gated. It is generally accepted that activation of β-adrenergic receptors in the nasal mucosa causes vasodilatation of the blood vessels in the mucosa and a decrease in blood flow.[18,19] On the other hand, whether the ocular circulation is influenced by β-adrenergic antagonists remains contradictory. The results of some studies have indicated that timolol deceases the ocular blood flow and vasoconstriction,[20,21] and other studies report an increase in blood flow and vasodilatation.[22,23] There are even some reports that timolol does not affect the ocular blood flow and vasoconstriction.[24]

From the results of our earlier study, we hypothesized that β-adrenergic receptors are involved in the control of the blood flow of the cavernous body of the NLD by altering its lumen width. To test this hypothesis, we performed dacryocystography to measure the lumen width before and after infusing the nasolacrimal duct with a β-adrenergic receptor antagonist.

METHODS

Subjects

Eighteen (18) patients (14 women and 4 men, age: 64.3 ± 17.4 years; mean ± standard deviation) were studied. Because X-rays are used for dacryocystography, we could not ethically use subjects without disease of the nasolacrimal drainage system as controls. Instead, we selected patients who had a unilateral stenosis or obstruction of the nasolacrimal drainage system or dacryocystitis with epiphora and mucoid discharge from the medial canthal region. These patients required dacryocystography on the symptomatic side to make a definitive diagnosis, and on the asymptomatic side to be certain that the lacrimal drainage system was patent and normal anatomically. All subjects were examined in the Department of Ophthalmology at the Saijo City Shuso Hospital between October 2005 and September 2006. Approval for this study was obtained from the Institutional Review Board of the Ehime University School of Medicine (Ehime, Toon City, Japan). The procedures used conformed to the tenets of the Declaration of Helsinki, and an informed consent was obtained from all subjects after the nature and possible consequences of the study were explained. Patients with any ocular surface diseases, glaucoma, nasal inflammatory diseases, abnormal eyelid position, a recent episode of ocular trauma, acute or chronic upper respiratory inflammation, or eye surgery were excluded. Patients with heart disease or bronchial asthma were also excluded because of the possible adverse reaction to the drug.

The nasolacrimal drainage system on the asymptomatic side was carefully examined in all patients, and no signs of epiphora and mucoid discharge were recognized. Dacryocystography showed no stenosis or obstruction or dilatation of the nasolacrimal system on this side. In addition, the tear meniscus height (TMH) was normal, and the lacrimal system was patent on nasolacrimal irrigation.

Dacryocystography

Dacryocystography was performed as we described in detail,[1] and all examinations were conducted by one of the authors (J.N.). Briefly, dacryocystography was performed on both sides to compare the differences between the asymptomatic and symptomatic sides, and to be certain that stenosis or obstruction was not present bilaterally.

Dacryocystography was performed with a digital radiographic system (ADR-1000A; Toshiba Medical Systems, Tokyo, Japan), and the images were stored electronically as a 1024 × 1024 pixel matrix in a 6-inch image intensifier (II) mode. Under these conditions, one pixel corresponded to approximately 0.118 mm.

To record the X-rays, the patient was placed in a supine position and both eyes were anesthetized topically with 0.4% oxybuprocain hydrochloride. Then, 0.5–1.0 mL of 61.2% iopamidol (Iopamiron 300; Nihon Schering K.K., Osaka, Japan), a water-soluble contrast medium, was infused slowly and steadily under fluoroscopic guidance from the upper punctum into the nasolacrimal drainage system through the canaliculus with a 27-G lacrimal cannula (outer diameter = 0.4 mm, inner diameter = 0.15 mm; Inami, Tokyo, Japan).

Dacryocystography was performed from the anteroposterior and oblique (45-degree lateral oblique) directions, and images were obtained before and after the application of the autonomic drug.

Effect of β-adrenergic antagonist

The effect of β-adrenergic antagonist was estimated as we reported.[1] Each subject received

100 μL of 0.5% timolol maleate, a β-adrenergic antagonist used as a topical medication for glaucoma (Timoptol; Santen Pharmaceutical, Osaka, Japan). After the control dacryocystography images were recorded, approximately 100 μL (corresponding to 2 or 3 drops) of timolol was infused into the asymptomatic side from the upper punctum into the nasolacrimal drainage system through the canaliculus, using a procedure similar to that used to infuse the contrast medium. During the infusion, the lower punctum was compressed to prevent a reflux of the agonist. The patients were asked not to blink and to keep their eyes closed for 15 min in order to reduce the outflow of the drug by lacrimal pumping. Fifteen (15) min later, dacryocystography was performed from the anteroposterior and oblique directions to obtain the post-treatment images.

The width of the lumen of the nasolacrimal drainage system was measured from the dacryocystography images before and after timolol. For all measurements, the electronic images were magnified by six times, based on the scale required to give 1-cm squares that were nontransparent to radiation. The lumen width was measured by identifying one edge of the duct and anchoring one end of an adjustable line at this point. Then, the other end of the line was dragged to the other edge of the duct. The length of the line was calculated automatically based on the number of pixels, and the data were displayed. Thus, at the measurement points, the edges of both sides of the nasolacrimal drainage system were dragged manually, and then the sizes were automatically calculated and displayed on the monitor screen of the digital radiographic system. In addition to the absolute size, the ratio of the change in the lumen was calculated as: ratio (%) = (width before − width after)/width before × 100.

The lumen width of the nasolacrimal drainage system was measured at five points: point 1, at the level of the internal common punctum, which is approximately the upper lacrimal sac (LS); point 2, 5 mm below point 1, which is approximately the middle of the LS; point 3, 10 mm below point 1, which is approximately the lower LS or upper membranous NLD; point 4, 15 mm below point 1, which is approximately the middle membranous NLD; and point 5, 20 mm below point 1, which is approximately the lower membranous NLD.

Statistical analyses

Paired Student *t* tests were used to compare the widths of the lumen before and after administration of the β-adrenergic antagonist at the five points, and one-way analysis of variance with a post hoc Tukey test was used to analyze the ratio of the lumen width after drug administration. A *P*-value of <0.05 was taken to be significant. In these calculations, the pixel-based width was used.

RESULTS

The results of dacryocystography on the symptomatic side are shown in Table 1. No complications were seen during the study, such as ocular hypertension, nasolacrimal stenosis/obstruction, or dacryocystitis.

Dacryocystography before β-adrenergic antagonist

The mean pretreatment lumen widths obtained from the anteroposterior images were 1.68 ± 0.8, 1.89 ± 1.0, 1.18 ± 0.6, 1.84 ± 0.9, and 2.54 ± 1.6 mm at points 1 through 5, respectively. The mean pretreatment lumen widths for these same points in the oblique images were 1.80 ± 0.9, 1.70 ± 1.0, 1.25 ± 0.9, 1.81 ± 1.0, and 2.64 ± 1.4 mm, respectively.

Effects of β-adrenergic antagonist

After timolol infusion, the width of the nasolacrimal drainage system lumen decreased significantly at points 3 through 5 in the anteroposterior images (Fig. 1, left panel; Table 2), and at points 4 and 5 in the oblique images (Fig. 1, right panel; Table 2). The ratio of the lumen widths before and after timolol administration was smaller

TABLE 1. RESULTS OF DACRYOCYSTOGRAPHY IN THE SYMPTOMATIC SIDES

	Number (%)
Nasolacrimal stenosis	6 (33.3)
Chronic dacryocystitis due to nasolacrimal obstruction	3 (16.7)
Functional nasolacrimal obstruction	3 (16.7)
Upper- and lower punctal obstruction	3 (16.7)
Internal common punctal obstruction	2 (11.0)
Canaliculitis	1 (5.6)
Total	**18 (100)**

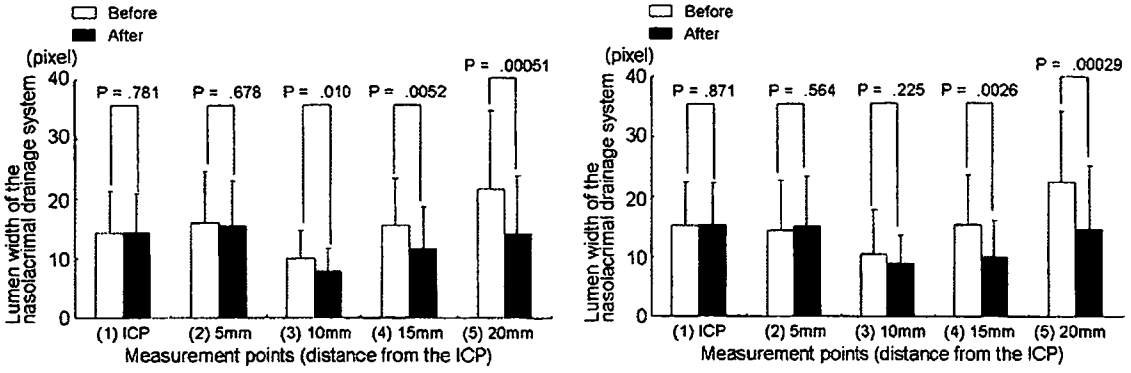

FIG. 1. Comparison of the width of the nasolacrimal drainage system lumen at five points before and after timolol. The anteroposterior images are shown in the left panel, and oblique images are shown in the right panel. Analysis using paired *t* tests. (1), point 1; (2), point 2; (3), point 3; (4), point 4; (5), point 5; ICP, internal common punctum.

at point 5 than at points 1 and 2 in the anteroposterior images (Fig. 2, left panel), and at point 5 than at points 2 and 3, and at 4 than at 2 in the oblique images (Fig. 2, right panel).

These findings indicate that timolol reduced the lumen width of the nasolacrimal drainage system significantly, but the changes were not uniform along the NLD. Our findings demonstrated that the changes in the width of the NLD were more marked in the middle and lower region of the NLD, while the lumen width of the LS was essentially unchanged (Fig. 3).

DISCUSSION

Our results demonstrated clearly that the width of the lumen of the NLD is significantly reduced by timolol, a nonselective β-adrenergic antagonist. This indicates that timolol had a vasodilatory effect on the blood vessels in the cavernous body of the NLD.

Paulsen and colleagues studied the anatomy of the nasolacrimal drainage system, and identified the specialized blood vessels (e.g., the barrier arteries, capacitance veins, throttle veins, and arteriovenous anastomosis).[5,6,10] Based on these findings, they hypothesized that the cavernous body[4–6] plays an important role in the regulation of tear outflow.[8] That is, swelling of the cavernous body occurs when the barrier arteries are opened and the throttle veins closed. The filling of the capacitance veins occurs at the same time with closure of the lumen of the lacrimal passage. In contrast, closure of the barrier arteries and opening of the throttle veins reduce the blood flow to the capacitance veins, and simultaneously allows the blood to flow out from these veins, resulting in shrinkage of the cavernous body and dilatation of the lumen of the lacrimal passage. In addition, the rapid filling of the capacitance veins is possible when the shunts of the arteriovenous anastomosis are open.[6,10] Paulsen and colleagues also suggested that this mechanism may be under autonomic control.

Adrenergic innervations to the blood vessels in the cavernous body can lead to shrinkage of the submucosal cavernous tissue, resulting in an

TABLE 2. COMPARISON OF THE LUMEN WIDTH OF THE NASOLACRIMAL DRAINAGE SYSTEM AT FIVE POINTS BEFORE AND AFTER TIMOLOL (PIXEL)

	Anteroposterior imaging		*Oblique imaging*	
	Before	*After*	*Before*	*After*
Internal common punctum	14.3 ± 7.0	14.4 ± 6.4	15.3 ± 7.3	15.4 ± 7.0
5 mm	16.0 ± 8.7	15.6 ± 7.4	14.4 ± 8.3	15.2 ± 8.1
10 mm	10.0 ± 4.7	7.8 ± 3.9*	10.6 ± 7.3	8.9 ± 4.7
15 mm	15.6 ± 7.8	11.6 ± 7.1**	15.3 ± 8.3	10.0 ± 6.0**
20 mm	21.6 ± 13.3	14.2 ± 9.7***	22.4 ± 11.8	14.7 ± 10.4***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

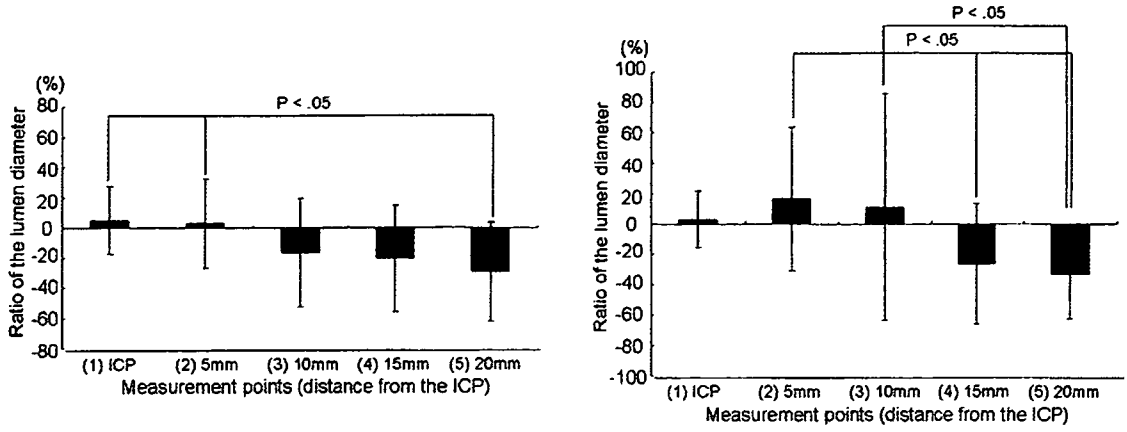

FIG. 2. Comparison of the changes in the ratios of the lumen width after timolol from anteroposterior images (left panel) and oblique images (right panel) with one-way analysis of variance with a post-hoc Tukey test. (1), point 1; (2), point 2; (3), point 3; (4), points 4; (5), points 5; ICP, internal common punctum.

opening of the lacrimal passage. In contrast, cholinergic innervation of the blood vessels of the cavernous tissue may induce a swelling of the tissue.[4,8,10] Our results supported their hypothesis and suggested that β-adrenergic receptors may also be involved in this mechanism.

Anatomically, both the mucosa of the nasolacrimal drainage system and the nasal mucosa have pseudostratified epithelium, goblet cells in the epithelium, mucus glands, resistance vessels (corresponding to the specialized vessels), capacitance vessels, arteriovenous anastomosis, and a cavernous body.[5,6,25] However, the epithelium of the nasolacrimal drainage system have microvilli,[5,26] whereas the epithelium of the nasal mucosa are lined by the kinocilia.[25,27] Therefore, it is suggested that both mucosa have many similarities, but are not comparable as the goblet

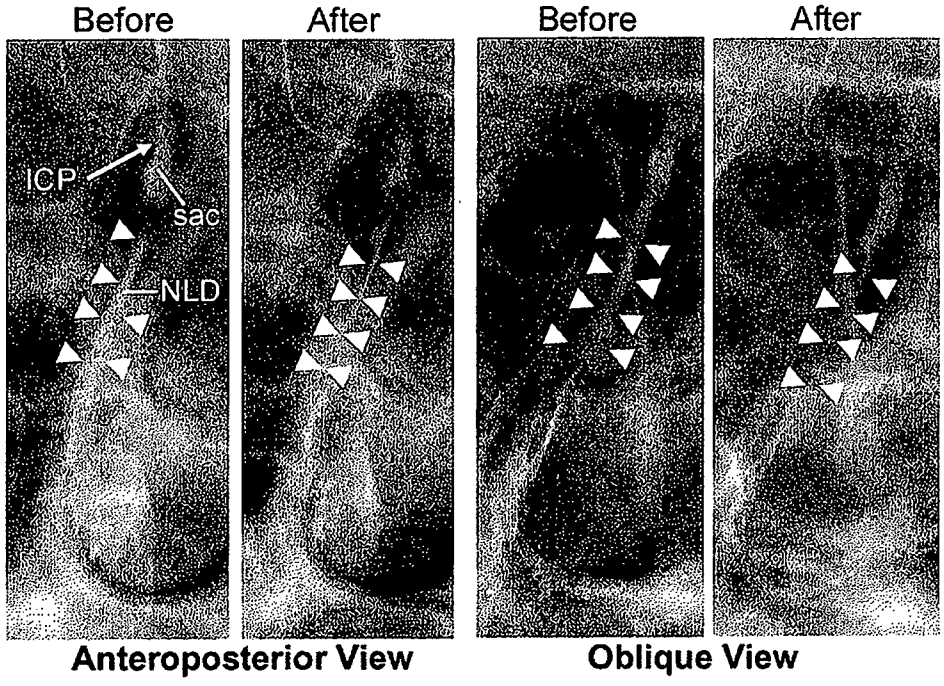

FIG. 3. Dacryocystography showing the reduction of the lumen width of the nasolacrimal drainage system after timolol administration in anteroposterior and oblique views (arrowheads), right side. ICP, internal common punctum; NLD, nasolacrimal duct.

cells. In addition, the intraepithelial distribution of mucus cells and the expression of mucin cytokeration are different in the two types of epithelium.[5,10,25–27]

Physiologically, the "erectile" effect of the cavernous body of the nasal mucosa plays an important role in nasal patency[28] similar to how the cavernous body of the nasolacrimal drainage system may be related to the lumen width of the system. The nasal cavernous body is under autonomic control, and the anatomic and physiologic roles of the nasal cavernous body for nasal patency have been extensively investigated.[15,16,18,19,28–30] Some studies have reported that the capacitance vessels are more prominently affected than the resistance vessels by the autonomic nerves,[28] whereas other studies showed that the responses of the resistance and capacitance vessels are different, depending on the type of agent.[30] In the nasolacrimal drainage system, there is no direct evidence for the existence of β-adrenergic receptors, and it remains to be determined which β-subtype is dominant, and which vessels, (e.g., specialized arteries, capacitance veins, cushion veins, and arteriovenous anastomosis) are predominantly influenced by timolol.

In the nasal mucosa, β-adrenergic stimulation induces a vasodilation, which was the opposite of our observations. This discrepancy may be attributed to: (i) some studies demonstrated that only β_2-adrenergic receptors are present,[31] but not β_1-subtypes in the nasal mucosa, and β_2-agonists were used in these nasal studies, but not β-antagonist, and (ii) differences in drug dosage and concentration. Braakman and associates[23] demonstrated that β-adrenergic antagonists induced a vasodilation only in very high concentrations, but not low; an indirect, hemodynamic mechanism may account for the increase of the ocular perfusion. Thus, the differences in the effects of timolol may depend on its concentration. Although it is not known how different concentrations of topically applied timolol affect the nasolacrimal drainage system, they may be markedly high. Unlike the nasolacrimal drainage system, in the study of the nasal mucosa, the use of a large dose or high concentrations may have confounding effects because they can be easily absorbed to induce hemodynamic side effects in humans.[19] Further immunohistochemical, pharmacologic, and physiologic studies are necessary to determine the distribution and quantification of the β-receptors to answer these questions.

One limitation of this study was the lack of appropriate normal controls who received only water and/or no drug. Therefore, whether timolol reduced the lumen width of the nasolacrimal drainage system through the β-adrenergic antagonistic effects is speculative. We believed that a cross-over study design with the use of water or vehicle on the same patient would have increased the statistical power of our conclusions; however, repeated X-ray radiation would have to be performed on the same patient.

We chose the 0.5% timolol eye drop solution because it is widely used in the management of glaucoma and has the highest concentration that can be purchased in Japan. The β-adrenergic antagonists are usually classified according to the presence or absence of cardioselectivity, that is, intrinsic sympathomimetic activity (ISA), and membrane stabilizing activity.[32] Because timolol is a nonselective β-adrenergic antagonist without ISA,[32] it is unlikely that the change of the lumen width of the NLD was related to the ISA of timolol. It is well known that some β-adrenergic antagonists possess membrane-stabilizing activity[33] that causes local anesthesia.[34] However, it is still debatable whether timolol possesses such activity.[32] Because carteolol and bupranolol have stronger corneal anesthetic activity than timolol,[35] further investigation using these other β-adrenergic antagonists would be necessary to clarify the relationship between the vasodilatory effect and local anaesthetic activity on the width of the lumen of the NLD.

Hester and associates[36] demonstrated that the β-adrenergic antagonists, betaxolol, carteolol, and timolol, have direct vasodilatory effects through the inhibition of Ca^{2+} channels. Therefore, although additional studies will be required to determine the effects of the Ca^{2+} channel blocking agents on the lumen width of the NLD, it may be possible that the vasodilatory effect of the timolol on the NLD may be through the blocking of Ca^{2+} channels.

The action of timolol is linked to its dopaminergic action, (i.e., timolol blocks dopaminergic function).[37,38] Dopamine is present endogenously and low concentrations cause vasodilation and decrease systemic[39] and ciliary blood flow via activation of dopaminergic receptors.[40] However, high doses cause vasoconstriction and increase the blood flow via activation of α-adrenergic receptors.[37,40] As the concentration of the timolol in the nasolacrimal drainage system in our proce-

dure may have attained high levels, the correlation of timolol and dopaminergic function may affect the vasodilation of the nasolacrimal drainage system.

Paulsen and colleagues demonstrated[41] that the drug and lipophilic components of the tear fluid can be absorbed by the mucosa of the nasolacrimal drainage system, and the absorbed timolol by the systemic circulation may also affect the vessels of the nasolacrimal drainage system.

Iodinated radiographic contrast media (RCM) are widely used for image enhancement in several diagnostic procedures.[42] Even nonionic RCM, such as iopamidol, induce adverse reactions, including allergic, renal, and cardiac effects.[42] Orally administrated Iopamidol is not absorbed[43] or only 1% was absorbed from gastrointestinal mucosa.[44] On the other hand, a change in vessel tone is a well-known side effect of RCM[45] after intravenous injection. Some studies have demonstrated the dose-dependent vasoconstrictive effect of iopamidol,[46] and this effect was not induced through the α_1 receptor.[47] In contrast, others reported a vasodilation after applying iopamidol.[48] Therefore, although the absorbed iopamidol from the mucosa of the nasolacrimal drainage system may only be limited and may have some vasoactive effects on the vessels of the system, further studies using appropriate controls will be needed.

Huang and Lee[49] reported that nasolacrimal occlusion by the punctal plug increased the efficacy of antiglaucoma eyedrops. It was also demonstrated that topical timolol decreased the tear turnover, which was probably related to the decreased tear production.[50] Moreover, Zimmerman and associates[51] reported that the nasolacrimal occlusion markedly decreased the systemic side effects.

The relationship between the diameter of the lumen of the nasolacrimal drainage system and the kinetics of tear flow has not yet been clearly determined. If a narrower lumen of the NLD is correlated with a lower tear clearance, the prior application of timolol may increase the efficacy and decrease the systemic side effects of antiglaucomatous drugs in the combination therapy. In contrast, if the narrower lumen facilitates tear outflow, timolol application may decrease the effects of the other drugs and perhaps may induce dry eye disorders. In the clinical situation, it is not clear that whether timolol affects tear outflow or has systemic and/or side effects, and we are not aware of any of these cases. It is also necessary to study these pharmacologic effects in the glaucoma patient with prolonged use of timolol or other adrenergic inhibitors.

During this study, all patients complained of tearing after the second dacryocystography. Ocular surface irritation induces reflex tear secretion[52,53] and decreased tear clearance.[54,55] Moreover, ocular stimulation induced a reflex secretion and, following the application of the topical anesthesia, decreases tear production.[56] Stimulations of the lid margin and cilia also increases the tear turnover rate.[56] We assume that these factors also affected our study (i.e., topical anesthesia) lid and cilia stimulation for infusion of the contrast medium and timolol from the upper punctum not only to the asymptomatic side, but also the symptomatic sides. Therefore, it is difficult to estimate the effect of timolol from the tearing and/or TMH. We believe that additional studies using suitable normal subjects that receive only water or vehicle is needed to exclude the influences of those factors.

CONCLUSIONS

In summary, our data showed that a β-adrenergic antagonist reduced the width of the nasolacrimal drainage system lumen, especially that of the NLD. This supports our previous observation that the tear drainage through the NLD may be influenced, in part at least, by the autonomic nervous system.

REFERENCES

1. Narioka, J., and Ohashi, Y. Changes in lumen width of nasolacrimal drainage system after adrenergic and cholinergic stimulation. *Am. J. Ophthalmol.* 141:689–698, 2006.
2. Bailey, J.H. Surgical anatomy of the lacrimal sac. *Am. J. Ophthalmol.* 6:665–671, 1923.
3. Lang, J. Inferior nasal meatus (anatomy of the routes of access). Clinical anatomy of the nose, nasal cavity and paranasal sinus. New York: Thieme Medical Publishers, Inc. 1989:99–102.
4. Paulsen, F., Hallmann, U., Paulsen, J., et al. Innervation of the cavernous body of the human efferent tear ducts and function in tear outflow mechanism. *J. Anat.* 197:177-187, 2000.
5. Paulsen, F., Thale, A., Kohla, G., et al. Functional

anatomy of human lacrimal duct epithelium. *Anat Embryol.* 198:1–12, 1998.
6. Paulsen, F., Thale, A., Hallmann, U., et al. The cavernous body of the human efferent tear ducts: Function in tear outflow mechanism. *Invest. Ophthalmol. Vis. Sci.* 41:965–970, 2000.
7. Paulsen, F., Thale, A., and Mentlein, R. What happens to tear inside the efferent lacrimal passage? An animal experimental study. *Graefe's Arch. Clin. Exp. Ophthalmol.* 238:496–499, 2000.
8. Ayub, M., Thale, A., Hedderich, J., et al. The cavernous body of the human efferent tear ducts contributes to regulation of tear outflow. *Invest. Ophthalmol. Vis. Sci.* 44:4900–4907, 2003.
9. Paulsen, F., Schaudig, U., and Thale, A. Drainage of tears: Impact of the ocular surface and lacrimal system. *Ocul. Surf.* 1:180–191, 2003.
10. Paulsen, F. The human nasolacrimal ducts. *Adv. Anat. Embryol. Cell Biol.* 170:1–106, 2003.
11. Grevers, G., and Herrmann, U. The "cavernous" tissue of the nasal mucosa. *Laryngol. Rhinol. Otol.* 66:152–156, 1987.
12. Grevers, G., and Kamargakis, W.N. Intervascular smooth muscle fibers and muscular bolsters in nasal swell bodies of humans. *Ann. Otol. Rhinol. Laryngol.* 104:144–148, 1995.
13. Garay, R. Mechanisms of vasomotor rhinitis. *Allergy* 59:4–10, 2004.
14. Whitnall, S.E. The anatomy of the human orbit and accessory organs of vision. 2nd ed. London: Oxford University Press, 1932. (Reprinted and published, New York: Robert E. Krieger, 1979:1–252.
15. Änggård, A. Parasympathetic control of blood circulation and secretion in the nasal mucosa. *Rhinology* 13:147–153, 1975.
16. Eccles, R., and Wilson, H. The autonomic innervation of the nasal blood vessels of the cat. *J. Physiol.* 238:549–560, 1974.
17. Schaeffer, J.P. The nasolacrimal passageways. The nose, paranasal sinus, nasolacrimal passageways, and olfactory organ in man. Philadelphia: P. Blakiston's Son and Co. 1920:237–260.
18. Lacroix, J.S., Kurt, A.M., Auberson, S., et al. Beta-adrenergic mechanisms in the nasal mucosa vascular bed. *Eur. Arch. Otorhinolarungol.* 252:298–303, 1995.
19. Konno, A., Terada, N., and Okamoto, Y. Responses of resistance and capacitance vessels in human nasal mucosa to β-receptor agonists. *ORL* 49:206–213, 1987.
20. Schmetterer, L., Strenn, K., Findl, O., et al. Effects of antiglaucoma drugs in ocular hemodynamics in healthy volunteers. *Clin. Pharmacol. Ther.* 61:583–595, 1997.
21. Chiou, G.C.Y., and Chen, Y.J. Effects of antiglaucoma drugs on ocular blood flow in ocular hypertensive rabbits. *J. Ocul. Pharmacol. Ther.* 9:13–24, 1993.
22. Hayashi-Morimoto, R., Yoshitomi. T., Ishikawa, H., et al. Effects of β antagonists on mechanical properties in rabbit ciliary artery. *Graefe's Arch. Clin. Exp. Ophthalmol.* 237:661–667, 1999.
23. Braakman, R., van der Lindon, P.J.W., Sipkema, P. Effects of topical β-blockers on the diameter of the isolated porcine short posterior ciliary artery. *Invest. Ophthalmol. Vis. Sci.* 40:370–377, 1999.
24. Fuchsjäger-Mayrl, G., Wally, B., Rainer, G., et al. Effects of dorzolamide and timolol on ocular blood flow in patients with primary open angle glaucoma and ocular hypertension. *Br. J. Ophthalmol.* 89:1293–1297, 2005.
25. Berger, G., Balum-Azim, M., and Ophir, D. The normal inferior turbinate: Histomorphometric analysis and clinical implications. *Laryngoscope* 113:1192–1198, 2003.
26. Paulsen, F., Thale, A., Maune S., et al. New insights into the pathophysiology of primary acquired dacryostenosis. *Ophthalmology* 108:2329–2336, 2001.
27. Boysen, M. The surface structure of the human nasal mucosa. *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol.* 40:279–294, 1982.
28. Lung, M.A., and Wang, J.C.C. Autonomic nervous control of nasal vasculature and airflow resistance in the anaesthetized dog. *J. Physiol.* 419:121–139, 1989.
29. Klaassen, A.B., van Megen, Y.J., Kuijpers, W., et al. Autonomic innervation of the nasal mucosa. *ORL J. Otorhinolaryngol. Relat. Spec.* 50:32–41, 1988
30. Malm, L. Resistance and capacitance vessels in the nasal mucosa. *Rhinology* 13:85–89, 1975.
31. Woodhead, C.J., and Nimmo, A.J. Beta adrenoceptors in human nasal mucosa. *J. Laryngol. Otol.* 105:632–634, 1991.
32. Frishman, W.H., and Kostis, J. The significance of intrinsic sympathomimetic activity in beta-adrenoceptor blocking drugs. *Cardiovasc. Rev. Rep.* 3:503–512, 1982.
33. Gill, E.W., and Vaughan Williams, E.M. Local anesthetic activity of the β-receptor antagonists, pronethalol. *Nature* 201:199, 1964.
34. Shand, D.G. Clinical pharmacology of beta-blocking drugs: Implications for the postinfarction patient. *Circulation* 67:I-2-5, 1983.
35. Novack, G.D. Ophthalmic beta-blockers since timolol. *Surv. Ophthalmol.* 31:307–327, 1987.
36. Hester, R.K., Chen, Z., Becker E.J., et al. The direct vascular relaxing action of betaxolol, carteolol, and timolol in porcine long posterior ciliary artery. *Surv. Ophthalmol.* 38:125–134, 1994.
37. Watanabe, K., and Chiou, G.C.Y. Action mechanism of timolol to lower the intraocular pressure in rabbits. *Ophthalmic Res.* 15:160–167, 1983.
38. Chiou, G.C.Y., and Chiou, F.Y. Dopaminergic involvement in intraocular pressure in the rabbit eye. *Ophthalmic Res.* 15:131–135, 1983.
39. Brodde, O.E. Vascular dopamine receptors: Demonstration and characterization by *in vitro* studies. *Life Sci.* 31:289–306, 1982.
40. Reitsamer, H.A., and Kiel, J.W. Effects of dopamine on ciliary blood flow, aqueous production, and intraocular pressure in rabbits. *Invest. Ophthalmol. Vis. Sci.* 43:2697–2703, 2002.
41. Paulsen, F., Föge, M.,Thale A., et al. Animal model for the absorption of lipophilic substances from tear

fluid by the epithelium of the nasolacrimal ducts. *Invest. Ophthalmol. Vis. Sci.* 43:3137–3143, 2002.
42. Sueyasu, M., Komasu, C., Hara T., et al. Enhancement by morphine of radiographic contrast media-induced histamine release in rat peritoneal mast cells. *Jpn. J. Pharmacol.* 74:217–220, 1997.
43. Miyazawa, T., Murayama, C., Ozawa, M., et al. Comparison of iopamidol and diatrizoate with iotrolan as a contrast medium for radiographic gastrointestinal tract examinations. *Invest. Radiol.* 26:S182–S184, 1991.
44. Rinetti, M., Sivelli, R., Palli, D. Blood and urinary levels of Iopamidol (Gastromiro "300"®) after contrast examination of the digestive tract. *Rays* 12:35–42, 1987.
45. Karstoft, J., Bååth, L., Jansen, I., et al. Vasoconstriction of isolated arteries induced by angiographic contrast media. *Acta. Radiol.* 36:312–316, 1995.
46. Rauch, D., Drescher, P., Pereira, F.J., et al. Comparison of iodinated contrast media-induced renal vasoconstriction in human, rabbit, dog, and pig arteries. *Invest. Radiol.* 32:315–319, 1997.
47. Drescher, P., Rauch, D., Knes, J.M., et al. Contrast medium induced renal vasoconstriction, role of alpha receptors. *Scand. J. Uro. Nephrol. Suppl.* 172:103–108, 1995.
48. Egashira, S., Tomoike, H., Nishijima, H., et al. Effects of ionic and nonionic contrast media on coronary diameter and blood flow in chronically instrumented dogs. *Am. Heart J.* 118:39–46, 1989.
49. Huang, T.C., and Lee, D.A. Punctal occlusion and topical medications for glaucoma. *Am. J. Ophthalmol.* 107:151–155, 1989.
50. Kuppens, E.V.M., Stolwijk, T.R., de Keizer, R.J., et al. Basal tear turnover and topical timolol in glaucoma patients and healthy controls by fluorophotometry. *Invest. Ophthalmol. Vis. Sci.* 33:3442–3448, 1992.
51. Zimmerman, T.J., Kooner, K.S., Kandarakis, A.S., et al. Improving the therapeutic index of topically applied ocular drugs. *Arch. Ophthalmol.* 102:551–553, 1984
52. Mishima, S., Gasset, A., Klyce S.D., et al. Determination of tear volume and tear flow. *Invest. Ophthalmol.* 5:264–276, 1966.
53. Jones, L.T. The lacrimal secretory system and its treatment. *Am. J. Ophthalmol.* 62:47–60, 1966.
54. Afonso, A.A., Monroy, D., and Stern, M.E. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. *Ophthalmology* 106:803–810, 1999.
55. Prabhasawat, P., and Tseng, S.C.G. Frequent association of delayed tear clearance in ocular irritation. *Br. J. Ophthalmol.* 82:666–675, 1998.
56. Jordan, A., and Baum, J. Basic tear flow: Does it exist? *Ophthalmology* 87:920–930, 1980.

Received: March 11, 2007
Accepted: June 27, 2007

Reprint requests: *Junji Narioka*
Department of Ophthalmology
Ehime University School of Medicine,
Shitsukawa, Toon City
Ehime, 791-0295
Japan

E-mail: nariokaj@m2.dion.ne.jp

Cytomegalovirus as an Etiologic Factor in Corneal Endotheliitis

Noriko Koizumi, MD, PhD,[1,3] Takashi Suzuki, MD, PhD,[2] Toshihiko Uno, MD, PhD,[2] Hidemi Chihara, MD,[1] Atsushi Shiraishi, MD, PhD,[2] Yuko Hara, MD,[2] Tsutomu Inatomi, MD, PhD,[1] Chie Sotozono, MD, PhD,[1] Satoshi Kawasaki, MD, PhD,[1] Kenta Yamasaki, MS,[1] Chikako Mochida, BPharm,[1] Yuichi Ohashi, MD, PhD,[2] Shigeru Kinoshita, MD, PhD[1]

Purpose: To investigate clinical manifestations and response to antiviral therapy of 8 patients with cytomegalovirus (CMV)-induced corneal endotheliitis who were diagnosed and treated at 2 university hospitals in Japan.

Design: Retrospective, consecutive, multicenter case series.

Participants: Eight eyes of 8 patients diagnosed with active CMV corneal endotheliitis at Kyoto Prefectural University of Medicine and Ehime University School of Medicine. The diagnosis was made based on the detection by polymerase chain reaction assay of CMV, but not herpes simplex virus (HSV) and varicella zoster virus (VZV) DNA, in the aqueous humor from the affected eye.

Methods: Retrospective review of the clinical manifestations and responses to antiviral treatment.

Main Outcome Measures: Patient profiles, including duration of corneal endotheliitis, systemic disease, intraocular pressure, and clinical manifestation of anterior and posterior segments. The clinical response to systemic and topical antiviral treatment was evaluated by slit-lamp examination. Corneal endothelial density was examined by specular microscopy.

Results: The average observation period after CMV detection was 10.4 months (range, 2–24 months). None of the patients had systemic immunodeficiency. Corneal manifestations included linear keratic precipitates associated with multiple coin-shaped lesions and local corneal stromal edema. Of the 8 patients, 4 had undergone penetrating corneal transplantation. Systemic ganciclovir therapy was used in 7 patients, and in 1 patient, valacyclovir was administered, with the corneal endotheliitis responding quickly to the early administration of galovir. At the final examination, 6 eyes had a clear cornea, but 2 eyes had bullous keratopathy.

Conclusions: Besides HSV and VZV, CMV must be considered as an etiologic agent in patients with corneal endotheliitis. Cytomegalovirus corneal endotheliitis may be a newly identified clinical entity of reactivated CMV in the anterior chamber of individuals free of accompanying systemic symptoms. *Ophthalmology 2008;115:292–297 © 2008 by the American Academy of Ophthalmology.*

The corneal endothelium comprises the innermost layer of the cornea and plays an important role in maintaining corneal clarity. Corneal endotheliitis, a specific inflammation of the corneal endothelium, is characterized by corneal edema associated with linear keratic precipitates and destruction of the endothelium and often leads to severe visual disturbance. Khodadoust and Attarzadeh,[1] who first reported bilateral recurrent corneal edema associated with linear keratic precipitates similar to endothelial rejection lines, proposed the designation *presumed autoimmune corneal endotheliopathy*. However, later observations that some patients with corneal endotheliitis responded poorly to corticosteroid therapy suggested an infectious disease origin.[2] Some reports suggested herpes simplex virus (HSV) as an etiologic agent for corneal endotheliitis,[3,4] and HSV and varicella zoster virus (VZV) have been detected in the corneal endothelium,[5] aqueous humor,[6–8] and trabeculae[8,9] of patients with corneal endotheliitis. An association between herpes virus and corneal endotheliitis was supported by the favorable response of corneal endothelial lesions to topical and systemic acyclovir treatment,[10] and corneal endotheliitis is now considered one of the clinical entities of corneal infection with herpes virus species.[11,12] However, the aqueous humor of patients with corneal endotheliitis

Originally received: January 27, 2007.
Final revision: April 4, 2007.
Accepted: April 6, 2007.
Available online: July 31, 2007. Manuscript no. 2007-125.

[1] Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

[2] Department of Ophthalmology, Ehime University School of Medicine, Ehime, Japan.

[3] Research Center for Regenerative Medicine, Doshisha University, Kyoto, Japan.

Drs Koizumi and Suzuki contributed equally as co–first authors.

Supported in part by research grants from the Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan.

The authors have no commercial or financial interests associated with the article.

Correspondence to Noriko Koizumi, MD, PhD, Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan. E-mail: nkoizumi@ophth.kpu-m.ac.jp.

ISSN 0161-6420/08/$–see front matter
doi:10.1016/j.ophtha.2007.04.053

often demonstrates negative results for HSV and VZV DNA, and they do not respond well to antiherpetic treatment. These cases are diagnosed as idiopathic corneal endotheliitis and their prognosis is poor because of progressive deterioration of the corneal endothelium.

Members of the authors' group from Kyoto Prefectural University of Medicine recently reported a patient with unilateral corneal endotheliitis without systemic immunodeficiency whose aqueous humor was found by polymerase chain reaction (PCR) assay to contain cytomegalovirus (CMV) DNA.[13] To the best of the authors' knowledge, this was the first report of CMV-induced corneal endotheliitis. Subsequently, other members of our group from Ehime University School of Medicine reported a second case of CMV corneal endotheliitis in a patient who had undergone keratoplasty.[14] Recently, other investigators have described CMV corneal endotheliitis diagnosed by nested multiplex PCR in human immunodeficiency virus-negative patients.[15] Cytomegalovirus is an opportunistic pathogen that induces various pathologic characteristics in immunosuppressed individuals. Although many late-phase AIDS patients experience CMV retinitis, with one article reporting a case of corneal stromal keratitis in CMV retinitis patients,[16] there have been few reports of primary CMV corneal infection that are not accompanied by CMV retinitis in either immunocompromised or immunocompetent hosts. The authors consider CMV to be an important etiologic factor of corneal endotheliitis and herein present clinical findings and responses to antiviral therapy of 8 patients diagnosed with CMV corneal endotheliitis based on the PCR detection of CMV DNA from aqueous humor.

Patients and Methods

The study population consisted of 8 consecutive patients whose active corneal endotheliitis was diagnosed and treated between November 2004 and May 2006 at the Departments of Ophthalmology of Kyoto Prefectural University of Medicine (patients 1–5) and Ehime University School of Medicine (patients 6–8). Of these 8 patients, patients 1 and 6 were previously reported as individual case reports by these 2 departments independently[13,14]; they are also included here with more detailed information of long-term observation. The study protocol was approved by the university ethical committees and informed consent was obtained from all patients. The cohort comprised 6 men and 2 women ranging in age from 51 to 83 years (mean, 68.5 years). Using a 30-gauge needle, 100 μl aqueous humor were aspirated from the affected eye and subjected to PCR assay.

In Kyoto Prefectural University of Medicine, DNA extraction and PCR condition were based on the previously reported method established by the authors' group for the diagnosis of herpes virus ocular diseases.[17–19] Aqueous humor samples were incubated at 55° C for 5 hours in a final reaction volume of 500 μl containing 0.5% sodium dodecyl sulfate, 100 μg/ml proteinase K, 1 mmol/l ethylenediaminetetraacetic acid, and 10 mmol/l TRIS hydrochloride (pH, 7.8). After glycogen was added as a carrier, the DNA was precipitated with ethanol and dissolved in 10 μl distilled water. The PCR reaction for each sample included 1 μl DNA and 24 μl PCR reaction buffer (10 mmol/l TRIS hydrochloride, 1.5 mmol/l magnesium chloride, 0.01% gelatin) in the presence of 80 μm each of all 4 triphosphates, 5 pmol each of oligonucleotide primers, and 1.25 U Taq DNA polymerase. The primers used for CMV allowed amplification of a 400-base pair sequence of CMV DNA that codes for a portion of a late antigen of CMV[20] (5′-CACCTGTCACCGCTGCTATATTTGC, 3′-CACCACGCAGCGGCCCTTGATGTTT). For HSV amplification, primer pairs previously shown to identify a specific 142-base pair segment of the DNA polymerase gene of the HSV genome[21] (5′-ATCCGAACGCAGCCCCGCTG, 3′-TCTCCGTCCAGTCGTTTATCTTC) were used. The primer pairs for VZV were used to amplify a 642-base pair target sequence that is incorporated in the EcoR1-D fragment of the VZV genome[22,23] (5′-TTCAGCCAACGTGCCAATAAA, 3′-GACGCGCTTAACGGAAGTAAC). Thirty-two independent repeated cycles of the PCR reaction were carried out at 94° C for 1 minute, 55° C for 1 minute, and 72° C for 1 minute. The authors confirmed that the method was sufficiently sensitive to detect HSV DNA in eyes with clinically diagnosed herpetic keratitis and uveitis and was reliable enough not to give false-positive results in normal subjects (reduced sensitivity PCR).[17–19]

In Ehime University, DNA extraction and viral PCR were performed by a Japanese company specializing in laboratory testing services (SRL, Inc., Tokyo, Japan).[14] DNA was extracted from specimens using QIAamp DNA Blood Mini kit (Qiagen, Inc., Valencia, CA). An 186-base pair segment of the major immediate-early gene of the CMV genome was amplified by PCR using primer pairs (5′-TTA GTG AAC CGT CAG ATC GC, 3′-GCA TGC ATA AGA AGC CAA GG). Herpes simplex virus DNA and VZV DNA were detected by PCR using primer pairs that identify a specific 234-base pair segment of the DNA polymerase gene of the HSV genome (5′-AGATGGCGAGCCACATCTC, 3′-CTCCGGATACGGTATCGTC) and a specific 216-base pair segment of the *ORF38* gene of the VZV genome (5′-TCACGAACCGTTGACAGGAC, 3′-CCACTACTCATTGTATCCGCG). The PCR cycling conditions were: 40 cycles of denaturation for 1 minute at 94° C, elongation for 2 minutes at 72° C, and annealing for 1 minute at 57° C for VZV or 58° C for CMV and HSV.[14]

Results

The aqueous humor collected from the affected eyes of patients 1 through 5 contained CMV, but not HSV or VZV DNA. For these 5 eyes, the specificity of the amplified band was confirmed by Southern blot analysis.[17] Under the same PCR conditions, none of 5 aqueous samples from cataract patients nor 7 aqueous samples from patients with uveitis demonstrated positive results for CMV, HSV, or VZV DNA. Likewise, the aqueous humor from the affected eyes of patients 6 through 8 contained CMV, but neither HSV nor VZV DNA were detected in aqueous humor samples of a patient without intraocular inflammation nor in 3 nonviral uveitis patients.

The clinical profiles of all 8 patients from the 2 centers is summarized in Table 1 (available at http://aaojournal.org). Of the 8 patients, 4 had never undergone corneal surgery, and of these 4 patients, 3 patients (patients 1, 7, and 8) had a history of chronic uveitis of unknown origin. The other 4 patients (patients 2, 4, 5, and 6) had previously received penetrating keratoplasty for endothelial dysfunction resulting from recurrent corneal endotheliitis (patient 4), for Fuchs' dystrophy (patient 2), or for corneal opacity (patients 5 and 6). None of the patients manifested systemic immune deficiency or CMV antigenemia. Serologic analysis of immunoglobulin M and immunoglobulin G showed that all 8 patients had previously been infected with CMV. The average follow-up period after CMV detection was 10.4 months (range, 2–24 months). Because patients 3, 4, 6, and 7 were followed up under an initial diagnosis of idiopathic corneal endotheliitis before the detection of CMV DNA, the average duration of corneal endotheliitis was 46.6 months (range, 2 months–20 years).

The clinical findings of CMV corneal endotheliitis are summarized in Table 2 (available at http://aaojournal.org). Typical findings, including linear keratic precipitates associated with multiple coin-shaped lesions and localized corneal stromal edema, were observed in 4 eyes (Fig 1A). According to the previously reported classification of HSV corneal endotheliitis,[11] corneal manifestations of these 8 cases were classified as linear corneal endotheliitis, but not disciform or diffuse corneal endotheliitis. Linear keratic precipitates were found at the leading edge of the corneal endotheliitis and advanced slowly centrally before ganciclovir treatment. After ganciclovir therapy, the corneal edema subsided quickly and the leading edge moved back to the peripheral cornea. In all 8 patients, coin-shaped (nummular) lesions were detected on the corneal endothelial surface. These consisted of medium-sized keratic precipitates arranged in a circumferential pattern. Coin-shaped lesions typically were detected on the central side (clear area) of the leading edge of the corneal edema (Fig 1A, C). In the area inside the coin-shaped lesions, corneal edema was prominent in cases without topical steroid administration (Fig 1C), whereas with topical steroid therapy, less corneal edema was detected and coin-shaped lesions were seen more clearly by retroillumination (Fig 1E). In the 4 patients who had undergone keratoplasty, keratic precipitates were seen on both donor and host corneas, thus distinguishing the corneal endotheliitis from endothelial rejection. Invariably, the corneal endotheliitis started in the peripheral area of the graft, including the surrounding host cornea. With the exception of patient 7, the anterior chamber reaction was minimal. None of the 8 patients had CMV retinitis at presentation. In 6 eyes, intraocular pressure (IOP) was elevated; these patients received a topical or systemic medication for IOP control.

Medications for corneal endotheliitis and final outcome are shown in Table 3 (available at http://aaojournal.org). Systemic antiviral therapy consisting of ganciclovir was delivered immediately after diagnosis in 6 patients (all except patients 2 and 3). Patient 2 started ganciclovir treatment 8 months after CMV detection, and patient 3 declined ganciclovir treatment and was treated with valacyclovir. Of the 8 patients, 6 patients (patients 1, 4, 5, 6, 7, and 8) responded quickly to systemic antiviral therapy. At midterm follow-up, 6 eyes retained a clear cornea; however, patients 2 and 3 experienced bullous keratopathy resulting from irreversible corneal endothelial dysfunction (Fig 1B).

Case Reports

Patient 4

A 51-year-old man began to experience recurrent episodes of bilateral corneal endotheliitis of unknown origin in the 1980s (Fig 1C). Aqueous humor samples subjected to PCR at the time of his previous attack returned negative results for HSV and VZV; CMV was not examined at that time. Despite HSV-targeting combination treatment consisting of oral acyclovir and topical acyclovir and corticosteroid, his corneal endotheliitis recurred often and he experienced bullous keratopathy. He had undergone penetrating keratoplasty in his right eye in 1984 and in both eyes in 1986; however, recurrent corneal endotheliitis with multiple coin-shaped lesions in both eyes (Fig 1D) culminated in graft failure. His visual acuity was hand movements in both eyes. Polymerase chain reaction analysis of aqueous humor samples collected during a second keratoplasty for his left eye in July 2005 at Kyoto Prefectural University of Medicine detected CMV DNA. Its specificity was confirmed by Southern blot analysis (Fig 2).

After surgery, his best-corrected visual acuity in the left eye improved to 20/50; however, 6 weeks later, he again experienced left corneal endotheliitis with multiple coin-shaped lesions on the endothelial surface of both donor and host sides. No cellular infiltration was detected in the corneal stroma. Based on the diagnosis of CMV corneal endotheliitis, 10 mg/kg ganciclovir daily for 10 days was administered intravenously. He responded quickly and has remained free of further episode. The graft placed in July 2005 was clear at 1 year of follow-up, and no CMV DNA was detected in aqueous humor from the left eye.

Patient 7

A 60-year-old man had been receiving treatment with corticosteroid eye drops since November 2000 for corneal subepithelial infiltrates of unknown origin in the right eye. Slit-lamp examination at Ehime University School of Medicine in May 2004 revealed white keratic precipitates lining a coin-shaped lesion in the lower nasal part of the cornea, and in October 2004, corneal edema and coin-shaped lesions were noted that were strongly suspicious of corneal endotheliitis (Fig 1E). Although topical acyclovir and oral valacyclovir were added to his corticosteroid eye drops, the area of stromal edema spread progressively and he experienced inflammation of the anterior chamber and anterior vitreous. In September 2005, he underwent pars plana vitrectomy to remove vitreous opacities. Vitreous samples collected during the operation demonstrated negative results for HSV and VZV DNA. Assay for CMV was not carried out at that time.

After surgery, the vitreous inflammation subsided; however, the corneal stromal edema progressed centrally (Fig 1F) and his IOP increased. Aqueous humor samples were subjected to PCR, and CMV DNA was detected. Under a diagnosis of corneal endotheliitis of CMV origin, systemic (10 mg/kg daily for 7 days) and topical (0.5% eye drops 8 times daily) ganciclovir were administered. In a rapid response to this treatment, the stromal edema disappeared and his IOP decreased. He was instructed to continue the topical application of ganciclovir, and there has been no recurrence for 8 months.

Discussion

The mechanisms regulating the latency and reactivation of CMV in the course of natural infection remain poorly understood. Cytomegalovirus DNA has been detected in human peripheral blood monocytes[24,25] and $CD34^+$ bone marrow progenitors.[26] The clinical observations that corneal endothelial lesions always start from the periphery and move toward the center of the cornea imply that tissues surrounding the cornea such as the trabecular meshwork or ciliary body may be the reservoir for CMV. The finding that bone marrow–derived cells migrate to the cornea[27] supports this hypothesis, as does the observation that murine CMV inoculated intraperitoneally reaches the cornea of nude mice via the bloodstream.[28]

Although the pathogenesis of CMV corneal endotheliitis is not yet understood, one must consider that anterior chamber-associated immune deviation (ACAID) occurs in the anterior chamber, a unique immune-privileged site.[29–32] Zheng et al,[33] who suggested that ACAID may play an important role in the pathogenesis of herpetic corneal endotheliitis, established an animal model by inducing ACAID in rabbits first inoculated with inactivated HSV and then infected intracamerally with live HSV. In these animals, endothelial lesions developed whose clinical appearance greatly resembled human corneal endotheliitis. Their findings raise the

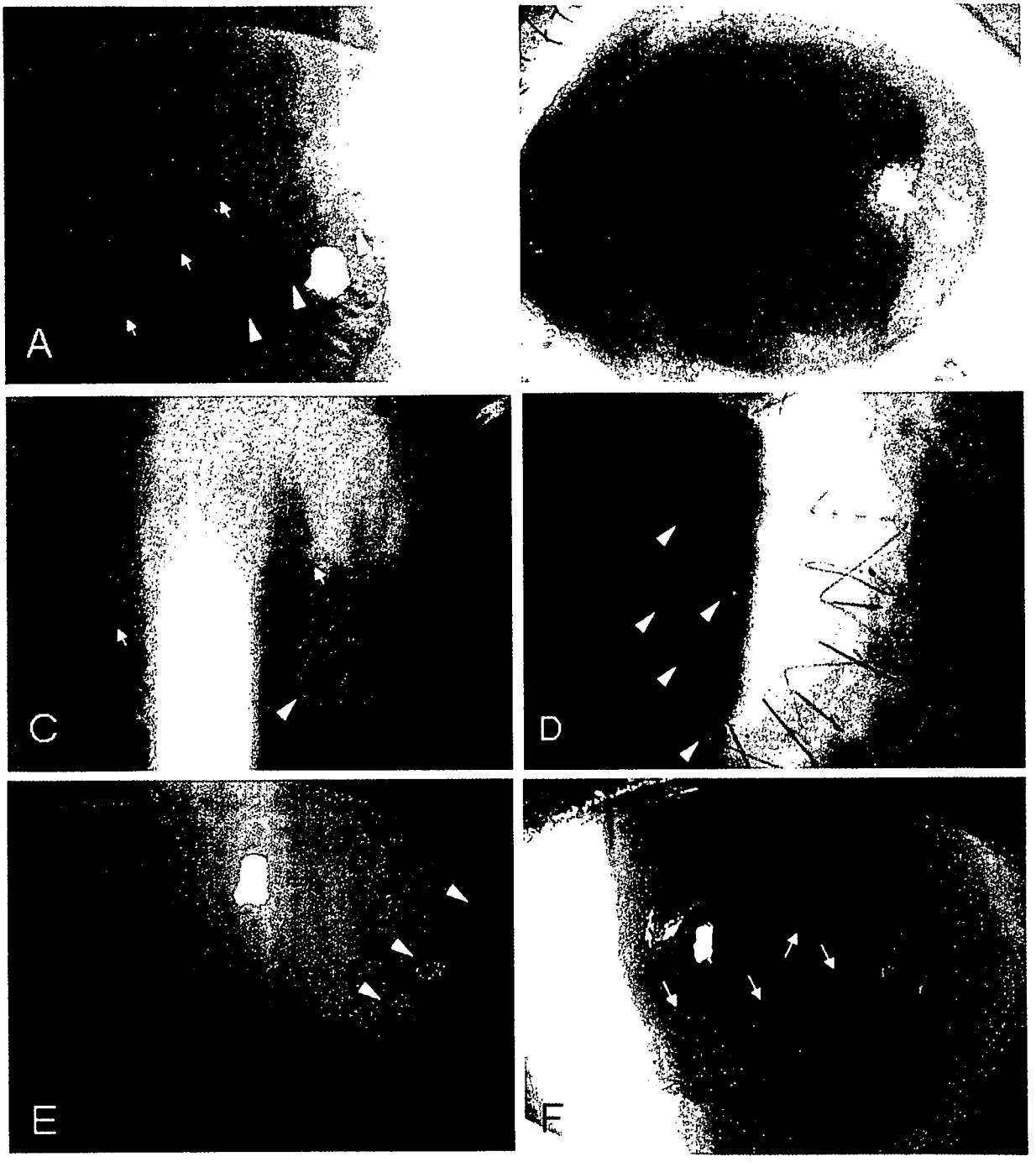

Figure 1. Clinical manifestations of cytomegalovirus corneal endotheliitis examined by slit-lamp biomicroscopy. **A**, Photograph from patient 1 showing localized corneal stromal edema in the upper half of the cornea. Note the linear keratic precipitates (arrows) and coin-shaped lesions (arrowheads). **B**, Photograph from patient 3 showing that the corneal endotheliitis failed to respond to more than 1 year of treatment with acyclovir. Because analysis demonstrated that the aqueous humor had negative results for herpes simplex virus (HSV) and varicella zoster virus (VZV) DNA at disease onset, a diagnosis of idiopathic corneal endotheliitis was made. One year after onset, polymerase chain reaction analysis identified cytomegalovirus (CMV) DNA in the aqueous humor. Corneal edema spread over the entire cornea, and eventually, bullous keratopathy developed in the patient. **C**, Photograph obtained at disease onset from patient 4 showing severe corneal edema in the upper half of the cornea. Note the linear keratic precipitates (arrows) and coin-shaped lesions (arrowhead). The patient had negative results for HSV and VZV DNA, and a diagnosis of idiopathic corneal endotheliitis was made. Eventually, bullous keratopathy developed in the eye. **D**, Photograph from patient 4 obtained 3 months after keratoplasty corneal endotheliitis recurred, accompanied by the typical coin-shaped lesions (arrowheads). **E**, Photograph from patient 7 obtained at disease onset. In October 2004, corneal endotheliitis was diagnosed based on the presence of coin-shaped lesions (arrowheads) accompanied by slight corneal stromal edema. The response to acyclovir and valacyclovir was insufficient, and the corneal endotheliitis worsened. **F**, Photograph from patient 7 obtained 11 months after disease onset. In September 2005, the corneal stromal edema became severe and involved approximately 75% of the cornea (arrows). Polymerase chain reaction analysis detected CMV DNA in the aqueous humor.

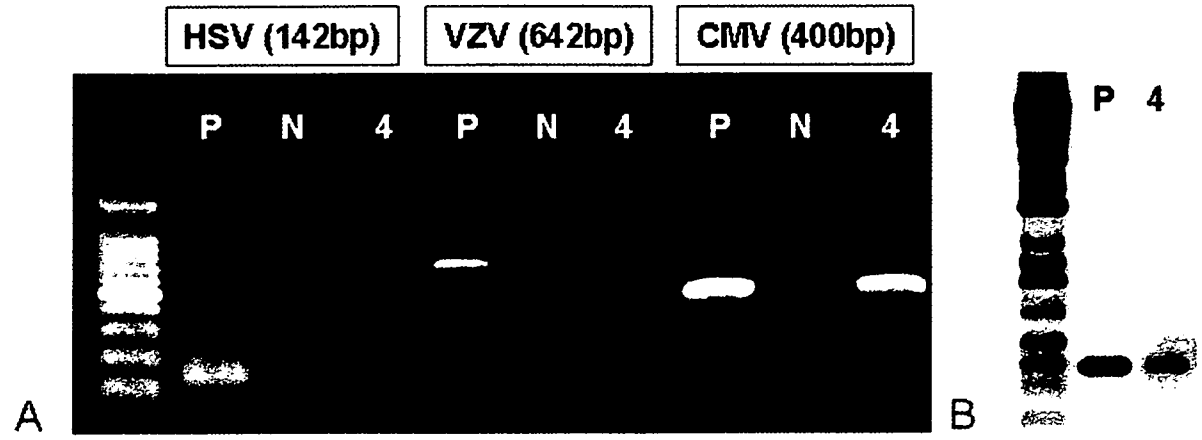

Figure 2. A, Representative polymerase chain reaction assays for DNA from herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV) that were performed at Kyoto Prefectural University of Medicine. B, Southern blot analysis results confirming the specificity of the amplified CMV DNA. 4 = aqueous humor sample from patient 4; N = negative control; P = positive control.

possibility that ACAID is the underlying pathogenic mechanism of herpetic corneal endotheliitis. If CMV is reactivated in the anterior chamber (including the corneal endothelium) and ACAID prevents the control of its proliferation by cell-mediated immunity, viral infection may develop in the corneal endothelium. The authors postulate that this was the scenario in their patients.

Cytomegalovirus corneal endotheliitis may represent a newly identified clinical entity of reactivated CMV in the anterior chamber of healthy subjects free of accompanying systemic symptoms. In patients with this disease, it is crucial to consider the possibility not only of HSV and VZV, but also of CMV infection, especially if there is a poor response to topical and systemic acyclovir or coin-shaped lesions develop. These patients should be switched to systemic ganciclovir to prevent further loss of corneal endothelial cells. It is noteworthy that the 2 patients in the current series (patients 2 and 3) who did not have ganciclovir treatment soon after CMV detection failed to maintain a clear cornea.

Coin-shaped lesions are observed commonly in patients with CMV-induced corneal endotheliitis, and clinical manifestations similar to the coin-shaped lesions have been described variously as keratic precipitates in a circular pattern or a ring configuration.[15] The authors suggest that these features are a characteristic sign of this condition and may represent the viral plaques generally seen in virus titration procedures. In fact, in patient 8, inspection under a confocal microscope disclosed a group of enlarged corneal endothelial cells with owl's eye appearance[34] around the lesion.[35]

Although it is reasonable to assume that CMV retinitis does not occur in patients with corneal endotheliitis because their immune system protects against the development of retinal pathologic features, the association of pars planitis and presumed immune corneal endotheliopathy has been reported in 4 patients with clinical manifestations resembling corneal endotheliitis.[36] Patient 7 in the current series, who had vitreous opacity as well as localized corneal edema associated with keratic precipitates at presentation, may fall into this category. This suggests that some patients with pars planitis of unknown origin may also have CMV infection.

During the course of this investigation, the authors encountered 2 immunodeficient patients with CMV corneal endotheliitis (unpublished data). They were not included in this study because the authors were unable to examine their aqueous humor for the presence of CMV DNA. However, the finding that both were positive for CMV antigenemia strongly suggests that CMV infection may have been an etiologic factor in their corneal endotheliitis, and the authors conclude that the increased incidence of human immunodeficiency virus infection and of organ and bone marrow transplantation may lead to a concomitant increase in CMV corneal endotheliitis in immunocompromised individuals.

References

1. Khodadoust AA, Attarzadeh A. Presumed autoimmune corneal endotheliopathy. Am J Ophthalmol 1982;93:718–22.
2. Ohashi Y, Kinoshita S, Mano T, et al. Idiopathic corneal endotheliopathy: a report of two cases. Arch Ophthalmol 1985;103:1666–8.
3. Vannas A, Ahonen R. Herpetic endothelial keratitis: a case report. Acta Ophthalmol (Copenh) 1981;59:296–301.
4. Sutcliffe E, Baum J. Acute idiopathic corneal endotheliitis. Ophthalmology 1984;91:1161–5.
5. Maudgal PC, Missotten L, De Clercq E, Descamps J. Varicella-zoster virus in the human corneal endothelium: a case report. Bull Soc Belge Ophtalmol 1980;190:71–86.
6. Ohashi Y, Yamamoto S, Nishida K, et al. Demonstration of herpes simplex virus DNA in idiopathic corneal endotheliopathy. Am J Ophthalmol 1991;112:419–23.
7. Robin JB, Steigner JB, Kaufman HE. Progressive herpetic corneal endotheliitis. Am J Ophthalmol 1985;100:336–7.
8. Mimura T, Amano S, Nagahara M, et al. Corneal endotheliitis and idiopathic sudden sensorineural hearing loss. Am J Ophthalmol 2002;133:699–700.
9. Amano S, Oshika T, Kaji Y, et al. Herpes simplex virus in the trabeculum of an eye with corneal endotheliitis. Am J Ophthalmol 1999;127:721–2.

10. Cheng CK, Chang SW, Hu FR. Acyclovir treatment for linear endotheliitis on grafted corneas. Cornea 1995;14:311–5.
11. Holland EJ, Schwartz GS. Classification of herpes simplex virus keratitis. Cornea 1999;18:144–54.
12. Liesegang TJ. Classification of herpes simplex virus keratitis and anterior uveitis. Cornea 1999;18:127–43.
13. Koizumi N, Yamasaki K, Kawasaki S, et al. Cytomegalovirus in aqueous humor from an eye with corneal endotheliitis. Am J Ophthalmol 2006;141:564–5.
14. Suzuki T, Hara Y, Uno T, Ohashi Y. DNA of cytomegalovirus detected by PCR in aqueous of patient with corneal endotheliitis following penetrating keratoplasty. Cornea 2007;26: 370–2.
15. Chee SP, Bacsal K, Jap A, et al. Corneal endotheliitis associated with evidence of cytomegalovirus infection. Ophthalmology 2007;114:798–803.
16. Inoue T, Hayashi K, Omoto T, et al. Corneal infiltration and CMV retinitis in a patient with AIDS. Cornea 1998;17:441–2.
17. Yamamoto S, Pavan-Langston D, Kinoshita S, et al. Detecting herpesvirus DNA in uveitis using the polymerase chain reaction. Br J Ophthalmol 1996;80:465–8.
18. Yamamoto S, Shimomura Y, Kinoshita S, et al. Detection of herpes simplex virus DNA in human tear film by the polymerase chain reaction. Am J Ophthalmol 1994;117:160–3.
19. Koizumi N, Nishida K, Adachi W, et al. Detection of herpes simplex virus DNA in atypical epithelial keratitis using polymerase chain reaction. Br J Ophthalmol 1999;83:957–60.
20. Demmler GJ, Buffone GJ, Schimbor CM, May RA. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. J Infect Dis 1988;158: 1177–84.
21. Powell KF, Anderson NE, Frith RW, Croxson MC. Non-invasive diagnosis of herpes simplex encephalitis [letter]. Lancet 1990;335:357–8.
22. Usui M, Usui N, Goto H, et al. Polymerase chain reaction for diagnosis of herpetic intraocular inflammation. Ocul Immunol Inflamm 1993;1:105–12.
23. Nishi M, Hanashiro R, Mori S, et al. Polymerase chain reaction for the detection of the varicella-zoster genome in ocular samples from patients with acute retinal necrosis. Am J Ophthalmol 1992;114:603–9.
24. Taylor-Wiedeman J, Sissons JG, Borysiewicz LK, Sinclair JH. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 1991;72:2059–64.
25. Larsson S, Soderberg-Naucler C, Wang FZ, Moller E. Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. Transfusion 1998;38:271–8.
26. Mendelson M, Monard S, Sissons P, Sinclair J. Detection of endogenous human cytomegalovirus in CD34+ bone marrow progenitors. J Gen Virol 1996;77:3099–102.
27. Nakamura T, Ishikawa F, Sonoda KH, et al. Characterization and distribution of bone marrow-derived cells in mouse cornea. Invest Ophthalmol Vis Sci 2005;46:497–503.
28. Hayashi K, Suwa Y, Shimomura Y, Ohashi Y. Pathogenesis of ocular cytomegalovirus infection in the immunocompromised host. J Med Virol 1995;47:364–9.
29. Streilein JW, Wilbanks GA, Taylor A, Cousins S. Eye-derived cytokines and the immunosuppressive intraocular microenvironment: a review. Curr Eye Res 1992;11(suppl):41–7.
30. Streilein JW. Ocular immune privilege and the Faustian dilemma. The Proctor lecture. Invest Ophthalmol Vis Sci 1996: 37:1940–50.
31. Streilein JW. Molecular basis of ACAID. Ocul Immunol Inflamm 1997;5:217–8.
32. Niederkorn JY, Streilein JW. Analysis of antibody production induced by allogeneic tumor cells inoculated into the anterior chamber of the eye. Transplantation 1982;33:573–7.
33. Zheng X, Yamaguchi M, Goto T, et al. Experimental corneal endotheliitis in rabbit. Invest Ophthalmol Vis Sci 2000;41: 377–85.
34. Herriot R, Gray ES. Images in clinical medicine: owl's-eye cells. N Engl J Med 1994;331:649.
35. Shiraishi A, Hara Y, Takahashi M, et al. Demonstration of "owl's eye" pattern by confocal microscopy in a patient with presumed cytomegalovirus corneal endotheliitis. Am J Ophthalmol 2007;143:715–7.
36. Khodadoust AA, Karnama Y, Stoessel KM, Puklin JE. Pars planitis and autoimmune endotheliopathy. Am J Ophthalmol 1986;102:633–9.

Table 1. Patient

Patient No.	Age/ Gender	Affected Eye	Type of Endotheliitis	History of Ocular Disease	Medications before CMV Detection			
					Antiviral Drug	*Steroid*	*Antibiotics*	*Antiglaucoma Drug*
1	51/M	Left	U	Anterior uveitis,† secondary glaucoma	—	0.1% betamethasone	—	2% carteolol, 0.005% latanoprost
2	73/F	Right	U	Keratoplasty for Fuchs dystrophy	—	0.1% dexamethasone	0.5% levofloxacin	—
3	83/F	Right	U	Idiopathic corneal endotheliitis,† ocular hypertension	0.3% acyclovir, valacyclovir	0.1% betamethasone	—	0.5% timolol
4	51/M	Left	B	Keratoplasty for BK due to idiopathic corneal endotheliitis,† ocular hypertension	0.3% acyclovir	0.1% dexamethasone	0.5% levofloxacin	2% carteolol
5	78/M	Right	U	Keratoplasty for corneal opacity, ocular hypertension	—	0.1% dexamethasone	0.3% ofloxacin	2% carteolol
6	77/M	Left	U	Keratoplasty for corneal opacity	0.3% acyclovir, valacyclovir	0.1% betamethasone	0.5% levofloxacin	—
7	60/M	Right	U	Uveitis,† idiopathic corneal endotheliitis,† secondary glaucoma	0.3% acyclovir, valacyclovir	0.1% betamethasone	—	Systemic acetazolamide
8	75/M	Right	U	Anterior uveitis,† secondary glaucoma	0.3% acyclovir, valacyclovir	0.1% betamethasone	—	1% brinzolamide, systemic acetazolamide

B = bilateral; BK = bullous keratopathy; CMV = cytomegalovirus; F = female; M = male; U = unilateral.
*Includes the period before CMV was examined.
†Herpes simplex virus and varicella zoster virus were not detected by polymerase chain reaction in aqueous humor and clinically resistant to acyclovir

Table 2. Clinical Findings of Cytomegalovirus (CMV) Corneal Endotheliitis

Patient No.	Affected Eye	Slit-Lamp Findings				Funduscopic Findings	IOP (mmHg)
		Coin-Shaped Lesions	*Linear KPs*	*Stromal Edema*	*AC Inflammation*	*CMV Retinitis*	
1	Left	+	+	2+	1+	−	18*
2	Right	+	+	2+	1+	−	17
3	Right	+	+	3+	−	−	12*
4	Left	+	−	1+	1+	−	15*
5	Right	+	−	2+	−	−	9*
6	Left	+	−	2+	−	−	9
7	Right	+	−	3+	3+	−	30†
8	Right	+	+	2+	−	−	32†

AC = anterior chamber; IOP = intraocular pressure; KPs = keratic precipitates.
Stromal edema and AC inflammation were evaluated from 0 to 3+ based on the slit-lamp examination.
*Controlled by topical medication.
†Controlled by systemic acetazolamide.

Data

Systemic Disease	CMV Antigenemia	Duration of Corneal Endotheliitis* (mos)	Follow-up Period after CMV Detection (mos)
None	Negative	24	24
Uterus cancer (treated)	Negative	15	15
None	Negative	36	12
None	Negative	240	10
Cerebral infarction	Negative	3	3
None	Negative	28	9
None	Negative	25	8
None	Negative	2	2

treatment.

Table 3. Topical and Systemic Medications for Corneal Endotheliitis and Clinical Outcome

		Systemic Medication		Topical Medication			Clinical Outcome		
Patient No.	Affected Eye	Antiviral Drug	Steroid	Antiviral Drug	Steroid	Antibiotics	Slit-Lamp Findings	Corneal Endothelial Cell Density (cells/mm^2)	VA
1	Left	Ganciclovir, 5 mg/kg/day ×7 days	Betamethasone, 1 mg/day	0.3% acyclovir, 5 times a day	0.1% fluoromethorone, 4 times a day	0.5% levofloxacin, thrice daily	Clear cornea	247	20/60 (cataract)
2	Right	Ganciclovir,* 10 mg/kg/day ×9 days	Betamethasone, 1 mg/day	0.3% acyclovir, thrice daily	0.1% dexamethasone, 4 times a day	0.5% levofloxacin, thrice daily	Bullous keratopathy	Undetectable	20/300
3	Right	Valacyclovir, 1500 mg/day	Betamethasone, 0.5 mg/day	0.3% acyclovir, 5 times a day	0.1% betamethasone, 4 times a day	—	Bullous keratopathy	Undetectable	20/400
4	Left	Ganciclovir, 10 mg/kg/day ×10 days	Betamethasone, 1 mg/day	0.3% acyclovir, 5 times a day	0.1% dexamethasone, 4 times a day	0.5% levofloxacin, thrice daily	Clear cornea	2600	20/60
5	Right	Ganciclovir, 5 mg/kg/day ×10 days	—	0.5% ganciclovir, 6 times a day	0.1% fluoromethorone, 4 times a day	0.5% ofloxacin, 4 times a day	Clear cornea	1400	20/60
6	Left	Ganciclovir, 10 mg/kg/day ×14 days	—	0.5% ganciclovir, 8 times a day	0.1% fluoromethorone, 4 times a day	0.5% levofloxacin, 4 times a day	Clear cornea	800	20/25
7	Right	Ganciclovir, 10 mg/kg/day ×7 days	—	0.5% ganciclovir, 8 times a day	0.1% dexamethasone, 6 times a day	—	Clear cornea	884	20/20
8	Right	Ganciclovir, 10 mg/kg/day ×15days	—	0.5% ganciclovir, 8 times a day	0.1% fluoromethorone, 4 times/day	0.3% gatifloxacin, 4 times a day	Clear cornea	650	20/20

VA = visual acuity.
*Systemic ganciclovir treatment was started at 8 mos after cytomegalovirus detection.

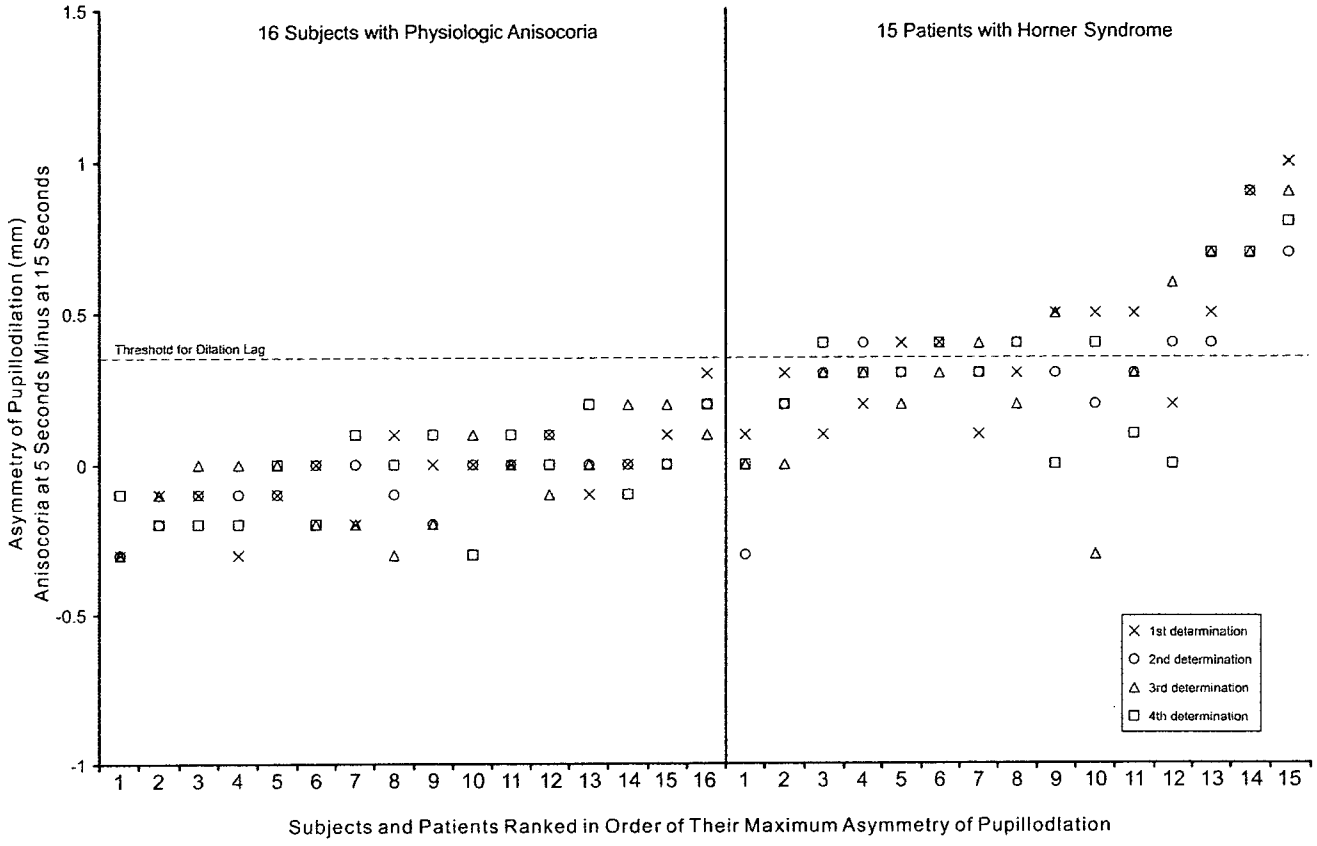

FIGURE 2. Scatterplot of calculated asymmetry of pupillodilation determined four times for 16 subjects with physiological anisocoria and 15 patients with Horner syndrome. Points above dotted line have asymmetry of ≥0.4 mm and thus meet criterion threshold used to define pupillary dilation lag. Dilation lag is only present among patients with Horner syndrome, but in most of these patients, it is only intermittently present over four recordings.

article (A.K.); critical revision of the article (A.K., F.X.B., S.V.C.); final approval of the article (A.K., F.X.B., S.V.C.), data collection (A.K., F.X.B., S.V.C.); provision of materials and patients (A.K., F.X.B.); literature search (S.C.); and administrative and technical support (F.X.B., S.C.).

Aki Kawasaki, MD, Hôpital Ophtalmique Jules Gonin, Lausanne, Switzerland, received an honorarium as speaker for Eli Lilly in 2006.

REFERENCES

1. Loewenfeld IE. Reflex dilation. In: Loewenfeld IE, editor. The pupil: anatomy, physiology and clinical applications. Detroit and Iowa City: Wayne State University Press and Iowa State University Press, 1993:318–394.
2. Kardon RH, Denison CE, Brown CK, Thompson HS. Critical evaluation of the cocaine test in the diagnosis of Horner syndrome. Arch Ophthalmol 1990;108:384–387.
3. Lam BL, Thompson HS, Corbett JJ. The prevalence of simple anisocoria. Am J Ophthalmol 1987;104:69–73.
4. Hong S, Narkiewicz J, Kardon RH. Comparison of pupil perimetry and visual perimetry in normal eyes: decibel sensitivity and variability. Invest Ophthalmol Vis Sci 2001;42:957–965.
5. Kawasaki A, Kardon RH. Evaluation of a simple photographic technique to detect pupillary dilation lag due to Horner syndrome. Neuro-ophthalmology 2004;28:215–219.
6. Pilley SF, Thompson HS. Pupillary "dilation lag" in Horner syndrome. Br J Ophthalmol 1975;59:731–735.

Demonstration of "Owl's Eye" Morphology by Confocal Microscopy in a Patient With Presumed Cytomegalovirus Corneal Endotheliitis

Atsushi Shiraishi, Yuko Hara, Miyabi Takahashi, Naoko Oka, Masahiko Yamaguchi, Takashi Suzuki, Toshihiko Uno, and Yuichi Ohashi

PURPOSE: To report confocal microscopic observations of characteristic corneal endothelial lesions in a patient with presumed cytomegalovirus (CMV) corneal endotheliitis.
DESIGN: Case report.
METHODS: A 77-year-old, immunocompetent man was admitted with corneal edema, keratic precipitates, and

Accepted for publication Nov 8, 2006.

From the Department of Ophthalmology, Ehime University School of Medicine, Shitsukawa, Toon, Ehime, Japan (A.S., Y.H., N.O., T.S., T.U., Y.O.), and Kurashiki Chuo Hospital, Kurashiki, Okayama, Japan (M.T.).

Inquiries to Atsushi Shiraishi, Department of Ophthalmology, Ehime University School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan; e-mail: shiraia@m.ehime-u.ac.jp

coin-shaped lesions in the right eye. Confocal microscopy was performed to examine the corneal endothelium. Polymerase chain reaction (PCR) was used to identify viral DNA in an aqueous humor sample.
RESULTS: CMV DNA was detected by PCR. Confocal microscopy showed large corneal endothelial cells with an area of high reflection in the nucleus surrounded by a halo of low reflection. This "owl's eye" morphology is characteristic of CMV infection. Topical and intravenous ganciclovir treatment resulted in rapid resolution of the corneal precipitates and edema, followed by disappearance of the owl's eye morphology.
CONCLUSIONS: Confocal microscopy can detect the owl's eye morphology in the corneal endothelium of patients with presumed CMV corneal endotheliitis.
(Am J Ophthalmol 2007;143:715–717.

CORNEAL ENDOTHELIITIS IS A CLINICAL ENTITY IN which the corneal endothelial cells are the primary target of a variety of infectious processes including herpes simplex virus (HSV) and varicella zoster virus (VZV).[1,2] It often leads to irreversible damage and severe visual disturbance. Koizumi and associates recently reported a case of corneal endotheliitis associated with cytomegalovirus (CMV) and emphasized the importance of recognizing this virus as a new etiologic agent for corneal endotheliitis.[3] We report the case of a patient with presumed CMV corneal endotheliitis in which in vivo confocal microscopy was used to identify "owl's eye" morphology in the enlarged corneal endothelial cells.

A 77-year-old, immunocompetent man was referred to us in May 2006 with redness, irritation, and blurred vision in his right eye. For seven months, he had suffered from intractable corneal edema, which did not respond to topical and systemic acyclovir (ACV) and topical corticosteroid. His best-corrected visual acuity was reduced to 20/25, and the intraocular pressure (IOP) was 32 mm Hg in the affected eye. Slit-lamp examination revealed localized edema in the superior nasal quadrant of the cornea associated with multiple keratic precipitates and scattered coin-shaped lesions on the endothelial surface (Figure 1). There were no signs of inflammation in the stroma or anterior chamber. The left eye was normal.

This study was approved by the Institutional Review Board of Ehime University. Based on the previous findings, we suspected that the corneal endotheliitis was caused by either an ACV-resistant HSV or other virus such as VZV and CMV. A sample of aqueous humor was aspirated from the patients' right eye after obtaining informed consent, and polymerase chain reaction testing was performed to search for HSV, VZV, or CMV DNA. A polymerase chain reaction product of 216 bp was identified as coming from CMV (Figure 2).

A confocal microscope, (Heidelberg Retina Tomograph II Rostock Cornea Module, Heidelberg Engineering GmbH, Dossenheim, Germany) was used to examine the corneal endothelium in vivo. When used to view one of the coin-shaped lesions located at the center of the cornea, confocal microscopy identified a group of large endothelial cells whose nuclei had an area of high reflection surrounded by a halo of low reflection. This owl's eye morphology (Figure 3) is a pathognomic sign of CMV infections.

The patient was treated with topical ganciclovir (0.5% solution, eight times per day) and intravenous ganciclovir (500 mg/day) for two weeks. After this treatment, the corneal edema, keratic precipitates, and IOP were markedly improved. The patient was given topical ganciclovir (0.5%, four times per day) thereafter,

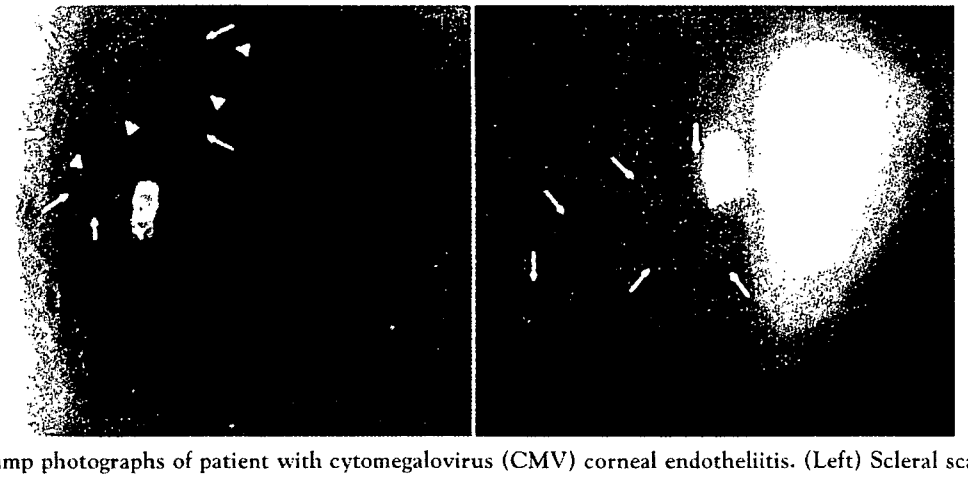

FIGURE 1. Slit-lamp photographs of patient with cytomegalovirus (CMV) corneal endotheliitis. (Left) Scleral scatter photograph demonstrating the localized edema in the upper nasal quadrant of the cornea (arrow heads) associated with keratic precipitates (arrows). (Right) Retro-illumination corneal photograph clearly demonstrating the coin-shaped lesions on the endothelial surface (arrows).

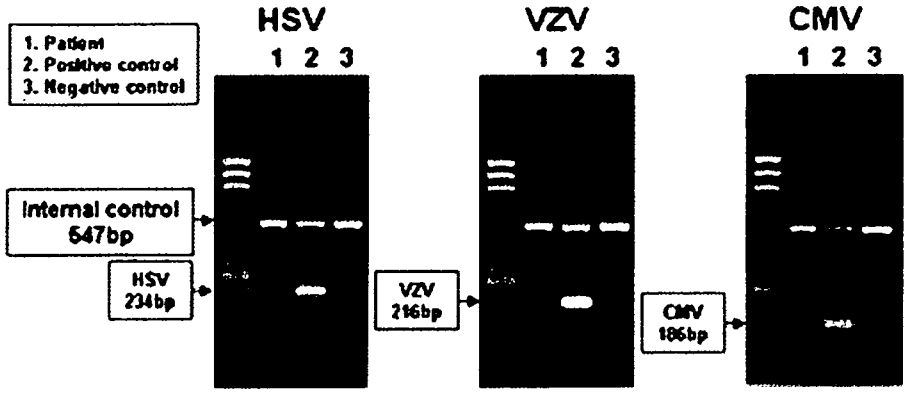

FIGURE 2. Polymerase chain reaction (PCR) results for DNA of herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV). The amplified CMV DNA was detected as 216 bp, but DNA for HSV and VZV were not isolated.

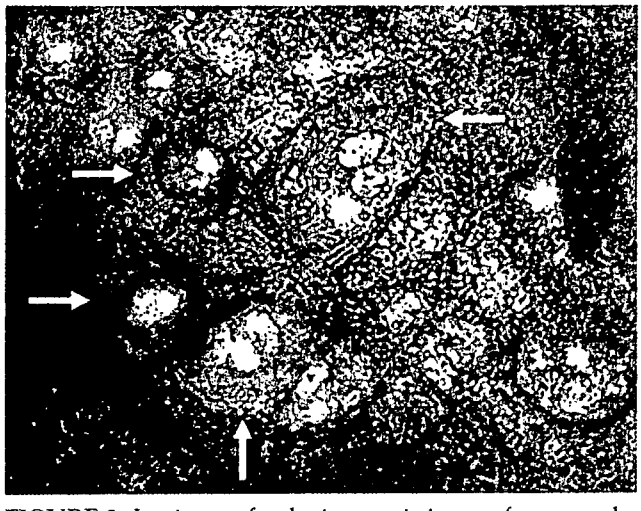

FIGURE 3. In vivo confocal microscopic image of cytomegalovirus (CMV) corneal endotheliitis at the level of corneal endothelium showing a group of large cells whose nuclei have a high reflection area surrounded by a halo of low reflection, which resembles an owl's eye.

and the owl's eye morphology has not reappeared in three months.

The clinical findings of coin-shaped lesions and peripherally located corneal edema, which are similar to the findings in Koizumi and associates' patient,[3] and the demonstration of CMV DNA in the aqueous humor suggest that the pathologic changes in our case were most likely associated with CMV.

CMV is an opportunistic pathogen causing a variety of infections, usually in newborns and immunocompromised hosts. However, a chronic form of infection can develop in the anterior chamber of the eye, which is known to be an immune privileged site of immunocompetent individuals.

The most telling finding in our case was the owl's eye morphology in the corneal endothelial cells detected by in vivo confocal microscopy. It is well known that owl's eye morphology is formed by a large, intranuclear inclusion body and is frequently seen in the pathologic specimens obtained during autopsy and biopsy. This is a hallmark finding of CMV infection.[4] Because biopsy of the corneal endothelial lesion was not practical, we could not verify that the lesions were actually caused by CMV; however, the rapid disappearance of these lesions after ganciclovir treatment suggests the accuracy of our diagnosis.

The newer confocal microscopes are valuable noninvasive examination tools that can provide useful information for the diagnosis of a variety of corneal diseases. It allowed us to identify owl's eye morphology in a patient with presumed CMV corneal endotheliitis.

THE AUTHORS INDICATE NO FINANCIAL SUPPORT OR Financial conflict of interest. Involved in design and interpretation of study (A.S., Y.H., M.H., N.O., M.Y., T.S., T.U., Y.O.); conduct of study (A.S., Y.O.); collection, management, analysis and interpretation of data, and preparation of the data (A.S., Y.H., M.H., N.O., M.Y., T.S., T.U., Y.O); and management, statistical analysis and interpretation of the data, and preparation of the manuscript (A.S., Y.H., M.H., N.O., M.Y., T.S., T.U., Y.O).

REFERENCES

1. Ohashi Y, Yamamoto S, Nishida K, et al. Demonstration of herpes simplex virus DNA in idiopathic corneal endotheliopathy. Am J Ophthalmol 1991;112:419–423.
2. Maudgal PC, Missotten L, De Clercq E, Descamps J. Varicella-zoster virus in the human corneal endothelium: a case report. Bull Soc Belge Ophthalmol 1980;190:71–86.
3. Koizumi N, Yamasaki K, Kawasaki S, et al. Cytomegalovirus in aqueous humor from an eye with corneal endotheliitis. Am J Ophthalmol 2006;141:564–565.
4. Herriot R, Gray ES. Images in clinical medicine. Owl's-eye cells. N Engl J Med 1994;331:649.