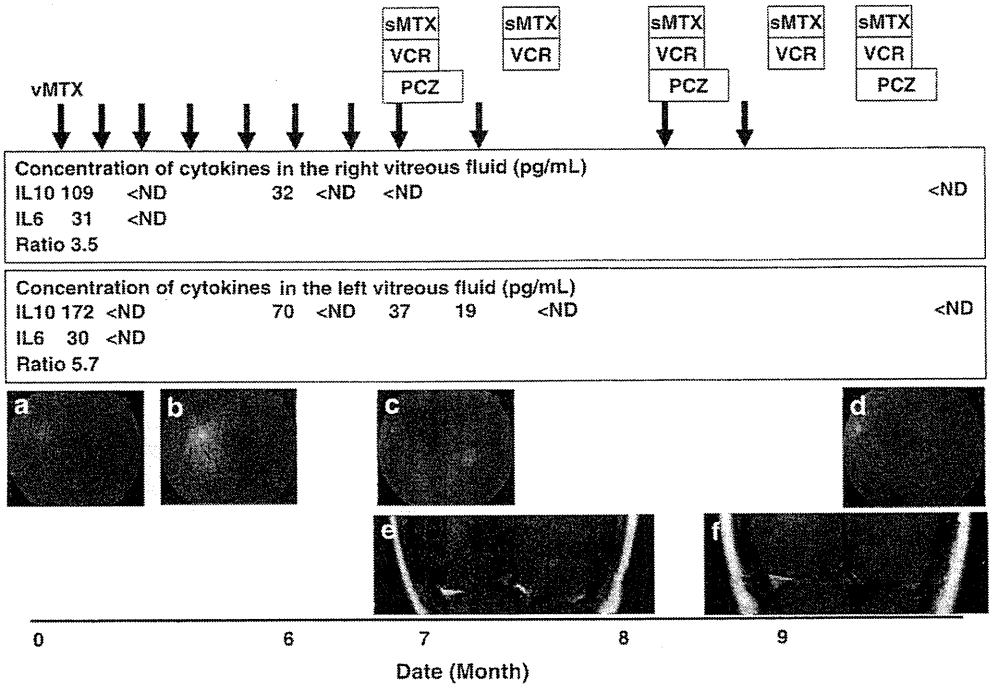

Fig. 1 Clinical course of Case 1. *vMTX* intravitreal methotrexate injection. *Black arrows* indicate each vMTX. *sMTX* systemic intravenous injection of methotrexate, *VCR* vincristine, *PCZ* procarbazine, *ND* not detected. **a** Left fundus findings at diagnosis. Severe opacity of the vitreous was detected. **b** Opacity was improved significantly after the first intravitreal MTX injection. **c** Recurrence of ocular lesion. Subretinal white masses were detected. **d** The opacity and white mass disappeared after finishing systemic intravenous and intravitreal injection of methotrexate. **e** Magnetic resonance imaging of the brain at recurrence of ocular lesion, gadolinium contrast, T1-weighted image. The enhanced lesion was detected at the posterior angle of the left lateral ventricle. **f** The lesion disappeared after finishing systemic intravenous and intravitreal injection of methotrexate

lesion disappeared (Fig. 1f) and was considered complete response. There has been no recurrence for more than 1 year.

3.2 Case 2

A 59-year-old woman was admitted to our hospital because of blurred vision in the right eye. She was congenially blind in her left eye because of vitreous opacity by an unknown etiology. An ophthalmological examination revealed uveitis and a white subretinal mass in the right eye (Fig. 2a). Vitrectomy on the right eye was performed, and the cytology was class 1. However, a monoclonal band for the IgH gene was detected by the PCR assay, and intravitreal concentrations of IL-10 and IL-6 were elevated (Fig. 2). The IL-10/IL-6 ratio was 7, and she was diagnosed with IOL. No abnormal lesions were detected by FDG-PET/CT or bone marrow aspiration and biopsy. However, soon after vitrectomy, she developed short memory disturbance and dysarthria. Brain MRI revealed a mass lesion in the left temporal lobe (Fig. 2c). Lumbar puncture was performed, and the number of cells was slightly elevated at 13/μL (class 3). We started vMTX followed by concurrent sMTX and vMTX administered into the right vitreous body on days 5, 11, 18, and 25. There were no adverse events during the treatment. After the third vMTX treatment, the IL-10 level in the vitreous fluid was undetectable, and ocular findings had significantly improved (Fig. 2b). MRI confirmed improvement of the CNS lesion (Fig. 2d). After additional radiation therapy to the whole brain (total 40 Gy), the CNS lesion disappeared, and there has been no recurrence for more than 1 year.

3.3 Case 3

A 53-year-old woman was admitted to our hospital because of bilateral blurred vision. Severe vitreous opacity was detected in the both eyes (Fig. 3a). She was diagnosed with uveitis. However, IOL was suspected because her lesions were refractory to steroid. She was admitted and vitrectomy on the right eye was performed. Cytological diagnosis could not be made because cells were highly degenerated. However, a monoclonal band for the IgH gene was detected by the PCR assay. In addition, a predominance of infiltrated cells for a κ-light chain expression was detected by flow cytometry (Fig. 3c). Intravitreal IL-10 and IL-6 concentrations were markedly elevated, and the IL-10/IL-6 ratio was 36.7 (Fig. 3). Thus, she was diagnosed with IOL. Vitrectomy on the left eye was performed in succession. IL-6 was slightly elevated without

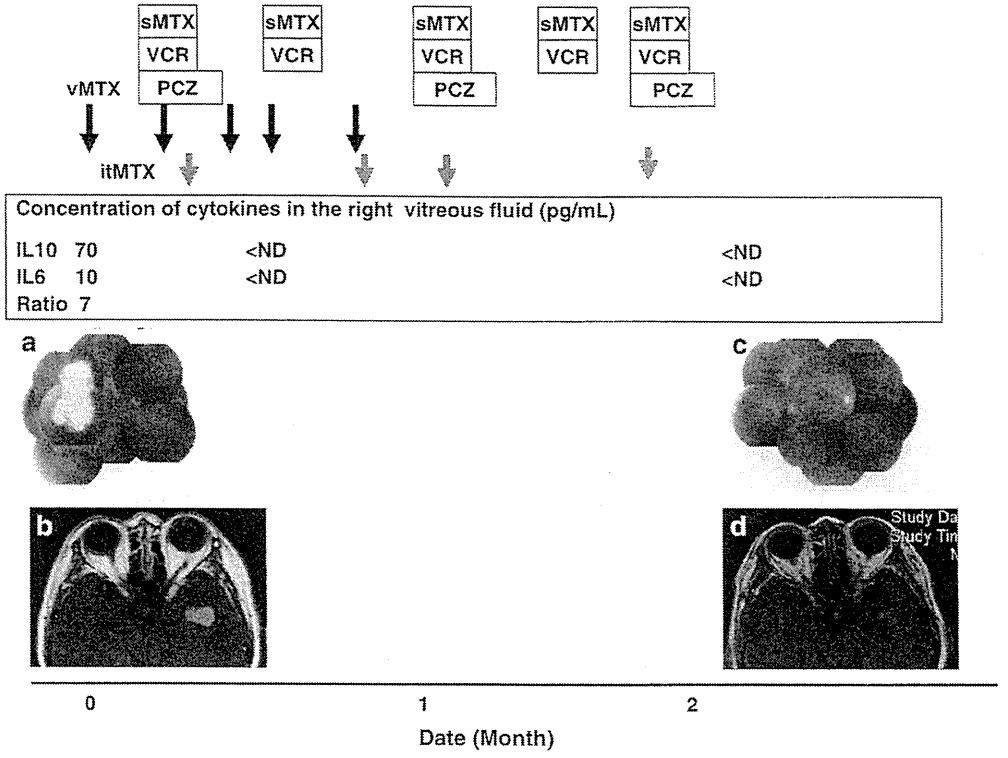

Fig. 2 Clinical course of Case 2. *vMTX* intravitreal methotrexate injection. *Black arrows* indicate each vMTX. *sMTX* systemic intravenous injection of methotrexate, *itMTX* intrathechal methotrexate injection. *Gray arrows* indicate each itMTX. *VCR* vincristine, *PCZ* procarbazine, *ND* not detected. **a** Right fundus findings at diagnosis. A subretinal white mass was detected in the right fundus. **b** White mass was reduced in size after finishing systemic intravenous and intravitreal injection of methotrexate. **c** Magnetic resonance imaging of the brain at diagnosis, gadolinium contrast, T1-weighted image. The enhanced lesion was detected at the left lateral lobe. **d** The lesion was improved significantly after finishing systemic intravenous and intravitreal injection of methotrexate

detection of IL-10. The rearrangement of IgH gene was detected. However, at the time when the second surgery was performed, she developed a rapidly progressive motor aphasia and loss of muscle strength in the right superior limb. Her brain MRI revealed multiple mass lesions in both parietal lobes (Fig. 3d). Lumbar puncture was normal. FDG-PET/CT, bone marrow aspiration, and biopsy were also normal. We started vMTX followed by concurrent sMTX and vMTX administered into the both vitreous cavities on days 8, 15, 29, and 48. All adverse events were grade 2 or less during the treatment, and no ocular complications occurred. After vMTX treatment (right eye, 2 times vMTX; left eye, 1 time vMTX), the IL-6 and the IL-10 in the vitreous fluid became undetectable, and the ocular (Fig. 3b) and brain MRI findings (Fig. 3e) improved. After additional radiation therapy to the whole brain (total 40 Gy), the CNS lesion disappeared, and there has been no recurrence for more than 5 months.

3.4 MTX concentrations and its half-life in the vitreous fluid

Among our patients, 2 (cases 2 and 3) received sMTX (second administration) followed by vMTX 2 h later. We measured intravitreal MTX concentrations in 3 eyes of these 2 patients at 2 time points; 2 h after sMTX (just before vMTX) and 24 h after adding vMTX to sMTX. The results were summarized in Table 1. The intravitreal MTX concentration 2 h after sMTX ranged widely in cases 2 and 3, which might be caused by differences of the time of administration. In addition, we analyzed the pharmacokinetics of MTX in the vitreous fluid. Since the vitreous volume was considered 4–5 mL, the MTX concentration just after intravitreal administration of 400 μg was estimated at 176–220 μM. By assuming the first-order elimination kinetics, the half-life of MTX in the vitreous fluid is estimated to be 12.4–14.8 h in case 2, 14.8–18.4 h in the right eye of case 3, and 16.8–21.5 h in the left eye of case 3.

4 Discussion

The optimal treatment for IOL has not been established, but local ocular irradiation achieves remission in most cases. However, complications such as retinopathy, keratitis, cataract, and optic nerve disturbances are inevitable [11]. In contrast, vMTX is highly effective and safe

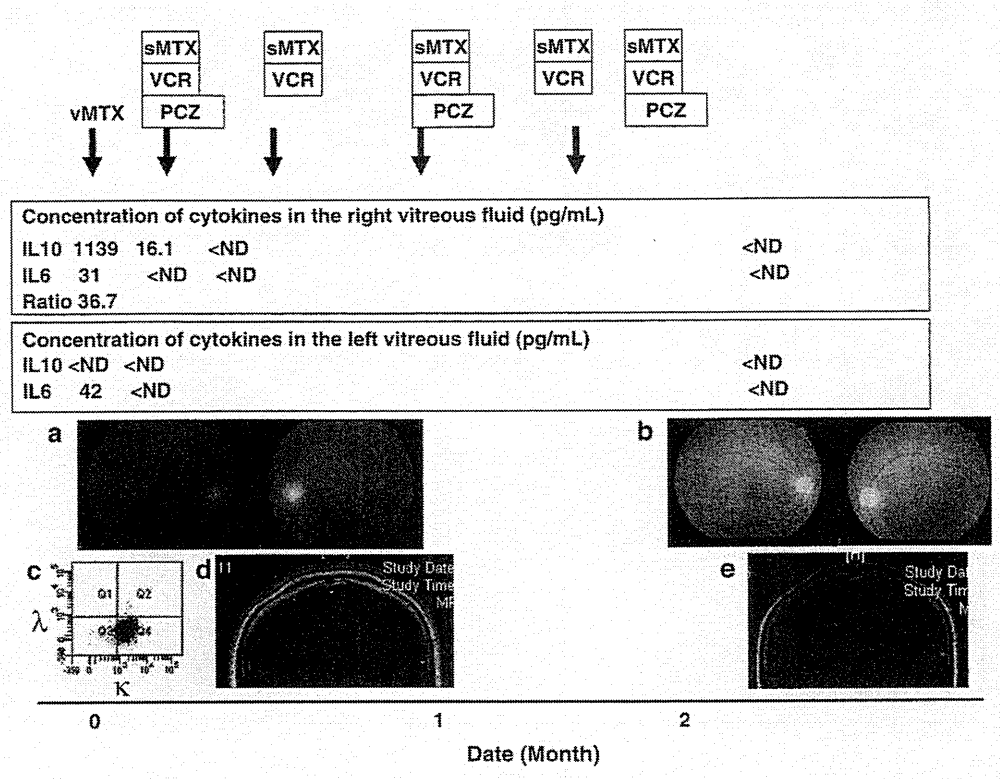

Fig. 3 Clinical course of Case 3. *vMTX* intravitreal methotrexate injection. *Black arrows* indicate each vMTX. *sMTX* systemic intravenous injection of methotrexate, *VC* vincristine, *PCZ* procarbazine, *ND* not detected. **a** Bilateral fundus findings at diagnosis. Severe opacity of the vitreous was detected. **b** Opacity was cleared after finishing systemic intravenous and intravitreal injection of methotrexate. **c** Flow cytometry of the right vitreous fluid. Dominant expression of κ-light chain on surface of the infiltrated cells was detected. **d** Magnetic resonance imaging of the brain at diagnosis, gadolinium contrast, T1-weighted image. The enhanced lesion was detected at the left lateral lobe. **e** The lesion was significantly improved after finishing systemic intravenous and intravitreal injection of methotrexate

Table 1 Concentration of methotrexate

	Vitreous 2 h after sMTX (μM)	Vitreous 24 h after adding vMTX to sMTX (μM)	Serum sMTX without vMTX	Serum sMTX with vMTX
Case 2	11.86	60.94	1.14 μM (24 h)	1.47 μM (24 h)
			0.12 μM (48 h)	0.09 μM (48 h)
Case 3 right	1.91	72.11	0.99 μM (24 h)	1.07 μM (24 h)
			0.16 μM (48 h)	0.16 μM (48 h)
Case 3 left	3.39	82.89		

MTX methotrexate, *sMTX* systemic intravenous MTX injection, *vMTX* intravitreal MTX injection

compared to irradiation. De Smet et al. investigated the effect of vMTX for 18 IOL patients. Among them, 16 were treated with 400 μg/dose of vMTX alone; the injection was initially given twice a week, followed by once a week for 1 month, and then monthly for 9 months to 1 year [6]. They reported that response rate was 100% (16/16) and that most complications were transient and reversible. However, it was written that most patients developed the CNS involvement. The results are summarized in Table 2. Ocular recurrences occurred 19% (3/16), but they responded well to a reinjection of vMTX. Recently, Frenkel et al. [7] reviewed the clinical courses of 26 IOL patients with or without CNS involvement treated by vMTX. They also concluded that IOL could be controlled effectively by vMTX with 95% of response rate for the local lesions without serious adverse event. No local recurrence was observed. However, they reported that 14 patients died of the CNS or systemic involvement. This might mean that vMTX was unable to control the CNS involvement. Actually, our case 1 developed a CNS relapse after achieving ocular response with clearance of IL-10 in the vitreous fluid by vMTX. On the other hand, Batchelor and colleagues [8] investigated the effect of sMTX on IOL. Nine patients (IOL alone in 1, IOL accompanied by CNS disease in 8) with IOL were treated with sMTX (8 g/m^2 MTX administered every 28 days for 11 doses). As shown

Table 2 Response of methotrexate administration in the reported cases

Administration	Patients	RR of ocular lesion	Ocular recurrence	RR of CNS lesion	CNS recurrence	Reference
vMTX	16	100%	19% (3/16)	ND	Most patients	[6]
vMTX	44 eyes of 26 patients (16 with CNS and systemic)	95% (CR 91%)	0	ND	ND	[7]
sMTX	9	78% (CR 67%)	86% (6/7)	100%	ND	[8]

MTX methotrexate, *sMTX* systemic intravenous MTX injection, *vMTX* intravitreal MTX injection, *CNS* central nervous system, *RR* response rate, *CR* complete response, *ND* not described

in Table 2, response rate for ocular lesion was 78% (7/9), which was lower than that of vMTX in the other reports, and the recurrence rate was 86% (6/7), which was distinctively higher than that of vMTX. Taken together, although sMTX was quite effective for CNS lesions, it was insufficient for most patients who had intraocular involvement.

To the best of our knowledge, there have been no reported cases in which systemic and intravenous MTX was administered simultaneously. Grim et al. [12] analyzed 176 patients with primary CNSL and ocular lesions. They concluded that median progression-free survival was prolonged in patients who were treated for both CNS and ocular involvement compared with those who did not receive dedicated ocular therapy, whereas overall survival was not [12]. However, CNS treatment in the report included not only MTX-based regimen but also CHOP and radiation therapy. From our results, concurrent administration of vMTX and sMTX may be appropriate and effective for IOL with CNS involvements, and study should be added to evaluate that.

We measured intravitreal MTX concentrations in 3 eyes of 2 patients at 2 different time points. As shown in Table 1, those who received sMTX had widely ranging values from 1.91 to 11.86 μM. Since it has been reported that the effective MTX concentration for lymphoma is >1 μM, these concentrations were high enough to control lymphoma cells despite the wide range. Batchelor et al. [8] measured serum and vitreous levels of MTX and determined that intravitreal MTX concentrations 4 h after sMTX also ranged widely (1.46–24.0 μM). However, they concluded that there was no significant correlation between treatment effectiveness and the MTX concentration. The reason for this wide range in intravitreal MTX concentrations after sMTX has not been clarified. The wide range of intravitreal MTX concentration 2 h after sMTX in cases 2 and 3 in the present report may have been caused because of differences in the duration of infusion. We administered systemic MTX for 6 h in case 2 and for 2 h in case 3. It is also notable that severe subretinal lesion was detected in case 2. The disease status, including a rupture of blood-retinal barrier, might have also caused the difference.

There have been a report analyzing pharmacokinetics of MTX in the vitreous fluid. De Smet and colleagues [13] reported a case with IOL who underwent repeated intravitreal injections of MTX and thiotepa. In the report vitreous samples were taken before each injection and MTX concentrations were determined. Then, they analyzed kinetics of MTX in the vitreous fluid by evaluating the correlation between time after previous injection and the concentration of MTX. They found a linear relationship between time and logarithmic plot of the concentration, suggesting a first-order elimination kinetics of MTX. According to this, the half-life of MTX in the vitreous fluid was estimated to be 12.4–21.5 h in our cases, which was much longer than that of blood level. Because there have been reported few eye complications [14], it was considered that the tolerability for vMTX was very high. Although the previous report demonstrated that MTX concentration in the vitreous fluid and its effect did not correlate with each other [8], administration strategies can be arranged in the future to obtain optimal effects, for instance by increasing intensity or density of vMTX.

In summary, our cases reveal that concurrent treatment with sMTX and vMTX may be effective for IOL with CNS involvement. More study is needed in order to examine efficacy as well as safety and to establish the optimal treatment for the disease.

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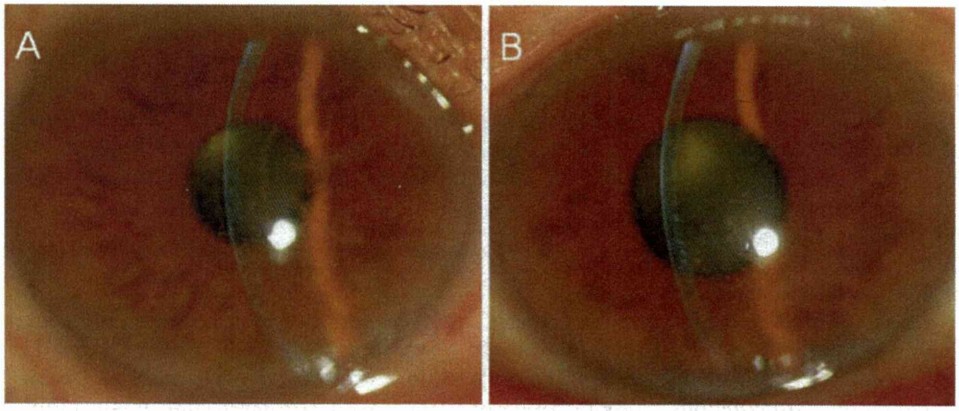

Figure 1A, B. Slit-lamp photographs demonstrating the corneal findings. **A** Several bifurcating, thick lattice lines in the superficial stroma are noted in the right eye. **B** Discrete and nodular opacities are noted in the deep stroma of the central cornea in the patient's left eye.

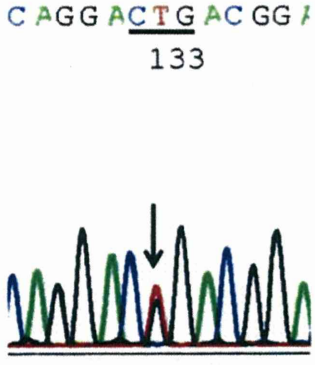

Figure 2. Results of the direct sequencing of exon 12 in the *TGFBI* gene. A heterozygous L527R mutation (CTG→CGG) is detected.

with the L527R mutation is unique in terms of its late onset, sporadic occurrence, and regional idiosyncrasy. It has been reported that the L527R mutation is descended from a founder mutation that occurred in a single Japanese ancestor.[5] Therefore, additional data are required to elucidate the clinical and genetic manifestations of lattice corneal dystrophy associated with the L527R mutation.

Keywords: lattice corneal dystrophy, L527R mutation, *TGFBI* gene

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Endogenous Candida Chorioretinitis in a Healthy Infant

Endogenous *Candida* endophthalmitis is often observed in patients with a history of recent major surgery, bacterial sepsis, use of systemic antibiotics, placement of central venous catheters, or a combination of these. Newborns, especially those born prematurely, do not have a sufficiently developed immune system against pathogens; however, they rarely develop endogenous fungal infections. Several infants with *Candida* chorioretinitis have been reported so far, all of whom had undergone surgery or total parenteral alimentation, received antibiotics, or were not full-term babies.[1] We report a case of a healthy infant with endogenous *Candida* chorioretinitis who had no risk factors for an opportunistic infection.

Case Report

A 41-week-gestation female infant (body weight, 2910 g) was delivered by Caesarean section because of delayed labor. No problems occurred during the developmental and perinatal periods. At 6 months of age, she became transiently febrile with temperatures above 38°C despite a lack of other systemic symptoms and was hospitalized for administration of transvenous antibiotics (piperacillin for 3 days and panipenem/betamipron for 10 days). After 2 weeks, she became

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afebrile, and a prominent conjunctival injection developed bilaterally. Slit-lamp biomicroscopy identified posterior synechiae and nodules in the iris of the left eye. Ophthalmoscopy identified multiple white lesions along the retinal vessels bilaterally and an exudative total retinal detachment in the left eye (Fig. 1). Fluorescein angiography (FA) showed anastomosis of the vessels and an avascular area in the peripheral retina of the right eye (Fig. 1). Whole-body computed tomography and biochemical, immunological, and culture examinations of blood and cerebrospinal fluid failed to detect any abnormalities. Polymerase chain reaction (PCR) analysis detected *Candida* DNA in the cerebrospinal fluid (2.4×10^2 copies/ml) and vitreous (9.4×10^4 copies/ml) obtained by needle aspiration. Immunological and culture examinations of blood and vaginal secretion from the mother failed to identify any signs of *Candida* infection. After administration of fluconazole (intravenous injection of 100 mg for 3 weeks, then 50 mg orally for 3 months), the retina of the left eye reattached and the volume of white lesions decreased, resulting in residual retinal scars (Fig. 2).

Comment

Endogenous *Candida* endophthalmitis is commonly seen in compromised hosts. Only two adult cases of *Candida* endophthalmitis without severe systemic disease have been reported, one in a patient with a common cold and the other

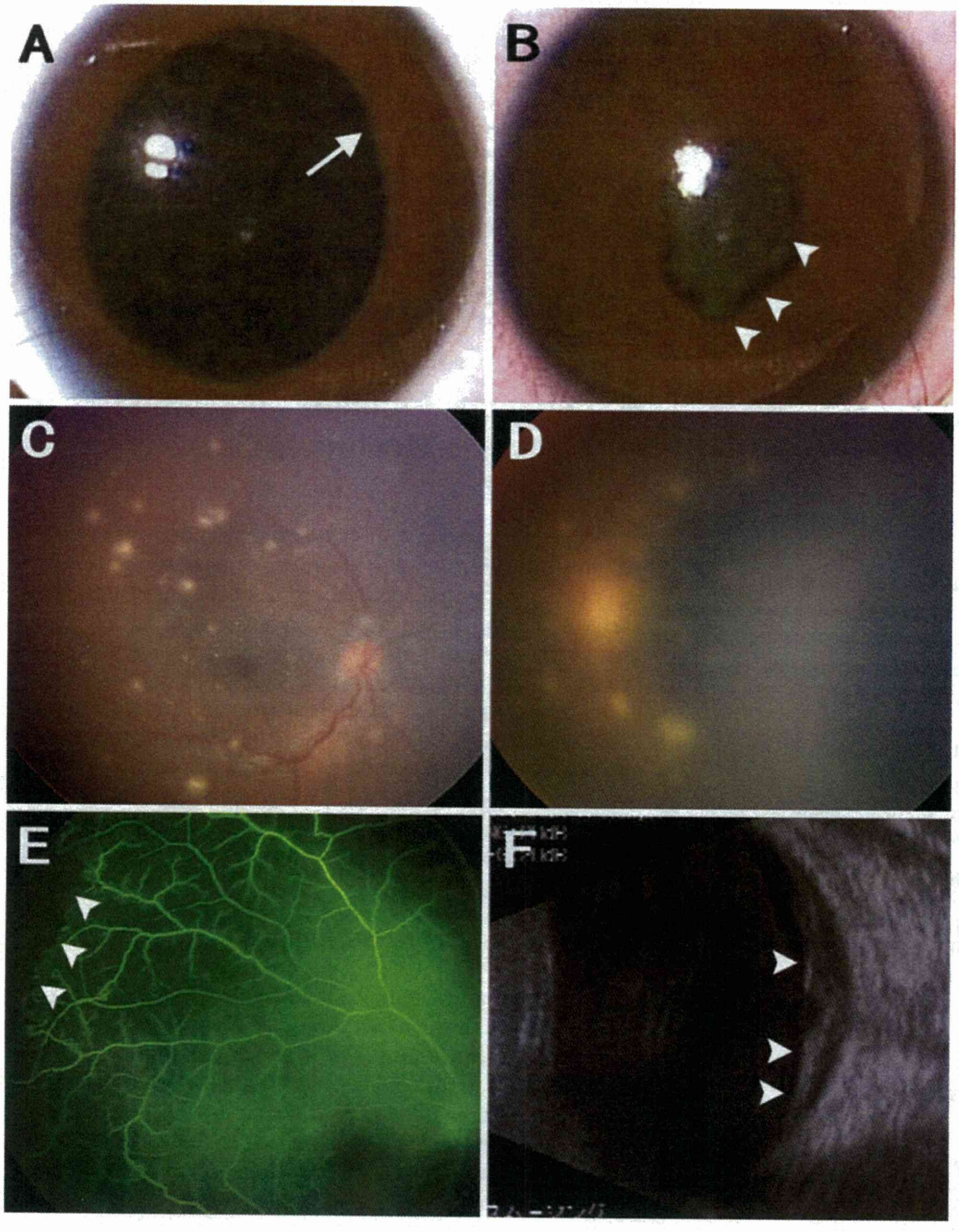

Figure 1A–F. Chorioretinitis before treatment. Photographs of the anterior segment (**A, B**) and fundus (**C, D**); fluorescein angiogram (**E**); and ultrasonogram (**F**). **A, C, E** Right eye; **B, D, F** left eye. **A** A slight posterior synechia of the iris margin (*arrow*) was observed. **B** An entire posterior synechia and nodules of the iris (*arrowheads*). **C** Multiple white lesions were seen along the retinal vessels. **D** Exudative retinal detachment and multiple white lesions were observed along the retinal vessels. **E** An avascular area in the peripheral retina (*arrowheads*) and anastomosis of vessels and leakage from retinal veins were observed, despite no staining of the white lesions. **F** Retinal detachment (*arrowheads*) was detected by ultrasonography.

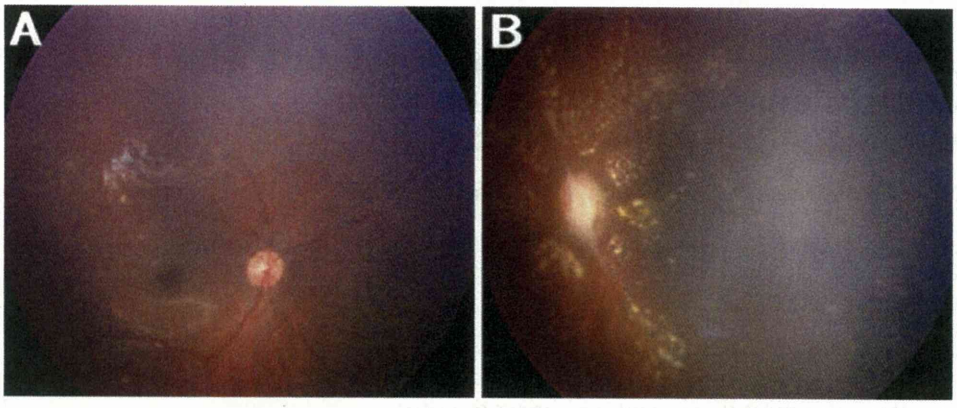

Figure 2A, B. Fundus photographs of the **A** right and **B** left eyes 1 month after administration of fluconazole. **A** The size of the white lesions had decreased significantly. **B** Retinal reattachment with hard exudates, white vessels, and optic disc pallor were observed.

in a patient with *Candida* vaginitis and onychomycosis; both patients were treated with antibiotics.[2,3] The current patient had no systemic abnormalities but evidently had endogenous *Candida* endophthalmitis because PCR analysis detected sufficient quantities of *Candida* DNA in the vitreous and the cerebrospinal fluid to diagnose the infection. FA findings of abnormal vasculature in the peripheral retina are usually seen in eyes with retinopathy of prematurity or familial exudative vitreoretinopathy, which prompted us to suspect that the *Candida* infection in the present case was congenital. Generally, a congenital *Candida* infection occurs by vertical transmission through the uterus or vagina and is associated with systemic involvement, including dermatitis, meningitis, anomaly of the brain, and oral mucositis.[4] However, the patient was delivered by Caesarean section, and no signs of *Candida* infection were detected in the mother. Thus, acquired *Candida* infection was the most likely diagnosis in the present case. Intravenous antibiotics delivered 2 weeks before the onset of bilateral endophthalmitis likely caused iatrogenic *Candida* infection because of inadvertent manipulation. Possible insufficient growth of the retinal vasculature might have facilitated the proliferation of *Candida* in the patient's retina.

To diagnose and treat such a difficult case, broad-range PCR for the 18S ribosomal RNA sequence is a good screening tool.[5] Moreover, real-time PCR can examine the quantity of the pathogen and determine its relation to the endophthalmitis. Early treatment of infectious endophthalmitis is essential in infants, in whom vision develops rapidly. Thus, a broad-range, real-time PCR system using ocular samples is useful when the patient has uveitis or endophthalmitis of unknown origin.

Keywords: *Candida* chorioretinitis, *Candida* infection, polymerase chain reaction

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Choroidal Neovascularization in a Child Following Laser Pointer-Induced Macular Injury

Laser pointer-induced macular injury is characterized by a decrease in visual acuity and metamorphopsia.[1] High-energy lasers can cause chorioretinal damage, which can lead to choroidal neovascularization (CNV) in animals.[2] Case reports of the development of a CNV following laser-induced macular injury have also been published.[3,4] We report the case of a child with a CNV that developed following a macular injury caused by repeated exposure to a green laser pointer. The prevalence of CNV in children is low, but it is still an important cause of visual impairment.[5] To the best of our knowledge, this is the first report of a child developing CNV following a macular injury caused by exposure to a green laser pointer.

A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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ABSTRACT

Aim The aim of the study was to investigate the correlation between the clinical manifestation and the cytomegalovirus (CMV) viral load in the aqueous humour of patients with CMV anterior uveitis.

Methods Seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis were enrolled. Presence of CMV, but not other human herpes viruses, was confirmed by multiplex polymerase chain reaction (PCR). Viral load was measured using real-time PCR. Clinical manifestations were examined using a slit-lamp microscope and ophthalmoscope, applanation tonometer and specular microscope.

Results All 11 patients had unilateral recurrent anterior uveitis with high intraocular pressure and mutton fat keratic precipitates with pigmentation. Stromal oedema of the cornea was found in CMV-associated endotheliitis, but not in CMV-associated iridocyclitis patients. A significant corneal endothelium cell loss was recorded in all 11 patients with CMV-associated endotheliitis and iridocyclitis patients. High viral loads of CMV were detected in the aqueous humour of all 11 patients. A significant association was found between the corneal endothelial cell loss intensity and CMV viral load in the aqueous humour.

Conclusion There is a significant correlation between the CMV viral load and corneal endothelial cell loss in both CMV-associated iridocyclitis and corneal endotheliitis.

INTRODUCTION

Cytomegalovirus (CMV) is a member of the human herpes virus family and is found in latent infections in the majority of the adult population. In immunocompromised hosts, the virus causes necrotising retinitis,[1] but has been thought not to cause any diseases in immunocompetent hosts. However, a previous study showed local production of anti-CMV antibodies in the aqueous humour of an immunocompetent patient with iridocyclitis with elevated intraocular pressure (IOP).[2] In addition, recent studies using qualitative PCR have demonstrated that genomic CMV DNA is present in the aqueous humour of immunocompetent patients with unilateral iridocyclitis[3–6] as follows. Markomichelakis *et al*[5] reported two cases of iridocyclitis with sectoral iris atrophy in which CMV was detected by PCR, and de Schryver *et al*[4] also reported five similar cases. In the recent report by Chee *et al*,[5] they studied if there was a relationship between the CMV viral load in the aqueous and clinical manifestation of the diseases such as either acute or chronic iridocyclitis, eg Posner–Schlossman syndrome and Fuchs heterochromic iridocyclitis. CMV genomic DNA was also detected in the aqueous humour of immunocompetent patients with another inflammatory condition of the eye, ie corneal endotheliitis, in three previous reports.[7–9] Corneal endotheliitis is an inflammatory condition at the corneal endothelium in which keratic precipitates (KPs) develop together with severe stromal oedema in the cornea, whereas iridocyclitis has cells and flare in the anterior chamber with or without KPs but no stromal oedema in the cornea.

The real-time PCR made it possible to measure the viral load quantitatively. Thus, the use of this assay makes it possible to determine the clinical significance of the viral infection in the pathogenesis of human diseases. Our previous report showed a high CMV genomic DNA load in the aqueous humour in an immunocompetent patient with unilateral iridocyclitis with high IOP.[6] However, the correlation between the viral load in the aqueous humour and the clinical manifestation of the disease (iridocyclitis versus corneal endotheliitis) was not investigated. Therefore, we examined if there was any correlation between the CMV viral load in the aqueous humour and the clinical manifestation of anterior inflammatory diseases associated with CMV. We showed a significant correlation between the CMV viral load in the aqueous humour and the endothelial cell damage of the cornea in patients with iridocyclitis and corneal endotheliitis associated with CMV.

MATERIALS AND METHODS

Subjects

Between 2006 and 2008, 11 patients with CMV-associated inflammation in the anterior segment of the eye, ie seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis, were enrolled. These patients were from Tokyo Medical and Dental University Hospital (Tokyo, Japan), Miyata Eye Hospital (Miyakonojo, Miyazaki, Japan) and Kyoto Prefectural University Hospital (Kyoto, Japan). Diagnosis was made based on clinical manifestations and the qualitative detection of the CMV genomic DNA in the aqueous humour by the multiplex PCR. The viral load in the aqueous humour was further measured quantitatively by the real-time PCR.

An aliquot of 0.1 ml of the aqueous humour was aspirated with a 30G needle after disinfection and

processed for PCR. Anti-viral therapy was not given before the PCR assay, but topical corticosteroids were given by local ophthalmologists to treat intense anterior uveitis. The interval between the disease onset and the aqueous humour sampling varied among the patients.

Polymerase chain reaction

The aqueous humour samples were centrifuged at 1000 *g* for 5 min and used for multiplex PCR and real-time PCR.[10 11] Multiplex PCR was designed to qualitatively measure the genomic DNA of eight human herpes viruses: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), CMV, and human herpes virus type 6 (HHV-6), type 7 (HHV-7) and type 8 (HHV-8). DNA was extracted from the aqueous humour samples using a DNA minikit (Qiagen, Valencia, California, USA). Multiplex PCR was performed using LightCycler (Roche, Basle, Switzerland). The primers of the glycoprotein gene sequences for CMV were TACCCCTATCGCGTG TGTTC (forward) and ATAG-GAGGCGCCACGTATTC (reverse). The probes used included 3′-fluorescein isothiocyanate: TCGTCGTAGCTACGCTTACAT and LcRed705-5′: ACACCACTTATCTGCTGGGCAGC. Specific primers for the virus were used in conjunction with Accuprim Taq (Invitrogen, Carlsbad, California, USA). PCR amplification conditions used in the current study have been reported previously.[12]

Real-time PCR was only performed for the HHV, with multiplex PCR used to detect the genomic DNA. Amplitaq Gold, with a Real-Time PCR 7300 system (ABI, Foster City, California, USA), was used to perform the procedure. The forward and reverse primers of immediate early (IE)-1 were CATGAAGGT-CTTTGCCCAGTAC and GGCCAAAGTGTAGGCTACAATAG, respectively. FAM-TGGCCCGTAGGTCATCCACACTAGG-TAMRA was used as the probe. The PCR amplification conditions used in the current study were previously reported by Sugita *et al*.[11] When more than 50 copies per tube (5×10^3/ml) were observed, the value of the sample's viral copy number was considered to be significant.

Clinical evaluation

Clinical manifestations of the eye were determined by a slit-lamp microscopic and ophthalmoscopic examination. Each patient underwent best corrected visual acuity (BCVA) measurement using a Japanese standard decimal visual acuity chart (Landolt ring chart) after treatment. Anterior chamber flare was measured by a laser flare photometer (FC-1000; Kowa Electronics, Nagoya, Japan). A photograph of the central cornea using a specular microscope (NONCON ROBO FA-3509; Konan Medical, Nishinomiya, Japan) was used for evaluation of the corneal endothelial cells. In cases of corneal endotheliitis, intense corneal oedema disturbed the measurements of the corneal endothelium, and we measured corneal endothelial cell counts after the inflammation was reduced by the treatment.

Evaluation of corneal endothelial cell loss

The relationship between the CMV viral load in the aqueous humour and the intensity of the corneal endothelial cell loss was assessed. The corneal endothelial cell loss was determined according to the following formula:

Corneal endothelial cell loss(%) =
100 − (endothelial cell counts in affected eye)/
(endothelial cell counts in the fellow eye)×100

Statistical analysis

Statistical analysis was performed using the Mann–Whitney U test. Statistical significance was set at $p<0.05$. Linear regression analysis was performed using the Spearman's correlation coefficient by rank test.

RESULTS

Clinical manifestations

Nine men and two women ranging in age from 23 to 71 years (mean age 60.6 years) were enrolled in the study. No abnormalities were found in the systemic investigations and laboratory tests. Serology examinations for human immunodeficiency virus were all negative. None of the patients had any history of eye surgery prior to the onset of uveitis. Clinical findings of the CMV-associated iridocyclitis patients (n=7) and corneal endotheliitis patients (n=4) are shown in table 1. A unilateral mild anterior uveitis with high IOP was noted in all 11 patients. There were no significant differences between the iridocyclitis and corneal endotheliitis groups in the cells and flare values in the anterior chamber, nor were there any differences noted for the elevated levels of IOP, KPs, gonioscopic findings and iris atrophy. Stromal oedema of the cornea was seen in all corneal endotheliitis but not in iridocyclitis patients. While the stromal oedema was diffuse in three out of the four patients, it was localised at upper cornea in one of the corneal endotheliitis patients. Representative cases for iridocyclitis and corneal endotheliitis are shown in figures 1 and 2, respectively. As for the IOP elevation, all 11 eyes required anti-glaucoma medications, with two eyes (cases 1 and 2) requiring trabeculectomy. With regard to the iris atrophy, no sectorial iris atrophy was seen in all 11 eyes, although four eyes (two each in the iridocyclitis and the corneal endotheliitis groups, respectively) presented diffuse iris atrophy.

Systemic valganciclovir therapy (1800 mg/day for longer than 3 weeks) in conjunction with topical corticosteroids and

Table 1 Clinical findings in patients with CMV anterior uveitis

Case	Age (years)	Sex	Eye	Diagnosis	Corneal oedema	KPs	Cells in AC	Flare in AC	IOP (mmHg)	Pigmentation in the AC angle	Iris atrophy
1	66	M	R	Iridocyclitis	-	Mutton-fat	1+	17	38	Depigmentation	None
2	62	M	R	Iridocyclitis	-	Mutton-fat	1+	26	40	PAS and pigment	Diffuse
3	56	M	L	Iridocyclitis	-	Mutton-fat	1+	13	44	Depigmentation	Diffuse
4	53	F	R	Iridocyclitis	-	Mutton-fat	1+	13	36	Depigmentation	None
5	71	M	L	Iridocyclitis	-	Mutton-fat	2+	28	25	PAS	None
6	63	M	R	Iridocyclitis	-	Fine	1+	Nt	50	Depigmentation	None
7	23	M	R	Iridocyclitis	-	Fine	1+	Nt	25	Depigmentation	None
8	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	2+	151	37	PAS	None
9	67	M	R	Endotheliitis	+ (diffuse)	Fine	1+	14	25	Depigmentation	Diffuse
10	64	F	L	Endotheliitis	+ (superior)	Fine	1+	21	28	Depigmentation	None
11	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	1+	12	43	PAS	Diffuse

Information from 11 patients with CMV anterior uveitis were reviewed. Data collected included intraocular pressure and clinical manifestation of the anterior segments in the affected eye.
AC, anterior chamber; F, female; KP, keratic precipitate; M, male; Nt, not tested; PAS, peripheral anterior synechia.

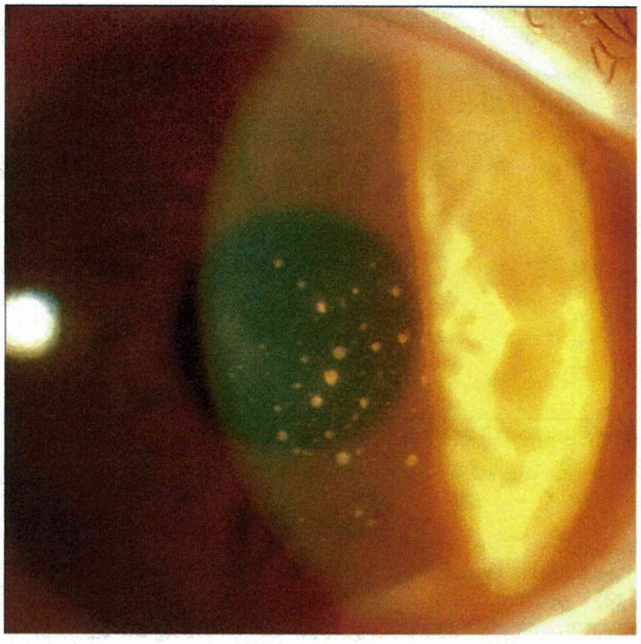

Figure 1 Case 4: Slit-lamp microscopy photo with cytomegalovirus-associated iridocyclitis. Mutton fat keratic precipitates with some pigmentation were scattered within the central area of the cornea. There was mild inflammation found within the anterior chamber.

anti-glaucoma agents effectively controlled the inflammation in the anterior segment of the eye as well as the high IOP.

Corneal endothelial cell loss

Specular microscopic examination revealed significant corneal endothelial cell loss (≥35%) in all 11 patients (table 2). Severe corneal endothelial cell loss larger than 70% was recorded in more than one-half of the endotheliitis group eyes. In contrast, this severe cell loss was observed in one of the seven patients with iridocyclitis.

There were several patients (cases 1, 8, 10 and 11; see table 2) with corneal endothelial cell counts <700 cells/mm^2. Among the patients, three cases had a low visual acuity between 0.3 and 0.6. However, one patient had a good visual acuity of 1.5.

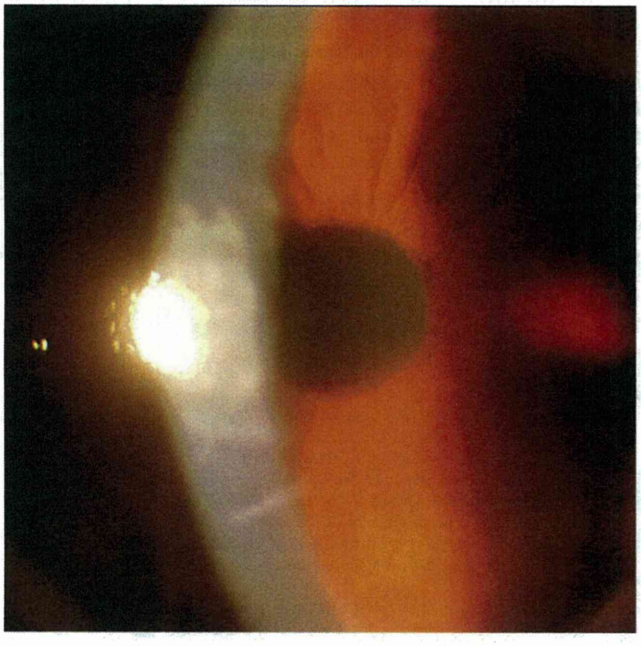

Figure 2 Case 8: Slit-lamp microscopy photo with cytomegalovirus-associated corneal endotheliitis. Diffuse corneal stromal oedema with folds in Descemet's membrane was observed.

PCR analysis of the aqueous humour samples

Multiplex PCR analyses confirmed the presence of CMV genomic DNA, but none of the other human herpes viruses (HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7 or HHV-8) in all 11 of the patients (table 2).

Quantitative real-time PCR detected significant viral loads of CMV genomic DNA in the aqueous humour of all 11 patients, with values ranging from 5.4×10^3 to 5.9×10^6 copies/ml (table 2). The mean values for the CMV viral load in the iridocyclitis and corneal endotheliitis groups were 9.4×10^5 and 1.2×10^6 copies/ml, respectively. The differences in CMV viral load between the two groups were not significant (p=0.571).

The corneal endothelial cell damage intensity was correlated to the CMV viral load in the aqueous humour. Results of the linear regression analysis demonstrated a positive correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036; figure 3).

However, there was no correlation between the interval from the disease onset to the aqueous sampling and the viral load in the aqueous humour (Spearman's correlation coefficient by rank test, r=0.445; p=0.159). Furthermore, the interval from the disease onset to the sampling was not correlated with the corneal endothelial cell damage intensity (Spearman's correlation coefficient by rank test, r=0.373; p=0.239). In addition, there was also no correlation between the viral load and many other ocular findings, such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP and post-treatment BCVA.

DISCUSSION

The present study analysed ocular manifestations and CMV viral loads in the aqueous humour of patients with CMV-associated iridocyclitis and corneal endotheliitis. Our major findings included: (1) presence of significant corneal endothelial cell loss in both corneal endotheliitis and iridocyclitis tested eyes; and (2) a significant correlation between corneal endothelial cell loss and CMV viral load in the aqueous humour.

Even though it has been demonstrated that viral infections play a significant role in many inflammatory diseases, a qualitative PCR method that is capable of determining the pathological role of these viral infections has yet to be elucidated. If the presence of viral DNA in an affected disease site could be proven, the quantitative determination and correlation with the clinical manifestations of the viral infection could lead to a much deeper understanding of the role of the virus as a pathogenic disease candidate. For example, we have previously reported on two intraocular inflammatory disorders: one involving uveitis associated with human T-cell leukaemia virus type 1 (HTLV-1)[13 14] and the other involving anterior uveitis associated with VZV.[14] In HTLV-1 uveitis, a significantly higher HTLV-1 viral load was detected in the peripheral blood mononuclear cells of the patients compared with asymptomatic HTLV-1 carriers.[13] This viral load was significantly correlated with the vitreous inflammation of the disease.[14] In our report on anterior uveitis associated with VZV, we demonstrated there was a high VZV viral load within the patient's aqueous humour. Furthermore, there was a significant correlation between the viral load and the intensity of the iris atrophy in these patients.[15]

Table 2 Virological analysis and corneal endothelial cell findings in patients with CMV anterior uveitis

Case	Herpes virus DNA		Endothelial cell count (cells/mm^2)		Corneal endothelial cell loss (%)†	Post-treatment BCVA	Interval from onset to sampling (months)
	CMV (copies/ml)	Others*	Affected eye	Fellow eye			
1	2.3×10^5	-	642	2738	77	0.4	96
2	5.5×10^3	-	1633	2869	43	0.8	8
3	1.3×10^4	-	1695	2789	39	1.5	48
4	6.5×10^4	-	1618	3576	55	1.5	24
5	3.5×10^5	-	1445	2608	38	1.2	14
6	5.9×10^6	-	919	2288	45	1.2	16
7	5.4×10^3	-	2512	3917	60	1.2	6
8	1.0×10^6	-	573	2427	76	0.6	12
9	2.8×10^4	-	1427	2262	35	0.7	5
10	1.2×10^4	-	593	2092	72	0.3	4
11	3.6×10^6	-	620	2674	77	1.5	20

Using aqueous humour samples, genomic DNA of the human herpes viruses was measured by qualitative multiplex PCR and quantitative real-time PCR. Corneal endothelial cell count was examined by specular microscopy.
*Herpes viruses excluding CMV, ie herpes simplex virus type 1 and type 2, varicella zoster virus, Epstein–Barr virus, and human herpes virus types 6, 7 and 8.
†Corneal endothelial cell loss was calculated as described in the methods section.
BCVA, best-corrected visual acuity (decimal fraction); CMV, cytomegalovirus.

Although we found that there was a positive correlation between the corneal endothelial cell loss and the CMV viral load in the aqueous humour, there was no correlation between the viral load and many other ocular signs such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP, post-treatment visual acuity and the interval from the disease onset to the aqueous sampling. These patients had been treated with topical corticosteroids (eg betamethasone) and anti-glaucoma agents (eg timolol and latanoprost) before they were referred to us by local ophthalmologists. These treatments are known to reduce the intensity of anterior uveitis, IOP and other ocular manifestations, but have no effect on recovering the corneal endothelial cell damage, because the corneal endothelial cell damage is barely reversible.

The cells and flare in the anterior chamber were mild in all 11 patients. A possible explanation why the intensity of the inflammatory reaction in the anterior chamber was so mild in this disease might be related to the involvement of the anterior chamber-associated immune deviation (ACAID).[16 17] In an experimental rabbit corneal endotheliitis model, eyes inoculated with inactivated HSV-1 prior to an active HSV-1 infection exhibited less severe inflammatory reactions and corneal endotheliitis. In addition, they also developed an immune deviation to HSV-1.[18] Although CMV-related ACAID has not been previously reported, real-time PCR in the present study demonstrated that CMV genomic DNA was present at high levels within the anterior chamber of the patients. Therefore, it may be that ACAID in response to CMV occurs in the eye, resulting in a relatively mild inflammatory reaction.

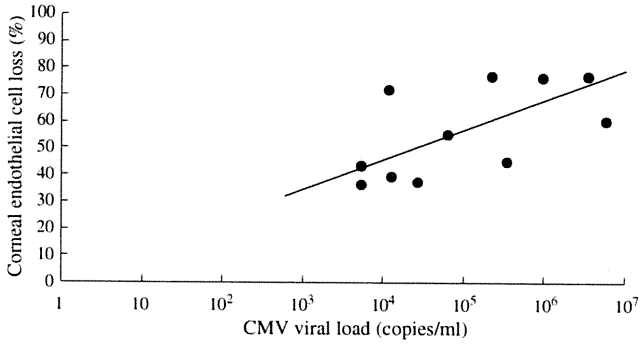

Figure 3 Correlation between cytomegalovirus (CMV) viral load and corneal endothelial cell damage. The CMV viral load was plotted on a logarithmic graph versus the corneal endothelial cell loss (%). The scatter plot shows significant correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036).

While our results showed CMV infection in the anterior segment of the eye caused inflammation and corneal endothelial cells loss in immunocompetent hosts, our study cannot answer many other questions. For example, why does CMV cause intraocular inflammation in immunocompetent hosts? Where does the CMV that is detected in the aqueous humour come from? And how is CMV able to cause inflammatory disorder only within the anterior segment of the eye? One possible explanation why our patients developed CMV anterior uveitis is that all our patients had been given topical corticosteroids for a long period of time. This may have contributed to induce local immunosuppressive condition in the anterior segment of the eye and resulted in reactivation of CMV.[8] Further clinical and experimental investigations are necessary to clarify these important questions.

In conclusion, significant corneal endothelial cell damage was detected in all CMV-associated iridocyclitis- and corneal endotheliitis-tested eyes. In addition, a significant correlation was found between corneal endothelial cell loss and the CMV viral load in the aqueous humour.

Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Ethics Committee of Tokyo Medical and Dental University.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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眼部帯状疱疹の涙液中の水痘・帯状疱疹ウイルスDNA量

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要約 **目的**：眼部帯状疱疹の涙液中の水痘・帯状疱疹ウイルス（varicella zoster virus：VZV）-DNA量の報告。**対象と方法**：対象は眼部帯状疱疹に伴う角結膜炎患者6例。両眼から涙液を採取し，マルチプレックス定性PCRでヘルペスウイルス属をスクリーニングし，リアルタイムPCRでVZV-DNA量を測定した。**結果**：患眼の涙液（n=6）から高値のVZV-DNA（平均 2.4×10^9 copy/ml）が検出された。僚眼の涙液（n=4）からもVZV-DNA（平均 1.0×10^6 copy/ml）が検出された。患眼のウイルス量は僚眼より有意に高値を示した。涙液中のウイルス量と眼所見とに有意な関係はなかった。**結論**：眼部帯状疱疹で，患眼および僚眼の涙液から高値のVZV-DNAが検出された。患眼のウイルス量は僚眼より有意に高かった。

DNA level of varicella zoster virus in the tear fluid in patients with herpes zoster ophthalmicus

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Abstract. **Purpose**：To report the DNA level of varicella zoster virus (VZV) in the tear fluid of patients with herpes zoster ophthalmicus. **Cases and Method**：This study was made on 6 consecutive patients with keratoconjunctivitis as manifestation of herpes zoster ophthalmicus. Genomic DNA of human herpes virus was quantitated in the tear fluid by two polymerase chain reaction (PCR) assays：qualitative multiplex PCR and quantitative real-time PCR. **Results**：Real-time PCR showed an average of 2.4×10^9 copy/ml of VZV-DNA in the tear fluid of 6 affected eyes. The uninfected fellow eyes of 4 patients showed an average of 1.0×10^6 copy/ml in the tear fluid. The difference was significant ($p=0.019$). **Conclusion**：VZV-DNA was detected in the tear fluid in affected and nonaffected eyes of patients with herpes zoster ophthalmicus. The titer was significantly higher in the infected than noninfected fellow eyes.

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緒言

水痘・帯状疱疹ウイルス（varicella zoster virus：以下，VZV）はヒトヘルペスウイルス属の3番目のウイルスで，水痘として初感染し，三叉神経や脊髄後根の神経節中に潜伏感染することが知られている[1]。ウイルスの再活性化により三叉神経第1枝・第2枝支配領域に発症するものを眼部帯状疱疹と呼んでおり，眼瞼炎，結膜炎，強膜炎，虹彩毛様体炎，続発緑内障，網膜疾患，外眼筋麻痺といったさまざまな眼合併症を引き起こす[2]。

これまでに眼部帯状疱疹の涙液中VZV-DNA

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表 1　眼部帯状疱疹に伴う角結膜炎患者の涙液中 VZV-DNA 量と初診時所見

症例	性別	年齢	検体採取前の治療	VZV-DNA (copy/ml) 患眼	VZV-DNA (copy/ml) 僚眼	眼圧 (mmHg) 患眼	眼圧 (mmHg) 僚眼	濾胞性結膜炎	角膜炎	鼻尖部発疹	虹彩毛様体炎
1	男性	68	−	7.1×10^{7}	4.0×10^{5}	未測定	未測定	+	点状表層	+	−
2	女性	60	−	3.6×10^{7}	3.3×10^{3}	21	15	+	偽樹枝状 点状表層	−	−
3	男性	39	−	4.0×10^{8}	未測定	24	17	+	点状表層	−	−
4	男性	66	+	1.1×10^{6}	未測定	17	17	+	点状表層	+	+
5	男性	63	−	1.4×10^{10}	3.9×10^{6}	18	16	+	点状表層	+	−(→+)
6	男性	74	−	1.2×10^{7}	4.0×10^{3}	17	15	+	点状表層	−	−

量を測定した報告は稀であり[3]，ウイルス量と眼所見との関連性についての報告はみられていない。

今回，筆者らは眼部帯状疱疹の両眼から涙液を採取して VZV-DNA 量を測定し，眼所見との関連性についても検討した。

対象と方法

対象は，2005 年 10 月～2008 年 4 月に東京医科歯科大学病院眼科を受診し，眼部帯状疱疹による角結膜炎を生じ，涙液を採取した 6 例 10 眼である。初診時年齢は 39～74 歳（平均 63 歳），男性 5 例，女性 1 例であった。対照検体として，帯状疱疹やヘルペス性眼疾患の既往のない白内障術前患者 5 例 10 眼（年齢は 60～94 歳，平均 76 歳），男性 1 例，女性 4 例からも同様の方法で涙液を採取した。検体はインフォームド・コンセントを行ったうえで採取した。

涙液は，生理食塩水 500 μl を用いて眼表面（角膜・結膜）を洗眼して滅菌注射器に回収する eye wash 法で採取した。検体は，LightCycler™(Roche 社）を用いたマルチプレックス定性 PCR（polymerase chain reaction）でヒトヘルペスウイルス 1～8 型 DNA（HHV1～8）のスクリーニングを行い，陽性検体のみリアルタイム定量 PCR でそのウイルス量を測定した。

6 例中 3 例で，初診時に血清中の抗 VZV-IgG，VZV-IgM 抗体価を酵素免疫測定法（enzyme immunoassay：EIA）で測定した。有意差解析には Mann-Whitney U 検定を用いた。

結　果

1．眼部帯状疱疹に伴う角結膜炎患者の涙液中の VZV-DNA 量

眼部帯状疱疹に伴う角結膜炎がみられた 6 名の患者の性，年齢，および採取された涙液中の VZV-DNA 量と採取時の治療開始の有無について表 1 にまとめた。

マルチプレックス定性 PCR で測定した患眼すべての涙液で VZV-DNA が陽性であった。リアルタイム定量 PCR では VZV-DNA が多く検出され，平均ウイルス量は 2.4×10^{9} copy/ml であった。4 例の僚眼からも涙液を採取し，その全例から VZV-DNA が検出され，ウイルス量は平均 1.0×10^{6} copy/ml であった。患眼と僚眼のウイルス量を比較すると，患眼が僚眼よりも有意に高値を示した（$p=0.019$）。VZV 以外のヒトヘルペスウイルス（HSV-1，HSV-2，EBV，CMV，HHV6，HHV7，HHV8）はすべて陰性であった。

対照の 5 例 10 眼から採取された涙液中のヒトヘルペスウイルス 1～8 型 DNA は，VZV も含めすべて陰性であった。

血清ウイルス抗体価を初診時に測定した 3 症例では，血清 VZV-IgM 抗体陰性，VZV-IgG 抗体陽性の既感染パターンを示した。

2．眼所見

眼部帯状疱疹に伴う角結膜炎がみられた 6 例の患者の初診時眼所見では，全例に濾胞性結膜炎と角膜炎を認め，角膜炎は主に点状表層角膜炎であったが，症例 2 では偽樹枝状角膜炎がみられた（表 1）。初診時の眼圧は，患眼平均 19 mmHg，

僚眼平均16 mmHgで，患眼で有意な上昇がみられた（p=0.023）。鼻尖部の発疹は6例中3例（50%）にみられ，そのうち2例では虹彩毛様体炎が経過中にみられた。涙液中の最もウイルス量の少なかった症例4では，前医で眼部帯状疱疹と虹彩炎に対し抗ウイルス薬治療が開始されていた。最もウイルス量の多い症例5では，初診時には眼内炎症所見はなかったが，経過観察中に虹彩炎が出現し，涙液だけではなく前房水からもVZV-DNAが3.7×10^4 copy/mlと高コピー数検出された。

治療は全症例で抗ウイルス薬（アシクロビル）の点滴静注と眼軟膏，ステロイド点眼を行い，比較的良好な経過で，眼瞼炎，結膜炎，角膜炎などは消退した。

考按

今回の検討では，眼部帯状疱疹に伴う角結膜炎患者の涙液中のウイルス量を測定し，患眼全例から非常に高いウイルス量のVZV-DNAが検出された。涙液中にVZV-DNAが検出される機序として，涙腺は三叉神経節から出る三叉神経第1枝である眼神経，三叉神経第2枝である上顎神経，顔面神経，頸部神経節由来の交感神経に支配されており，三叉神経節に潜伏感染したVZVが再活性化により下行性に涙液中に到達した経路が考えられた。また，感染した角結膜の細胞由来のVZVも含まれている可能性が考えられた。

眼部帯状疱疹の僚眼からも涙液中に高いウイルス量のVZV-DNAが検出された。これまでの報告では，Robertら[3]が眼部帯状疱疹の4例中，患眼4眼中4眼（100%），僚眼4眼中1眼（25%）の涙液からVZV-DNAが検出されたと報告しているが，僚眼からは1例と少ないことから，コンタミネーションの可能性が否定できないと考察している。しかし，筆者らは測定した僚眼すべてから高コピー数のDNAが検出されたため，単なるコンタミネーションの可能性は低いと考えた。

単純ヘルペスウイルスでは，病変形成を伴うことなく涙液や唾液中などにウイルスが排泄される無症候性排泄が知られており，下村[4]はHSV-1では無症候性排泄が12.4%みられたと報告している。一方，健常人における涙液中のVZV-DNA検出率は，Robertら[3]の報告では162眼中0眼（0%），本検討でも10眼中0眼（0%）と検出されておらず，無症候性排泄により健眼の涙液中VZV-DNAが高コピー数を示した可能性は低いと考えた。

眼部帯状疱疹の僚眼から涙液中にVZV-DNAが検出されたこれまでの報告としては，弘重ら[5]はRamsay Hunt症候群（第7・8脳神経領域の帯状疱疹）の15症例の涙液と唾液におけるVZV-DNAの検討で，涙液と唾液からVZV-DNAが検出され，患側だけでなく健側からも検出されたことを報告している。本検討の結果と合わせ，帯状疱疹患者では患側だけでなく健側でもVZVの再活性化が生じている可能性も示された。

帯状疱疹は一般的に加齢，ストレス，悪性腫瘍などVZVに対する特異的な細胞性免疫が低下したときにVZVの局所での再活性化が生じ，患側の皮膚や神経に炎症が出現すると考えられている[1]。

僚眼からも涙液中にVZV-DNAが検出されるもう1つの可能性として，帯状疱疹発症前後にVZVがDNAレベル（あるいはウイルス粒子レベル）で感覚神経内を逆向性に軸索流に乗って伝播し，三叉神経は中枢レベルで交叉するため反対側へ到達し，僚眼涙液中に分泌されるという機序が考えられた。実際，患側に比べて10^3〜10^4レベルでDNAコピー数が少なく，ウイルス量が少ないため健側では発症しないのではないかと推測された。

以上から，眼部帯状疱疹患者では，患眼涙液中に多量のウイルスDNAが検出され，その炎症の病態形成に関与していると思われた。また，患眼だけではなく僚眼からも涙液中にVZV-DNAが検出されていたが，患眼よりも低いウイルス量であった。今後，症例数を増やしてさらなる検討が必要と考えられた。

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べらどんな

墨汁

6・3・3 の新学制は昭和 22 年に始まったが，そのときに占領軍から指令が出た。「道」がつく科目はすべて学校から追放せよというのである。

剣道や柔道ならわかるが，書道もこれに該当することになった。やっと昭和 28 年から小中学校で書道を教えることが認められたが，それよりも前の世代の方々は筆でものを書くことを習わなかったのである。

眼科は墨とかなりの縁がある。網膜の動脈には約 30 μm の幅の無血管帯があることや，網膜の血管そのものが 2 層ないし 3 層で層状になっていることは，眼科医の常識になっている。

これを見事に証明したのはマイケルソン（Isaac C. Michaelson）である。哺乳類の網膜の血管構築を論じた "Retinal Circulation in Man and Animals"（1954）は，146 ページの小さな本であるが，現在でも精読する価値がある名著である。

マイケルソンは，網膜の血管構築を検索する手段として India ink 法を使った。摘出した眼球の血管に墨汁を注入し，これを伸展標本として顕微鏡で観察するのである。

ずっと長いあいだ，インドには特殊な墨があるのだと思っていたが，そうではなかった。墨は中国と日本の特産品だからである。マイケルソン先生も中国製の墨を使ったらしい。

東洋から渡来したものには「インド」と呼ぶ習慣があった。コンサイスに使う薄い紙は，インドとは関係がないのに India paper である。サツマイモを唐芋，カボチャを南瓜と言うのと似ている。

西洋でも羊皮紙などに字を書くときにはインクを使った。煤よりも蛸や烏賊の墨が愛用されたらしい。淡い黒褐色のことをセピアというのは，ラテン語の「烏賊の墨」からきている。イタリア語の seppia は「烏賊そのもの」である。

日本では ink のことをインキまたはインクという。「インク」のほうが正しそうだが，「大日本インキ化学工業」など，会社名ではインキが圧倒的に多い。

これはどうも日本語の癖らしい。ヨードチンキもその例である。これは英語の tincture に相当する。この系統の単語は多く，stain, extinct などもその例だし，赤ワインもスペインでは vino tinto である。どれもラテン語の動詞 tingere「染める」から来ている。

GEN

角膜炎を伴わない単純ヘルペスウイルス1型虹彩毛様体炎の3例

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Three Cases of Herpes Simplex Virus-1 Anterior Uveitis in the Absence of Keratitis

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虹彩毛様体炎患者の前房水より単純ヘルペスウイルス1型（HSV-1）DNAがpolymerase chain reaction（PCR）法で高値を示し，HSV-1による虹彩毛様体炎と考えられたが，経過中に角膜病変がみられなかった3例を経験した．3例ともに虹彩毛様体炎のため東京医科歯科大学眼科を受診し，PCR検査で前房水より高コピー数のHSV-1 DNAが検出された．角膜知覚低下が全例に，高眼圧と隅角色素沈着が3例中2例にみられた．いずれの症例もバラシクロビル内服，アシクロビル眼軟膏，ステロイド点眼，眼圧降下薬で軽快したが，経過観察中に角膜病変および虹彩萎縮はみられなかった．ヘルペスウイルスは虹彩毛様体炎の重要な原因ウイルスであるが，角膜病変や虹彩萎縮などの特徴的な所見がない場合，診断に苦慮することも少なくない．その疑いがあるときには，早期から眼内液のウイルス学的な検査が重要であると思われた．

Herpes simplex virus (HSV) is well known as causing unilateral anterior uveitis characterized by keratitis, mutton-fat precipitates, iridocyclitis, ocular hypertension and iris atrophy. We report here on 3 cases of unusual unilateral anterior uveitis caused by HSV. Polymerase chain reaction was performed to determine the genomic DNA of the human herpes virus in the aqueous humor；HSV-1 DNA was detected in all 3 patients. All patients reported diminished corneal sensation, 2 of the 3 also showing intraocular pressure elevation and moderate pigmentation. However, none showed iris atrophy or keratitis. They were treated with oral valacyclovir, topical acyclovir and corticosteroids. Unilateral anterior uveitis may result from infection by HSV-1 even in the absence of keratitis and iris atrophy. Virological analysis of intraocular fluid, followed by anti-viral treatment, is therefore recommended from the early stage of this disease.

〔Atarashii Ganka (Journal of the Eye) 27(2)：252～255, 2010〕

Key words：ポリメラーゼ連鎖反応，単純ヘルペスウイルス1型，虹彩毛様体炎，角膜炎．polymerase chain reaction (PCR), herpes simplex virus-1 (HSV-1), iridocyclitis, keratitis.

はじめに

ヘルペス性虹彩毛様体炎は，前部ぶどう膜炎の約5～10%を占めるとされており[1]，原因ウイルスとして，単純ヘルペスウイルス（HSV），水痘・帯状疱疹ウイルス（VZV），サイトメガロウイルス（CMV）が報告されている[2,3]．HSVは角膜，前房，虹彩，線維柱帯など多くの眼内組織に炎症を起こすことが知られており，角膜に樹枝状角膜炎や角膜内皮炎などの炎症を伴う場合は角膜ぶどう膜炎（keratouveitis）とよばれる．HSVはHSV-1とHSV-2の2つのサブタイプが存在するが，この角膜ぶどう膜炎の原因ウイルスはほとんどがHSV-1と考えられている．一方，VZVが原因の前部ぶどう膜炎では角膜に炎症所見はなく経過中に虹彩萎縮や麻痺性散瞳などの合併症を伴うことが多い．HSVによる虹彩毛様体炎でもこのような虹彩萎縮がみられることがある．近年，CMVが前部ぶどう膜炎に関与することを示す報告[3,4]があり，このような症例ではPosner-Schlossman症候群に類似

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した高眼圧を伴う虹彩炎か角膜内皮炎の所見を示す．このように，ヘルペスウイルスが原因の前部ぶどう膜炎は，経過中に角膜病変や虹彩萎縮といった特徴的な所見を伴わない場合，臨床的な診断に苦慮することも少なくない．今回筆者らは，経過中に角膜病変や虹彩萎縮などのHSVに特徴的とされる眼所見を伴わない虹彩毛様体炎の前房水よりHSV-1 DNAがpolymerase chain reaction（PCR）検査で陽性を示し，HSV-1虹彩毛様体炎と考えられた3症例を経験したので報告する．

I 症　　例

2007年2月から2008年3月の期間に，東京医科歯科大学病院眼科（以下，当科）を受診し，虹彩毛様体炎の前房水よりHSV-1 DNAが検出され，HSV-1虹彩毛様体炎と考えられた3症例．

〔**症例1**〕 58歳，女性．

現病歴：左眼の視力低下，充血，霧視が出現したため近医を受診し，左眼視力低下（0.6），眼圧上昇（36 mmHg），虹彩炎のためフルオロメトロン点眼，アセタゾラミド内服を開始されたが，改善しないため2007年2月に当科紹介受診となった．

初診時眼所見：視力は右眼0.9（1.2×＋0.25D○cyl－0.75D Ax70°），左眼0.15（0.7×－1.25D○cyl－1.00D Ax75°），眼圧は右眼15 mmHg，左眼25 mmHg．左眼の前眼部に多数の色素を含む豚脂様角膜後面沈着物，cell 2＋程度の中等度の虹彩炎がみられたが，角膜炎はみられなかった（図1）．中間透光体，眼底に異常所見はなく，患眼の隅角に軽度の色素沈着がみられた．角膜知覚は健眼と比べて患眼で低下を示していた．上記の特徴的な眼所見に加えステロイド点眼で改善しないことよりヘルペス性虹彩毛様体炎を疑い，インフォームド・コンセントのもと前房水を0.1 m*l*採取し，定性PCRでヘルペスウイルス1型から8型のスクリーニングを行った．その結果，HSV-1 DNAが陽性を示し，定量PCRでDNAコピー数を測定したところ1.7×10^6 copy/m*l*と高コピー数検出された．

経過：治療はバラシクロビル1,000 mg/日の内服を3週間，アシクロビル眼軟膏1日5回，ベタメタゾン点眼1日4回で徐々に改善し，眼圧上昇はアセタゾラミド1日750 mg内服で速やかに軽快した．経過中に角膜炎，虹彩萎縮，麻痺性散瞳の出現はみられず，右眼視力（1.5×＋0.50D○cyl－0.50D Ax70°）に改善した．

〔**症例2**〕 57歳，男性．

現病歴：前日から右眼の充血，頭痛が出現し，増悪したため2007年2月当科を受診した．

初診時眼所見：視力は右眼0.2（0.4×－0.50D○cyl－1.00D Ax110°），左眼0.7（1.2×＋0.50D○cyl－1.00D Ax20°），眼圧は右眼47 mmHg，左眼18 mmHg．右眼に強い毛様充血，cell 1＋の軽い虹彩炎がみられたが，角膜炎や角膜後面沈着物はみられなかった（図2）．中間透光体，眼底に異常所見はなく，隅角所見に左右差はみられなかったが，角膜知覚は患眼で低下していた．3年前に外眼角部のヘルペスの既往がありヘルペス性虹彩毛様体炎を疑い，前房水を採取し同様にPCRを試行した．その結果HSV-1 DNAが1.2×10^4 copy/m*l*と高コピー数検出された．

経過：治療はバラシクロビル1,000 mg/日の内服を4週間，アシクロビル眼軟膏1日3回，ベタメタゾン点眼1日4回で徐々に改善し，眼圧上昇はD-マンニトールの点滴静注，アセタゾラミド1日750 mg内服で速やかに軽快した．経過中

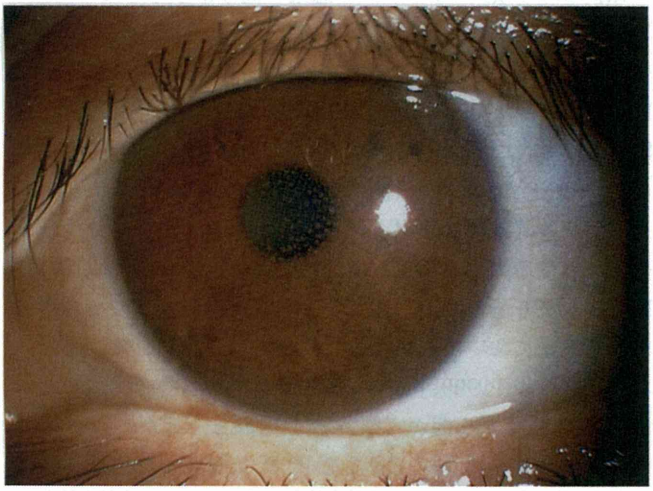

図1　症例1の初診時前眼部写真
多数の色素を含む豚脂様角膜後面沈着物と虹彩炎がみられていた．

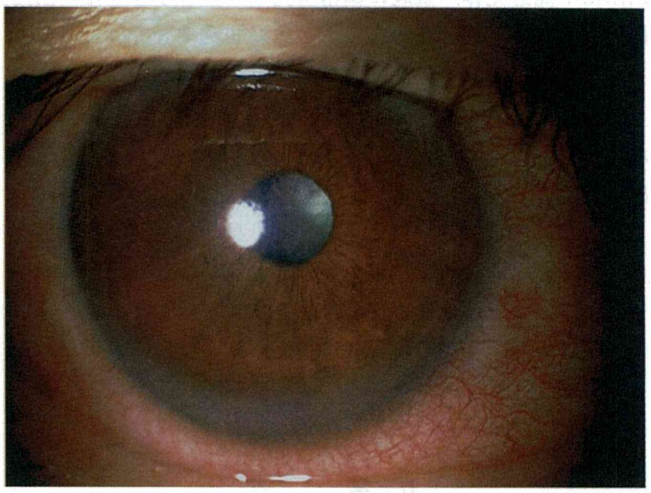

図2　症例2の初診時前眼部写真
強い毛様充血，軽度の虹彩炎がみられたが，角膜炎や角膜後面沈着物はみられなかった．