Human Herpesvirus 7: Antigenic Properties and Prevalence in Children and Adults

LINDA S. WYATT,1 WILLIAM J. RODRIGUEZ,2 N. BALACHANDRAN,3 AND NIZA FRENKEL1*

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

Children’s Hospital National Medical Center and George Washington University, Washington, D.C. 20010

and Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas 66103

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The recent isolation of human herpesvirus 7 (HHV-7) from activated CD4+ T lymphocytes of a healthy individual raises questions regarding the prevalence of this virus in humans and its immunological relationship to previously characterized human herpesviruses. We report that HHV-7 is a ubiquitous virus which is immunologically distinct from the highly prevalent T-lymphotropic HHV-6. Thus, (i) only two of six monoclonal antibodies to HHV-6 cross-reacted with HHV-7-infected cells, (ii) Western immunoblot analyses of viral proteins revealed different patterns for HHV-6 and HHV-7-infected cells, (iii) tests of sequential serum samples from children revealed seroconversion to HHV-6 without concomitant seroconversion to HHV-7, and (iv) in some instances HHV-7 infection occurred in the presence of high titers of HHV-6 antibodies, suggesting the lack of apparent protection of children seropositive for HHV-6 against subsequent infection with HHV-7.

On the basis of the analyses of sera from children and adults it can be concluded that HHV-7 is a prevalent human herpesvirus which, like other human herpesviruses, infects during childhood. The age of infection appears to be somewhat later than the very early age documented for HHV-6.

We have recently isolated a previously unrecognized human herpesvirus from CD4+ T cells purified from peripheral blood lymphocytes (PBL) of a healthy individual following incubation of the cells under conditions leading to T-cell activation. The virus was found to be distinct from previously characterized human herpesviruses and was therefore designated human herpesvirus 7 (HHV-7) (7). Comparative analyses of HHV-7 with DNA of previously identified human herpesviruses revealed limited homology between HHV-7 DNA and the DNA of HHV-6, the T-lymphotropic herpesvirus which was initially isolated from patients with AIDS and patients with lymphoproliferative disorders (5, 11, 15). HHV-6 was later found to be the causative agent of exanthem subitum (ES or roseola infantum), a childhood disease characterized by spiking fever and skin rash (19).

The isolation of the seventh human herpesvirus from peripheral blood raises questions regarding its prevalence in humans and regarding its potential association with disease. Prior to undertaking studies regarding these questions, it was important to determine whether HHV-6 and HHV-7 are antigenically related and whether they produce cross-reacting antibodies because assessment of antibody status to HHV-7 could be complicated if this was the case. We now report that HHV-7 differs from HHV-6 on the basis of reactivity with monoclonal antibodies (MAbs) to HHV-6 and Western immunoblot analyses using human sera. Examination of sequential serum samples from children who have seroconverted to these viruses clearly showed that the viruses cause distinct infections in their human hosts. On the basis of a limited survey, it can be concluded that HHV-7 is a prevalent virus which infects children at a young age. As previously observed by others (1, 3, 8, 10, 12, 16, 20), seroconversion to HHV-6 occurs prior to 24 months of age. In contrast, HHV-7 infects children somewhat later in childhood.

MATERIALS AND METHODS

Viruses and cells. HHV-6 strain Z29 was obtained from Carlos Lopez (Centers for Disease Control) and HHV-6 strain U1102 was obtained from Robert Honess (National Institute for Medical Research, Mill Hill, London, England). Their propagation in PBL was previously described (4, 17). The isolation and propagation of HHV-7(RK) in cord blood lymphocytes (CBL) were previously described (7). The isolation of HHV-7 strains OT3 through OT5 from peripheral blood of healthy individuals will be separately described (6).

IFA with MAbs. Virus-infected PBL or CBL were harvested at the peak of cytopathic effect 5 to 9 days postinfection. In the majority of cases the multiplicity of infection ranged from 0.01 to 0.05 50% tissue culture infective dose per cell. The infected cells were fixed with acetone and were used in an indirect immunofluorescence assay (IFA) as described previously (17), using MAbs derived against the GS strain of HHV-6 (11). These MAbs were derived and characterized previously by Balachandran et al. (2). Results obtained with a human serum, positive for both HHV-6 and HHV-7 antibodies by IFA, revealed that 40 to 60% of the cells were infected in the preparations used for IFA.

IFA with human sera. Cells infected with HHV-6(Z29) or HHV-7(RK) were fixed with acetone on glass slides. The fixed cells were incubated first for 1 h at 37°C with twofold dilutions of human sera and then for 45 min with fluorescein-conjugated F(ab')2 fragment of rabbit immunoglobulins to human immunoglobulin G (gamma chains) (Dako Corp.). All sera were incubated in parallel with the HHV-6(Z29) and HHV-7(RK)-infected cells and the corresponding mock-infected PBL or CBL. The fluorescence was visualized on coded slides, and the titer was defined as the reciprocal of the last dilution which gave a positive (though weak) response when compared with the conjugate and mock-infected control. Titers of >20 and >80 were considered positive for HHV-6 and HHV-7, respectively (see Results).

Source of human sera. Sera from 25 healthy children ranging in age from newborn to 58 months were collected.
TABLE 1. Reactivity of HHV-6 MAbs with HHV-7a

<table>
<thead>
<tr>
<th>MAb</th>
<th>Reacting protein(s)</th>
<th>Reactivityb with:</th>
<th>U1102</th>
<th>Z29</th>
<th>OT4</th>
<th>OT5</th>
<th>OT3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HHV-6</td>
<td>HHV-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2D6</td>
<td>gp82k-105k</td>
<td>+ + 0 0 0 0 NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A5D5</td>
<td>gp116k, gp64k, gp54k</td>
<td>+ + 0 0 0 0 NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7A2</td>
<td>gp102k</td>
<td>+ + + 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9A5D12</td>
<td>p41k, p110k</td>
<td>+ + + + + NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12B3G4</td>
<td>p135k</td>
<td>+ + + + + NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A6</td>
<td>p180k</td>
<td>+ + 0 0 0 0 NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a HHV-6 strains U1102 and Z29 and HHV-7 strain RK were tested with ascitic fluids at dilutions of 1:10, 1:100, and 1:1,000. The remaining HHV-7 strains were tested at the following dilutions: 1:100 for 2D6, 6A5D5, 12B3G4, and 4A6; 1:1,000 for 7A2 and 9A5D12.
b +, reactivity; 0, lack of reactivity; ±, weak and variable reactivity; NT, not tested.

one to three times during 1978 to 80. These children were from a middle class northern Virginia population. Sequentially drawn serum samples from three additional children were received from Andre Nahmias (Emory University). Paired acute and convalescent-phase sera from three ES patients were obtained from Koichi Yamamishi (Osaka University, Osaka, Japan). Twenty-six serum samples from adults were tested, including samples from 15 parents of the children described above.

Western blot analyses. Proteins from HHV-6(Z29)- and HHV-7(RK)-infected CBL were separated in sodium dodecyl sulfate-9.5% polyacrylamide gels, using N,N,N-diallyltartardiamide as the cross-linker, and electrophobted onto polyvinylidene difluoride membrane in 25 mM Tris-192 mM glycine–20% methanol buffer (pH 8.3). After being blocked for 1 h at 37°C in phosphate-buffered saline (PBS)-3% bovine serum albumin (BSA), the blot was incubated for 1 h with a 1:50 dilution of serum or plasma, washed in PBS-0.4% Tween, and then reacted for 1 h with 125I-protein A (0.1 μCi/ml) in PBS-1% BSA-0.2% Tween. The blots were then rinsed again prior to autoradiography at ~80°C with intensifying screens. 14C and prestained molecular weight markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were included in the gel.

RESULTS

Reactivity of HHV-6 MAb with HHV-7. In the first set of studies we determined the reactivity of HHV-6 MAb with HHV-7 (Table 1). Six MAbS (2) produced against the GS strain of HHV-6 (11) were tested against HHV-6 and HHV-7-infected PBL or CBL. These MAbS were directed against six different polypeptides and included three MAbS reactive with major glycoproteins (2). Four HHV-7 strains, including the prototype strain RK and three additional strains which were recently isolated in our laboratory (6), were tested. In addition, two HHV-6 strains, U1102 and Z29, were employed as controls. The U1102 strain was isolated by Downing et al. (5) from a patient with AIDS in Uganda, and the Z29 strain was isolated by Lopez et al. (9) from a patient with AIDS in Zaire. These L strains were chosen to represent two diverse categories of HHV-6 strains, as judged by both restriction enzyme and antigenic analyses (13).

The results of the studies are shown in Table 1 and can be summarized as follows. (i) As recently reported by us, four of the six MAbS (MAbS 6A5D5, 7A2, 9A5D12, and 12B3G4) reacted well with the U1102 and Z29 HHV-6 strains, whereas two of the MAbS (2D6 and 4A6) reacted strongly with U1102 but failed to react by IFA with the Z29 strain (17). (ii) MAbS 2D6, 6A5D5, 7A2, and 4A6 failed to react with the HHV-7 strains tested. (iii) Only MAbS 9A5D12 and 12B3G4, directed against p41k and p110k and against p135k, respectively, reacted weakly with HHV-7-infected PBL, and this reaction varied with the PBL used for antigen preparation. We conclude on the basis of these results that HHV-6 and HHV-7 are antigenically distinct for the antigens or epitopes to which MAbS 2D6, 6A5D5, 7A2, and 4A6 are directed. The two viruses may exhibit limited cross-reactivity with regard to the antigens or epitopes to which the 9A5D12 and 12B3G4 MAbS are directed.

HHV-6 and HHV-7 antibody titers in healthy adults. Sera from 26 adults were assayed by IFA for antibodies to HHV-6 and HHV-7, using acetone-fixed infected cells (Table 2). Sera from 24 of 26 individuals (92%) were positive for both HHV-6 and HHV-7 antibodies. Serum from one individual was positive for HHV-6 but negative for HHV-7, whereas another serum was negative for HHV-6 but positive for HHV-7 antibodies. Several lines of evidence suggest that the HHV-6 and HHV-7 titers represent the presence of antibodies specific for these viruses rather than cross-reactivity. First, one would expect that if the titers reflected solely cross-reactivities, the HHV-6 titers would correlate well with those for HHV-7. This was not the case. Second, the results of the studies with children described below have shown that cross-reactivity does not account for titers higher than 80. Because 96% of the adults had HHV-7 titers of ≥160, we conclude that HHV-7, like HHV-6, is a prevalent virus in adults.

Seroconversions to HHV-6 or HHV-7 in healthy children. Because the majority of sera from adults showed positive reaction with HHV-7, it was important to determine whether the immunofluorescence observed with HHV-7-infected-cell antibodies represented the presence of antibody specific for the virus or cross-reactivity with HHV-6 antibodies also present in the sera. We chose to test for cross-reacting antibodies by assaying seroconversions to HHV-6 and HHV-7 in sequential serum samples from children. It was anticipated that for some of the children, the initial sample would be negative whereas a subsequent sample would reveal seroconversion for one or both viruses, reflecting an infection with that virus. Cross-reactivity would then be expected to result in a simultaneous increase in both HHV-6 and HHV-7 titers.

Figure 1 shows the antibody response of six children in which sequential serum samples showed seroconversion to HHV-6 or HHV-7 in the course of this study. The results can be summarized as follows. (i) As seen in the example of child 2 and as will be discussed below for additional children, titers of maternal antibodies to both HHV-6 and HHV-7 of ≥640 were present at birth. (ii) Children 1 to 4 seroconverted for HHV-6 in the course of the study, with seroconversion occurring at an early age. Specifically, seroconversion was
observed between 7 and 10 months in child 1, 11 and 20 months in child 2, 8 and 14 months in child 3, and 7 and 11 months in child 4. (iii) In those children (children 1 to 4) there was no increase in HHV-7 titers. We conclude on the basis of these data that seroconversion to HHV-6 and HHV-7 occurred independently and that the antibody responses to HHV-6 and HHV-7 were distinct. Thus, the high anti-HHV-6 titers in all six children were not reflected in high titers for HHV-7. In one case (child 1) a titer of ≥1,280 for HHV-6 was accompanied by a titer of 40 to HHV-7, indicating that there may be a limited cross-reactivity between the two viruses. In all other cases (children 2 to 4) the HHV-7 titer remained ≤20. With regard to the remaining children in the study, in 6 of 11 with high titers of antibodies to HHV-6 when they were first tested, low-level titers to HHV-7 were seen (>20 but ≤80).
TABLE 3. Antibody titers to HHV-6 and HHV-7 in paired sera from ES patients

<table>
<thead>
<tr>
<th>Serum</th>
<th>Titer (reciprocal)</th>
<th>HHV-6</th>
<th>HHV-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-acute</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>1-convalescent</td>
<td>320</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>2-acute</td>
<td>&lt;20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2-convalescent</td>
<td>≥1,280</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>3-acute</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>3-convalescent</td>
<td>640</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>4-acute</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>4-convalescent</td>
<td>160</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

observations suggest that the level of cross-reactivity does not exceed titers of 80 in the IFA employed in this study. (iv) Children 5 and 6 were positive for HHV-6 in the first drawing and remained seropositive during the second drawing. Thus, child 5 had HHV-6 titers of ≥640 at 37 and 52 months, whereas the HHV-6 titers of child 6 were 320 at 49 and 68 months. Despite the continued presence of high-titer HHV-6 antibodies in these children, they seroconverted to HHV-7, suggesting the lack of protection to subsequent infection with HHV-7. This observation further supports the conclusion that HHV-6 and HHV-7 are immunologically distinct.

Seroconversions to HHV-6 and HHV-7 in paired sera from ES patients. Further evidence for the immunological distinction between HHV-6 and HHV-7 came from analyses of paired sera of ES patients. Because HHV-6 was shown to be the causative agent of the disease, we anticipated seroconversion for HHV-6 in these patients. Sera from three ES patients in Japan (obtained from K. Yamanishi, Osaka University) and one ES patient from the United States (obtained in Children’s Hospital, Washington, D.C.) were tested. In the case of the last patient, virus was recovered in our laboratory and shown to be HHV-6 by restriction enzyme analyses (13). As summarized in Table 3, three of the four paired sera showed seroconversion to HHV-6 while the HHV-7 titers remained ≤80. Convalescent-phase serum of patient 2 showed titers of ≥1,240 for HHV-6 and 320 for HHV-7. At present it is not clear whether the relatively high HHV-7 titer in this patient represents cross-reactivity or whether this child had concurrent HHV-6 and HHV-7 infections.

Age of acquisition of antibodies to HHV-6 and HHV-7. Figure 1 shows results for sequential serum samples which exhibited seroconversion for HHV-6 and for HHV-7. A summary of the age of acquisition of antibodies to HHV-6 and HHV-7 determined by utilizing the last serum sample from each of the 25 healthy children in this study is given in Figure 2. The number of sera tested in each time interval is given below the graph. Maternal antibodies to HHV-6 and HHV-7 were present in all 11 children tested at birth to 2 months. Of the four children who had sera collected between 9 and 14 months of age, one had antibodies to HHV-6 while none had antibody titers to HHV-7. Of six children who were tested in the interval from 15 to 25 months, five were positive for HHV-6 and none were positive for HHV-7. Sera collected from 5 children 26 to 44 months old and 10 children 45 to 75 months old were 100% positive for HHV-6 and 40 and 70% positive for HHV-7, respectively. Thus, almost all children tested in this study had acquired antibodies to HHV-6 by 25 months, while none had acquired antibodies to HHV-7. The percentage of children who acquired antibodies to HHV-7 gradually increased through early childhood. It thus appears that HHV-7 infects in early childhood but at a later time than HHV-6. Studies with large numbers of sera are currently under way to further confirm this result.

Reactivity of HHV-6 and HHV-7 sera by Western blotting. Figure 3 shows a Western blot obtained by using plasma from a healthy individual. The human plasma reacted with a major band(s) which in our gel system migrated at 104 and 111 kDa. These values are within the range previously reported by others (2, 18). In contrast, the major antigenic protein for HHV-7 is a 91-kDa protein. It is noteworthy that similar blotting patterns were observed when a commercially available preparation of pooled human gamma globulin was tested (data not shown), indicating that the general patterns exemplified in Fig. 3 represented a general pattern for human sera against HHV-6(Z29) and HHV-7(RK). However, Western blot analyses could not be done using the children’s sera, because they were available only in limited amounts. Current efforts are designed to develop a mini-Western blot assay.

DISCUSSION

HHV-7 was first isolated from PBL of a healthy individual (7). Analyses of viral DNA revealed that the virus was different from previously characterized human herpesviruses. Specifically, HHV-7 DNA did not hybridize with probes derived from herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus and was found to be distinct from HHV-6 DNA because (i) analysis with 21 restriction enzymes revealed very different patterns, (ii) large probes of HHV-6 and HHV-7 DNA either did not cross-hybridize or hybridized only weakly to the heterologous DNA. Thus far, hybridization of HHV-6 and HHV-7 probes to the heterologous DNAs have revealed maximal homology corresponding to 42 of 124 kb tested. Because the analyses of HHV-6 and HHV-7 DNAs showed limited
sequence homology, it was important to determine the degree of immunological cross-reactivity between the two viruses prior to analyses of the prevalence of HHV-7 in children and adults.

Several lines of evidence suggest that the two viruses are antigenically distinct. First, no cross-reactivity was observed with four of six MAbs tested, including three which were directed against viral glycoproteins. The remaining two MAbs (9A5D12 and 12B3G4) reacted with HHV-7. However, the resultant fluorescence was rather weak and variable. It should be noted that bright fluorescence was obtained with these samples when a human serum positive for HHV-7 was used. The weak and variable fluorescence observed with these MAbs could represent imperfect conservation of similar antigenic epitopes in the two viruses, resulting in less efficient reaction. Alternatively, it could reflect low concentrations of the proteins in the HHV-7 samples used for the IFAs. In further studies designed to clarify this question, infected cells from two duplicate infections with HHV-7(RK) were fixed with acetone or with acetone-methanol at daily intervals postinfection. In both cases the immunofluorescence observed with the 9A5D12 and 12B3G4 MAbs was low and variable. Further studies are currently in progress to determine the source of this variability.

Second, the results of the analyses employing Western blots have shown that whereas the major immunogenic protein(s) of HHV-6 migrated at 104 and 111 kDa, the major immunogenic protein of HHV-7 was 91 kDa. Moreover, the same major bands were observed when pooled human gamma globulin was tested, confirming that the distinction was not unique to the serum tested.

Third, in two cases, seroconversion for HHV-7 was documented in the presence of high antibodies to HHV-6. This observation is all the more significant in light of previous reports (14, 21) that children who seroconverted to HHV-6 have neutralizing antibodies to the virus. However, on the basis of the results presented above, prior infection with HHV-6 did not appear to confer protection against a subsequent HHV-7 infection, confirming the uniqueness of immunological responses to the two viruses. This observation is not unexpected since none of the three HHV-6 MAbs directed against viral glycoproteins reacted with HHV-7. Further analyses using more extensive sampling of the population are currently under way to substantiate this finding.

Finally, analyses of the children's sera in this study revealed that seroconversion to HHV-6 was not associated with a parallel increase in anti-HHV-7 titers, and vice versa. Examination of sera obtained within the window of ages during which HHV-6 infection is prevalent and HHV-7 infection is low allowed us to establish boundaries of cross-reactivity not exceeding 1:80 in our test.

Taken together, our results confirmed the results of others (1, 3, 8, 10, 12, 16, 20) that HHV-6 is a very prevalent virus in the population and that the majority of children have acquired antibodies to HHV-6 by their second year of life. Yet, by 15 to 25 months, the same children were found to be seronegative for HHV-7. Only later in childhood was a rise in HHV-7 titers observed. In fact, a number of children 4 to 6 years of age remained seronegative for HHV-7 in the presence of high HHV-6 titers.

The finding that HHV-6 and HHV-7 are immunologically distinct extends the results of our previous studies, which have led to the conclusion that HHV-7 should be classified as a distinct herpesvirus (7). As additional strains of HHV-6 and HHV-7 are being characterized, a certain hierarchy in the relatedness between these lymphotropic herpesviruses is emerging. In fact, our recent analyses of viral isolates obtained from ES patients have revealed that HHV-6 strains themselves fall into two separate categories, differing in the restriction enzyme patterns of DNAs, reactivity with HHV-6 MAbs, and most likely, also disease patterns (13). Thus, restriction enzyme patterns of viral DNAs from 20 different isolates of HHV-6 recently isolated from PBL and from ES patients were well conserved but were very different from the corresponding patterns of U1102 DNA. Furthermore, all of these Z29-like viruses did not react with MAbs 2D6 and 4A6 (13, 17; additional data not shown). Thus far no specific serological assays are available to distinguish these classes of viruses. Inasmuch as all ES patient strains isolated thus far are Z29-like viruses, it is reasonable to suggest that the early seroconversion seen in the IFAs done in this study as well as the early studies by Suga et al. (14) and Yoshikawa et al. (21) reflect early infection with a Z29-like virus. In contrast, the age of infection by and prevalence of the U1102-like strains is at present unknown because of the cross-reactivity between the U1102- and Z29-like viruses. Clearly HHV-7 infection occurs at a later age than that by
the ZZ9-like viruses. More studies are required to further assess the hierarchy of relatedness among these newly emerging groups of lymphotropic herpesviruses.

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