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Detection of Human Herpesvirus 6 and Human Herpesvirus 7 in the Submandibular Gland, Parotid Gland, and Lip Salivary Gland by PCR

EIJI SADA,[1]* MASAKI YASUKAWA,[2] CHIZURU ITO,[3] AKIRA TAKEDA,[4] TAKAHIKO SHIOSAKA,[1] HIROAKI TANIOKA,[3] AND SHIGERU FUJITA[2]

Department of Medical Technology, Ehime Medical College of Health Science, Tobe,[1] and First Department of Internal Medicine[2] and Department of Oral and Maxillofacial Surgery,[3] Ehime University School of Medicine, Shigenobu, Ehime, and Division of Clinical Immunology, Jichi Medical School, Tochigi,[4] Japan

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In order to define the major sites of persistence of human herpesvirus 6 (HHV-6) and HHV-7, PCR with DNAs from more than 100 specimens of 3 different salivary glands was performed. HHV-6 DNA was detected in 52 (88.1%) of 59 submandibular gland, 17 (63.0%) of 27 parotid gland, and 9 (52.9%) of 17 lip salivary gland specimens. On the other hand, HHV-7 DNA was detected in 59 (100%) of 59 submandibular gland, 23 (85.2%) of 27 parotid gland, and 10 (58.8%) of 17 lip salivary gland specimens. These findings demonstrate that salivary glands are a site of persistent infection of both HHV-6 and HHV-7 and that among the three types of salivary gland examined, the submandibular gland is the primary one in which these herpesviruses, especially HHV-7, persist.

The great majority of individuals are infected with human herpesvirus 6 (HHV-6) and HHV-7 during childhood (13, 14), and like other herpesviruses, these are thought to persist as latent infections throughout life. The frequent isolation of HHV-6 from the saliva of healthy individuals suggests that salivary glands are major sites harboring persistent HHV-6 infection (3, 5, 6, 9, 10). However, several recent studies demonstrating that HHV-7, rather than HHV-6, is frequently isolated from saliva (2, 4, 7, 12) contradict previous reports. In the present study, to clarify the major sites of persistent infection with HHV-6 and HHV-7, the presence of HHV-6 and HHV-7 genomes in three different types of salivary gland, the submandibular, parotid, and lip salivary glands, was examined by the highly sensitive nested PCR method.

A total of 103 salivary gland specimens, including 59 submandibular glands, 27 parotid glands, and 17 minor salivary glands (lip salivary glands), were obtained at surgery or autopsy from adult patients with oral cancer, sialoadenitis, pleomorphic adenoma of the salivary glands, and other diseases. All the specimens contained normal salivary gland tissue. None of the patients were severely immunocompromised.

The detection of viral genomes in the specimens was performed by nested PCR as follows. DNA was extracted from each sample by a standard method using phenol-chloroform and was precipitated with ethanol. Specific primers were synthesized in a DNA synthesizer (Applied Biosystems, Foster City, Calif.) on the basis of the published sequences (1, 8). The sequences of the outer pair for HHV-6 DNA were 5′-GTGT TTCCATTGTACTGAAACCGGT-3′ and 5′-TAAACATCA ATGCGTTGCATACAGT-3′, and those of the inner pair were 5′-CCTTGTGTAGGTGGTCGAATGCGAC-3′ and 5′-ACAGCGCAGCAACATGTTTCAGAGC-3′. The sequences of the outer pair for HHV-7 DNA were 5′-TATCCCAGCTG TTTTCATATAGTAAC-3′ and 5′-GCCTTGCGGTAGCAC TAGATTTTTTG-3′, and those of the inner pair were 5′-CA GAAATGATAGACAGATGTTGG-3′ and 5′-TAGATTTTT TGAAAAAGATTTAATAAC-3′. These primers appeared to specifically amplify HHV-6 DNA or HHV-7 DNA but not DNAs of herpes simplex virus type 1 and type 2, Epstein-Barr virus, or human cytomegalovirus (data not shown). The expected sizes of the nested PCR amplification products for HHV-6 and HHV-7 were 492 and 124 bp, respectively. In order to confirm the presence of intact DNA in a sample, PCR for exon 50 of the dystrophin gene was also performed for each sample with primers 5′-CACCAAATGGATTAAGATGTTC ATGAAT-3′ and 5′-TCTCTCTCACCCAGTCATCACTTCA TAG-3′, producing a 271-bp product. The PCR was performed in a microcentrifuge tube with a thermal cycler (Takara Shuzo, Shiga, Japan). The incubation mixture in a total volume of 20 μl contained 0.2 μg of denatured DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 1 U of *Taq* DNA polymerase (Takara Shuzo), and the outer primers at a concentration of 1.0 μM. The sample DNA was subjected to 20 amplification cycles as a primary PCR. After the first round of amplification, 2 μl of the PCR product was added to 18 μl of reaction mixture containing 1.0 μM inner primers with the same ingredients as the first PCR mixture. A nested PCR was performed for 30 amplification cycles. The amplified products were subjected to electrophoresis on a 2.5% agarose gel, and DNA was visualized by UV fluorescence after staining with ethidium bromide. Each sample was assayed at least twice, and negative samples were analyzed five times to confirm the absence of virus DNA. The Z-test of two independent proportions using Yates' correction for continuity was used to compare the proportions of samples in which virus DNA was detectable to those in which it was undetectable.

The representative PCR data are shown in Fig. 1. Dystrophin DNA was clearly amplified in all the samples analyzed, indicating that DNA in each sample was intact. Lane 3 showed a positive reaction for both HHV-6 and HHV-7 DNAs. Lanes 4 and 5 were positive for HHV-7 DNA and HHV-6 DNA, respectively. Lane 6 was negative for either HHV-6 or HHV-7.

* Corresponding author. Mailing address: Department of Medical Technology, Ehime Medical College of Health Science, Tobe, Ehime 791-21, Japan. Phone: 81-89-958-2111. Fax: 81-89-958-2177.

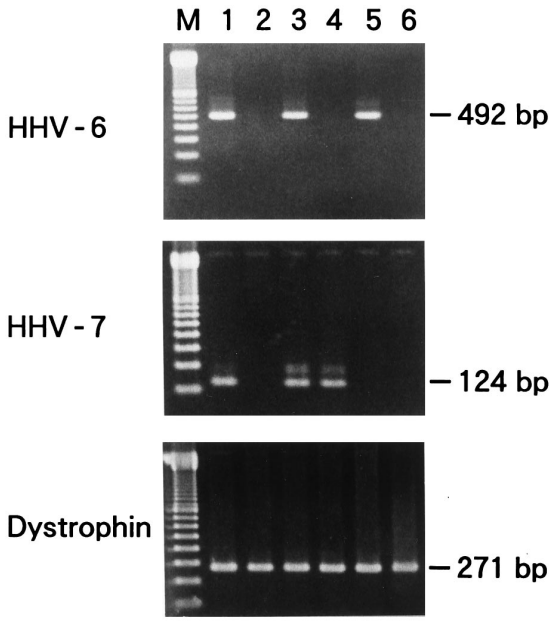

FIG. 1. Detection of HHV-6, HHV-7, and dystrophin DNAs by PCR. PCR products were subjected to electrophoresis on 2.5% agarose gel and stained with ethidium bromide. Lane M, size marker; lane 1, positive control; lane 2, negative control; lanes 3 to 6, samples.

Table 1 shows a summary of the PCR results for HHV-6 and HHV-7 DNAs in 103 specimens of three types of salivary gland. Among the three types, the submandibular gland appeared to be the site in which both the HHV-6 and HHV-7 genomes were most frequently detected. The proportion of specimens positive for HHV-6 DNA and HHV-7 DNA was significantly highest for the submandibular gland among the three types of salivary gland ($P < 0.05$ and $P < 0.05$). It was noteworthy that HHV-7 DNA was amplified in all 59 specimens of submandibular gland examined. On the other hand, the HHV-6 and HHV-7 genomes were detected in 17 (63.0%) of 27 and 23 (85.2%) of 27 parotid glands, respectively. These data are compatible with the recent study by Di Luca et al. (4), in which eight parotid glands were analyzed for HHV-6 and HHV-7 DNAs. The proportions of lip salivary glands positive for HHV-6 DNA and HHV-7 DNA were 9 (52.9%) of 17 and 10 (58.8%) of 17, respectively.

Although previous studies showing the highly frequent isolation of HHV-6 and HHV-7 from the saliva of healthy individuals have suggested that salivary glands are a site for persistence of these viruses (2–7, 9, 10, 12), any differences in their tropism among different salivary glands remain unclear. In the present study, we demonstrated that among three types of salivary gland, the submandibular gland is the primary one in which HHV-6 and HHV-7 persist.

TABLE 1. Frequency of HHV-6 and HHV-7 detected in salivary glands

Virus	No. of samples (%) in which virus was detected[a]		
	Submandibular gland ($n = 59$)	Parotid gland ($n = 27$)	Lip salivary gland ($n = 17$)
HHV-6	52 (88.1)	17 (63.0)	9 (52.9)
HHV-7	59 (100)	23 (85.2)	10 (58.8)

[a] The values are the numbers of samples in which viral DNA was amplified. The percentages of positive samples are shown in parentheses.

Human salivary glands can be classified on the basis of their secretory products, the principal types of secretion being serous secretions and mucous secretions. The parotid gland is a purely serous gland, whereas the submandibular gland is mixed, producing both serous and mucous secretory products. On the other hand, the minor salivary glands are variable, being predominantly mucus producing, but some are mixed. Furthermore, salivary glands differentiate from different origins: the submandibular gland arises from endoderm, and the parotid gland arises from ectoderm (11). Therefore, such differences in the characteristics of salivary glandular cells may affect the tropisms of HHV-6 and HHV-7.

In summary, we have shown that the HHV-6 and HHV-7 genomes are frequently present in salivary glands, especially the submandibular gland. Taken together with recent studies demonstrating that HHV-7 is frequently isolated from saliva, whereas HHV-6 is rarely present in saliva (10, 12, 13), it is suggested that HHV-7 productively infects salivary glands, whereas HHV-6 shows latent infection with a low level of replication in these organs.

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