Virological Analysis in Patients with Human Herpes Virus 6–Associated Ocular Inflammatory Disorders

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Purpose. To determine whether human herpes virus 6 (HHV-6) genomic DNA and mRNA can be detected in ocular samples from patients with inflammatory disorders, and whether viral replication is involved in the development of inflammation in the eye.

Methods. After informed consent was obtained, ocular fluid samples (aqueous humor and vitreous fluids) were collected from 350 patients with uveitis or endophthalmitis. Corneal samples were also collected from 65 patients with corneal infections. Multiplex PCR was performed to screen ocular samples from the patients for HHV-1 to HHV-8. Samples were also assayed for HHV-6 DNA using quantitative real-time PCR. Primers for nested RT-PCR were designed to detect amplification of mRNA (HHV-6 A IE1 U90).

Results. PCR results indicated a total of seven patients with uveitis or endophthalmitis (7/350, 2% +) and a single patient with corneal inflammatory disease were positive for HHV-6 DNA (1/65, 1.5% +). These eight patients had high copy numbers of HHV-6 DNA, with values ranging from 4.0×10^3 to 5.1×10^6 copies/mL. Real-time PCR analysis indicated that two of these cases were HHV-6 variant A and six cases were variant B. In addition, HHV-6 mRNA was clearly detected in vitreous cells collected from one of the patients, suggesting that viral replication may occur in the eye.

Conclusions. Our results indicate that HHV-6 infection/reactivation is implicated in ocular inflammatory diseases. (www.umin.ac.jp/ctr/index/htm number, R000002708.) (*Invest Ophthalmol Vis Sci.* 2012;53:4692–4698) DOI:10.1167/iovs.12-10095

Human herpesvirus 6 (HHV-6) is the causative agent of exanthema subitum in children and has been associated with a number of inflammatory and neurological disorders worldwide. It has been implicated in hepatitis, pneumonitis, and severe infections of the central nervous system in both immunosuppressed and immunocompetent patients. HHV-6 can reactivate from its latent form after primary infection. In the case of eye diseases, it has been implicated in AIDS-associated retinitis,[1–3] uveitis,[4–8] corneal inflammation,[9] and optic neuropathy.[10–12] Two variants of HHV-6 have been identified. HHV-6A is less often associated with disease and has a greater predilection for neural cells than HHV-6B.[13] Although HHV-6A DNA is frequently found in the nervous system of infected adults, HHV-6B DNA is rarely present in ocular fluids, although it is found in most documented primary HHV-6 infections.

Diagnosis of clinically relevant HHV-6 can be challenging due to the high prevalence of infection and viral persistence. Detection of viral nucleic acids may indicate active or latent infections, depending on the clinical setting and specimens tested. Quantitative PCR methods have been established to detect active infections. Detection of HHV-6 DNA in plasma or serum is indicative of active replication and is therefore more directly interpretable.[14,15] Using these PCR techniques, several investigators previously reported that HHV-6 genomic DNA is found in ocular inflammatory diseases, including infectious uveitis and endophthalmitis[1–8]; however, involvement of HHV-6 in ocular infections has not yet been clearly demonstrated.

Therefore, we designed experiments to investigate whether ocular samples from patients with various ocular inflammatory disorders contain HHV-6 genomic DNA, whether ocular samples from noninflammatory patients also contain HHV-6 DNA, whether positive cases are either HHV-6 variant A or B, and whether HHV-6 mRNA as well as a high copy numbers of HHV-6 DNA can be detected in positive samples.

Materials and Methods

Subjects

The first patient group was examined between 2006 and 2010 at the Tokyo Medical and Dental University Hospital, Kyoto Prefectural University Hospital, and Shinkawabashi Hospital in Japan. After informed consent was obtained, ocular fluid samples were collected from patients with uveitis (infectious and noninfectious) or endophthalmitis. This group included consecutive patients with uveitis or endophthalmitis ($n = 350$), including a previously HHV-6–positive severe panuveitis case.[7] Corneal tissues were also collected from patients with ocular surface diseases (e.g., keratitis, $n = 65$). At this time, we excluded ocular tumor diseases (e.g., intraocular lymphoma) from the patient group.

In addition to the patient group, we also analyzed samples from a control group. A total of 100 samples (50 aqueous humor and 50 vitreous fluids) were collected from patients who did not have any type of ocular inflammation (age-related cataract, macular edema, retinal

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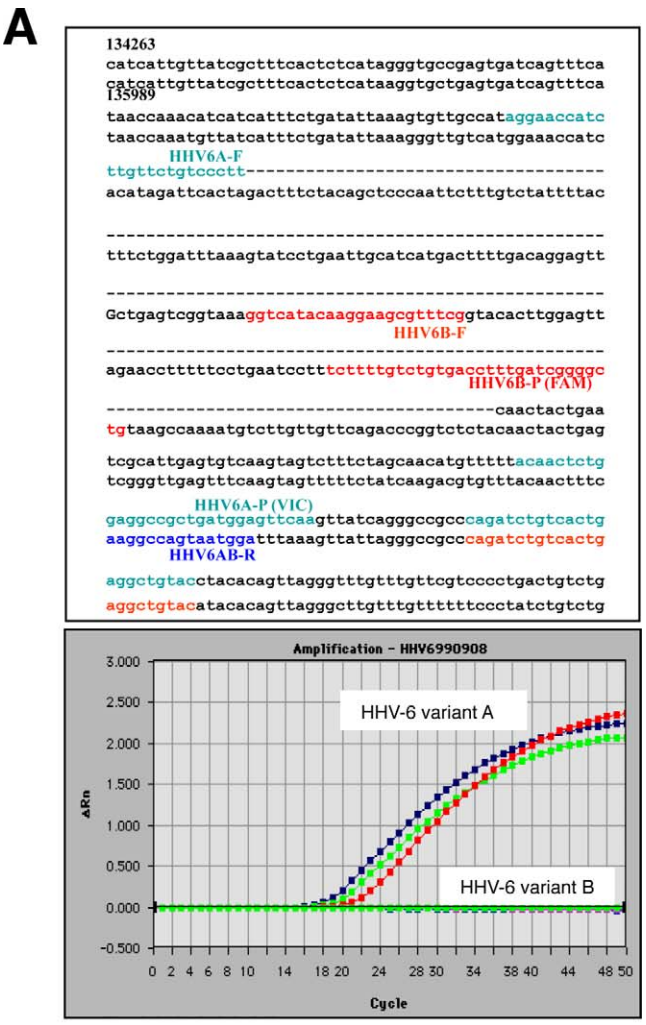

Figure 1. Amplification of HHV-6–specific DNA and mRNA. (**A**) TaqMan probes and primers used to amplify HHV-6 DNA (HHV-6A and HHV-6B). HHV-6 subtypes were identified using PCR with variant-specific primers and probes (*lower graph*). (**B**) Nested RT-PCR primers were designed to amplify HHV-6A mRNA.

detachment, idiopathic macular hole, or idiopathic epiretinal membrane).

The research followed the tenets of the Declaration of Helsinki and all study protocols were approved by the Institutional Ethics Committee of Tokyo Medical and Dental University. A clinical trial registration was conducted and information is available at www.umin.

Table 1. Clinical Findings in Patients with HHV-6–Associated Ocular Inflammatory Disorders

Case	Age / Sex	Eye	Initial Diagnosis	VA	IOP	Cornea	AC	KPs	VO	Fundus	Bacterial Examination*	Final Diagnosis
1	75 / Male	R	Pan-uveitis	0.02	15	None	Hypopyon	Mutton fat	Grade III	Retinal exudates	Culture (-) / PCR (-)	Ocular toxocariasis
2	64 / Female	L	Corneal endotheliitis	0.5	33	Edema	Cell 2+	Mutton fat	None	None	PCR (-)	HSV-1 corneal endotheliitis
3	70 / Male	L	Bacterial endophthalmitis	sl-	35	None	Hypopyon	Fine	Grade III	Retinal necrosis	Culture (+) / PCR (+)	Endogenous endophthalmitis
4	74 / Female	R	Idiopathic uveitis	0.8	16	None	Cell1+	None	Grade II	None	PCR (+)	Late postoperative endophthalmitis
5	79 / Female	L	Bacterial endophthalmitis	mm	19	None	Hypopyon	Fine	Grade II	Retinal exudates, hemorrhage	Culture (+) / PCR (+)	Acute postoperative endophthalmitis
6	71 / Female	L	Necrotic retinitis	0.04	12	None	None	None	None	Retinal necrosis, hemorrhage	PCR (-)	Cytomegalovirus retinitis
7	24 / Female	L	Posner-Schlossman syndrome	1.2	24	None	Cell 1+	Mutton fat	None	None	PCR (-)	Idiopathic uveitis
8	22 / Male	R	Karatitis	0.7	15	Infiltration	Cell1-	None	None	None	Culture (-) / PCR (+)	Bacterial karatitis

* Bacterial examination: Results for bacterial culture and/or PCR (bacterial 16S rDNA).
AC, anterior chamber; KPs, keratic precipitates; VA, visual acuity by Landolt Chart; VO, vitreous opacity.

Table 2. Virological Analysis and Treatment in Patients with HHV-6–Associated Ocular Inflammatory Disorders

Case	Ocular Sample	HHV Genome	Viral Copy No. by Real-Time PCR	HHV-6A or B	Treatment
1	Aqh	HHV-6	HHV-6: 2.4×10^6 copies/mL	HHV-6A	PSL, PPV, VCV, VGV
	VF	HHV-6, EBV	HHV-6: 2.0×10^4copies/mL, EBV: <50 copies/mL		
2	Aqh	HHV-6, HSV-1	HHV-6: 7.5×10^3copies/mL, HSV-1: 2.8×10^5 copies/mL	HHV-6B	VGV
3	VF	HHV-6	HHV-6: 5.1×10^6 copies/mL	HHV-6B	PPV, SA, IAI
4	VF	HHV-6	HHV-6: 1.1×10^4 copies/mL	HHV-6B	PPV, VGV
5	VF	HHV-6	HHV-6: 1.1×10^6 copies/mL	HHV-6B	PPV, SA, Betametasone
6	VF	HHV-6, CMV	HHV-6: 4.4×10^4 copies/mL, CMV: 1.6×10^6 copies/mL	HHV-6A	VGV
7	Aqh	HHV-6	HHV-6: 4.0×10^3 copies/mL	HHV-6B	None
8	Cornea	HHV-6	HHV-6: 3.9×10^6 copies/μg · DNA	HHV-6B	Antibiotics

Aqh, aqueous humor; IAI, intravitreal antibiotic injection; PPV, pars plana vitrectomy; PSL, prednisolone; SA, systemic antibitotics; VCV, valacyclovir; VF, vitreous fluids; VGV, valganciclovir.

ac.jp/ctr/index/htm with study number of R000002708. The study started in April 2006 and terminated in April 2010.

PCR

DNA was extracted from samples using an E21 virus minikit (Qiagen, Valencia, CA) installed on a robotic workstation for automated purification of nucleic acids (BioRobot E21, Qiagen). HHV genomic DNA in ocular samples was detected using two independent PCR assays: a qualitative multiplex PCR and a quantitative real-time PCR.[16]

The multiplex PCR was designed to qualitatively measure genomic DNA of eight human herpes viruses as follows: herpes simplex virus type 1 (HSV-1), type 2 (HSV-2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpes virus 6 (HHV-6), 7 (HHV-7), and 8 (HHV-8). PCR was performed using a LightCycler (Roche, Rotkrevz, Switzerland). Primers for HHV-6 were as follows: Forward – ACCCGAGAGATGATTTTGCG and Reverse – GCAGAAGA-CAGCAGCGAGAT. Probes were used as follows: 3′FITC-TAAG-TAACCGTTTTCGTCCCA and LcRed705-5′-GGGTCATTTATGTTATAGA. These primers and probes do not distinguish between HHV-6A and B. PCR conditions, primers, and probes specific for other HHV have been described previously.[17]

Real-time PCR was performed for detection of HHV only, following identification of genomic DNA by multiplex PCR. Real-time PCR was performed using Amplitaq Gold and the Real-Time PCR 7300 system (ABI, Foster City, CA). The sequence of the HHV-6 primers and probes are as follows: Forward – GACAATCACATGCCTGGATAATG and Reverse – TGTAAGCGTGTGGTAATGTACTAA. The probe was AG-CAGCTGGCGAAAAGTGCTGTGC. The primers and probes of other herpes viruses and the PCR conditions have been described previously.[16,17] These primers and probes do not distinguish between HHV-6A and B. TaqMan probes and primers used in the HHV-6 DNA amplifications, HHV-6 type A and HHV-6 type B, are shown in Figure 1A. The value of viral copy number in the sample was considered to be significant when more than 50 copies/mL were observed.

RT-PCR

The primers for nested RT-PCR were designed to detect mRNA (HHV-6 A IE1 U90 immediate early) as follows: first PCR Forward – GATGAACGTATGCAAGACTACC and ATGAACATGGATTGTTGCTG and Reverse – CAGCGGACTGAGCAGCTA; nested PCR Forward – CCGATCCAATGATGGAAGAA and Reverse – CAGCGGACTGAGCAGC-TA (Fig. 1B). A one-step RT-PCR was performed on 100 ng of total RNA with 0.5 μM of each primer and SuperScript III One-Step RT-PCR with platinum Taq (Life Technologies Co., Tokyo, Japan) in a final volume of 50 μL. Samples were reverse transcribed for 30 minutes at 54°C and amplified for 40 cycles consisting of denaturation for 15 seconds at 94°C, annealing for 30 seconds at 54°C, and polymerization for 20 seconds at 72°C. Following identification of a PCR product of 340 bp, nested PCR was performed on 1 μL of the first PCR solution using 0.5 μM of each primer and 200 mM deoxynucleotide triphosphates and 1.25 U of Taq DNA polymerase (Thermo Fisher Scientific, Tokyo, Japan). Monoclonal antibody (anti-taq high: Toyobo Life Science, Tokyo, Japan) was used at 0.25 μg in a buffer containing 75 mM Tris-HCl (pH = 8.8), 0.01% Tween-20, 20 mM $(NH_4)_2SO_4$, and 1.5 mM $MgCl_2$ in a final volume of 50 μL. Twenty cycles of amplification consisting of denaturation for 15 seconds at 94°C, annealing for 30 seconds at 55°C, and polymerization for 15 seconds at 72°C were performed to give a positive PCR product of 198 bp.

All ocular samples were tested for the presence of β-actin as an internal control. β-Actin mRNA RT-PCR was performed on 100 ng of total RNA with 0.5 μM each primer and SuperScript III One-Step RT-PCR with platinum Taq in a final volume of 50 μL (Forward-CTTCCTTCCTGGGCAT and Reverse-TCTTCATTGTGCTGGGT). Samples were reverse transcribed for 30 minutes at 55°C followed by 40 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C, and polymerization for 1 minute at 72°C on a thermal cycler TP-400 instrument (Takara Bio Inc., Tokyo, Japan). Raji cell lines were used as a positive control, and MOLT-4 cells were used as a negative control. PCR products were analyzed using 2% agarose gel electrophoresis and ethidium bromide staining and the positive product was 215 bp.

Results

Detection of HHV-6 Genomic DNA in Patients with Uveitis, Endophthalmitis, and Ocular Surface Diseases

We first performed multiplex PCR to screen for 8 HHVs after collecting intraocular samples from patients with various ocular inflammatory diseases. PCR results indicated that 7 (2%) of 350 patients with uveitis or endophthalmitis were positive for HHV-6 DNA. In addition, 1 (1.5%) of 65 patients tested positive for HHV-6 in a corneal tissue sample. These HHV-6–positive cases together with clinical findings are summarized in Tables 1 and 2. These eight HHV-6–positive patients were clinically suspected to have HHV-6–associated infectious diseases based on the detection of HHV-6 genome in ocular fluid or corneal tissue samples. HHV-6 DNA was not detected in any of the 100 control samples that were collected from patients without ocular inflammation.

The clinical features observed in HHV-6–positive cases at their initial presentation are summarized in Table 1. Almost all of the patients with uveitis and endophthalmitis had active ocular inflammation, that is, there were anterior chamber cells (except case 6), keratic precipitates (except cases 4 and 6), vitreous opacity (except cases 2 and 7), and fresh retinal exudates/necrosis (except cases 2, 4, and 7). In the single patient with HHV-6$^+$ keratitis (case 8 in Table 1), corneal

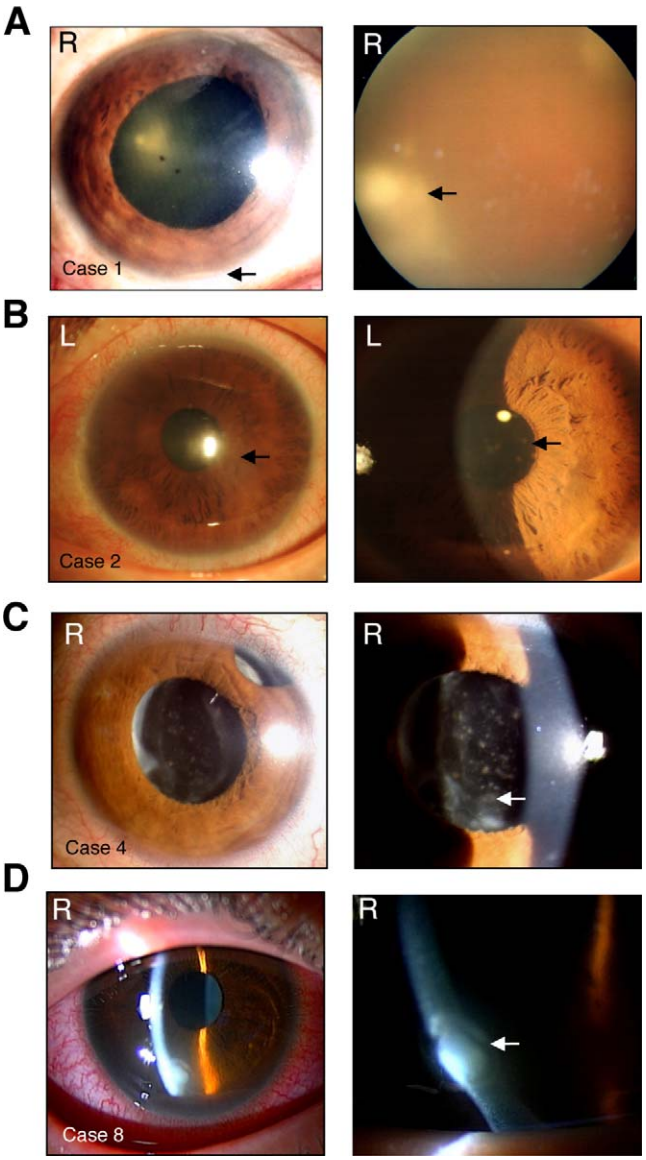

Figure 2. Slit-lamp and fundus photographs for HHV-6 infections. (**A**) Case 1: A case of ocular toxocariasis. Slit-lamp examination of right eye (RE) disclosed ciliary injection, moderate mutton-fat keratic precipitates (KPs), and severe anterior chamber cells with hypopyon (*arrow*). Funduscopic examination of the RE revealed dense vitreous opacities and yellowish white massive retinal lesions (*arrow*) in the peripheral fundus. HHV-6 DNA was detected in both aqueous humor and vitreous samples. (**B**) Case 2: A case of HSV-1–associated corneal endotheliitis. Slit-lamp examination of left eye (LE) disclosed pigmented mutton-fat–like KPs with high intraocular pressure, mild anterior chamber cells, and small-size corneal stromal edema (*arrow*). HSV-1 and HHV-6 DNA were detected in aqueous humor, but other HHV-DNA, such as VZV and CMV, was not detected. (**C**) Case 4: A case of late postoperative endophthalmitis. This patient with Vogt-Koyanagi-Harada disease had postcataract surgery 6 months earlier. Slit-lamp examination of RE disclosed ciliary injection and mild anterior chamber cells. White plaque (*arrow*) on the intraocular lens and mild inflammation were seen, and an aqueous humor sample was obtained. HHV-6 DNA and *Propionibacterium acnes* DNA were detected in the aqueous humor sample. The final diagnosis was *P. acnes*–associated late postoperative endophthalmitis. (**D**) Case 8: A case of bacterial keratitis. Slit-lamp examination of RE disclosed keratitis (*arrow*) with ciliary injection. A corneal infiltration with epithelial defect was observed and a high copy number of HHV-6 DNA was detected in corneal tissue samples.

infection, such as corneal epithelial ulcer and ciliary injection, was indicated. Representative findings including slit-lamp or fundus photographs for HHV-6–positive cases are shown in Figure 2. In addition, ocular samples from all patients were subjected to bacterial examinations, including conventional bacterial culture and bacterial broad-range PCR (bacterial 16S rDNA)[18] (Table 1). The final diagnoses were as follows: case 1, ocular toxocariasis; case 2, HSV-1 corneal endotheliitis; case 3, endogenous endophthalmitis; case 4, late postoperative endophthalmitis; case 5, acute postoperative endophthalmitis; case 6, CMV retinitis; case 7, idiopathic uveitis; case 8, bacterial keratitis (Table 1).

We next summarized the virological analysis of ocular samples from these eight HHV-6–positive patients (3 aqueous humor, 5 vitreous fluids, and 1 corneal tissue) in Table 2. Multiplex PCR was used to detect HHV infection (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-7, and HHV-8). HHV-6 was found together with EBV (only case 1), HSV-1 (only case 2), or CMV (only case 6). Figure 3 is representative of the results of the multiplex PCR where HHV-6 DNA was detected in aqueous and vitreous fluid from case 1. HHV DNA in nine ocular samples from eight cases was also measured by real-time PCR. These patients had high copy numbers of HHV-6 DNA, with values ranging from 4.0×10^3 to 5.1×10^6 copies/mL (Table 2), suggesting that viral replication may occur in the eye. Following diagnosis, 4 patients received antiviral treatment (i.e., valacyclovir or valganciclovir), which controlled their ocular inflammation (Table 2).

Detection of HHV-6 Variant A or B in Patients with HHV-6–Associated Ocular Inflammatory Disorders

HHV-6 can be classified into two groups: a variant A (HHV-6A) and a variant B (HHV-6B).[13] Distinguishing between HHV-6 subtypes is mainly accomplished using PCR techniques, including melting curve[19] or variant-specific primers.[20] Therefore, we next determined whether the HHV-6–positive cases were HHV-6A or B using real-time PCR. In this study, we designed a probe and primers for use in the HHV-6 DNA amplification. The paired primers and TaqMan probes used for detection of HHV-6A and HHV-6B are shown in Figure 1A. By using several different primers and probes, we were able to detect each of these HHV-6 types separately (Fig. 1A). The PCR results from case 1 showed that intraocular samples included HHV-6A but not HHV-6B DNA (Fig. 4). Final analysis with quantitative PCR indicated that two of the cases were positive for HHV-6A and six cases were positive for HHV-6B (Table 2).

Detection of HHV-6 mRNA in Intraocular Samples

RT-PCR has previously been used on mRNA from peripheral blood mononuclear cells to detect actively replicating virus.[21] We therefore tested ocular samples for the presence of HHV-6 mRNA. Various samples, such as aqueous humor, vitreous fluid, retinal membrane tissues, and collected vitreous cells from an HHV-6A–positive case (case 1), were available for the RT-PCR assay. We designed primers to amplify mRNA using a nested RT-PCR (HHV-6 A IE1 U90, Fig. 1B). As revealed in Figure 5, HHV-6A mRNA was clearly detected in vitreous cell samples, but other ocular samples from the same patient were all negative.

Discussion

In this study, we demonstrate that seven patients with uveitis or endophthalmitis were positive for HHV-6 DNA. In addition, one patient with infectious keratitis was also found to be HHV-6–positive. These patients had high copy numbers of HHV-6

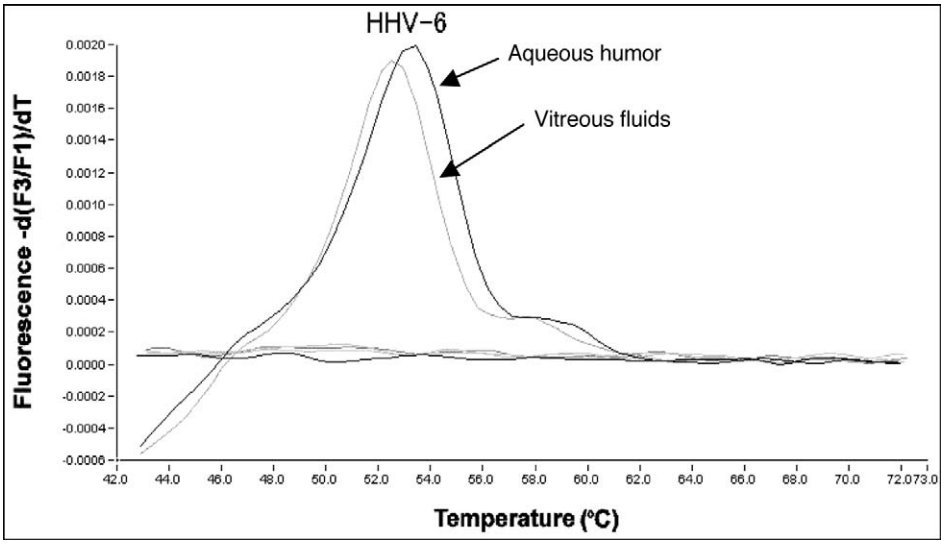

Figure 3. Results for multiplex PCR in a patient with HHV-6–positive uveitis. A significant positive curve was seen at 52°C, indicating detection of HHV-6 genomic DNA in the ocular fluids (case 1). DNA from other herpes viruses, such as HSV1, HSV2, VZV, EBV, CMV, HHV7, and HHV8, was not detected in this sample.

DNA, and two cases were found to be HHV-6 type A and six cases were type B. In addition, HHV-6 mRNA was detected in intraocular samples from HHV-6–positive patients, suggesting that viral replication or reactivation may occur in the eye.

Recently, Cohen et al.[5] reported that HHV-6A DNA could be detected by PCR in vitreous fluid from a patient with CMV-associated retinitis when vitreous fluids were assayed from 101 patients with ocular inflammation for HHV-6A, HHV-6B, and HHV-7. HHV-6B DNA was also detected in vitreous fluid from a patient with idiopathic uveitis in the absence of CMV DNA. This study suggests that HHV-6A and HHV-6B DNA are detectable in approximately 1% of vitreous samples from patients with ocular inflammation. In our study, we show that HHV-6 DNA was detectable in 2% of ocular samples from patients with intraocular inflammation following screening for HHV-1 to –8 infection using multiplex PCR.

In a previous study,[16] we found that intraocular HHV DNA was detectable in a wide range of herpes virus–associated uveitis cases when analysis was performed using multiplex PCR. PCR is a valuable tool for the diagnosis of herpetic uveitis and it is now possible to exclude nonherpetic uveitis patients using this method. Moreover, de Boer et al.[8] previously found that in patients with herpetic anterior uveitis, PCR was more frequently positive than the Goldmann-Witmer coefficient. HHV-6 has been implicated in ocular inflammation, most remarkably when the posterior segment of the eye was affected.[6,7,10–12] On the other hand, the role of HHV-6 as a cause of anterior uveitis is inconclusive and further studies are required. As revealed in this study, we found three cases of anterior inflammatory diseases including keratitis and five cases of pan- or posterior inflammatory diseases in the eye.

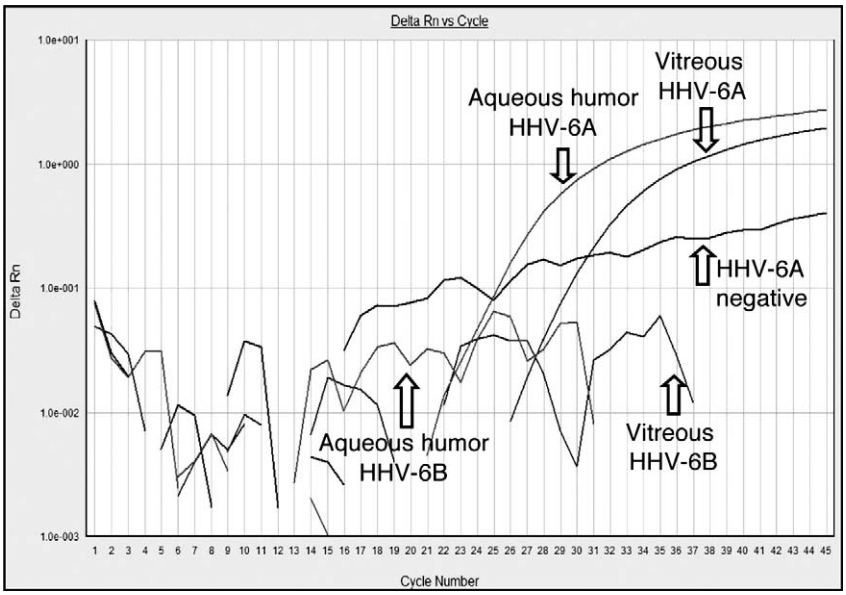

Figure 4. Detection of HHV-6 DNA by quantitative real-time PCR. The real-time PCR results for the samples from case 1 showed that intraocular samples, such as aqueous humor and vitreous fluids, contained a high copy number of HHV-6A DNA, but not HHV-6B DNA.

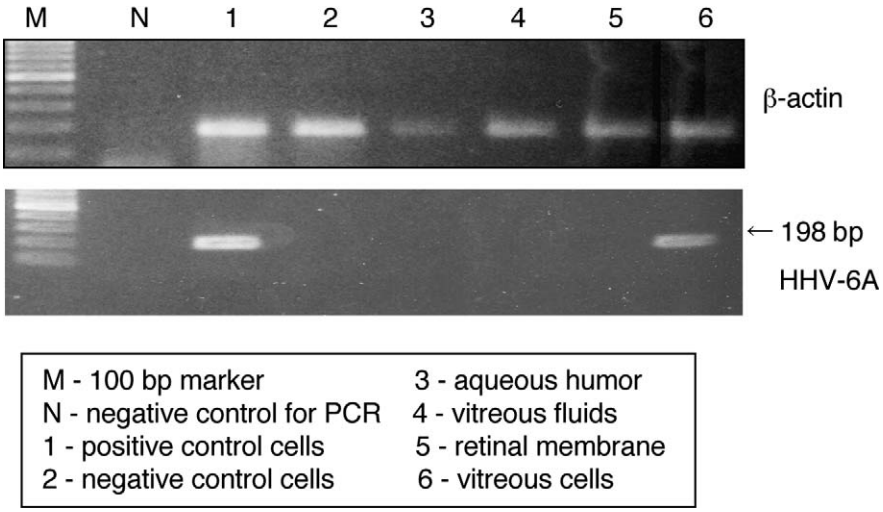

Figure 5. Detection of HHV-6 mRNA in intraocular samples. HHV-6A mRNA was detected in samples from vitreous cells, but other ocular samples, such as aqueous humor, vitreous fluids, and retinal membrane tissues were all negative (*lower image*). All samples, including control RNA, were positive for β-actin (*upper image*).

The detection of HHV-6 in the eye might not be clinically relevant. HHV-6 can latently reside in cells of the lymphoid and myeloid lineage and it may have entered the inflamed eye via immune cells, similar to EBV and human immunodeficiency virus.[3,22,23] Thus, HHV-6 DNA has been detected in circulating T cells, monocytes, and leukocytes and may simply have been carried into the eyes in the inflammatory cells as a result of destruction of the blood-retina barrier. Our data indicate that most HHV-6 DNA in intraocular fluids of inflamed eyes might be a consequence of the release of HHV-6 DNA from resident ocular cells caused by intraocular inflammation. A high copy number of HHV-6 DNA was detected in patients with severe ocular inflammation, pan- or posterior uveitis, or endophthalmitis (Tables 1 and 2). This is supported by the findings of Arao et al.,[24] who showed that HHV-6 can infect human retinal pigment epithelial cells.

We detected HHV-6 in only one patient with an ocular surface inflammatory disorder. The patient was a young healthy donor suffering from atopic dermatitis. Okuno et al.[9] recently reported that 14 of 22 patients with corneal inflammation were positive for HHV-6, suggesting that the association of HHV-6 with disease was more frequent than with other herpes viruses, such as HSV-1. Thus, HHV-6 may be another sole causative agent of corneal inflammation.

HHV-6 reactivation frequently accompanies CMV reactivation,[25] and the presence of HHV-6A DNA in the eye may simply reflect the immunocompromised state of the patient. Case 6 in this study was a patient with CMV retinitis who was also found to be HHV-6A DNA-positive; however, with the exception of this patient, our HHV-6 PCR-positive patients were neither young nor immunosuppressed. We previously used multiplex PCR to search for HHV-6 in ocular fluids from 100 patients with uveitis and detected HHV-6A DNA in one patient with severe unilateral uveitis (case 1).[7] This patient's ocular fluid also contained antibodies to *Toxocara canis* larvae and we finally diagnosed ocular toxocariasis and HHV6-related panuveitis.[7] In this study, 7 patients were found to have other infectious agents, including bacteria, other herpes viruses (HSV-1), and parasites (*Toxocara*); however, it is unclear whether HHV-6 was the predominant pathogen. It is assumed that HHV-6 infections play a secondary role in the pathogenesis of ocular inflammation. Therefore, we tested intraocular samples for the presence of HHV-6 mRNA. Additional tests for HHV-6 RNA or protein in ocular tissues would have been more definitive and provided evidence of HHV-6 replication. We found HHV-6A mRNA and a high copy number of HHV-6 DNA in the same sample from a patient with ocular toxocariasis (case 1). As far as we know, this is the first report of detection of both HHV-6 DNA and mRNA in an ocular sample. The RT-PCR assay can reliably differentiate between latent and actively replicating HHV-6 and its use should allow an insight into the pathogenesis of this ubiquitous virus as previously reported.[21]

In conclusion, ocular samples collected from patients with infectious ocular disorders can contain a high copy number of HHV-6 DNA. The HHV-6–positive case was found to have HHV-6 DNA and mRNA in the inflamed eye. We are currently conducting experiments to determine whether HHV-6 type A and type B can infect ocular cells, such as retinal pigment epithelium, in vitro. Infected ocular cells can produce inflammatory cytokines and chemokines that differ from those in normal uninfected cells.

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