ABSTRACT

Aims: To investigate the correlation between the clinical manifestation and the cytomegalovirus (CMV) viral load in the aqueous humor of patients with CMV anterior uveitis.

Methods: A total of 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 manifestation was 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 (IOP) and mutton fat keratic precipitates with pigmentation. Stromal edema 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 humor of all 11 patients. A significant association was found between the corneal endothelial cell loss intensity and CMV viral load in the aqueous humor.

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 necrotizing 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 humor of an immunocompetent patient with iridocyclitis with elevated intraocular pressure (IOP) [2]. In addition, recent studies using qualitative polymerase chain reaction (PCR) have demonstrated that genomic CMV genomic DNA is present in the aqueous humor of immunocompetent patients with unilateral iridocyclitis [3-6] as follows. Markomichelakis et al [3] 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, e.g., Posner-Schlossman syndrome and Fuchs heterochromic iridocyclitis. CMV genomic DNA was also detected in the aqueous humor of immunocompetent patients with another inflammatory condition of the eye, i.e. corneal endotheliitis, in three previous reports [7-9]. Corneal endotheliitis is an inflammatory condition at the corneal endothelium and to develop keratic precipitates (KPs) together with severe stromal edema in the cornea, whereas iridocyclitis has cells and flare in the anterior chamber with or without KPs but no stromal edema in the cornea.

The real-time PCR made it possible to measure quantitatively the viral load. 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 humor in an immunocompetent patient with unilateral iridocyclitis with high

IOP [6]. However, the correlation between the viral load in the aqueous humor and the clinical manifestation of the disease (iridocyclitis versus corneal endotheliitis) is not investigated. Therefore, we examined if there was any correlation between the CMV viral load in the aqueous humor and the clinical manifestation of anterior inflammatory diseases associated with CMV. We shall show a significant correlation between the CMV viral load in the aqueous humor 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, a total of 11 patients with CMV-associated inflammation in the anterior segment of the eye, i.e. seven patients with CMV-associated iridocyclitis and four patients 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 humor by the multiplex PCR. The viral load in the aqueous humor was further measured quantitatively by the real-time PCR.

An aliquot of 0.1 ml of the aqueous was aspirated with a 30G needle after disinfection and processed to 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 sampling varied among the patients.

The Institutional Ethics Committees of Tokyo Medical and Dental University approved the

research, which followed the tenets of the Declaration of Helsinki. After each patient provided informed consent, a 0.1 ml aliquot of aqueous humor was collected.

Polymerase Chain Reaction

The aqueous humor samples were centrifuged at 3000 rpm for 5 min and used for the following PCR assays: multiplex PCR and real-time PCR [10, 11]. Multiplex PCR was designed to qualitatively measure the genomic DNA of 8 human herpes viruses: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), CMV, human herpes virus type 6 (HHV-6), type 7 (HHV-7) and type 8 (HHV-8). DNA was extracted from the aqueous humor samples using a DNA minikit (Qiagen, Valencia, CA, USA). Multiplex PCR was performed using LightCycler (Roche, Basle, Switzerland). The primers of the glycoprotein gene sequences for CMV were TACCCCTATCGCGTG TGTTC (forward) and ATAGGAGGCGCCACGTATTC (reverse). The probes used included 3'FITC: TCGTCGTAGCTACGCTTACAT and LcRed705-5': ACACCACTTATCTGCTGGGCAGC. Specific primers for the virus were used in conjunction with Accuprim Taq (Invitrogen, Carlsbad, CA, USA). PCR amplification conditions used in the current study have been previously reported [12].

Real-time PCR was only performed for the human herpes virus (HHV), with multiplex PCR used to detect the genomic DNA. Amplitaq Gold, with a Real-Time PCR 7300 system (ABI, Foster City, CA, USA), was used to perform the procedure. The forward and reverse primers of IE-1 were CATGAAGGTCTTTGCCCAGTAC 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/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 VA chart (Landolt ring chart) at after treatment. Anterior chamber flare was measured by a laser flare photometer (FC-1000®, Kowa Electronics, Japan). A photograph of the central cornea using a specular microscope (NONCON ROBO FA-3509®, Konan Medical, Japan) was used for evaluation of the corneal endothelial cells. In cases of corneal endothiliitis, intense corneal edema 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 humor 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

A total of 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 for 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 for 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 edema of the cornea was seen in all corneal endotheliitis but not in iridocyclitis patients. While the stromal edema was diffuse in three out of the four patients, it was localized at upper cornea in one of the corneal endotheliitis patients. Representative cases for iridocyclitis and corneal endotheliitis are shown in Figs. 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 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% or more) in all 11 patients (Table 2). Severe corneal endothelial cell loss larger than 70% was recorded in more than half of the endotheliitis group eyes. In contrast, this severe cell loss was observed in one of the seven patients with iridocyclitis.

There are several patients (case 1, 8, 10, and 11 in Table 2) with corneal endothelial cell counts less than 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.

PCR Analysis of the Aqueous Humor Samples

Multiplex PCR analyses confirmed the presence of CMV genomic DNA, but not any 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 humor 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 humor. 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; $P = 0.036$; Fig. 3).

However, there was no correlation between the interval from the disease onset to the aqueous sampling and the viral load in the aqueous humor (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 analyzed ocular manifestations and CMV viral loads in the aqueous humor 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 humor.

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 pathologic 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 to 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 leukemia 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 when compared to 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 humor. Furthermore, there was a significant correlation between the viral load and the intensity of the iris atrophy in these patients [15].

Although we found that there was a positive correlation between the corneal endothelial cell loss and the CMV viral load in the aqueous humor, 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 (e.g., betamethasone) and anti-glaucoma agents (e.g., 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 effects on recovering the corneal endothelial cell damage, because the corneal endothelial cell damage is well known to hardly to recover once it occurs.

The cells and flare in the anterior chamber are mild in all 11 patients. A possible explanation why the intensity of the inflammatory reaction in the anterior chamber is 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 genomic DNA of CMV was present at

high levels within the anterior chamber of the patients. Therefore, it may very well be that ACAID in response to CMV could possibly occur in the eye, resulting in a relatively mild inflammatory reaction.

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 humor 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. This may attributed to induce local immunosuppressive condition in the anterior segment of the eye and to result in reactivation of CMV [19]. 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 humor.

Funding: None.

Competing interests: None.

Ethics approval: Ethics approval was provided by the Institutional Ethics Committee of Tokyo Medical and Dental University.

Patient consent: Obtained.

FIGURE LEGENDS

Figure 1. Case 4: Slit-lamp microscopy photo with CMV-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.

Figure 2. Case 8: Slit-lamp microscopy photo with CMV-associated corneal endotheliitis.

Diffuse corneal stromal edema with folds in Descemet's membrane was observed.

Figure 3. Correlation between 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 scatterplot 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).

REFERENCES

1. Yoser SL, Forster DJ, Rao NA. Systemic viral infections and their retinal and choroidal manifestations. *Surv Ophthalmol* 1993;**37**:313-352.
2. Mietz H, Aisenbrey S, Ulrich Bartz-Schmidt K, et al. Ganciclovir for the treatment of anterior uveitis. *Graefes Arch Clin Exp Ophthalmol* 2000;**238**:905-909.
3. Markomichelakis NN, Canakis C, Zafirakis P, et al. Cytomegalovirus as a cause of anterior uveitis with sectoral iris atrophy. *Ophthalmology* 2002;**109**:879-882.
4. de Schryver I, Rozenberg F, Cassoux N, et al. Diagnosis and treatment of cytomegalovirus iridocyclitis without retinal necrosis. *Br J Ophthalmol* 2006;**90**:852-855.
5. Chee SP, Jap A. Presumed fuchs heterochromic iridocyclitis and Posner-Schlossman syndrome: comparison of cytomegalovirus-positive and negative eyes. *Am J Ophthalmol* 2008;**146**:883-889.
6. Kawaguchi T, Sugita S, Shimizu N, et al. Kinetics of aqueous flare, intraocular pressure and virus-DNA copies in a patient with cytomegalovirus iridocyclitis without retinitis. *Int Ophthalmol* 2007;**27**:383-386.
7. 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.
8. Chee SP, Bacsal K, Jap A, et al. Corneal endotheliitis associated with evidence of cytomegalovirus infection. *Ophthalmology* 2007;**114**:798-803.
9. Koizumi N, Suzuki T, Uno T, et al. Cytomegalovirus as an etiologic factor in corneal endotheliitis. *Ophthalmology* 2008;**115**:292-297.
10. Sugita S, Shimizu N, Kawaguchi T, et al. Identification of human herpes virus 6 in a patient with severe unilateral panuveitis. *Arch Ophthalmol* 2007;**125**:1426–1427.
11. Sugita S, Shimizu N, Watanabe K, et al. Use of multiplex PCR and real-time PCR to detect

human herpes virus genome in ocular fluids of patients with uveitis. *Br J Ophthalmol* 2008;**92**:928-932.

12. Schaade L, Kockelkorn P, Ritter K, et al. Detection of cytomegalovirus DNA in human specimens by LightCycler PCR. *J Clin Microbiol* 2000;**38**:4006-4009.
13. Ono A, Mochizuki M, Yamaguchi K, et al. Increased number of circulating HTLV-1 infected cells in peripheral blood mononuclear cells of HTLV-1 uveitis patients: a quantitative polymerase chain reaction study. *Br J Ophthalmol* 1995;**79**:270-276.
14. Ono A, Mochizuki M, Yamaguchi K, et al. Immunologic and virologic characterization of the primary infiltrating cells in the aqueous humor of human T-cell leukemia virus type-1 uveitis. Accumulation of the human T-cell leukemia virus type-1-infected cells and constitutive expression of viral and interleukin-6 messenger ribonucleic acids. *Invest Ophthalmol Vis Sci* 1997;**38**:676-689.
15. Kido S, Sugita S, Horie S, et al. Association of varicella zoster virus load in the aqueous humor with clinical manifestations of anterior uveitis in herpes zoster ophthalmicus and zoster sine herpete. *Br J Ophthalmol* 2008;**92**:505-508.
16. Streilein JW, Wilbanks GA, Taylor A, et al. Eye-derived cytokines and the immunosuppressive intraocular microenvironment: a review. *Curr Eye Res* 1992;**11 Suppl**:41-47.
17. Streilein JW. Ocular immune privilege and the Faustian dilemma. The Proctor lecture. *Invest Ophthalmol Vis Sci* 1996;**37**:1940-1950.
18. Zheng X, Yamaguchi M, Goto T, et al. Experimental corneal endotheliitis in rabbit. *Invest Ophthalmol Vis Sci* 2000;**41**:377-385.
19. Chee SP, Bacsal K, Jap A, et al. Corneal endotheliitis associated with evidence of cytomegalovirus infection. *Ophthalmology* 2007;**114**:798-803.

Table 1. Clinical findings in patients with CMV anterior uveitis

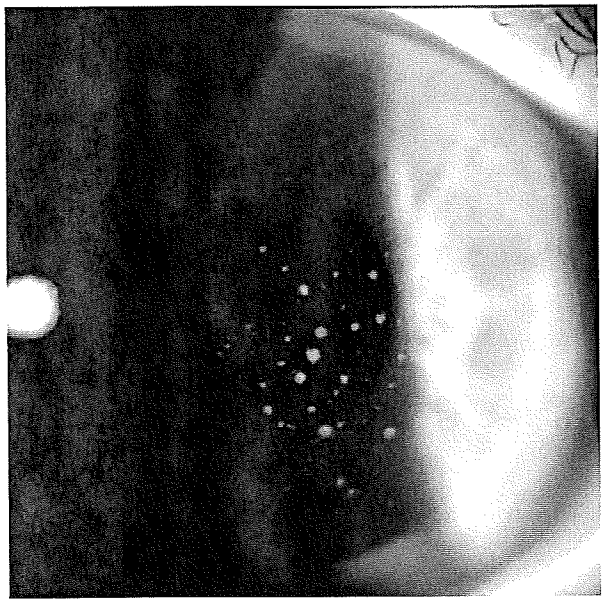
Case	Age (years)	Sex	Eye	Diagnosis	Corneal edema	KPs	Cells in AC	Flare in AC	IOP (mmHg)	Pigmentation in the AC angle	Iris atrophy
1	66	M	R	Iridocyclitis	-	Mutton-fat	1+	17	38	Depigmentation	None
2	62	M	R	Iridocyclitis	-	Mutton-fat	1+	26	40	PAS & pigment	Diffuse
3	56	M	L	Iridocyclitis	-	Mutton-fat	1+	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

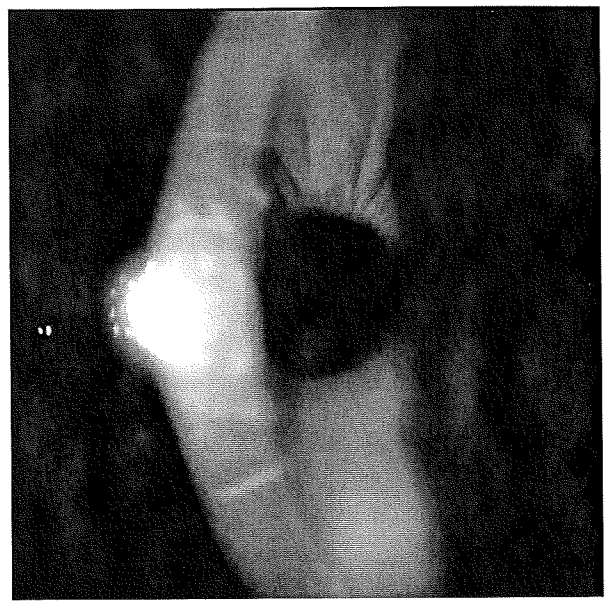
Information of 11 patients with CMV anterior uveitis was reviewed. Data collected included intraocular pressure (IOP) and clinical manifestation of the anterior segments in the affected eye. M, male; F, female; KPs, keratic precipitates; AC, anterior chamber; PAS, peripheral anterior synechia; Nt, not tested.

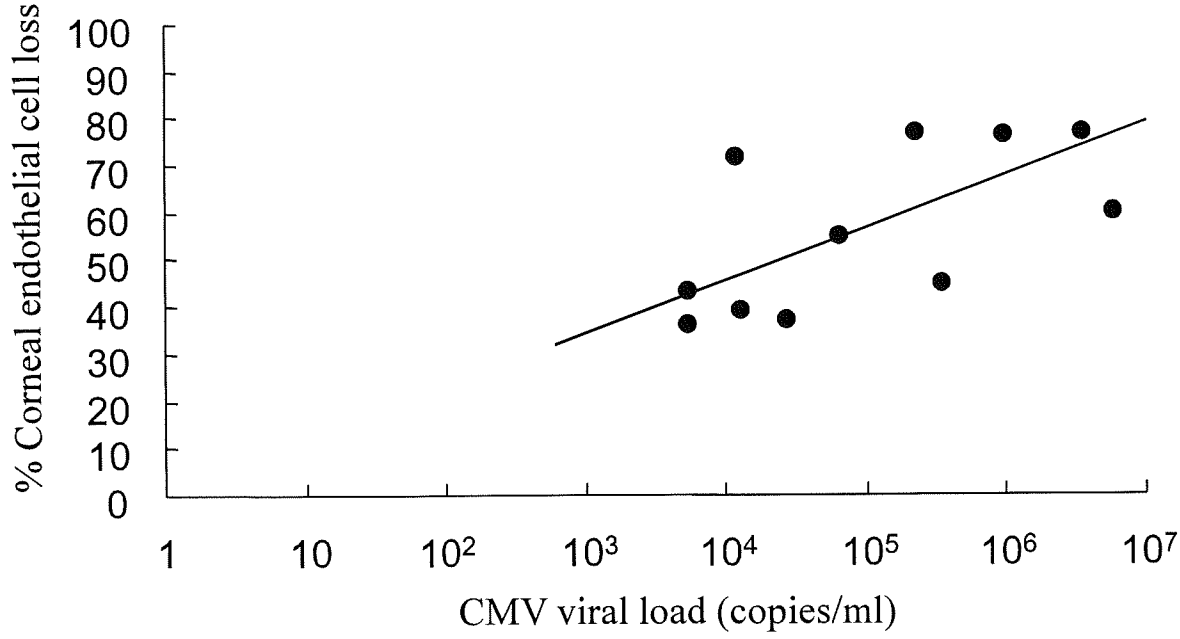
Table 2. Virological analysis and corneal endothelial cell findings in patients with CMV anterior uveitis

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 humor samples, genomic DNA of the human herpes viruses was measured by qualitative multiplex PCR & quantitative real-time PCR. Corneal endothelial cell count was examined by specular microscopy. *Herpes viruses excluding CMV, i.e., herpes simplex virus type 1 and type 2, varicella zoster virus, Epstein-Barr virus, 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).







Int J Hematol (2009) 89:238–242
DOI 10.1007/s12185-008-0234-9

CASE REPORT

Cord blood transplantation using minimum conditioning regimens for patients with hematologic malignancies complicated by severe infections

Takeshi Yamashita · Chiharu Sugimori · Ken Ishiyama · Hirohito Yamazaki · Hirokazu Okumura · Yukio Kondo · Akiyoshi Takami · Shinji Nakao

Received: 2 September 2008 / Revised: 13 November 2008 / Accepted: 18 November 2008 / Published online: 25 December 2008

Abstract Patients with severe infections are thought to be ineligible for cord blood stem cell transplantation (CBT) because the conventional 5–6 day-conditioning regimens potentially makes them susceptible to fatal infections by the time neutrophil engraftment occurs. Two patients were treated with minimum conditioning regimens consisting of 30 mg/m^2 fludarabin (Flu) and 2 g/m^2 cyclophosphamide (CY) on day-1 and total body irradiation (TBI) of 2 or 4 Gy on day −1 or 0 followed by single unit CBT. The reasons for adopting such weak regimen were febrile neutropenia due to the rejection of the first cord blood (CB) graft given to a patient with follicular lymphoma resistant to chemotherapy and pulmonary aspergillosis in another patient with AML who relapsed after CBT. The AML patient received 40 mg/m^2 of melphalan on day-2 to reduce the leukemia burden. Both patients achieved 100% donor chimerism by day 19 and day 20 after CBT without an apparent exacerbation of the infections and remained in remission at 23 and 18 months after the CBT. These findings suggest that the 1–2 day regimens excluding antihuman thymocyte globulin may be sufficiently potent to ensure engraftment of CB in immunocompromised patients and safely administered even when patients are complicated by active infections.

Keywords Cord blood transplantation · Active infection · Minimum intensity conditioning regimen

T. Yamashita · C. Sugimori · K. Ishiyama · H. Yamazaki · H. Okumura · Y. Kondo · A. Takami · S. Nakao (✉)
Cellular Transplantation Biology,
Kanazawa University Graduate School of Medical Science,
13-1 Takaramachi, Kanazawa 920-8641, Japan
e-mail: snakao@med3.m.kanazawa-u.ac.jp

1 Introduction

Cord blood (CB) is becoming a major source of allogeneic hematopoietic stem cell transplantation [1, 2]. The success of reduced intensity CB transplantation has accelerated the use of CB for treatment of aged patients with hematologic malignancies [3]. However, patients complicated by severe documented infections are still considered ineligible for cord blood transplantation (CBT) even if reduced intensity regimens are adopted because the preconditioning causes severe neutropenia which usually lasts until day 20 after transplantation [2, 3] and exacerbates infections leading to treatment related-death. As a result, some patients with hematologic malignancies who failed to achieve remission after chemotherapy or those who failed to engraft after allogeneic stem cell transplantation cannot benefit from CBT.

One possible measure to solve this problem is to shorten the time for preconditioning in addition to reducing the intensity. Since most conventional preconditioning regimens take more than 4 days, they need to be started at least 5 days prior to the day of transplantation [4]. Shortening the time for preconditioning to 1 or 2 days may help patients to survive a neutopenic period from the start of preconditioning to neutrophil engraftment. Goggins et al. used a 1-day conditioning regimen consisting of fludarabin (Flu), alemtuzumab and cyclophosphamide (CY) to treat five leukemia patients with allogeneic peripheral blood stem cell transplantation (PBSCT) and observed stable engraftment in three patients. A similar 1-day regimen consisting of Flu, CY and antihuman thymocyte globulin (ATG) was used to treat a myelodysplastic syndrome (MDS) patient with a second allogeneic PBSCT (K. Mochizuki et al., in preparation). The patient suffered from a high fever suggestive of bacteremia due to

persistent neutropenia following the rejection of the first PBSC graft. The second PBSC of another HLA-identical sibling from the original donor successfully engrafted and the patient has been in remission for more than 4 years. However, all of these cases used PBSC grafts containing a high number of hematopoietic stem cells as well as T cells which are thought to be helpful to accelerate the engraftment of donor stem cells and rapid neutrophil recovery. It is still unclear whether CB can engraft after such a very weak regimen and eventually rescue neutropenic patients complicated by severe infections.

This report describes two patients with a devastating condition who were successfully treated with a minimum intensity regimen of 1–2 days followed by single unit CBT.

2 Patients

2.1 Patient 1

In January 2005, a 56-year-old man was diagnosed to have a clinical stage IV follicular lymphoma. He achieved only PR after standard chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone and was refractory to other chemotherapy regimens for salvage. He underwent CBT following a reduced conditioning regimen consisting of cladribine, CY and 4 Gy of total body irradiation (TBI). His neutrophil count remained at 0 on day 21 after the CBT. A chimerism analysis of the bone marrow cells preformed on the same day revealed 100% cells to be recipient-type, thus indicating graft rejection. There was no sign of autologous hematologic recovery and a high fever persisted. There was no sign of an autologous hematologic recovery and a high fever persisted despite the administration of meropenem 1.0 g twice daily and micafungin 300 mg daily. The patient's CRP rose to 25.9 mg/dl on day 25. On day 27 after CBT, he received 30 mg/m^2 Flu and 2 g/m^2 CY followed by 2 Gy of TBI in the morning of the next day. HLA 2 locus-mismatched CB containing 2.6 $\times$ 10^7/kg cells and 9.8 $\times$ 10^6 CD34$^+$ cells/kg was infused 13 h after the completion of CY infusion. Clinical data including HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. The high fever started abating on day 16 after the second CBT and his neutrophil count surpassed 0.5 $\times$ 10^9/l on day 19. A chimerism analysis performed on day 26 revealed the 100% of the peripheral blood leukocytes were donor-type. Although grade I GVHD occurred, it resolved without treatment. CT scanning on day 33 after the second CBT showed a marked reduction of cervical lymph node swelling in comparison to that at 29 days before the first CBT (Fig. 1). He remains well in partial remission 30 months after the second CBT.

Table 1 Clinical data and HLA alleles of the patients and cord blood donors

	Sex	Blood type	HLA-A	HLA-B	HLA-DR
Patient 1	M	O+	0206/3303	3901/4403	1302/1501
First CB for patient 1	M	O+	0201/3303	3501/4403	1302/1501
Second CB for patient 1	M	A+	1101/3303	3901/4403	0803/1501
Patient 2	F	A+	2402/–	3501/4001	0901/1302
First CB for patient 2	M	AB+	0201/2402	3501/4006	0901/1302
Second CB for patient 2	M	B+	2402/–	4001/4006	0901/1501

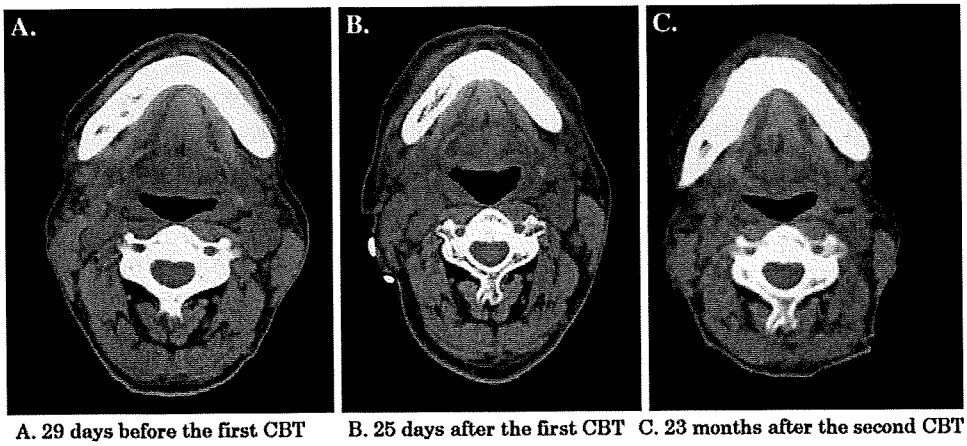


Fig. 1 Changes in the cervical lymphoma lesions after CBT in patient 1. CT scan on 23 months after the second CBT showed a marked reduction in size of the cervical lymph nodes in comparison to those before the first and the second CBT

A. 29 days before the first CBT **B. 25 days after the first CBT** **C. 23 months after the second CBT**

2.2 Patient 2

In April 2005, a 66-year-old female was diagnosed to have AML evolving from MDS. Chemotherapy consisting of idarubicin (IDA) and cytosine arabinoside (Ara-C) failed to induce remission and severe pancytopenia persisted. She underwent CBT following a conditioning regimen with fludarabine, melphalan, rabbit ATG and 4 Gy of TBI. The CB was 2-loci mismatched and contained 2.9×10^7/kg cells. Engraftment was confirmed on day 18 and she achieved complete remission. However, the AML relapsed in 18 months after the CBT. Remission induction with IDA and Ara-C only induced marrow hypoplasia with 33% residual leukemia cells. On day 18 of the chemotherapy, invasive aspergillosis developed in the left lung. Liposomal amphotericin B, 2.5 mg/kg daily, was administered from the same day without any appreciable effects. The neutrophil count remained at 0 on day 22 of the chemotherapy. She received melphalan 40 mg/m^2 to reduce leukemic cell burden, followed by 30 mg/m^2 Flu and 2 g/m^2 CY on the next day. In the morning of the following day, she received 4 Gy of TBI and underwent a second CBT 12 h after the completion of CY infusion. The CB was 2-loci mismatched, and contained 2.9×10^7/kg cells and 1.9×10^6 CD34$^+$ cells/kg. HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. Liposomal amphotericin B was switched to voliconazole, 4.0 mg/kg daily, on day 48 after the second CBT due to a rise in the creatinine level. Although her pulmonary aspergillosis was transiently exacerbated on day 6 after the second CBT, the high fever abated on day 17 and engraftment of donor cells was confirmed on the same day. The aspergillosis lesion was encapsulated with time after the second CBT (Fig. 2).

She underwent a left upper lobectomy on day 113 and presently remains in CR at 24 months after the second CBT.

3 Discussion

Treatment of the patients with hematologic malignancies complicated by severe neutropenic infections with no hope of prompt hematologic recovery is challenging. Although immunoablative conditioning followed by allogeneic stem cell transplantation is the only measure to rescue patients with such devastating conditions, this treatment may also tend to sometimes hasten the patients' death by aggravating the preexisting infections. Even if reduced intensity regimens are adopted, severe neutropenia which lasts from the day of preconditioning until 2–3 weeks after SCT greatly increases the risk of infectious death [3, 5, 6]. In order to solve this dilemma, Goggins et al. pioneered a very weak conditioning regimen, known as the 1-day regimen [7]. They treated five infirmed patients with 30 mg/m^2 Flu, 2 g/m^2 CY, 20 mg/kg alemtuzumab, TBI 2 Gy on day-1 and infused PBSC from family donors who were HLA 1–3 loci mismatched. Engraftment occurred in three patients, two of whom achieved long-term remission. According to their protocol, an MDS patient who suffered febrile neutropenia due to rejection of the first PBSCT was treated with Flu (30 mg/m^2), CY (2 g/m^2), horse ATG (15 mg/kg) and TBI (2 Gy) followed by PBSCT from a second HLA-identical sibling donor. The neutrophil count promptly recovered and the patient achieved complete donor chimerism. This experience indicated that the alemtuzumab in the 1 day regimen can be replaced with low dose ATG and that the minimum conditioning regimen coupled with PBSCT from a second donor can overcome the rejection after SCT.

Cord blood transplantation is associated with a higher incidence of engraftment failure [8–12] and a slower neutrophil recovery [2, 9, 13] than BMT or PBSCT due to the low number of hematopoietic stem cells and mature T cells in the CB graft. The disadvantages of CBT has precluded the use of CB for treatment of patients with very low intensity regimens for allogeneic stem cell transplantation such as 2 Gy TBI alone [14] or ATG + total lymphoid

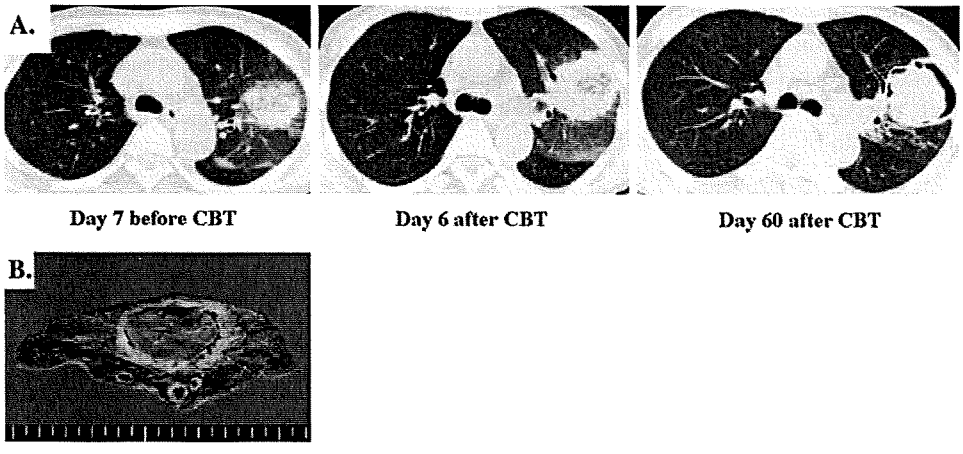


Fig. 2 Pulmonary aspergillosis lesion of patient 2. **a** Changes in the CT findings before and after CBT. **b** *Left upper* lung resected on day113 after CBT

irradiation regimens [15]. However, there were no options other than CBT for the two current patients because they did not have matched family donors and could not afford to wait until an HLA-matched unrelated donor was available. ATG was not included in the conditioning regimen for those patients because they could have succumbed to their infections which became exacerbated by the administration of ATG. Despite their devastating conditions and the elimination of ATG from the conditioning regimen, both patients achieved engraftment of CB without any apparent exacerbation of their infections or the development of severe GVHD. Therefore, in vivo purging of T cells using anti-T cell antibodies may not be a prerequisite for engraftment of CB after the 1–2-day regimen. However, it should be noted that both patients had been previously treated with conditioning regimens for allo-SCT. Prior conditioning regimens used for the first CBT may therefore be necessary for patients to take CB following such a minimum conditioning regimen. Other reduced-intensity regimens have been successfully used as preconditioning for a second transplantation using CB to treat graft rejection after allo-SCT [16–20]. However, all such regimens were administered for over 5 days and were not as weak as the regimens we used for the above described two patients.

Sustained engraftment of CB after the weak regimen in the current patients may therefore have important implications in the management of patients with hematologic malignancies refractory to chemotherapy. Patients who fail chemotherapy often suffer from severe infections due to persistent neutropenia and are therefore excluded as candidates for hematopoietic stem cell transplantation, particularly CBT, which is associated with delayed neutrophil recovery. Following very weak preconditioning, the patients not only circumvented life threatening infections but also achieved hematologic remission possibly with the help of the graft-versus-leukemia/lymphoma effects of CBT. CB can be utilized for patients with severe complications because of its easy accessibility and prompt availability [21]. Therefore, CBT following the minimum intensity conditioning may provide a chance to achieve complete chimerism in patients suffering from severe infections associated with profound neutropenia due to graft rejection or chemotherapy for leukemic relapse after the first allo-SCT.

References

1. Laughlin MJ, Barker J, Bambach B, Koc ON, Rizzieri DA, Wagner JE, et al. Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med. 2001;344:1815–22. doi:10.1056/NEJM200106143442402.
2. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood. 2007;109:1322–30. doi:10.1182/blood-2006-04-020172.
3. Miyakoshi S, Yuji K, Kami M, Kusumi E, Kishi Y, Kobayashi K, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. Clin Cancer Res. 2004;10:3586–92. doi:10.1158/1078-0432.CCR-03-0754.
4. Ooi J, Iseki T, Takahashi S, Tomonari A, Takasugi K, Uchiyama M, et al. Unrelated cord blood transplantation after myeloablative conditioning in patients over the age of 45 years. Br J Haematol. 2004;126:711–4. doi:10.1111/j.1365-2141.2004.05130.x.
5. Komatsu T, Narimatsu H, Yoshimi A, Kurita N, Kusakabe M, Hori A, et al. Successful engraftment of mismatched unrelated cord blood transplantation following reduced intensity preparative regimen using fludarabine and busulfan. Ann Hematol. 2007;86:49–54. doi:10.1007/s00277-006-0190-5.
6. Misawa M, Kai S, Okada M, Nakajima T, Nomura K, Wakae T, et al. Reduced-intensity conditioning followed by unrelated umbilical cord blood transplantation for advanced hematologic malignancies: rapid engraftment in bone marrow. Int J Hematol. 2006;83:74–9. doi:10.1532/IJH97.05124.
7. Goggins TF, Rizzieri DA, Prosnitz R, Gasparetto C, Long G, Horwitz ME, et al. One day preparative regimen for allogeneic non-myeloablative stem cell transplantation (NMSCT) using 3–5/6 HLA matched related donors. Blood. 2003;102(11):476b–7b.
8. Wolff SN. Second hematopoietic stem cell transplantation for the treatment of graft failure, graft rejection or relapse after allogeneic transplantation. Bone Marrow Transplant. 2002;29:545–52. doi:10.1038/sj.bmt.1703389.
9. Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood. 2004;104:3813–20. doi:10.1182/blood-2004-03-1001.
10. Narimatsu H, Kami M, Miyakoshi S, Murashige N, Yuji K, Hamaki T, et al. Graft failure following reduced-intensity cord blood transplantation for adult patients. Br J Haematol. 2006;132:36–41. doi:10.1111/j.1365-2141.2005.05832.x.
11. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio AR, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med. 1998;339:1565–77. doi:10.1056/NEJM199811263392201.
12. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood. 2002;100:1611–8.
13. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med. 2004;351:2265–75. doi:10.1056/NEJMoa041276.
14. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390–400. doi:10.1182/blood.V97.11.3390.
15. Lowsky R, Takahashi T, Liu YP, Dejbakhsh-Jones S, Grumet FC, Shizuru JA, et al. Protective conditioning for acute graft-versus-host disease. N Engl J Med. 2005;353:1321–31. doi:10.1056/NEJMoa050642.