MAJOR ARTICLE

Analysis of Shedding of 3 β-Herpesviruses in Saliva from Patients with Connective Tissue Diseases

Tetsushi Yoshikawa,[1] Masaru Ihira,[3] Hiroaki Taguchi,[2] Shunji Yoshida,[2] and Yoshizo Asano[1]

Departments of [1]Pediatrics and [2]Internal Medicine, Fujita Health University School of Medicine, and [3]Department of Medical Information Technology, Fujita Health University College, Toyoake, Aichi, Japan

Background. Whether an association exists between infection with β-herpesviruses and connective tissue diseases remains unclear, as are the mechanisms for the regulation of these infections in the salivary glands.

Methods. Human herpesvirus (HHV)–7 was isolated and viral DNA was quantified by real-time polymerase chain reaction (PCR) in serially collected saliva samples, to determine whether viral load correlated with infectivity. Then, to examine the role played by β-herpesviruses in connective tissue diseases, cytomegalovirus, HHV-6, and HHV-7 DNA loads were examined by real-time PCR in serially collected saliva samples from 21 patients with connective tissue diseases.

Results. Although subjects with frequent HHV-7 shedding were more likely to have a high viral load than were other subjects, high viral loads were detected in saliva samples from a portion of the subjects with low viral shedding rates. No significant difference between the quantity of HHV-7 DNA in saliva samples from which active virus was isolated and that amplified from samples without detectable virus was observed. Patients with adult-onset Still disease consistently had high HHV-7 DNA loads, in contrast to patients with other connective tissue diseases ($P = .0003$) and healthy adults ($P = .0224$). The mean HHV-6 ($P = .012$) and HHV-7 ($P < .0001$) DNA loads in patients with connective tissue diseases were lower than those in healthy adults.

Conclusion. These data suggest that a number of host factors in patients with adult-onset Still disease may function to accelerate HHV-7 replication in the salivary glands.

Human herpesvirus (HHV)–7 was originally isolated from peripheral-blood mononuclear cells (PBMCs) from healthy adults in 1990 [1]. This virus, a member of the β-herpesvirinae subfamily, is closely related to HHV-6. Primary infection with either of these 2 viruses causes exanthem subitum [2, 3], a common febrile disease of childhood [2, 3]. Because most infants and young children acquire infections with both of these viruses [4, 5], both are ubiquitous among adult populations. The epidemiologic profile of each virus is similar to that of cytomegalovirus (CMV), the original member of the β-herpesvirinae subfamily. After primary infection, herpesviruses persistently infect the host throughout its lifetime. The salivary glands have been proposed as reservoir sites for persistent infection with β-herpesviruses; HHV-7 can frequently be isolated from saliva [6, 7]. Using longitudinal viral-isolation analysis, we recently demonstrated that HHV-7 shedding in saliva varies among individuals [8]. This finding suggests that host factors may play an important role in the regulation of viral replication in the salivary glands. It will be significant to determine whether the kinetics of shedding of the other β-herpesviruses in saliva are similar to that of HHV-7, because it will help to illuminate the mechanisms that control viral replication in the salivary glands.

The role played by such viral infections in the pathogenesis of autoimmune diseases remains unclear. Herpesviruses, including Epstein-Barr virus [9] and CMV [10], interact with the immune system in a variety of ways and establish latency in either lymphocytes or monocytes/macrophages while retaining the potential for reactivation. In addition to these viruses, HHV-6

Received 18 April 2005; accepted 3 June 2005; electronically published 28 September 2005.

Presented in part: 29th International Herpesvirus Workshop, Reno, Nevada, 25–30 July 2004 (abstract 9.16).

Potential conflicts of interest: none reported.

Financial support: Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant-in-aid to the 21st Century Center of Excellence Program of Medicine and the Open Research Center, Fujita Health University).

Reprints or correspondence: Dr. Tetsushi Yoshikawa, Dept. of Pediatrics, Fujita Health University School of Medicine, Toyoake, Aichi 4701192 Japan (tetsushi@fujita-hu.ac.jp).

The Journal of Infectious Diseases 2005; 192:1530–6

0022-1899/2005/19209-0005$15.00

Table 1. Characteristics of the patients with connective tissue diseases.

Patient (age [in years], sex)	Disease	Disease activity	Steroids	Pulse therapy	Immunosuppressive drugs	Positivity/antibody titer		
						CMV	HHV-6	HHV-7
1 (45, F)	MCTD	Inactive	No	No	No	+	+	32
2 (46, F)	MCTD	Inactive	Yes	No	No	+	+	32
3 (52, F)	Polymyositis	Onset	Yes	Yes	No	+	+	NS
4 (59, F)	Systemic sclerosis	Inactive	No	No	No	+	NS	NS
5 (59, F)	Systemic sclerosis	Inactive	No	No	No	+	+	16
6 (55, M)	Rheumatoid arthritis	Inactive	Yes	No	MTX	+	+	32
7 (66, F)	Rheumatoid arthritis	Inactive	Yes	No	MTX	+	+	128
8 (68, M)	Rheumatoid arthritis	Inactive	Yes	No	No	+	+	16
9 (28, F)	SLE	Inactive	Yes	No	No	+	+	256
10 (43, F)	SLE	Relapse	Yes	No	No	+	+	16
11 (62, F)	SLE	Inactive	Yes	No	No	+	+	16
12 (39, F)	SLE	Inactive	Yes	No	No	+	NS	NS
13 (32, F)	SLE	Relapse	Yes	Yes	No	ND	NS	NS
14 (20, F)	SLE	Inactive	Yes	No	No	−	+	NS
15 (20, M)	AO Still disease	Relapse	Yes	Yes	MTX, CyA, AZP	ND	+	16
16 (23, F)	AO Still disease	Relapse	Yes	No	No	+	+	32
17 (33, F)	AO Still disease	Relapse	Yes	Yes	MTX, CyA	−	+	32
18 (33, M)	AO Still disease	Onset	Yes	Yes	No	+	+	8
19 (32, F)	AO Still disease	Onset	Yes	No	No	+	+	64
20 (36, M)	AO Still disease	Onset	Yes	No	No	+	+	64
21 (55, F)	AO Still disease	Onset	Yes	No	MTX	+	+	16

NOTE. AO, adult onset; AZP, azathioprine; CMV, cytomegalovirus; CyA, cyclosporin A; F, female; HHV, human herpesvirus; M, male; MCTD, mixed connective tissue disease; MTX, methotrexate; ND, not done; NS, nonspecific fluorescence; SLE, systemic lupus erythematosus.

and HHV-7 latently infect PBMCs [11–13], periodically reactivating in immunosuppressed patients [14]. It has recently been suggested that these 2 viruses may modulate the immune response [15–19]; an association between HHV-6 and multiple sclerosis, an autoimmune-mediated demyelinating disease of the central nervous system, has been intensely debated in recent years [20, 21]. Thus, it is important to determine the role played by these 2 β-herpesviruses in the pathogenesis of connective tissue diseases. Furthermore, it is well known that patients with adult-onset Still disease, which is one of the popular connective tissue diseases, often have hypercytokinemia. Because hypercytokinemia might play an important role in the control of viral replication in the salivary glands, analysis of the relationship between connective tissue diseases (Still disease, in particular) and infection with β-herpesviruses would provide us with important information that will elucidate the host factors that control viral replication.

In the present study, we sought to identify host factors involved in the regulation of 3 β-herpesviruses (HHV-7, in particular) in the salivary glands. Because isolating HHV-7 from saliva is difficult and analyzing large numbers of samples is time-consuming, we first determined whether real-time polymerase chain reaction (PCR), instead of viral isolation, could be used for monitoring viral replication in the salivary glands. In addition to exploring the host factors that control viral replication in the salivary glands, we also sought to elucidate the association between infection with β-herpesviruses and autoimmune connective tissue diseases.

SUBJECTS, MATERIALS, AND METHODS

Subjects and sample preparation. The subjects included 19 healthy adults (9 men and 10 women between 21 and 29 years old), 7 patients with adult-onset Still disease, and 14 patients with other connective tissue diseases (such as systemic lupus erythematosus [6], rheumatoid arthritis [3], systemic sclerosis [2], poliomyelitis [1], and mixed connective tissue disease [2]). The patients' characteristics, such as disease activity and treatment (including receipt of several immunosuppressive drugs), are summarized in table 1. All subjects consented to participation in this study. Saliva samples were serially collected from the healthy adults weekly for 6 weeks and from the patients with connective tissue diseases monthly for 6 months. Two milliliters of saliva from the healthy adults was used for viral isolation and measurement of HHV-7 DNA load by real-time PCR. A total of 114 saliva samples from the healthy adults were analyzed in this study. One milliliter of saliva from the patients with connective tissue diseases was used to measure the viral DNA load of each of 3 β-herpesviruses (CMV, HHV-6, and HHV-7) by real-time

PCR. A total of 126 saliva samples were prospectively collected from the patients and were used to measure viral DNA load.

Isolation of HHV-7 from saliva samples. For the healthy adults, HHV-7 shedding in saliva was evaluated by use of the viral-isolation method. HHV-7 was isolated by cocultivation of saliva with preactivated cord-blood mononuclear cells, as described elsewhere [6]. Briefly, 1 mL of saliva was diluted 1:2 in RPMI 1640 and then centrifuged at 2000 *g*. Supernatants were filtered through a 0.45-μm-pore filter, were inoculated onto preactivated cord-blood mononuclear cells, and were centrifuged at 2000 *g* for 60 min, to enhance viral absorption. After centrifugation, cells were cultured in growth medium at 37°C in 5% CO_2. Viral isolation was considered to be achieved when large, round cell formation and specific immunofluorescence staining with a monoclonal antibody to HHV-7 (KR4; provided by T. Okuno, Department of Microbiology, Hyogo College of Medicine, Hyogo, Japan) were observed.

Serological assays. Specific anti–HHV-6 and anti–HHV-7 IgG antibody titers were measured by indirect immunofluorescence, as described elsewhere [4, 5], with the FG-1 HHV-6 strain and the Sato HHV-7 strain as standard antigens, respectively. An antibody titer was defined as the reciprocal of the serum dilution necessary to obtain specific fluorescence. Anti-CMV IgG antibody titers were measured by ELISA in a commercial laboratory (SRL, Tokyo, Japan).

DNA extraction and real-time PCR. DNA samples extracted from 200 μL of saliva by use of a QIAamp Blood Kit (QIAGEN) were stored at −20°C. Real-time PCR was used to quantitate CMV, HHV-6, and HHV-7 DNA copy numbers. Details on the real-time PCR methods used to assess CMV and HHV-6 DNA loads have been described elsewhere [22]. The validity of this real-time PCR technique to quantify HHV-7 DNA was established by use of the *U31* gene, which encodes the HHV-7 tegument protein, as a positive control sequence. The forward (5′-AAAGAATGGTTTTGTTCAACTCCAA-3′) and reverse (5′-ACATTCACTTTGCGTGCATTTTC-3′) primers and the probe (5′-[FAM]-TCATCGAGAACATAGGAGAAGCTCC-AGCA-[TAMRA]-3′) sequence were designed by use of Primer Express (version 3.0; PE Applied Biosystems) [23]. PCRs were performed by use of the TaqMan PCR Kit (PE Applied Biosystems). Standard curves for each viral DNA assessment were constructed on the basis of the cycle-threshold (C_T) values obtained from serial dilution of plasmid DNA containing the target sequence. To construct plasmids containing the target sequence, viral DNAs were amplified from the AD-169 CMV strain, the Z29 HHV-6 strain, or the RK HHV-7 strain with the primers designed for real-time PCR. Amplified PCR products were then subcloned by use of the pGEM-T Vector System (Promega), in accordance with the manufacturer's protocol. The C_T values for each sample were plotted on a standard curve, allowing automatic calculation of the DNA copy number by use of Sequence Detector (version 1.6; PE Applied Biosystems). Each sample was tested in duplicate; the mean of the 2 values defined the sample copy number.

Statistical analysis. Viral loads were compared by the Mann-Whitney *U* test, by use of StatView (version J-5.0; SAS Institute).

RESULTS

Association between HHV-7 isolation and viral DNA load, as determined by real-time PCR. It is complicated and time-consuming to isolate HHV-7; for this reason, if HHV-7 DNA load, as determined by real-time PCR, correlates well with viral isolation, measurement of viral DNA load would be a valuable tool for monitoring active viral infection. Because the amount of HHV-7 DNA in saliva from healthy adults is stable in each individual, 6 saliva samples serially collected over a course of 6 weeks from each healthy adult in the present study were used to analyze the correlation between viral isolation and viral DNA load. During the observation period, 114 saliva samples were collected from 19 healthy adults, all of whom were seropositive for HHV-7. The frequency of HHV-7 shedding (calculated as the number of saliva samples from which active virus was isolated per the 6 samples collected from the subject during the observation period) in the saliva samples varied between individuals, as was expected (figure 1). Virus could not be isolated from any of the 6 saliva samples from 1 (5.2%) of the healthy adults. Two (10.5%) of the healthy adults excreted significant amounts of virus in all saliva samples. The saliva samples from the healthy adults with frequent viral shedding (5 or 6 positive results; figure 2*A*) generally had higher viral DNA loads than did the saliva samples from the healthy adults with intermediate viral shedding (3 or 4 positive results) (figure 2*B*). Several healthy adults with infrequent viral shedding (0–2 positive results), however, had high viral DNA loads (figure 2*C*). There were no statistical differences in HHV-7 DNA load between the

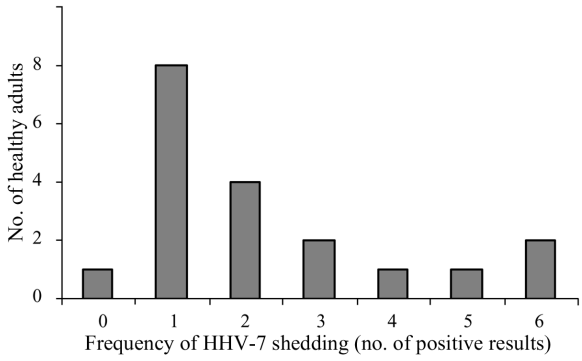

Figure 1. Frequency of human herpesvirus (HHV)–7 shedding in 6 saliva samples serially collected from healthy adults during the 6-week observation period. Virus was isolated from saliva by cocultivation with preactivated cord-blood mononuclear cells.

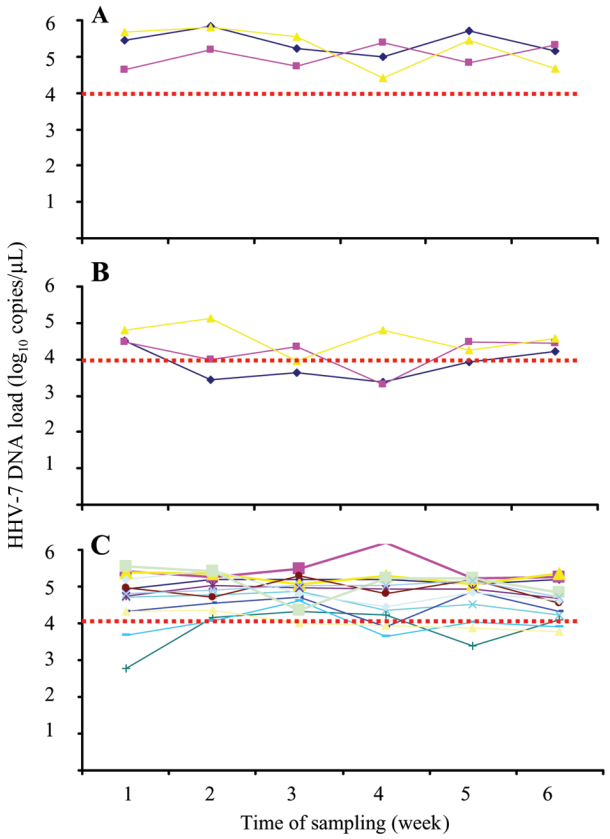

Figure 2. Association between frequency of human herpesvirus (HHV)–7 shedding and DNA load in saliva samples from healthy adults ($n = 19$). Each line denotes the HHV-7 DNA loads of 1 healthy adult. *A,* Frequent shedding (5 or 6 positive results during the 6-week observation period; $n = 3$); *B,* Intermediate shedding (3 or 4 positive results; $n = 3$); *C,* Infrequent shedding (0–2 positive results; $n = 13$). The dotted red lines are included to facilitate comparison among the groups and do not indicate data.

saliva samples from which active virus was isolated and those from which virus was not isolated ($P = .2837$) (table 2).

Viral DNA load for 3 β-herpesviruses in saliva. The kinetics of changes in viral DNA load for the 3 β-herpesviruses were compared among the healthy adults, the patients with adult-onset Still disease, and the patients with other connective tissue diseases (figure 3). It is likely that HHV-7 DNA load differed among the 3 groups. In contrast to HHV-7 DNA load, both HHV-6 and CMV DNA load varied significantly during the observation period. HHV-7 DNA load was higher than HHV-6 DNA load for all 3 groups. CMV DNA was detected in only 2 patients with other connective tissue diseases and 2 patients with adult-onset Still disease. No CMV DNA was detected in the saliva samples from the healthy adults, despite their CMV seropositivity.

To compare the viral DNA loads among the 3 groups, we calculated the mean viral DNA copy numbers in the 6 saliva samples serially collected from each subject. The mean viral DNA loads for the subjects were then compared by the Mann-Whitney *U* test (table 3). Because no CMV shedding was detected in any of the healthy adults, only the HHV-6 and HHV-7 DNA loads were analyzed. HHV-7 DNA load was significantly higher in the patients with adult-onset Still disease (mean ± SD, 649,149 ± 751,504 copies/mL) than in either the healthy adults (mean ± SD, 119,382 ± 119,878 copies/mL; $P = .0224$) or the patients with other connective tissue diseases (mean ± SD, 10,733 ± 21,896 copies/mL; $P = .0003$). There were no statistically significant differences, however, in HHV-6 DNA load between the patients with adult-onset Still disease (mean ± SD, 875 ± 1120 copies/mL) and the healthy adults (mean ± SD, 312 ± 269 copies/mL; $P = .6438$) or between the patients with adult-onset Still disease and the patients with other connective tissue diseases (mean ± SD, 489 ± 1325 copies/mL; $P = .0862$). The patients with other connective tissue diseases had significantly lower viral DNA loads than did the healthy adults for both HHV-6 (mean ± SD, 489 ± 1325 vs. 312 ± 269 copies/mL; $P = .0120$) and HHV-7 (mean ± SD, 10,733 ± 21,896 vs. 119,382 ± 119,878 copies/mL; $P < .0001$). Because the diseases of a subset of the patients with other connective tissue diseases (11/14 patients) were inactive, we compared HHV-6 and HHV-7 DNA loads between patients in an active disease period and those in an inactive disease period, to determine the effect of disease activity on viral DNA load. We could not identify a statistical difference in either HHV-6 or HHV-7 DNA load between the patients in an active disease state and those in an inactive disease state (mean ± SD for HHV-6, 521 ± 902 vs. 480 ± 1456 copies/mL; $P = .6854$) (mean ± SD for HHV-7, 25,772 ± 42,219 vs. 6631 ± 13,432 copies/mL; $P = .5858$), although the number of patients in an active disease state was small.

DISCUSSION

Clinical isolation of HHV-7 requires the cocultivation of samples with preactivated cord-blood mononuclear cells, a time-consuming and troublesome process, especially for the analysis of a large number of samples. Real-time PCR often replaces viral isolation in the analysis of HHV-7 infection. However, information on the association between viral DNA load in saliva and viral isolation has been limited. We attempted to correlate viral DNA load with viral isolation in serially collected saliva samples from healthy adults. The kinetics of changes in HHV-7 DNA load in saliva observed in this study

Table 2. Comparison of human herpesvirus (HHV)–7 DNA load in saliva samples positive or negative by the viral-isolation assay.

Isolation of HHV-7	HHV-7 DNA load in saliva, mean ± SD, copies/mL	*P*
Yes	143,766 ± 168,839	.2837
No	105,158 ± 191,571	

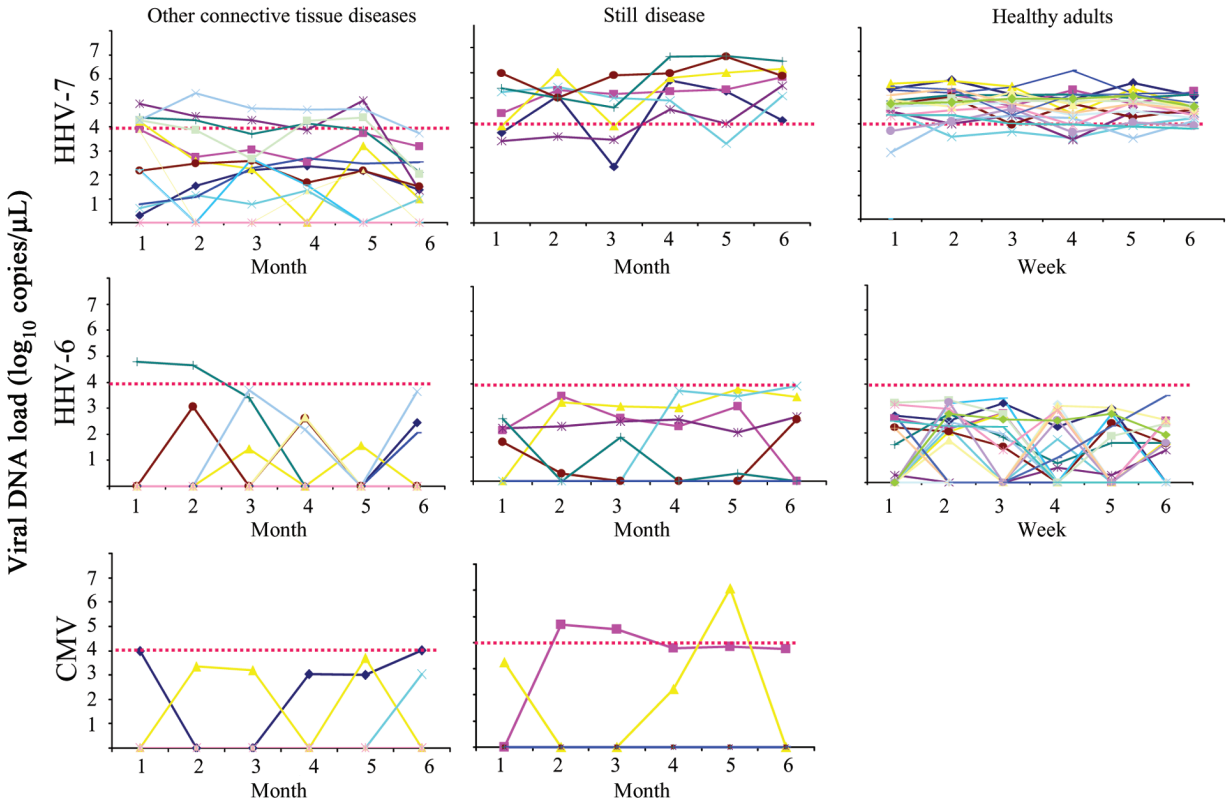

Figure 3. Comparison among cytomegalovirus (CMV), human herpesvirus (HHV)–6, and HHV-7 DNA loads in saliva samples from healthy adults, patients with adult-onset Still disease, and patients with other connective tissue diseases. The kinetics of changes in viral DNA load in serially collected saliva samples were also examined. Because no CMV DNA could be detected in any of the samples from the healthy adults, data on CMV DNA loads for this group are not shown in this figure. The dotted red lines are included to facilitate comparison among the groups and do not indicate data.

were consistent with the results of 2 previous longitudinal studies (in which samples were collected for 1 and 3 months) [24, 25], demonstrating that HHV-7 DNA load in saliva varies between individuals. Because 3 healthy adults with frequent viral shedding appeared to excrete higher levels of viral DNA than did 3 healthy adults with intermediate viral shedding (figure 2*A* and 2*B*), those with frequent viral shedding likely excrete higher copy numbers of viral DNA in their saliva than do those with lower levels of viral shedding. Several healthy adults with infrequent viral shedding, however, excreted significant quantities of viral DNA in saliva, particularly the 3 individuals. Despite this trend, there was no statistical difference in HHV-7 DNA load between the saliva samples from which active virus was isolated and those from which no virus was isolated ($P = .2837$) (table 2). Although the number of subjects was smaller than that examined in this study, similar findings have been reported by Fujiwara et al. [25]. This discrepancy may be the result of the presence of a substance that inhibits viral isolation or of large volumes of fragmented viral DNA in the saliva of certain individuals. To better understand the viral life cycle, further study is necessary to elucidate the molecular mechanisms underlying this discrepancy between viral isolation and real-time PCR analysis. These findings suggest that, although real-time PCR can be used for monitoring viral replication in the salivary glands, it might be inappropriate for an analysis of viral transmission because of the lack of a strong correlation between infectious viral particles and viral DNA load in some saliva samples.

In the present study, we measured the viral DNA loads of 3 β-herpesviruses in saliva samples from healthy adults and patients with connective tissue diseases by real-time PCR. We sought to evaluate viral replication in the salivary glands to establish an association between viral infection and connective tissue disease, potentially implicating the host factors involved in the control of tissue viral replication. We believe that real-time PCR is appropriate to address these questions.

Although these 3 β-herpesviruses share many genetic and biological similarities, the kinetics of changes in viral DNA load in saliva differed between CMV, HHV-6, and HHV-7 (figure 3). Although the quantity of HHV-7 DNA in saliva differed among subjects, the majority of the patients with connective tissue diseases and the healthy adults excreted consistent amounts of viral DNA in saliva. Both the frequency of HHV-6 DNA excretion and the HHV-6 DNA loads, however, were low in comparison

Table 3. Comparison of mean human herpesvirus (HHV) DNA loads (HHV-6 and HHV-7) in saliva samples from healthy adults, patients with adult-onset Still disease, and patients with other connective tissue diseases.

Virus, group	Viral DNA load in saliva, mean ± SD, copies/mL	*P*		
		Healthy vs. Still	Still vs. other	Healthy vs. other
HHV-6		.6438	.0862	.0120
Healthy	312 ± 269			
Adult-onset Still disease	875 ± 1120			
Other connective tissue diseases	489 ± 1325			
HHV-7		.0224	.0003	<.0001
Healthy	119,382 ± 119,878			
Adult-onset Still disease	649,149 ± 751,504			
Other connective tissue diseases	10,733 ± 21,896			

to those for HHV-7. CMV DNA was detected in saliva samples from only a few patients with connective tissue diseases, likely in samples collected after immunosuppression. Several investigators have reported frequencies of viral detection in either healthy control subjects or immunosuppressed patients, such as patients with AIDS [26, 27]. Because differences in the ethnicities of subjects or the details of PCR methods might affect viral DNA detection in saliva samples, it is difficult to compare directly all of these observations. Although our findings are similar to those reported by Lucht et al. [27], they compared healthy control subjects and patients with AIDS by qualitative PCR. In addition, although the number of subjects was limited, Fujiwara et al. [25] reported that HHV-7 copy numbers in saliva were higher than those for HHV-6. To our knowledge, ours is the first report analyzing longitudinal DNA loads in saliva for 3 β-herpesviruses. In conjunction with previous studies, our results should be helpful in examining the kinetics of changes in viral load in patients with other diseases.

In the present study, the patients with adult-onset Still disease constantly excreted a significantly higher number of copies of HHV-7 DNA in their saliva than did the patients with other connective tissue diseases and the healthy adults (figure 3 and table 3). These data suggest that a number of host factors in patients with adult-onset Still disease may function to accelerate HHV-7 replication in the salivary glands. Patients with Still disease exhibit a characteristic hypercytokinemia. Proinflammatory cytokines, such as tumor necrosis factor–α, play an important role in CMV reactivation by inducing the transcription of CMV immediate early genes via induction of NF-κB [28–31]. Although the mechanisms underlying both HHV-7 reactivation and the regulation of HHV-7 immediate early genes are not well understood, the advent of hypercytokinemia during adult-onset Still disease may control the up-regulation of HHV-7 immediate early gene expression, resulting in the reactivation of the virus. Impairment of host immune responses against HHV-7 may accelerate viral replication in the salivary glands. Although CMV-specific T cell–mediated immunity is important in the prevention of symptomatic viremia, it is not important in the local control of viral replication [32–34]. To elucidate the mechanisms underlying the up-regulation of HHV-7 replication in the salivary glands of patients with adult-onset Still disease, we explored both the virological and immunological aspects of this regulation. No activation of CMV infection was demonstrated in the patients with adult-onset Still disease in the present study. Although the sensitivity of real-time PCR for CMV might be insufficient, low rates of CMV excretion in saliva have been demonstrated by other investigators [27]. Therefore, it is possible that the salivary glands are not the main site of persistent infection of the virus. Further in-depth analysis is needed to elucidate an association between CMV infection and connective tissue disease.

Both HHV-6 and HHV-7 DNA loads in saliva were significantly lower in the patients with other connective tissue diseases than in the patients with adult-onset Still disease and the healthy adults (figure 3 and table 3). Because the majority of patients (11/14) with other connective tissue diseases were in an inactive disease period, we examined the effect of disease activity on viral DNA load in saliva and observed that disease activity did not affect either HHV-6 or HHV-7 DNA load in saliva. Thus, a portion of the host factors involved in other connective tissue diseases may prevent replication of these β-herpesviruses in the salivary glands. Because multiple factors, including age, underlying disease, and treatment protocols, may be involved in the control of viral replication in the salivary glands, a detailed multivariate analysis of a large number of patients should be undertaken in the future. The mechanisms underlying the regulation of β-herpesvirus in the salivary glands are not well understood. Future work is necessary to elucidate these mechanisms, which hopefully will provide a greater understanding of the viral life cycle and the clinical course of connective tissue diseases.

Acknowledgments

We thank Akiko Yoshikawa and Maki Sawamura, for technical support.

References

1. Frenkel N, Schirmer EC, Wyatt LS, et al. Isolation of a new herpesvirus from human CD4+ T cells. Proc Natl Acad Sci USA **1990**; 87:748–52.
2. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **1988**; 1:1065–7.
3. Tanaka K, Kondo T, Torigoe S, Okada S, Mukai T, Yamanishi K. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). J Pediatr **1994**; 125:1–5.
4. Yoshikawa T, Suga S, Asano Y, Yazaki T, Kodama H, Ozaki T. Distribution of antibodies to a causative agent of exanthem subitum (human herpesvirus-6) in healthy individuals. Pediatrics **1989**; 84:675–7.
5. Yoshikawa T, Asano Y, Kobayashi I, et al. Seroepidemiology of human herpesvirus 7 in healthy children and adults in Japan. J Med Virol **1993**; 41:319–23.
6. Wyatt LS, Frenkel N. Human herpesvirus 7 is a constitutive inhabitant of adult human saliva. J Virol **1992**; 66:3206–9.
7. Hidaka Y, Liu Y, Yamamoto M, et al. Frequent isolation of human herpesvirus 7 from saliva samples. J Med Virol **1993**; 40:343–6.
8. Ihira M, Yoshikawa T, Ohashi M, et al. Variation of human herpesvirus 7 shedding in saliva. J Infect Dis **2003**; 188:1352–4.
9. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol **2003**; 24:584–8.
10. Sekigawa I, Nawata M, Seta N, Yamada M, Iida N, Hashimoto H. Cytomegalovirus infection in patients with systemic lupus erythematosus. Clin Exp Rheumatol **2002**; 20:559–64.
11. Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K. Latent human herpesvirus 6 infection of human monocytes/macrophages. J Gen Virol **1991**; 72:1401–8.
12. Katsafanas GC, Schirmer EC, Wyatt LS, Frenkel N. In vitro activation of human herpesviruses 6 and 7 from latency. Proc Natl Acad Sci USA **1996**; 93:9788–92.
13. Wilborn F, Schmidt CA, Lorenz F, et al. Human herpesvirus type 7 in blood donors: detection by the polymerase chain reaction. J Med Virol **1995**; 47:65–9.
14. Yoshikawa T. Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. Br J Haematol **2004**; 124:421–32.
15. Lusso P, Malnati MS, Garzino-Demo A, Crowley RW, Long EO, Gallo RC. Infection of natural killer cells by human herpesvirus 6. Nature **1993**; 362:458–62.
16. Furukawa M, Yasukawa M, Yakushijin Y, Fujita S. Distinct effects of human herpesvirus 6 and human herpesvirus 7 on surface molecule expression and function of CD4+ T cells. J Immunol **1994**; 152:5768–75.
17. Isegawa Y, Ping Z, Nakano K, Sugimoto N, Yamanishi K. Human herpesvirus 6 open reading frame U12 encodes a functional beta-chemokine receptor. J Virol **1998**; 72:6104–12.
18. Nakano K, Tadagaki K, Isegawa Y, Aye MM, Zou P, Yamanishi K. Human herpesvirus 7 open reading frame U12 encodes a functional beta-chemokine receptor. J Virol **2003**; 77:8108–15.
19. Smith AP, Paolucci C, Di Lullo G, Burastero SE, Santoro F, Lusso P. Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6. J Virol **2005**; 79:2807–13.
20. Cermelli C, Berti R, Soldan SS, et al. High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection. J Infect Dis **2003**; 187:1377–87.
21. Goodman AD, Mock DJ, Powers JM, Baker JV, Blumberg BM. Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions. J Infect Dis **2003**; 187:1365–76.
22. Tanaka N, Kimura H, Hoshino Y, et al. Monitoring four herpesviruses in unrelated cord blood transplantation. Bone Marrow Transplant **2000**; 26:1193–7.
23. Yoshikawa T, Ihira M, Akimoto S, et al. Detection of human herpesvirus 7 DNA by loop-mediated isothermal amplification. J Clin Microbiol **2004**; 42:1348–52.
24. Kidd IM, Clark DA, Ait-Khaled M, Griffiths PD, Emery VC. Measurement of human herpesvirus 7 load in peripheral blood and saliva of healthy subjects by quantitative polymerase chain reaction. J Infect Dis **1996**; 174:396–401.
25. Fujiwara N, Namba H, Ohuchi R, et al. Monitoring of human herpesvirus-6 and -7 genomes in saliva samples of healthy adults by competitive quantitative PCR. J Med Virol **2000**; 61:208–13.
26. Gautheret-Dejean A, Aubin JT, Poirel L, et al. Detection of human betaherpesvirinae in saliva and urine from immunocompromised and immunocompetent subjects. J Clin Microbiol **1997**; 35:1600–3.
27. Lucht E, Brytting M, Bjerregaard L, Julander I, Linde A. Shedding of cytomegalovirus and herpesviruses 6, 7, and 8 in saliva of human immunodeficiency virus type 1–infected patients and healthy controls. Clin Infect Dis **1998**; 27:137–41.
28. Stein J, Volk HD, Liebenthal C, Kruger DH, Prosch S. Tumor necrosis factor alpha stimulates the activity of the human cytomegalovirus major immediate early enhancer/promoter in immature monocytic cells. J Gen Virol **1993**; 74:2333–8.
29. Fietze E, Prosch S, Reinke P, et al. Cytomegalovirus infection in transplant recipients: the role of tumor necrosis factor. Transplantation **1994**; 58:675–80.
30. Docke WD, Prosch S, Fietze E, et al. Cytomegalovirus reactivation and tumor necrosis factor. Lancet **1994**; 343:268–9.
31. Simon CO, Seckert CK, Dreis D, Reddehase MJ, Grzimek NK. Role for tumor necrosis factor alpha in murine cytomegalovirus transcriptional reactivation in latently infected lungs. J Virol **2005**; 79:326–40.
32. Chen SF, Tu WW, Sharp MA, et al. Antiviral CD8 T cells in the control of primary human cytomegalovirus infection in early childhood. J Infect Dis **2004**; 189:1619–27.
33. Jonjic S, Mutter W, Weiland F, Reddehase MJ, Koszinowski UH. Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes. J Exp Med **1989**; 169:1199–212.
34. Lucin P, Pavic I, Polic B, Jonjic S, Koszinowski UH. Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. J Virol **1992**; 66:1977–84.