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Human Herpesvirus 7 Is a Constitutive Inhabitant of Adult Human Saliva

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We report the frequent isolation of human herpesvirus 7 from the saliva of healthy adults. Virus isolates recovered from different individuals exhibited minimal restriction enzyme polymorphism, which was mostly confined to heterogenous (*het*) sequences in the genome. DNAs of isolates recovered from the same individual over a period of several months showed the same characteristic *het* fragments, indicating the stability of the *het* sequences upon virus replication and shedding in vivo. In contrast to the results of previous reports, human herpesvirus 6, the causative agent of roseola infantum, could not be isolated from the saliva specimens, raising questions regarding oral transmission of human herpesvirus 6 and human herpesvirus 7 to young children.

Two human herpesviruses, human herpesvirus 6 (HHV-6) (25) and human herpesvirus 7 (HHV-7) (8), have been identified in recent years as infectious agents associated with T cells in peripheral blood. HHV-6 is a prevalent virus which infects more than 90% of all children before 2 years of age (17, 23, 27, 30). Infection ranges from asymptomatic to causing clinical disease. The virus causes roseola infantum, usually a benign, self-limiting disease characterized by spiking fever and rash (33). The less common consequences of infection include diarrhea, bulging fontanelles, bronchial pneumonia, convulsions, hepatocellular dysfunction, and rare encephalitic complications (1). The association of HHV-6 with fatal fulminant hepatitis has recently been reported (2), as has the association of HHV-6 with fatal hemophagocytic syndrome (14). Moreover, HHV-6 infections associated with disease have been reported for bone marrow and organ transplant patients undergoing immunosuppressive therapy (4, 22, 34). HHV-7 is a newly recognized herpesvirus which was isolated in our laboratory from the $CD4^+$ T cells of a healthy individual (8). Seroconversion for HHV-7 occurs at a somewhat later age than for HHV-6 (32). It is as yet unknown whether the virus causes disease.

Several recent reports have concluded that HHV-6 could be recovered from the saliva samples of a great majority of healthy individuals (12, 17, 24), and it was proposed that HHV-6 is transmitted by saliva to babies following the decline in maternal antibodies. We now report that it is HHV-7 which can be isolated from the saliva specimens of a great majority of healthy adults. The virus was identified as HHV-7 by restriction enzyme analyses of viral DNA and by its reactivity with monoclonal antibodies (MAbs). No HHV-6 could be recovered from the same saliva specimens.

In these studies, cell-free, filtered saliva samples from 26 adults were mixed with cord blood mononuclear cells (CBMC) which had been preactivated with phytohemagglutinin. Saliva samples for virus isolation were diluted 1:3 in RPMI containing 20% inactivated fetal calf serum, 32 U of interleukin-2 (Advanced Biotechnologies) per ml, and 50 μg of gentamicin per ml. After centrifugation at 2,000 × *g* for 10 min and filtration through a 0.45-μm-pore-size filter, the samples were mixed 1:1 with 10^6 phytohemagglutinin-preactivated CBMC per ml. Viral cytopathic effects were noted 9 to 14 days after the initial inoculation. In the final experiments, approximately 75% of the tested saliva specimens yielded virus.

To determine the nature of the saliva virus isolates, ^{32}P-labeled DNAs from CBMC infected with 13 saliva isolates (S1 through S13) were prepared as previously described (5) and analyzed with restriction enzymes. Two HHV-7 strains (RK and OT5) isolated from the peripheral blood of healthy individuals (7, 8) were included in the analyses. Additionally, the Z29 strain (20) and U1102 strain (6) were included as HHV-6 markers. We have previously shown that these two viruses represent two distinct categories of HHV-6 strains which differ by restriction enzyme analyses and their antigenic reactivities (29, 31).

The results revealed that all 13 saliva isolates corresponded to HHV-7 rather than to HHV-6. This is most clearly exemplified in the *Sal*I digests, in which the large fragments produced by the enzyme exhibit the typical patterns of HHV-7 DNA rather than those of HHV-6 strain Z29 or U1102. *Sal*I digestion of HHV-7 DNA typically yields few fragments of relatively large sizes, whereas digestion of DNAs from the HHV-6 strains Z29 and U1102 yields smaller-sized fragments, as shown in Fig. 1. Limited restriction enzyme polymorphism was noted upon the digestion of viral DNAs of the saliva isolates with some of the enzymes producing smaller fragments, such as the *Hin*dIII pattern. As shown in Fig. 2, the fragmentation patterns of the saliva isolates resemble those of HHV-7 DNA, especially for the smaller-sized fragments below the 4.8-kb size region. The patterns are distinct from the *Hin*dIII patterns of the Z29 and U1102 strains of HHV-6.

Examination of the restriction enzyme patterns suggested that the variations involved a specific subset(s) of the HHV-7 DNA sequences, designated heterogeneous (*het*) in Fig. 2. It is noteworthy that a similar type of heterogeneity has been found in HHV-6 strains and has been shown to affect predominantly the subsets of sequences located within the terminal repeat (19, 21, 28, 29).

To further characterize the saliva isolates, we have analyzed by immunofluorescence assays cells infected with the saliva-derived viruses by using HHV-6 MAbs. We have previously shown that the HHV-6 strains Z29 and U1102 and the HHV-7 strain RK differed with respect to their reactivities with the MAbs which were produced by Balachandran et al. (3) against the GS strain of HHV-6 (31, 32). As

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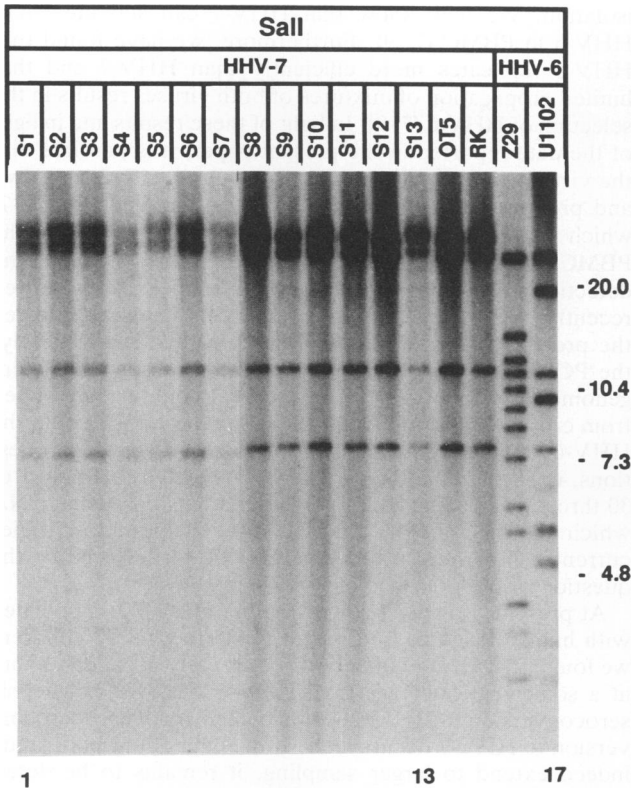

FIG. 1. *Sal*I digestion of HHV-6 and HHV-7 DNAs. The saliva virus isolates are HHV-7 strains. CBMC were infected with the 13 saliva isolates; the two HHV-7 strains, RK and OT5, which were isolated originally from PBMC (7, 8); and the two diverse prototype strains of HHV-6, Z29 and U1102 (29, 31). ^{32}P-labeled DNAs were digested with the *Sal*I enzyme.

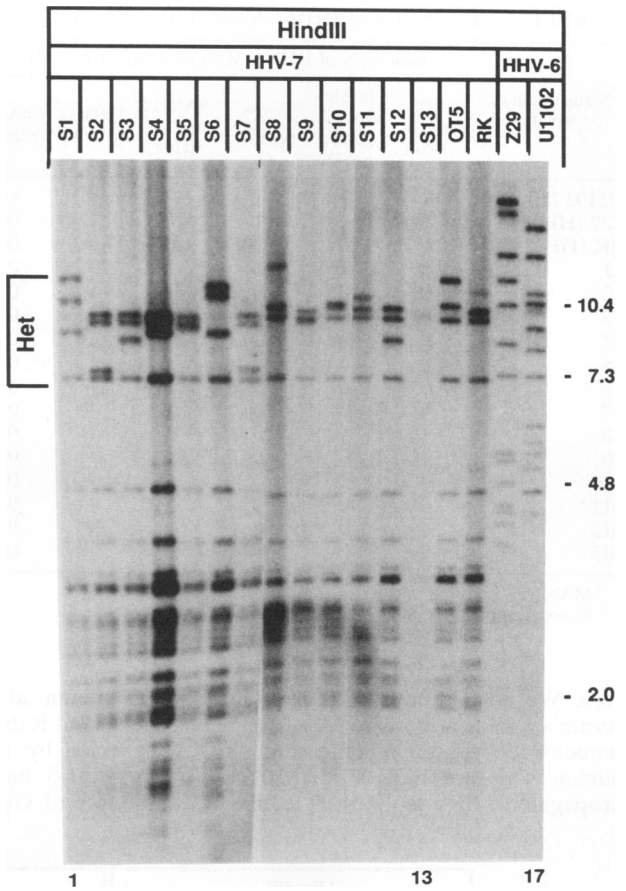

FIG. 2. *Hin*dIII digests of DNAs from the saliva isolates. All procedures were as described in the legend to Fig. 1. The variable fragments are designated heterogeneous (*het*).

summarized in Table 1, the 13 saliva isolates exhibited the reactivity patterns of HHV-7, insofar as cells infected with each of these isolates were not stained with the MAbs 2D6, 6A5D5, 7A2, and 4A6; reacted with MAb 12B3G4; and reacted variably with MAb 9A5D12.

To determine whether HHV-7 was continuously shed in saliva, we have repeatedly collected saliva samples from four individuals over a period spanning 17 to 123 days. The resultant viruses were analyzed by restriction enzymes to determine whether the same virus strain was shed repeatedly in the saliva of these individuals. In addition, some of the viruses were propagated in two different CBMC (designated I and II) to unambiguously determine whether the viruses indeed originated in the saliva samples rather than in the CBMC used for virus propagation. The results (Fig. 3) revealed that the viruses originated in the saliva samples rather than in the CBMC cultures. Thus, the *het* fragments were distinct and depended on the originating saliva rather than on the cord blood lymphocytes used for the propagation (e.g., compare lanes 1 and 2 with lanes 4 and 5 for the *Hin*dIII patterns in Fig. 3). Interestingly, multiple isolates obtained from the same individual had the same restriction enzyme pattern. Thus, the same virus strain was persistently shed in saliva over the period spanned in this experiment (up to 123 days). Moreover, the *het* fragments appeared to be stable in vivo, and the S8, S9, S10, and S11 isolates retained their characteristic *het* fragments over 105, 123, 13, and 17 days, respectively.

The repeated recovery of HHV-7 isolates with the same characteristic *het* fragments revealed the persistent presence of cell-free, infectious HHV-7 in the saliva of healthy adults. At present, it is unknown which cells harbor the virus; nor is it known whether individuals who shed virus in their saliva carry virus in their peripheral blood mononuclear cells (PBMC) and in what state the virus is present in the lymphocytes. In fact, the first HHV-7 strain to be recognized (strain RK) was isolated from $CD4^+$ lymphocytes of the peripheral blood of a healthy individual after activation of the cells with anti-CD3 MAbs in conjunction with interleukin-2 or with anti-CD28 antibody (8). As no virus could be recovered from the nonactivated quiescent cells, we have suggested that the virus was latent in these cells. Further experiments to clarify these questions are currently in progress.

Several investigators have recently reported the isolation of HHV-6 from the saliva of healthy adults (12, 17, 24) as well as the detection of viral DNA by polymerase chain reaction (PCR) in oropharynx and saliva samples (10, 15, 16). The reason(s) for our failure to isolate saliva-borne HHV-6 is at present unclear. It might, however, be useful to consider the earlier reports in light of more recent studies concerning the relevant properties of HHV-6 and HHV-7. Thus, Harnett et al. (12), who reported the isolation of HHV-6 by cocultivation of saliva samples with CBMC, were screening the resultant viruses with HHV-6-positive human

TABLE 1. Reactivities of HHV-6 MAbs with saliva isolates[a]

Virus strain or isolate	Reactivity of HHV-6 MAb (reacting protein[s])[b]:					
	2D6 (gp82k-105k)	6A5D5 (gp116k, gp64k, gp54k)	7A2 (gp102k)	9A5D12 (p41k, p110k)	12B3G4 (p135k)	4A6 (p180k)
U1102 (HHV-6)	+	+	+	+	+	+
Z29 (HHV-6)	0	+	+	+	+	0
RK (HHV-7)	0	0	0	±	+	0
S1	0	0	0	0	+	0
S2	0	0	0	0	+	0
S3	0	0	0	0	+	0
S4	0	0	0	0	+	0
S5	0	0	0	0	+	0
S6	0	0	0	0	+	0
S7	0	0	0	0	+	0
S8	0	0	0	0	+	0
S9	0	0	0	0	+	0
S10	0	0	0	+	+	0
S11	0	0	0	+	+	0
S12	0	0	0	+	+	0
S13	0	0	0	+	+	0

[a] MAbs are from reference 3.
[b] 0, nonreactivity; +, reactivity; ±, variable reactivity.

sera. We now know that a great majority of human adult serum samples are positive also for HHV-7 (32), and it thus remains to be seen whether the viruses identified by the human sera are indeed HHV-6. Levy et al. (17) have propagated saliva isolates in PBMC in the course of virus isolation. We now know that HHV-7 can activate latent HHV-6 in PBMC (7, 9). Furthermore, we have found that HHV-6 replicates more efficiently than HHV-7 and that limited propagation of mixtures of both viruses results in the selection of HHV-6 (7, 9). In light of these results and in light of the data reported in the present paper, it is possible that the virus which was isolated by Levy et al. (17) from saliva and propagated in PBMC cultures was originally HHV-7, which was then outreplicated by HHV-6 rescued from the PBMC. Finally, it remains to be determined whether the detection of HHV-6 DNA in saliva samples, as reported recently by Jarrett et al. (15) using PCR analyses, reflected the presence of infectious HHV-6 in saliva. Alternatively, the PCR signal might have reflected the presence of latent genomes in cells present in saliva, or it could have resulted from cross-reactivity of the PCR primers used with both the HHV-6 and the HHV-7 DNA. With regard to these questions, note that Kido et al. (16) have reported that only 1 of 30 throat swab samples from healthy adults contained DNA which could be amplified by HHV-6 PCR primers. Studies currently in progress are designed to further clarify the question of the presence of HHV-6 in saliva.

At present, it is not known whether HHV-7 is associated with human disease. In a recent pilot study of 25 children, we found that HHV-7 infection occurs early in childhood but at a somewhat later age than HHV-6 (32). Thus, whereas seroconversion to HHV-6 occurs by 2 years of age, seroconversion to HHV-7 occurs later. If the data of our pilot study indeed extend to larger sampling, it remains to be determined why infection with HHV-7 occurs later in life. It is

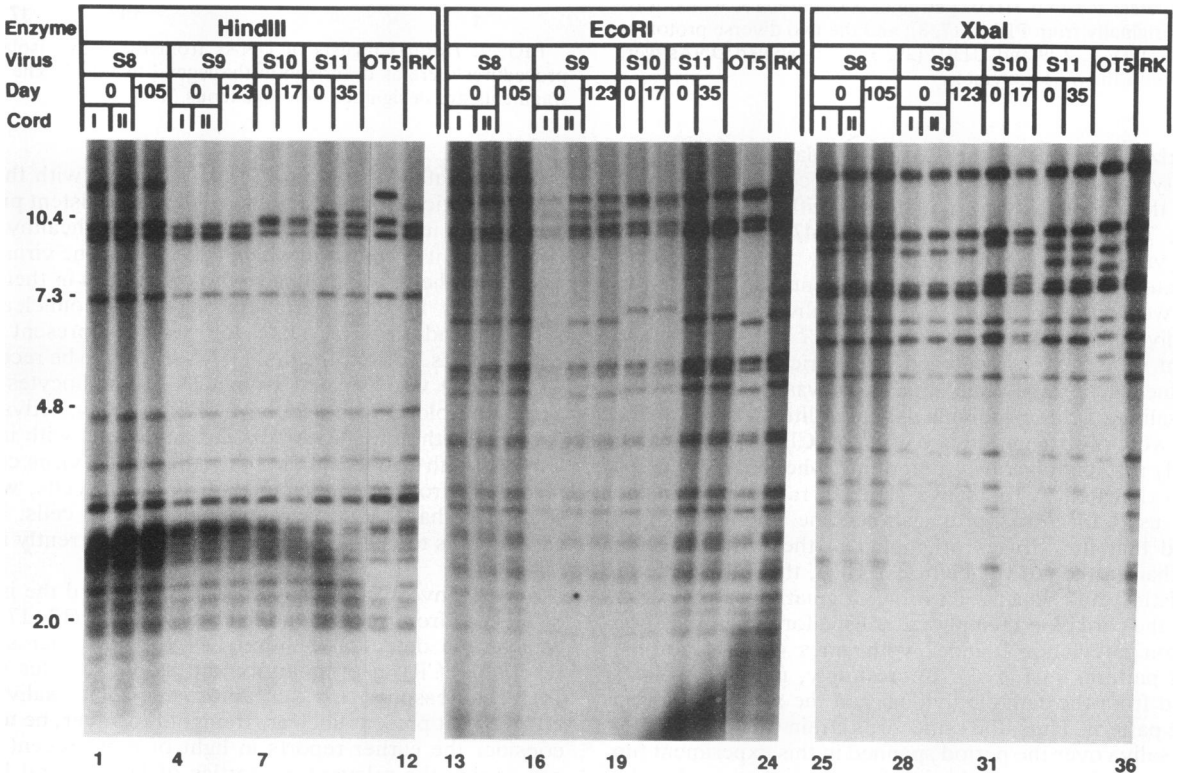

FIG. 3. Analyses of sequential isolates of saliva viruses from the same individual. Saliva samples from healthy adults, designated S8, S9, S10, and S11, were taken over a period of 105, 123, 17, and 35 days, respectively. The saliva samples S8 and S9 collected on day 0 of the study were cultivated on two different CBMC (I and II).

possible that HHV-7 which is present in saliva is not readily infectious to the infant. In this context, it is noteworthy that it has been shown that despite the presence of human immunodeficiency virus in saliva (11, 13, 18, 26), transmission by the oral route has not been documented. Further experiments are required to clarify the questions regarding the transmission of HHV-6 and HHV-7 to young children.

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REFERENCES

1. **Asano, Y., T. Nakashima, T. Yoshikawa, S. Suga, and T. Yazaki.** 1991. Severity of human herpesvirus-6 viremia and clinical findings in infants with exanthem subitum. J. Pediatr. **118:**891–895.
2. **Asano, Y., T. Yoshikawa, S. Suga, T. Yazaki, K. Kondo, and K. Yamanishi.** 1990. Fatal fulminant hepatitis in an infant with human herpesvirus-6 infection. Lancet **335:**862–863.
3. **Balachandran, N., R. E. Amelse, W. W. Zhou, and C. K. Chang.** 1989. Identification of proteins specific for human herpesvirus 6-infected human T cells. J. Virol. **63:**2835–2840.
4. **Carrigan, D. R., W. R. Drobyski, S. K. Russler, M. A. Tapper, K. K. Knox, and R. C. Ash.** 1991. Interstitial pneumonitis associated with human herpesvirus-6 infection after marrow transplantation. Lancet **338:**147–149.
5. **DiLuca, D., G. Katsafanas, E. C. Schirmer, N. Balachandran, and N. Frenkel.** 1989. The replication of viral and cellular DNA in human herpesvirus 6-infected cells. Virology **175:**199–210.
6. **Downing, R. G., N. Sewankambo, D. Serwadda, R. Honess, D. Crawford, R. Jarrett, and B. E. Griffin.** 1987. Isolation of human lymphotropic herpesviruses from Uganda. Lancet **ii:**390.
7. **Frenkel, N., G. Katsafanas, E. C. Schirmer, and L. S. Wyatt.** Unpublished data.
8. **Frenkel, N., E. C. Schirmer, L. S. Wyatt, G. Katsafanas, E. Roffman, R. M. Danovich, and C. H. June.** 1990. Isolation of a new herpesvirus from human CD4+ T cells. Proc. Natl. Acad. Sci. USA **87:**748–752.
9. **Frenkel, N., and L. S. Wyatt.** HHV-6 and HHV-7 as exogenous agents in human lymphocytes. Dev. Biol. Stand., in press.
10. **Gopal, M. R., B. J. Thomson, J. Fox, R. S. Tedder, and R. W. Honess.** 1990. Detection by PCR of HHV-6 and EBV DNA in blood and oropharynx of healthy adults and HIV-seropositives. Lancet **335:**1598–1599.
11. **Groopman, J. E., S. Z. Salahuddin, M. G. Sarngadharan, P. D. Markham, M. Gonda, A. Sliski, and R. C. Gallo.** 1984. HTLV-III in saliva of people with AIDS-related complex and healthy homosexual men at risk for AIDS. Science **226:**447–449.
12. **Harnett, G. B., T. J. Farr, G. R. Pietroboni, and M. R. Bucens.** 1990. Frequent shedding of human herpesvirus 6 in saliva. J. Med. Virol. **30:**128–130.
13. **Ho, D. D., R. E. Byington, R. T. Schooley, T. Flynn, T. Rota, and M. Hirsch.** 1985. Infrequency of isolation of HTLV-III virus from saliva in AIDS. N. Engl. J. Med. **313:**1606.
14. **Huang, L.-M., C.-Y. Lee, K.-H. Lin, W.-M. Chuu, P.-I. Lee, R.-L. Chen, J.-M. Chen, and D.-T. Lin.** 1990. Human herpesvirus-6 associated with fatal haemophagocytic syndrome. Lancet **336:**60–61.
15. **Jarrett, R. A., D. Clark, S. F. Josephs, and D. E. Onions.** 1990. Detection of human herpesvirus-6 DNA in peripheral blood and saliva. J. Med. Virol. **32:**73–76.
16. **Kido, S., K. Kondo, T. Kondo, T. Morishima, M. Takahashi, and K. Yamanishi.** 1990. Detection of human herpesvirus 6 DNA in throat swabs by polymerase chain reaction. J. Med. Virol. **32:**139–142.
17. **Levy, J. A., F. Ferro, D. Greenspan, and E. T. Lennette.** 1990. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. Lancet **335:**1047–1050.
18. **Levy, J. S., L. S. Kaminsky, W. J. W. Morrow, K. Steimer, P. Luciw, D. Dina, J. Hoxie, and L. Oshiro.** 1985. Infection by the retrovirus associated with acquired immunodeficiency syndrome. Ann. Intern. Med. **103:**694–699.
19. **Lindquester, G. J., and P. E. Pellett.** 1991. Properties of the human herpesvirus 6 strain Z29 genome: G+C content, length, and presence of variable-length directly repeated terminal sequence elements. Virology **182:**102–110.
20. **Lopez, C., P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, J. Black, D. Warfield, and P. Feorino.** 1988. Characteristics of human herpesvirus-6. J. Infect. Dis. **157:**1271–1273.
21. **Martin, M. E. D., B. J. Thomson, R. W. Honess, M. A. Craxton, U. A. Gompels, M.-Y. Liu, E. Littler, J. R. Arrand, I. Teo, and M. D. Jones.** 1991. The genome of human herpesvirus 6: maps of unit-length and concatemeric genomes for nine restriction endonucleases. J. Gen. Virol. **72:**157–168.
22. **Okuno, T., K. Higashi, K. Shiraki, K. Yamanishi, M. Takahashi, Y. Kokado, M. Ishibashi, S. Takahara, T. Sonoda, K. Tanaka, K. Baba, H. Yabuuchi, and T. Kurata.** 1990. Human herpesvirus 6 infection in renal transplantation. Transplantation **49:**519–522.
23. **Okuno, T., K. Takahashi, K. Balachandra, K. Shiraki, K. Yamanishi, M. Takahashi, and K. Baba.** 1989. Seroepidemiology of human herpesvirus 6 infection in normal children and adults. J. Clin. Microbiol. **27:**651–653.
24. **Pietroboni, G. R., G. B. Harnett, M. R. Bucens, and R. W. Honess.** 1988. Antibody to human herpesvirus 6 in saliva. Lancet **i:**1059.
25. **Salahuddin, S. Z., D. V. Ablashi, P. D. Markham, S. F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B. Kramarsky, and R. C. Gallo.** 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **234:**596–601.
26. **Salahuddin, S. Z., P. D. Markham, M. Popovic, M. G. Sarngadharan, S. Orndorff, A. Fladagar, A. Patel, J. Gold, and R. C. Gallo.** 1985. Isolation of infectious human T-cell leukemia/lymphotropic virus type III (HTLV-III) from patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) and from healthy carriers: a study of risk groups and tissue sources. Proc. Natl. Acad. Sci. USA **82:**5530–5534.
27. **Saxinger, C., H. Polesky, N. Eby, S. Grufferman, R. Murphy, G. Tegtmeir, V. Parekh, S. Memon, and C. Hung.** 1988. Antibody reactivity with HBLV (HHV-6) in U.S. populations. J. Virol. Methods **21:**199–208.
28. **Schirmer, E. C., R. M. Danovich, G. Katsafanas, and N. Frenkel.** Unpublished results.
29. **Schirmer, E. C., L. S. Wyatt, K. Yamanishi, W. J. Rodriguez, and N. Frenkel.** 1991. Differentiation between two distinct classes of viruses now classified as human herpesvirus 6. Proc. Natl. Acad. Sci. USA **88:**5922–5926.
30. **Ueda, K., K. Kusuhara, M. Hirose, K. Okada, C. Miyazaki, K. Tokugawa, M. Nakayama, and K. Yamanishi.** 1989. Exanthem subitum and antibody to human herpesvirus-6. J. Infect. Dis. **159:**750–752.
31. **Wyatt, L. S., N. Balachandran, and N. Frenkel.** 1990. Variations in the replication and antigenic properties of human herpesvirus 6 strains. J. Infect. Dis. **162:**852–857.
32. **Wyatt, L. S., W. J. Rodriguez, N. Balachandran, and N. Frenkel.** 1991. Human herpesvirus 7: antigenic properties and prevalence in children and adults. J. Virol. **65:**6260–6265.
33. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **i:**1065–1067.
34. **Yoshikawa, T., S. Suga, Y. Asano, T. Nakashima, T. Yazaki, R. Sobue, M. Hirano, M. Fukuda, S. Kojima, and T. Matsuyama.** 1991. Human herpesvirus-6 infection in bone marrow transplantation. Blood **78:**1381–1384.