JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1996, p. 3223–3225 Vol. 34, No. 12
0095-1137/96/$04.00+0

Presence of Human Herpesvirus 6 Variants A and B in Saliva and Peripheral Blood Mononuclear Cells of Healthy Adults

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Received 10 July 1996/Returned for modification 8 August 1996/Accepted 26 September 1996

Saliva and peripheral blood mononuclear cells (PBMCs) from 44 healthy young adults were tested for human herpesvirus 6 variants A and B (HHV-6A and -6B) DNA by a sensitive nested PCR. HHV-6B infection was ascertained in 98% of the subjects, and 95% were found to excrete variant B in their saliva. HHV-6A was found in the PBMCs of 16%, but was not detected in saliva samples.

Human herpesvirus 6 (HHV-6), first isolated by Salahuddin et al. in 1986 (18), is the causative agent of exanthema subitum, a common febrile illness associated with rash in infancy (23). Results obtained by seroepidemiological investigations demonstrated that more than 90% of children become infected with HHV-6 in the first 2 years of life (19). Like all other herpesviruses, HHV-6 establishes a persistent infection for which detailed information on the sites and cell types of virus persistence is still scarce. Since HHV-6 can be detected in saliva by PCR technique in up to 90% of healthy individuals (2, 12), the salivary glands may be one of the major sites of virus persistence (10). HHV-6 can also be found in peripheral blood mononuclear cells (PBMCs) of healthy and immunosuppressed individuals with varying frequencies (2, 4, 12, 21). The virus exists in two variants, A and B, which differ in their biological, immunological, and molecular characteristics (1). Studies investigating the differences in epidemiology and pathogenicity of the two variants demonstrated that variant B is the one usually detected in saliva (7, 11, 15) and in PBMCs during HHV-6 primary infection (5). Variant A was initially found only in PBMCs of immunosuppressed patients and in those suffering from lymphoproliferative disorders. Only recently could the presence of both variants be established in the PBMCs of healthy adults by applying a highly sensitive and variant-specific PCR technique (6, 21). Whether HHV-6A is transmitted by saliva is still unclear. We therefore decided to investigate the presence of both variants in saliva samples and in the PBMCs of young healthy adults by using a highly sensitive and variant-specific PCR method.

Forty-four healthy adults (22 male, 22 female) with a mean age of 23 years (range, 19 to 38 years) were studied. Saliva and EDTA-blood were collected simultaneously. Two hundred microliters of native saliva (cells and fluid) was stored at −20°C. Plasma was separated by centrifugation and stored for antibody detection at −20°C. PBMCs were separated by Ficoll-Paque density gradient centrifugation. Cells were stained with Turk solution and counted in a Fuchs-Rosenthal counting chamber. Between 10^6 and 10^7 cells (mean, 4×10^6) were obtained, resuspended in 200 μl of phosphate-buffered saline and stored at −20°C. DNA extraction from saliva and PBMCs was performed with the QIAamp blood kit (Qiagen, Inc., Chatsworth, Calif.) according to the manufacturer's instructions. DNA was resuspended in 200 μl of distilled water and was used for all experiments listed below.

The outer and inner PCR primer pairs cover a region of the HHV-6 immediate-early gene where variant B has an insertion of 226 bp compared with variant A, thereby enabling the two variants to be distinguished by the size of the PCR fragments (21, 22) (Fig. 1). For the first PCR amplification an aliquot (10 μl) of the purified DNA was added to a reaction mixture yielding a total volume of 50 μl, including 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 1 U of *Taq* polymerase, the deoxynucleoside triphosphates dATP, dCTP, dGTP and dUTP (200 μM each), and 50 pmol of the outer primer pair. Subsequently, 2 μl of the amplicon was used for the nested PCR with the same reaction mixture except that it contained 25 pmol of the inner primer pair. The thermocycling procedure consisted of denaturation at 94°C for 7 min in the first step and for 3 min in the nesting procedure; 35 cycles of denaturation at 94°C, annealing at 55°C, and extension at 68°C, each for 1 min; and then a final extension at 72°C for 7 min. Amplified samples were visualized on a 3:1 NuSieve gel. HHV-6A and -B standard dilutions as positive controls and numerous negative water controls were included in every run.

With the nested PCR, up to 10^{-2} infectious units for HHV-6A and up to 10^{-1} infectious units for HHV-6B could be detected (Fig. 1). In both cases this corresponds to a sensitivity of 10 genome copies for HHV-6A and -B. Complete HHV-6A and -B genome DNA standards were purchased from Advanced Biotechnologies Incorporated, Columbia, Md. Infectious units were determined as follows: fresh phytohemagglutinin-stimulated cord blood mononuclear cells were infected by centrifugal enhancement (17) with 10-fold dilutions of HHV-6A (strain GS, kindly provided by R. Gallo) and -B (strain Z-29, ATCC VR-1348). The development of cytopathic effect was observed by light microscopy for 14 days, and results obtained at the final reading were confirmed by immunofluorescence staining with variant-specific monoclonal antibodies (monoclonal antibody to HHV-6 p38/41, B0130 and to HHV-6 gp110, B0155; Universal Biotechnology, Rockville, Md.). The highest dilution which gave a positive result was considered one infectious unit. There was no cross-reactivity against herpes simplex virus 1/2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, and HHV 7 (strain RK, kindly provided by Niza Frenkel).

HHV-6 serology was performed by an indirect immunofluorescence assay. HSB-2 cells (human T-cell lymphoblast line; ATCC CCL 120.1) infected with HHV-6A (strain GS) served as antigen. Fluorescein isothiocyanate-conjugated goat immunoglobulin G (IgG) to human IgG (Cappel) in a dilution of

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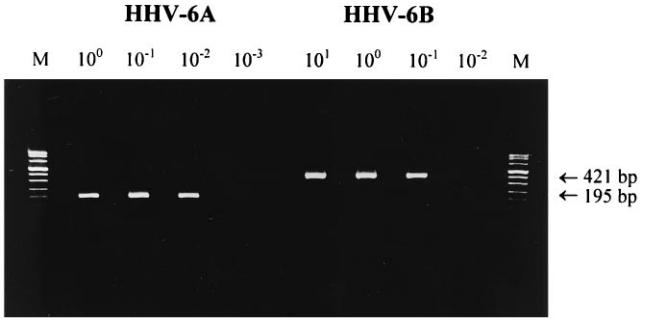

FIG. 1. Detection of HHV-6 variants by PCR. The number of infectious units used in the PCR is shown above each lane. Marker lanes are indicated by M.

1:100 was used as conjugate. IgG titers of 1:10 or higher were considered positive.

Using the highly sensitive nested PCR, we were able to demonstrate that 42 (95%) of the 44 healthy adults secreted HHV-6 in their saliva (Table 1). Detection rates for HHV-6 in saliva specimens range from 3 to 90% in the literature (2, 7, 11, 12, 15). Since the amount of HHV-6 DNA per milliliter of saliva can vary among individuals from 2×10^2 to 2×10^5 (2, 9), the detection rate is greatly influenced by the sensitivity of the method applied rather than by geographic variations (14). Only in the saliva of two seropositive young adults was no HHV-6 DNA detectable. Spiking experiments demonstrated that the addition of 10 μl of each of the two processed saliva samples to the reaction mixture did not reduce the sensitivity of HHV-6A and -B detection and ruled out a nonspecific inhibition (data not shown). For one of these two individuals, HHV-6 DNA could be detected in the PBMCs, so failure to detect HHV-6 DNA in the saliva of this individual indicates that the DNA level was below the detection limit of our assay. The other young adult was the only one in whom HHV-6 infection was not proven by the detection of viral DNA. Since serological results can be influenced by the presence of cross-reactive antibodies (13, 16), it remains unclear whether this person was infected with HHV-6 at all.

HHV-6 antibody titers of ≥10 were detectable in 41 (93%) of the subjects (Table 1). The mean titer of the positive samples was 1:120 (range, 1:10 to 1:640). In two subjects the antibody titers could not be evaluated because of nonspecific fluorescence, and in one subject the antibody titer was below 1:10. In these three individuals HHV-6 infection was confirmed by the presence of HHV-6 DNA in saliva; for one, it was also confirmed by detection of HHV-6 DNA in the PBMCs.

Analyzing our results with regard to the presence of variant-specific DNA in saliva and PBMCs, we found that 43 (98%) of the 44 healthy young adults were infected with variant B. Of these, 25 had variant B detectable in both saliva and PBMCs, 17 only in saliva, and 1 only in PBMCs. Seven of these 43 were also infected with variant A. These results are summarized in Table 2. The positive amplification results obtained with saliva samples were exclusively due to the presence of variant B. Since the amplification of variant A is not influenced by the presence of variant B, as shown by mixing experiments (data not shown), the failure to detect variant A in saliva cannot be due to the methodology applied. In addition, HHV-6A was easily detectable in 16% of the PBMC specimens of the subjects, who excreted only variant B in their saliva. Our results therefore clearly demonstrate that individuals infected with both variants do not secrete variant A in their saliva in a detectable concentration (10 genome copies per 10 μl), a finding which to our knowledge has not been described previously. This finding also fits well with the observation that only variant B is detectable in salivary glands (7) and is in agreement with the results of two recently published investigations in which it was shown that only variant B was present in saliva specimens (7, 15). Nevertheless, the presence of variant A has been reported in 1 of 66 saliva specimens in one study (11) and in 6 of 52 throat swabs in another (20). Since an infection rate of 71% with variant A has been reported for healthy individuals when lung tissue was investigated (3), the presence of variant A in a single saliva sample and in the throat swabs might reflect contamination of this material with respiratory tract secretion. Sporadic shedding of HHV-6A, which is known to occur for Epstein-Barr virus infection, cannot be ruled out, although the lack of this variant in salivary glands (7) argues against this theory.

HHV-6 DNA was detectable in the PBMCs of 28 (64%) of the 44 healthy young adults (Table 1). Variant B only was found in the PBMCs of 21, variant A only in 2, and both variants in 5 (Table 2). Detection rates higher than 16% for variant A in PBMCs have been reported only immunocompromised patients and persons suffering from chronic fatigue syndrome (8, 21), most likely a consequence of endogenous reactivation. Considering that the infection rate for HHV-6A is 71% in healthy young adults, established by testing lung tissue (3), we do not think that testing PBMCs provides reliable information on the actual frequency of infection with this variant in healthy young people.

In summary, nearly all healthy young adults tested were infected with HHV-6 variant B and 95% secreted the virus in their saliva, confirming saliva as the predominant route of transmission of HHV-6B. Although infection with variant A was proven in 16% of our study group, this variant was never detected in the saliva specimens. The routes for HHV-6A transmission therefore remain to be determined. Transmission of variant A by secretions of the respiratory tract represents an interesting hypothesis which remains to be tested.

TABLE 1. HHV-6 seroprevalence and HHV-6 DNA in saliva and PBMCs

HHV-6 antibody titer[a]	*n*	No. of study subjects with HHV-6 DNA in[b]:			
		Saliva		PBMCs	
		Pos	Neg	Pos	Neg
≥1:10	41	39	2	27	14
<1:10	1	1	0	0	1
n.e.[c]	2	2	0	1	1
Total	44	42	2	28	16

[a] Titers of ≥1:10 were considered positive; those of <1:10 were considered negative.
[b] Pos, positive; neg, negative.
[c] n.e., not able to be evaluated.

TABLE 2. HHV-6A and -B DNA in saliva and PBMCs

HHV-6 variant in saliva[a]	*n*	No. of study subjects with indicated HHV-6 variant(s) in PBMCs			
		HHV-6B	HHV-6A	HHV-6A + B	Neg[b]
HHV-6B	42	21	2	4	15
Neg[b]	2	0	0	1	1
Total	44	21	2	5	16

[a] HHV-6A DNA was not detectable in saliva.
[b] Neg, negative (i.e., neither HHV-6 variant was detected).

We gratefully acknowledge Steven L. Allison for helpful discussion and critical reading of the manuscript.

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