comigrated on agarose gels (Fig. 1). This is remarkable since the digestion patterns of these two strains with other enzymes are similar, and hybridization analyses have indicated a high degree of cross homology (>90%) with less than 3% base mismatch (16).

The variability in restriction endonuclease cleavage sites is certainly greater among *H. saimiri* strains than that previously observed among strains of *Herpesvirus tamarinus*, a herpes simplex-related virus also indigenous to squirrel monkeys (7). The variability in cleavage patterns also appears to be considerably greater than previously published for herpes simplex virus (5, 18), Epstein-Barr virus (2, 12, 22), equine herpesvirus (23), cytomegalovirus (13), and varicella-zoster virus (20).

Unlike some other viruses, herpesviruses are not easily spread from individual to individual through coughing, sneezing, etc., and it has been hypothesized that the spread of herpes simplex virus within a region or community may be restricted, being limited to family members and close contacts (21). To obtain evidence on the frequency of offspring infection from the mother, *H. saimiri* isolates obtained during the 1st year of life from three colony-born squirrel monkeys (101, 108, and 109) were compared with isolates obtained simultaneously from their mothers (104, 106, and 107). Offspring were nursed by their mothers in the same Peruvian group cage. None of the three colony-born squirrel monkeys yielded the same strain of virus isolated from the mother. Isolate 109, although different from isolate 107 obtained from the offspring's mother, was the same as isolate 106 obtained from another mother (Table 1 and Fig. 1). These results may not be surprising, since a considerable amount of interaction occurs between offspring and all the other animals in a group cage. However, we cannot be sure that these offspring were not infected by their mothers at some time, since results presented below indicate that squirrel monkeys may be infected with more than one strain of *H. saimiri* or readily become reinfected with a different strain of *H. saimiri*.

It was not possible to group the *H. saimiri* isolates into subtypes based on similarities in the restriction endonuclease fragmentation patterns. Furthermore, no characteristic features were noted in isolates obtained from squirrel monkeys imported from the different geographic regions.

Squirrel monkeys 197-71, 488-77, and 484-77

were bled for additional virus isolations at 22, 16, and 22 months, respectively, after the original isolations. Isolates obtained from animal 484-77 were identical at the two different times, but different strains of virus were obtained from animals 197-71 and 488-77 at the two bleedings (data not shown). The second bleeding of 197-71 yielded an isolate apparently identical to isolate 105 from squirrel monkey 201-71; although these two animals were imported at the same time, there is no record of their being housed together at NERPRC. The second bleeding of 488-77 yielded an isolate different from any other isolate we have examined. The number of cleavage site differences observed in isolates from animals 197-71 and 488-77 at different times is too large to be reasonably accounted for by alterations of a virus strain while present in the animal. Marmosets infected with an attenuated strain of 11 stably retain restriction endonuclease cleavage sites as long as 4 years after the original infection (9; data not shown). Thus, these results indicated that animals 197-71 and 488-77 had been infected with more than one strain of *H. saimiri*. We cannot determine from these data whether each animal was infected with two or more strains of virus before our original bleeding, with only one strain predominating at any one time, or whether the animals were reinfected exogenously after the original bleeding, with the reinfesting virus becoming predominant. Upon examination of herpes simplex virus type 2 recrudescence lesions, two of eight patients yielded different strains of virus on successive isolations (4). These results with *H. saimiri* and herpes simplex virus do not necessarily mean that the immune system is inefficient in preventing exogenous reinfection by a different strain of herpesvirus. It is possible that seronegative individuals can be initially infected with several different strains of virus during the susceptible period, but only one of these strains may predominate during a virus isolation. Further work is needed to distinguish these possibilities.

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