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Human Herpesvirus 6 (HHV-6) Variant B Accounts for the Majority of Symptomatic Primary HHV-6 Infections in a Population of U.S. Infants

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Two variants of human herpesvirus 6 were identified. To assess their epidemiology and disease association, we analyzed viral isolates and/or uncultured peripheral blood mononuclear cells from 76 infants with symptomatic primary infection. In 97% of cases, human herpesvirus 6 variant B was detected, but variant A was not.

Human herpesvirus 6 (HHV-6) infects most children by the age of 3 years, causing a number of clinical manifestations, including roseola (8, 11). Recently, two distinct variants of HHV-6 have been identified (1, 2, 10). In the study described here we examined the viral genomes present in 76 infants with symptomatic primary HHV-6 infection to determine the relative frequency of infection by HHV-6 variants A and B.

HHV-6 isolates were obtained from children 2 years of age or less who were brought to the Emergency Department of Strong Memorial Hospital (Rochester, N.Y.) with acute febrile illnesses (temperature, ≥38°C). The infants studied were defined as having primary HHV-6 infection on the basis of serologic evidence and transient viremia (8).

Virus was isolated from patient peripheral blood mononuclear cells (PBMCs), and an aliquot of PBMCs was also frozen at the time of virus isolation for subsequent analysis by polymerase chain reaction (PCR). Viral isolates were propagated in phytohemagglutinin-stimulated cord blood mononuclear cells, and infection of HSB-2 cells was assessed as described previously (4).

Differentiation between the A and B variants of HHV-6 was carried out by using an *Hin*dIII restriction site polymorphism (2, 4). In the case of viral isolates, DNA was amplified by PCR, digested with *Hin*dIII, and analyzed as described previously (4). A nested PCR protocol was used to analyze HHV-6 genomes in patient PBMCs (7). First-round amplification of an 830-bp fragment of HHV-6 DNA was performed as described previously (2, 4), and 1 μl of this first-round PCR product was then reamplified by using internal primers (HS6AE, 5′-CGG CCATTTAACGGAACCCTAG-3′; HS6AF, 5′-TCCAGAG AAAGGGTGTTGCG-3′). Second-round amplification used 50 pmol of each primer in a final volume of 50 μl which contained 1.5 mM Mg; primers were annealed for 1 min at 60°C, and a total of 40 cycles of amplification were performed. A total of 10 μl of second-round PCR product was then digested with *Hin*dIII and analyzed by gel electrophoresis.

Digestion results were confirmed in many cases by DNA dot blot analysis with variant A- or variant B-specific oligonucleotide probes, which differed at a single residue (in boldface; A-probe, 5′-ATTCCAAGTTTTATGA; B-probe, 5′-ATTCCAAGCTTTATGA-3′). This base change creates the *Hin*dIII site (underlined) used to differentiate HHV-6 variants A and B (2). Briefly, 20 μl of denatured second-round PCR product was dotted onto nitrocellulose by using a vacuum filtration apparatus. Blots were then hybridized in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt's solution–25 mM KPO_4 (pH 7.4)–50 μg of denatured salmon sperm DNA per ml, including approximately 2×10^6 cpm of oligonucleotide probe per ml treated with kinase, overnight at 37°C. Six washes were done in 1× SSC–1% sodium dodecyl sulfate for 5 min each at 40°C, and autoradiography was performed overnight at −70°C.

HHV-6 isolates from a total of 60 infants with symptomatic primary infection were segregated into groups on the basis of two criteria which we have shown to be useful in categorizing viral strains (4); namely, their ability to replicate in HSB-2 cells and the presence of a well-characterized restriction site polymorphism in the viral genome. All 60

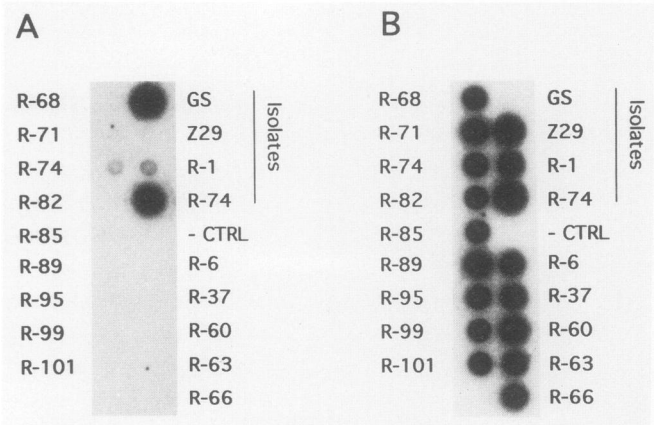

FIG. 1. Analysis of the HHV-6 genomes present in selected HHV-6 isolates and PBMCs from children with primary HHV-6 infection. Dots correspond to PCR-amplified DNAs from established viral isolates (marked) or from patient PBMC specimens; a negative control reaction lacking template DNA is also shown (−CTRL). Amplified DNAs were hybridized to either a variant A-specific or a variant B-specific oligonucleotide probe (A and B, respectively).

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TABLE 1. Categorization of HHV-6 strains found in vivo and after propagation in vitro[a]

Sample	Isolate		Variant in PBMCs	Sample	Isolate		Variant in PBMCs
	Growth in HSB-2 cells	Variant			Growth in HSB-2 cells	Variant	
GS	+	A	A	R-80	–	B	B
Z29	–	B	B	R-81	–	B	B
R-1	+[b]	B, a[b]	NS	R-82	–	B	B
R-3	–	B	NS	R-83	–	B	B
R-4	–	B	NS	R-84	–	B	B
R-5	–	B	B	R-85	[b]	[b]	B
R-6	–	B	B	R-86	–	B	B
R-15	–	B	B	R-87	–	B	B
R-22	–	B	B	R-88	–	B	B
R-23	–	B	B	R-89	[b]	[b]	B
R-24	–	B	B	R-90	–	B	B
R-25	–	B	B	R-91	–	B	B
R-26	–	B	B	R-92	–	B	NS
R-37	–	B	B	R-93	–	B	NS
R-38	–	B	NS	R-94	–	B	NS
R-39	–	B	NS	R-95	[b]	[b]	B
R-41	–	B	B	R-96	–	B	NS
R-42	–	B	NS	R-97	–	B	B
R-58	–	B	NEG	R-98	–	B	B
R-59	–	B	B	R-99	–	B	B
R-60	[b]	[b]	B	R-100	–	B	B
R-61	–	B	B	R-101	–	B	B
R-62	–	B	B	R-102	[b]	[b]	B
R-63	–	B	B	R-103	[b]	[b]	B
R-64	–	B	B	R-104	–	B	B
R-65	[b]	[b]	B	R-105	–	B	B
R-66	[b]	[b]	B	R-106	–	B	B
R-67	–	B	NS	R-107	–	B	B
R-68	[b]	[b]	B	R-108	–	B	B
R-69	[b]	[b]	B	R-109	–	B	B
R-70	–	B	B	R-110	NS	NS	B
R-71	[b]	[b]	B	R-111	–	B	B
R-72	[b]	[b]	B	R-112	–	B	NEG
R-73	–	B	B	R-113	–	B	NS
R-74	+[b]	A, B[b]	B, a	R-114	–	B	NT
R-75	–	B	B	R-115	–	B	NT
R-76	–	B	B	R-116	–	B	NT
R-77	–	B	B	R-117	–	B	NT
R-78	[b]	[b]	NS				
R-79	–	B	NS				

[a] NS, no sample available; NT, not tested; NEG, negative.
[b] Data were reported elsewhere (4). Unless otherwise indicated, these isolates did not grow in HSB-2 cells and contained variant B genomes only.

isolates tested were identified as HHV-6 variant B (Table 1). When added to the 15 isolates previously tested, 13 of which were HHV-6 variant B (4), this gives an overall total of 73 variant B isolates of HHV-6 from 75 infants tested (97%). Only isolates R-1 and R-74 contained variant A genomes, and even these comprised a mixture of A and B variant genotypes (4).

To address the possibility that in vitro cultivation of HHV-6 might lead to "unnatural" selection, we also examined the HHV-6 population in vivo by amplifying viral DNA directly from patient PBMCs. A nested PCR protocol was designed, and *Hin*dIII digestion was again used to segregate viral genomes into two groups (Table 1). This nested PCR protocol successfully amplified DNAs from both HHV-6 variant B and HHV-6 variant A (all three isolates of HHV-6 variant A tested [GS, R-1, and R-74] were positive) (Table 1; Fig. 1). When patient PBMCs were subjected to the PCR analysis, HHV-6 DNA was detected in 58 of 60 samples, but only a single specimen (R-74) was found to contain HHV-6 variant A genomes (no sample was available for patient R-1; Table 1).

These findings were confirmed in many cases by a second assay in which variant A-specific or variant B-specific oligonucleotides were used to probe PCR-amplified HHV-6 DNA. These data are presented in Fig. 1, which also incorporates data for several controls (i.e., prototype variant A [GS] and prototype variant B [Z29] strains plus the mixed isolates R-1 and R-74 [4]). Interestingly, PBMCs from patient R-74 contained a preponderance of HHV-6 variant B genomes (Fig. 1), whereas the isolate established from this infant contained roughly equivalent levels of variant A and B genotypes (4). This suggests that the propagation of HHV-6 in vitro can indeed lead to some degree of unnatural selection.

Our analysis of HHV-6 genomes from 76 infants with symptomatic primary viral infection indicate that the vast majority (97%) of such children harbor only HHV-6 variant B. HHV-6 variant A was very rare in the infants who we

studied, yet this variant made up most of the isolates established from adults (1, 2, 5, 9). Several factors might contribute to this apparent discrepancy. First, most of the HHV-6 isolates that have been derived from adults to date have come from immunocompromised individuals or patients with other disorders who may be unrepresentative of the population at large (1, 2, 5, 9). Second, it is possible that HHV-6 variant A may have a greater propensity for latency, reactivation, or sequelae (3, 6) in adults. Third, the peak age for primary infection with HHV-6 variant A may be later than that for infection with HHV-6 variant B, and finally, initial infection with HHV-6 variant A may be clinically inapparent or may cause nonfebrile illnesses. In any event, the prevalence and sequelae of infection with HHV-6 variant A remain unclear, and additional studies will be required to resolve these issues.

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