Variation of Human Herpesvirus 7 Shedding in Saliva

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Variation of Human Herpesvirus 7 (HHV-7) shedding in saliva obtained from healthy adults was performed for 6 months; virus was isolated in 92 (34.1%) of the 270 saliva samples obtained. Frequency of HHV-7 shedding in serially obtained saliva samples varied among subjects. Associations between frequency of HHV-7 shedding and age, sex, and virus antibody titer were analyzed, and, although sex was not associated with frequency of shedding, young age (P = .031) and low antibody titer (P = .006) were correlated with frequent viral shedding (5 or 6 times/6 months).

Human herpesvirus (HHV) 7 was originally isolated from peripheral blood mononuclear cells from healthy adults in 1990 [1]. This virus belongs to the b-herpesvirinae subfamily and is closely related to HHV-6, on the basis of biological and molecular analyses. Primary infection with these 2 viruses causes exanthem subitum, which is a common febrile disease in childhood [2, 3]. Primary HHV-7 infection is generally considered to be a benign and self-limited disease and rarely affects the central nervous system [4]. Because most children have a primary HHV-7 infection between 2–5 years old [5, 6], this virus is ubiquitous among the adult population. As with other HHVs, HHV-7 can reactivate in immunosuppressed patients. It has been suggested that HHV-7 reactivation is associated with cytomegalovirus disease in organ-transplant recipients [7]. Moreover, recently, fatal encephalitis due to HHV-7 infection was reported in a bone marrow–transplant recipient [8]. Thus, elucidation of the route and source of virus transmission is important for control of infection in hospitals.

Although horizontal transmission of HHV-7 has been suggested to occur as a result of close contact within a household [9], the portal of entry of the virus into the human host, the site of primary infection, and the site of latency have yet to be fully elucidated. Salivary glands have been proposed as sites for persistent viral infection, because of the high frequency of virus isolation from saliva samples [10, 11]. Thus, it has been speculated that HHV-7 shedding in saliva might play an important role in virus transmission. However, at present, frequency of HHV-7 shedding in seropositive individuals has only been studied at a single time point [10, 11]; indeed, longitudinal viral shedding in saliva has not been studied. Thus, in the present study, we monitored HHV-7 shedding in saliva samples serially obtained from seropositive adults, for 6 months. We found that frequency of viral shedding in saliva varies among individuals.

Materials and methods. Subjects included 45 healthy adults (22 men and 23 women). The median age of the men was 31 years (range, 26–57 years), and that of the women was 36 years (range, 23–43 years). All subjects consented to their participation in this study. Saliva samples were serially obtained from the subjects, every month for 6 months. One milliliter of saliva was collected in a sterile tube and was used immediately for virus isolation. A total of 270 saliva samples were obtained, from which we attempted to isolate the virus. At the beginning of the study, 2 mL of serum sample was obtained from all subjects. Serum was stored at −20°C until measurement of HHV-7 antibody titer by immunofluorescence assay.

HHV-7 isolation was performed by cocultivation with preactivated cord blood mononuclear cells, as described elsewhere [6]. In brief, 1 mL of saliva was diluted 1:2 with RPMI 1640 medium and was centrifuged at 2000 g. The supernatant was filtered through a 0.45-μm filter, was inoculated into preactivated cord blood mononuclear cells, and was centrifuged at 2000 g for 60 min to enhance absorption. After centrifugation, the cells were cultured in growth media and were incubated at 37°C in 5% CO2. Virus isolation was considered to be positive if the following 2 findings were present: (1) round, large cell formation of the cultured cells and (2) specific immunofluorescence staining with a monoclonal antibody to HHV-7, KR4 (provided by Dr. T. Okuno, Department of Microbiology, Hyogo College of Medicine, Hyogo, Japan), which does not cross-react with HHV-6.

Serosatus of all subjects was examined by immunofluores-
Table 1. Association between human herpesvirus 7 (HHV-7) shedding in saliva and virus antibody titers.

<table>
<thead>
<tr>
<th>Frequency of HHV-7 shedding, no. of positive samples/no. of trials</th>
<th>Subjects (M/F), no.a</th>
<th>Age, mean ± SD, years</th>
<th>HHV-7 antibody titer, mean ± SD, GMT (log10)</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/6</td>
<td>10 (4/6)</td>
<td>33.4 ± 5.3</td>
<td>…</td>
<td></td>
</tr>
<tr>
<td>1/6 and 2/6</td>
<td>19 (9/10)</td>
<td>32.5 ± 5.4</td>
<td>1.94 ± 0.45; 0.33; 0.059</td>
<td></td>
</tr>
<tr>
<td>3/6 and 4/6</td>
<td>11 (6/5)</td>
<td>35.5 ± 9.2</td>
<td>1.86 ± 0.42; 0.049</td>
<td></td>
</tr>
<tr>
<td>5/6 and 6/6</td>
<td>5 (3/2)</td>
<td>28.2 ± 3.9</td>
<td>1.63 ± 0.34; 0.006</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. GMT, geometric mean titer.

a Sex was not associated with the frequency of HHV-7 shedding in saliva.
b Mean HHV-7 antibody titers of each group were compared with those of subjects without viral shedding, by Student’s t test.

Discussion. In addition to being the etiologic agent for exanthem subitum, HHV-7 is being recognized as an important infectious agent in immunocompromised hosts, especially organ-transplant recipients [7]. To prevent nosocomial infection, it is important to elucidate details of virus transmission. HHV-7 is frequently isolated from saliva samples obtained from healthy adults [10, 11]. Because most healthy adults are seropositive against the virus [5, 6], it is speculated that horizontal transmission of the virus via saliva is the main route of viral infection. Although frequent HHV-7 shedding in saliva has been demonstrated on the basis of virus isolation at 1 time point [10, 11], longitudinal analysis of viral shedding in seropositive individuals has not been performed before the present study, in which we show that frequency of viral shedding, during a 6-month observation period, varies among individuals and 1.63, respectively. Furthermore, the mean HHV-7 antibody titer was significantly lower in subjects with frequent viral shedding than in subjects without viral shedding (P = .006).
(figure 1). Although the observation periods of the 2 previous longitudinal studies (1 month and 3 months) [12, 13] are shorter than that for the present study, it has been demonstrated that HHV-7 DNA copy number in saliva also varies among individuals. Moreover, Fujikawa et al. [13] have demonstrated that the level of HHV-7 DNA in saliva tends to be higher in saliva samples from which virus was isolated than in virus-negative samples. The results of these previous molecular-based studies support the present virus-isolation study. To our knowledge, this is the first report that demonstrates variation of HHV-7 shedding in saliva, on the basis of longitudinal virus-isolation analysis. Furthermore, our report is based on virus isolation, and not simply viral DNA, making it a reliable basis for the development of infection-control strategies.

It is of interest to know whether persistent infection of HHV-7 or reinfection with another strain of HHV-7 occurred in these subjects. Although molecular-epidemiological analysis was not performed on these isolates, Wyatt and Frenkel [10] have demonstrated that multiple isolates obtained from the same individual have the same restriction-enzyme pattern. They suggested that the same virus strain is shed persistently in saliva. To confirm their hypothesis, in a future study, we should perform molecular-epidemiological analysis in several isolates from single individuals.

In addition to their clinical implications, the results of the present study are important from a virology standpoint. Similar to HHV-7, human cytomegalovirus, another β-herpesvirinae subfamily member, is frequently recovered from saliva obtained from seropositive adults. However, variation in frequency of viral shedding in saliva, among individuals, has not been demonstrated. Therefore, the results of the present study would be helpful for considering mechanisms of persistent infection of these viruses, in the salivary gland. Although it has been demonstrated by quantitative polymerase chain reaction that women have a significantly higher median blood HHV-7 load than do men [12], in the present study, sex was not found to correlate with the frequency of viral shedding in saliva. Because not only study design, but also method of viral quantification, is different between these 2 studies, more-detailed analysis is needed to clarify the effects of sex on viral shedding. We also found that younger subjects are more likely to excrete the virus into saliva; the reasons for this effect of age on viral shedding are unclear and should also be studied further.

Although the numbers of subjects in the present study are limited, mean HHV-7 antibody titers in subjects with frequent viral shedding (5/6 and 6/6) were significantly lower than those in subjects without viral shedding (P = .0059). Although HHV-7–neutralizing antibody titers were measured in only selected subjects, because of the difficulty of measuring, similar statistical difference was demonstrated between the 2 groups (data not shown). These results suggest that host immune response against HHV-7 may play an important role in controlling viral shedding in saliva. It seems that a strong immune response against the virus can suppress virus replication in the salivary gland. Univariate analysis for determining the risk factors for HHV-7 shedding in saliva was performed in the present study because of the limited number of cases. However, to elucidate the real factors related to viral shedding in saliva, in future experiments, multivariate analysis is necessary. Moreover, another major problem is to determine whether frequency of HHV-7 shedding in saliva is correlated with any of its clinical aspects. Sequential analysis of HHV-7 shedding in saliva from patients with several concurrent diseases is presently under way.

Acknowledgments

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References