Table 1 Serum ECP and cytokine production in pre- and post-treatment of prednisolone (1.5 mg/kg/day)

	Pre-treatment	Day 7	Day 14	Day 21	Day 28	Disease control†
ECP (μg/L)‡	23.3	6.0	15.2	15.2	4.0	30.2 ± 4.4
IL-5 (pg/mL)§	5.0	2.2	2.9	0.8	4.1	9.6 ± 1.9
GM-CSF (pg/mL)	66.8	79.0	6.4	ND	6.4	126.6 ± 41.0
IL-4 (pg/mL)	78.9	41.8	12.2	ND	ND	26.2 ± 4.2
IL-13 (pg/mL)	99.8	14.6	19.1	6.6	11.2	5.1 ± 0.3
IL-10 (pg/mL)	7.7	4.6	5.1	6.3	10.2	28.9 ± 3.8
IFN-γ (pg/mL)	3.9	10.9	ND	ND	ND	114.7 ± 11.9
IL-2 (pg/mL)	ND	15.6	ND	ND	ND	14.9 ± 5.1

†Acute asthma (mean ± SEM, $n = 32–75$) as the disease control. ‡ECP was determined using fluoro-immunoassay (Pharmacia-Upjohn, Uppsala, Sweden). §Cytokines were determined using the Bioplex Multiplex Human Cytokine Assay kit (Bio-Rad Laboratories, Hercules, CA, USA). ECP, eosinophil cationic protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; ND, not detected.

A (9 mg/kg/day) to improve autoimmune manifestations. Four months after admission, the patient underwent allogeneic SCT with a full-matched unrelated cord blood unit, because a matched related donor was unavailable.[3] The conditioning regimen consisted of fludarabine at 25 mg/m^2 daily on days −7 to −3, melphalan at 70 mg/m^2 daily on days −4 to −3, and antithymocyte globulin at 10 mg/kg daily on days −2 and −1. The patient was treated with cyclosporin at 3 mg/kg and methylprednisolone at 1 mg/kg for GvHD prophylaxis. The total number of infused cells was 5.29×10^7/kg. The patient was successfully treated by altering the degree of immunosuppression and donor lymphocyte infusion (DLI) using donor cord-blood-derived activated CD4$^+$ T cells for mixed chimerism after unrelated cord-blood transplantation using reduced-intensity conditioning. Full donor chimerism in the bone marrow was also achieved on day +68.[3] Although the patient contracted mycobacterium avium complex infection, anti-mycobacterium therapy using ethambutol, rifampin, and azithromycin, prevented recurrence of high fever and produced a stable state after transplantation.

We analyzed eosinophilia, eosinophil activity, and production of several cytokines in this syndrome. Although our case before prednisolone (1.5 mg/kg/day) treatment presented with marked hypereosinophilia (21 800/μL) almost 50 times higher than that in disease control, namely, acute asthma (4.0 ± 0.4 years old), the eosinophil cationic protein (ECP) level was almost comparable to that of acute asthma (eosinophil counts, 21 800 vs 442.3 ± 70.0/μL; ECP levels, 23.3 vs 30.2 ± 4.4 ng/mL), as shown in Table 1. Figure 1 shows that the expression of CD69, an activation marker of eosinophils,[4] on peripheral eosinophils in our case was minimal compared with a patient with severe atopic dermatitis as positive control. Serum concentrations of T helper (Th)2 cytokines including interleukin (IL)-4 and IL-13 before treatment were markedly elevated compared with those of acute asthma (IL-4, 78.9 vs 26.2 ± 4.2; IL-13, 99.8 vs 5.1 ± 0.8 pg/mL). In contrast, the concentrations of the eosinophil-active cytokines IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) were not very high compared with those of acute asthma (IL-5, 5.0 vs 9.6 ± 1.9 pg/mL; GM-CSF, 66.8 vs 126.6 ± 41.0 pg/mL). The concentrations of the Th1 cytokines interferon (IFN)-γ and IL-2 were not elevated. After the treatment, the concentrations of Th2 cytokines decreased, whereas those of Th1 cytokines transiently increased. As shown in Table 2, when peripheral blood

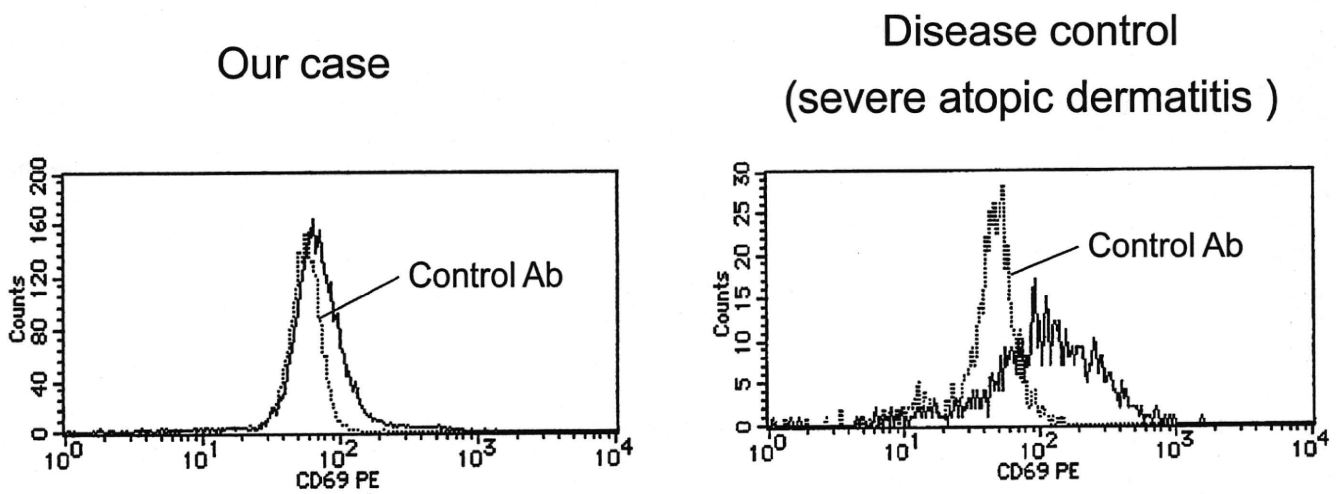

Fig. 1 Surface expression of cluster of differentiation (CD)69 antigen in eosinophils. Expression of CD69 in peripheral blood eosinophils in our case and that in a disease control were determined by flow cytometry and compared with that of an isotype-matched control antibody. Disease control represented a patient with severe atopic dermatitis (5 months, male). He also showed peripheral blood eosinophilia (21 762/μL) and highly elevated serum immunoglobulin E (17 867 IU/mL). Ab, antibody.

Table 2 Cytokine production in the peripheral mononuclear leukocytes

	IL-5	IL-4	IL-13	IFN-γ	IL-2
Pre-treatment	21.4†	1476.9	5201.0	6664.9	4372.6
Day 7 of treatment‡	7.2	43.8	302.0	792.7	4787.8
Day 14 of treatment‡	12.3	27.2	4.0	7.8	7.9

†pg/mL. ‡Prednisolone (1.5 mg/kg/day). The cells stimulated by phorbol 12-myristate 13-acetate and ionomycin for 24 h. IFN, interferon; IL, interleukin.

mononuclear cells (PBMC) from the patient were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 24 h,[5] the concentrations of IL-4, IL-13, IFN-γ and IL-2 markedly increased; however, that of IL-5 was not so high compared with that of acute asthma (IL-5, 21.4 vs 37.6 ± 7.1 pg/mL). The capacity to produce these cytokines was diminished after immunosuppressive therapy.

Discussion

We measured the serum level of ECP, a marker of eosinophil activation, and found that its level was not so high, suggesting that despite the prominent eosinophilia, marked activation of eosinophils was not observed in our case. The amount of ECP released in cell culture from hypereosinophilic syndrome (HES) patients is higher than that in those with other hypereosinophilic conditions (including OS); therefore, eosinophils in HES are more aggressive toward tissues than those in other conditions.[6] These results were also supported by the finding that the upregulation of CD69, an activated surface marker of eosinophils, on peripheral eosinophils is minimal compared with that in patients with atopic dermatitis. Secondly, we investigated cytokine profiles in both serum and PBMC from our patient. Th2-type lymphocytes might be activated because of eosinophilia and elevated IgE concentration in typical OS. However, reports on cytokine production in OS remain controversial. For example, *in vitro* stimulation of lymphocytes from an OS patient induced IL-4 and IL-5 secretion in the serum,[7] while others showed high concentrations of serum IL-5 in OS.[8] In contrast, other investigators failed to detect Th2 cytokines (IL-4, IL-5, and IL-13), but detected Th1 cytokines (tumor necrosis factor [TNF]-α, IFN-γ, and IL-1β) in an OS patient with reverse transcription–polymerase chain reaction (RT–PCR) for cytokine mRNA expression.[9] Our case showed high concentrations of Th2 cytokines, especially IL-4 and IL-13, in both serum and PBMC, but serum IL-5 concentration was not very high. In OS, oligoclonal expansion of T lymphocyte induces Th1/Th2 cytokine paradigm. Thus, regulations of cytokine production appear to be different depending on each case. In fact, not all OS cases show hypereosinophilia. On the other hand, marked eosinophilia can be induced without eosinophilic activation.

How can we explain an atypical cytokine profile and discrepancies between marked eosinophilia and less eosinophil activation? Accumulating evidence suggests that eosinophil differentiation and infiltration are regulated by IL-5, GM-CSF and IL-13, while eosinophil effector functions, such as degranulation, are regulated by IL-5 and GM-CSF but not by IL-13.[10–12] Thus, discrepancies between marked eosinophilia and less eosinophil activation in the present case might be explained, at least in part, by the atypical cytokine profile where IL-13 concentration is markedly elevated but that of IL-5 and GM-CSF were not in this patient. Another possible reason is that our case showed oligoclonal expansion of T lymphocytes with multiple second-site mutations leading to typical OS with *RAG1*-deficient SCID.[2,13] The patient is homozygous for a single-base C deletion predicted to cause frameshift mutation and premature termination of *RAG1*. Six compensatory second-site mutations were found in revertant T cells, showing an activated phenotype with a restricted TCR repertoire, expanding in peripheral blood, and possibly contributing to the modification of his clinical features, suggesting that revertant T-cell mosaicism is responsible for OS phenotypes switching from T-B-SCID.[13] In contrast, we speculate that *RAG1* activity in this patient is completely defective in B cells as well as granulocytes, reflecting the impaired B-cell differentiation.[2] Thus, it is suggested that the production of IgE was undetectable, although the production of IL-4 and IL-13 was increased in this patient. Therefore our patient may not show typical cytokine production and hyper IgE, even though he showed typical clinical features of OS.

Although the detailed mechanisms of eosinophilia, minimal activation of eosinophils and atypical cytokine profile were not completely clarified in this case, the mechanism of eosinophil proliferation might be different from that of eosinophil activation and an appropriate balance of cytokine production might be involved in this reaction.

Acknowledgments

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

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ABSTRACT

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

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

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Subjects

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

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

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

Polymerase chain reaction

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

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

Clinical evaluation

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

Evaluation of corneal endothelial cell loss

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

$$\text{Corneal endothelial cell loss}(\%) = 100 - (\text{endothelial cell counts in affected eye})/(\text{endothelial cell counts in the fellow eye})\times 100$$

Statistical analysis

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

RESULTS

Clinical manifestations

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

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

Table 1 Clinical findings in patients with CMV anterior uveitis

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

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

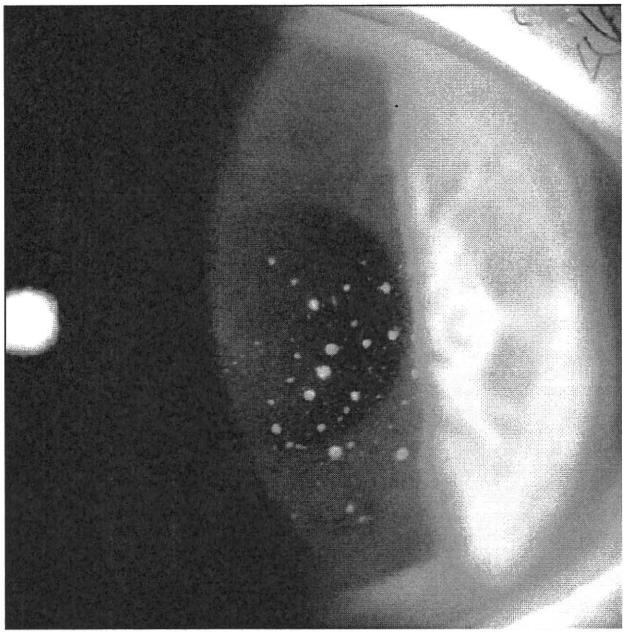

Figure 1 Case 4: Slit-lamp microscopy photo with cytomegalovirus-associated iridocyclitis. Mutton fat keratic precipitates with some pigmentation were scattered within the central area of the cornea. There was mild inflammation found within the anterior chamber.

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

Corneal endothelial cell loss

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

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

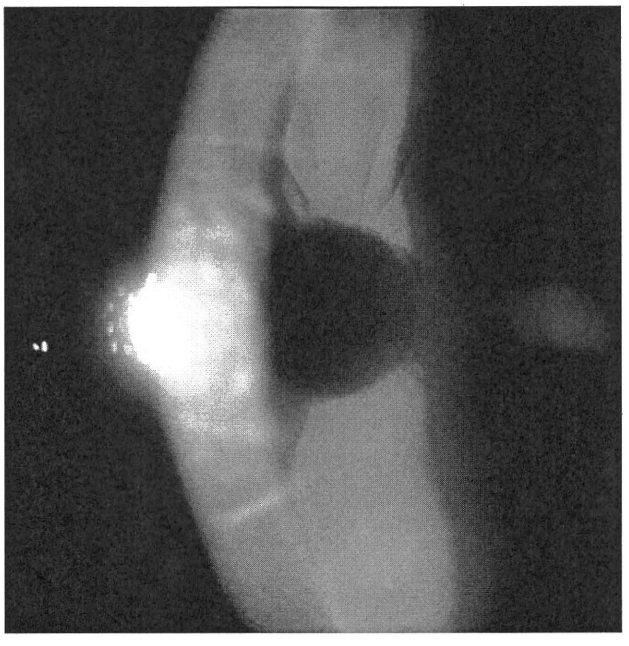

Figure 2 Case 8: Slit-lamp microscopy photo with cytomegalovirus-associated corneal endotheliitis. Diffuse corneal stromal oedema with folds in Descemet's membrane was observed.

PCR analysis of the aqueous humour samples

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

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

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

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

DISCUSSION

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

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

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

Case	Herpes virus DNA		Endothelial cell count (cells/mm²)		Corneal endothelial cell loss (%)†	Post-treatment BCVA	Interval from onset to sampling (months)
	CMV (copies/ml)	Others*	Affected eye	Fellow eye			
1	2.3×10^5	-	642	2738	77	0.4	96
2	5.5×10^3	-	1633	2869	43	0.8	8
3	1.3×10^4	-	1695	2789	39	1.5	48
4	6.5×10^4	-	1618	3576	55	1.5	24
5	3.5×10^5	-	1445	2608	38	1.2	14
6	5.9×10^6	-	919	2288	45	1.2	16
7	5.4×10^3	-	2512	3917	60	1.2	6
8	1.0×10^6	-	573	2427	76	0.6	12
9	2.8×10^4	-	1427	2262	35	0.7	5
10	1.2×10^4	-	593	2092	72	0.3	4
11	3.6×10^6	-	620	2674	77	1.5	20

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

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

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

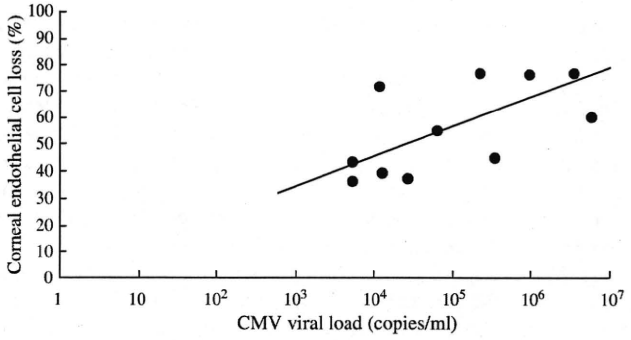

Figure 3 Correlation between cytomegalovirus (CMV) viral load and corneal endothelial cell damage. The CMV viral load was plotted on a logarithmic graph versus the corneal endothelial cell loss (%). The scatter plot shows significant correlation between the CMV viral load and the corneal endothelial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

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

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

Competing interests None.

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

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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

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Fatal degeneration of specialized cardiac muscle associated with chronic active Epstein–Barr virus infection

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Key words ATG, CAEBV, cardiac muscle, Epstein-Barr virus, hematopoietic stem cell transplantation.

Chronic active Epstein–Barr virus infection (CAEBV) is a life-threatening disorder characterized by prolonged fever, wasting, hepatosplenomegaly, and cytopenia, in addition to abnormal EBV antibody titers and the presence of EBV antigens or EBV-DNA in tissue.[1] The ultimate prognosis of CAEBV is very poor, and patients with CAEBV often develop a progressive cellular and humoral immunodeficiency with pancytopenia and hypoglobulinemia that renders them susceptible to opportunistic infections or B- or T-cell lymphoproliferative disease.[2] An effective treatment for CAEBV has yet to be established, although allogeneic hematopoietic stem cell transplantation (HSCT) was recently reported to be effective in eradicating EBV-infected lymphocytes.[3,4] Allogeneic HSCT is, however, accompanied by a considerable risk of therapy-related death. Kimura *et al.* reported that approximately one-half of patients with CAEBV who underwent HSCT died within 60 days.[5]

We here report a patient with CAEBV who developed fatal acute circulatory failure during the preconditioning of HSCT, and in whom autopsy showed degeneration of specialized cardiac muscle and severe large-vessel arteritis associated with CAEBV.

Case report

A 12-year-old boy was admitted to a regional hospital with a 2 year history of fever, wasting, and a short stature (−3.0 SD). He was diagnosed with CAEBV on clinical signs and the presence of EBV genome in peripheral blood, and was admitted to hospital in April 2005. He was allergic to mosquito bites. On examination, lymph node adenopathy was noted. No skin lesion was observed. On brain CT, bilateral calcification of the basal ganglia was noted. Echocardiography showed dilatation of the lumens of the coronary arteries. The leukocyte count was 2.2×10^9/L; hemoglobin, 12.5 g/dL; and platelet count, 61×10^9/L. Biochemical analysis was as follows: aspartate aminotransferase, 28 IU/L; alanine aminotransferase, 14 IU/L; lactate dehydrogenase, 265 IU/L; C-reactive protein, 0.63 mg/dL; soluble interleukin-2 receptor, 1200 U/mL (normal, <519 U/mL); viral capsid antigen (VCA)-IgG, ×320; EA-IgG, ×10; EBNA, ×10. Natural killer activity was 55%. The quantity of EBV genome DNA was increased (2.6×10^3 copies/μg DNA) on polymerase chain reaction in peripheral blood mononuclear cell (PBMC), particularly in CD4+T cells (4.3×10^4 copies/μg DNA), but not in CD8+T cells or CD56+NK cells. Southern blot of the peripheral blood cells using an EBV terminal probe indicated monoclonal proliferation of EBV-infected lymphocytes. Flow cytometry showed the expression of the perforin protein in PBMC. Chromosome analysis of peripheral blood showed the normal male karyotype.

The high load of EBV genome DNA had persisted for over 6 months. Therefore, HSCT from a human leukocyte antigen (HLA)-matched sibling donor was planned in October 2005. Just before bone marrow transplantation, the quantity of EBV-DNA was consistently high in peripheral blood. No chemotherapy was performed prior to bone marrow transplantation. Echocardiography showed normal cardiac function. The exercise electrocardiogram (ECG) identified no abnormal findings.

The preparative conditioning regimen consisted of fludarabine (30 mg/m^2 × 4, day −7, −6, −5, −4), anti-thymocyte globulin (15 mg/kg × 5, day −7, −6, −5, −4, −3), and melphalan (70 mg/m^2 × 2, day −3, −2). Because anti-thymocyte globulin had been administered, he had suffered from high fever, skin rash, systemic arthralgia, and abdominal pain. He had wine-colored urine, but not microhematuria. He was diagnosed as having serum sickness and disseminated intravascular coagulation syndrome, and treated with gabexate mesilate, fresh frozen plasma, platelet transfusions, and methylprednisolone. Therefore, subsequent conditioning was all stopped on day −6 before transplantation. Blood examination showed elevation of liver enzymes, creatinine kinase, and coagulopathy. Fifty-two hours after the beginning of conditioning for transplant (on day −5), he suddenly developed acute circulatory failure. Just before cardiac arrest, echocardiogram showed normal cardiac function. Transient ventricular fibrillation was observed during resuscitation, but he soon developed cardiac arrest. Cardiac pulmonary resuscitation was unsuccessfully performed, and he died of sudden circulatory failure.

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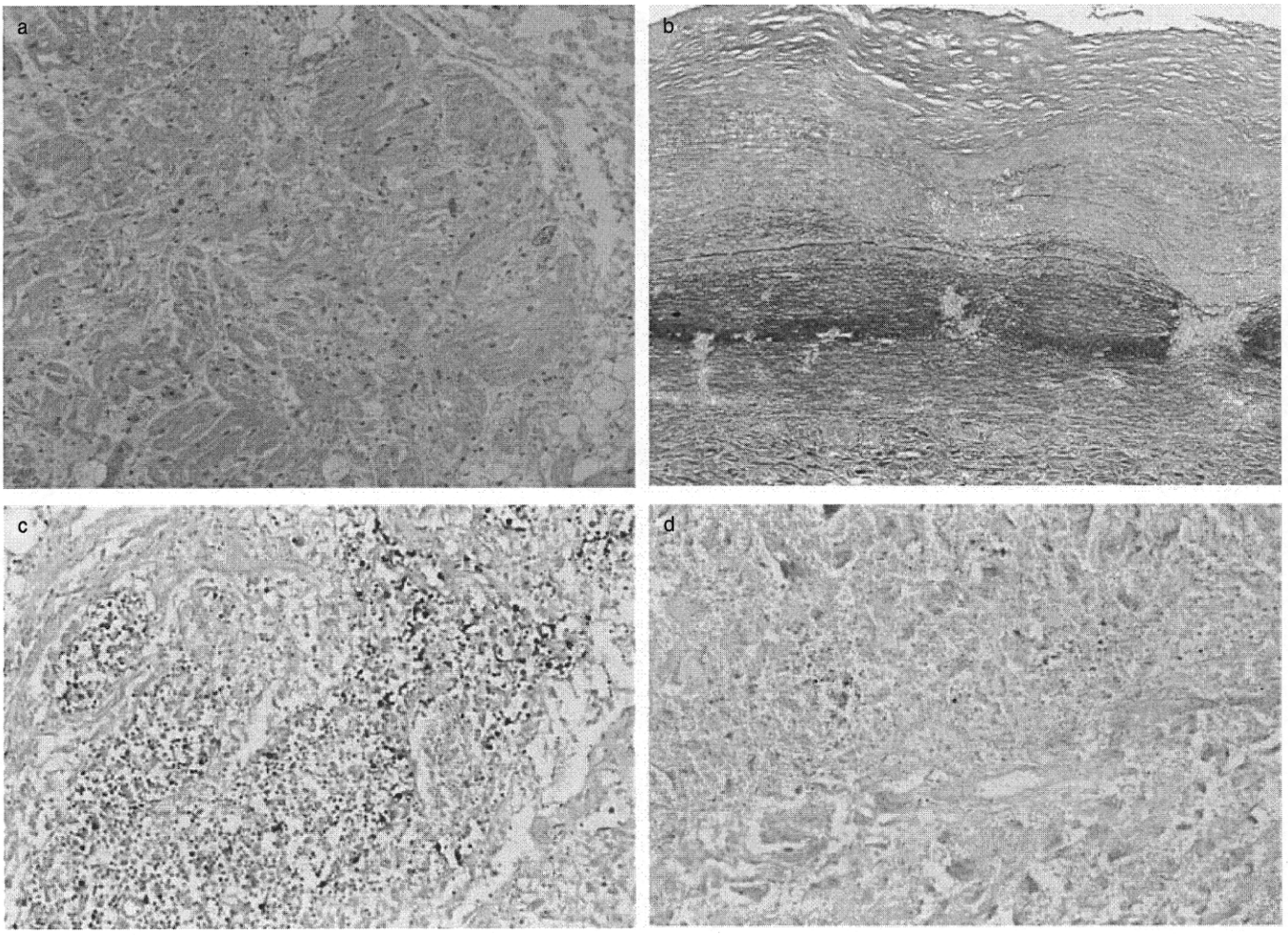

Fig. 1 (a) Histopathology showed degeneration of specialized cardiac muscle (HE), and mesoarteritis characterized by moth-eaten-appearing destruction of the medial elastic laminae, with (b) lymphocyte infiltration around the vasa vasorum and severe intimal thickening (EVG). (c) *In situ* hybridization demonstrated the presence of EBV-RNA (EBER-1) in the nuclei of lymphocytes in lymph nodes and (d) degenerated cardiac muscles.

At autopsy, histopathology showed not only EBV-associated hemophagocytic syndrome, but also degeneration of specialized cardiac muscle (Fig. 1a), mesoarteritis characterized by moth-eaten-appearing destruction of the medial elastic laminae (Fig. 1b), with $CD4^{+}$T lymphocyte infiltration around the vasa vasorum and severe intimal thickening. *In situ* hybridization demonstrated the presence of EBV-RNA (EBER-1) in the nuclei of lymphocytes in lymph nodes (Fig. 1c), around the vessels and degenerated cardiac muscles (Fig. 1d). Hemorrhagic tendency associated with disseminated intravascular coagulation syndrome was also noted at autopsy, although fatal intracranial hemorrhage and myocardial infarction were not documented. These findings suggested that hemophagocytic syndrome and degeneration of specialized cardiac muscles might have caused the fatal arrhythmia, considered to be associated with the EBV infection.

Discussion

In the current case, coronary artery dilatations and large-vessel arteritis were noted at autopsy. Arteritis has been reported in patients with CAEBV.[6] Coronary arteries and large vessels are often involved, and the presence of arteritis associated with EBV infection led to poor prognoses in those reported patients.[6–8] The vulnerability of systemic vessels caused by severe arteritis might be involved in the poor response to cardiopulmonary resuscitation. *In situ* hybridization showed that EBV-infected lymphocytes were associated with mesoarteritis, characterized by moth-eaten-appearing destruction of the medial elastic laminae and severe intimal thickening. Taken together, a comparatively high load of EBV-DNA persisting prior to transplantation might have been one of the factors promoting arteritis in this patient.

To our knowledge this is the first case reported in the literature in which the degeneration of specialized cardiac muscle was documented at autopsy in a patient with CAEBV. Transient ventricular fibrillation was also detected on ECG during cardiopulmonary resuscitation. The degeneration of specialized cardiac muscle might lead to fatal arrhythmia and acute circulatory failure. *In situ* hybridization also showed that EBER-positive

cells were scattered around cardiac muscle, suggesting that persistent EBV infection was involved in the degeneration of specialized cardiac muscle, Atrioventricular block has also been reported in EBV myocarditis.[9] These findings suggest that prolonged EBV infection could lead to the degeneration of specialized cardiac muscle over a long period.

Allogeneic HSCT has been reported to offer a good prognosis for those with CAEBV.[2,3] A national survey, however, performed in Japan found a high risk of HSCT-related mortality.[5] Recently, successful treatment of CAEBV infection using reduced-intensity stem cell transplantation (RIST) with fludarabine and melphalan with or without anti-thymocyte globulin (ATG) has been reported,[10,11] which could control CAEBV by reconstituting host immunity against EBV. Regimen-related toxicity is expected to be more effectively alleviated using a reduced intensity conditioning regimen for transplantation than by conventional myeloablative conditioning in those with an impaired residual function of organs. In contrast, it is well known that the risk of EBV-related complications after transplantation might increase with the additional use of ATG in non-myeloablative conditioning.[12] In the current case we adapted fludarabine-based, reduced-intensity conditioning with ATG for transplantation, because it was predicted that the residual cardiac function might be partly impaired by coronary giant aneurysms. The additional use of ATG, however, in fludarabine-based conditioning might have been involved in the development of hemophagocytic syndrome during preconditioning for transplantation, although it was uncertain whether the serum sickness induced by the use of ATG was associated with the degeneration of cardiac muscle. To answer the many remaining questions, such as the best transplantation method for CAEBV, the optimal timing of transplantation, and the most effective conditioning regimen, a multicenter-based clinical trial is needed.

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Congenital upper thoracic spondyloptosis with multiple other associated anomalies

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Key words congenital abnormalities, diagnosis, scapula, spine, spondyloptosis.

Spondyloptosis indicates any slip greater than 100% of a vertebral body on another or an extreme degree of spondylolisthesis. It is rare and there are isolated case reports in the literature.[1,2] It frequently involves the L5–S1 level. It is attributed to congenital dysplasia of the articular process.[3]

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Presented here is the case of an infant with severe spinal cord compression related to T2–3 spondyloptosis and multisegmental vertebral congenital anomaly. Multisystem anomalies such as Sprengle deformity, situs inversus totalis, and right renal agenesis were also demonstrated radiologically. To our knowledge this is a unique case of thoracic spondyloptosis.

Case report

A 9-month-old male infant presented with a significant delay in head holding. He was the result of the first pregnancy of young

P735

The effectiveness of liposomal amphotericin in the treatment of invasive fungal infections is not affected by prior azole administration: the Ambi-Prof study

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It is a matter of debate whether mold-active azole prophylaxis may reduce the effectiveness of Liposomal Amphotericin (L-AmB).

Objectives: This retrospective study was aimed to determine the non-inferiority of prior azole administration in the treatment of Invasive Fungal Infections (IFI) with L-AmB in hematologic and allogeneic HSCT patients.

Methods: Patients who met the EORTC/MSG criteria for IFI and received treatment with L-AmB were eligible and distributed in two arms according to: (A) mold-active azole exposure prior to L-AmB, and (B) fluconazole or no prior azole. Patients were stratified according to the type of IFI and evaluated for disease related risk factors and comorbidities. The primary endpoints were favorable response and survival at the end of antifungal therapy, at 4 and 12 weeks.

Results: From Feb/2008 to Sep/2009, 182 consecutive patients were recruited from 26 institutions. The median age was 45 years (range 1–78). Most had acute leukemia (AL) or myelodysplasia (MDS) (129; 70.0%). Baseline disease was treated for induction, in remission, or refractory/relapse status in 23.6%, 45.0% and 31.4%, respectively. A 40.1% of patients had allogeneic HSCT. Severe comorbidity and prior IFI were present in 20.3% and 14.8%, respectively. Arm A included 100 patients with prior itraconazole 39%, voriconazole 35% and posaconazole 26%. Arm B included 82 patients with fluconazole 49% or no azole 51%. Patients characteristics were not different in both arms, except for more AL or MDS ($P=0.002$) and prolonged neutropenia in arm A ($P=0.021$), and more use of high dose steroids in arm B ($P=0.01$). The rates of possible, probable and proven IFI were 52.7%, 28.6% and 18.7%, respectively (Table 1). Aspergillosis was the proven IFI in 28 of 35 cases. L-AmB was given 3 mg/kg/d for a median of 18±17 days in A and 15±13 in B. The favorable response rate to L-AmB was 75% and 74.4% in both groups, with no differences in the responses at the end of treatment, at 4 weeks or at 12 weeks. The response rates for possible and probable/proven IFI were similar in both groups (Table 2).

Conclusions: Prior exposure to mold-active azoles does not affect the effectiveness of L-AmB for the treatment of IFI in this high risk patient population, indicating that concerns for sequential administration are no longer justified.

Table 1
Patients characteristics

	Group A prior azole N=100	Group B no azole N=82	Total N=182	p value*
	n (%)	n (%)	n (%)	
Disease				
AML, ALL, MDS	80 (80.0)	49 (59.8)	129 (70.0)	0.002
Other	20 (20.0)	33 (40.2)	53 (29.1)	
Phase of the disease				
Induction	23 (23.5)	19 (23.8)	42 (23.6)	ns
Remission	41 (41.8)	39 (48.8)	80 (45.0)	
Refractory/Relapse	34 (34.6)	22 (22.7)	55 (31.4)	
Allogeneic HSCT	44 (44.0)	29 (35.4)	73 (40.1)	ns
Severe comorbidity	22 (22.0)	15 (18.3)	37 (20.3)	ns
Prior IFI	18 (18.0)	9 (11.0)	27 (14.8)	ns
Type of IFI at L-AmB				
Possible	53 (53.0)	43 (52.4)	96 (52.7)	ns
Probable	29 (29.0)	23 (29.1)	52 (28.6)	
Proven	18 (18)	16 (19.5)	34 (18.7)	
Neutropenia≥10 days	71 (84.5)	41 (68.3)	112 (61.5)	0.021
High dose steroids	18 (18.0)	28 (34.1)	46 (25.3)	0.013

* Group A vs B

Table 2
L-AmB treatment outcome

	Group A prior azole N=100	Group B no azole N=82	Total N=182	p value*
	n (%)	n (%)	n (%)	
Favorable response†	75 (75.0)	61 (74.4)	136 (75.0)	ns
Possible IFI	44 (63.0)	32 (74.4)	76 (79.2)	ns
Proven or Probable IFI	31 (66.0)	29 (74.4)	60 (69.8)	ns
End of treatment				
Favorable Response	73 (73.0)	55 (67.1)	128 (70.3)	ns
Survival	58 (58.0)	70 (85.4)	152 (64.1)	ns
At 4 weeks				
Favorable Response	57 (57.0)	50 (61.0)	107 (58.8)	ns
Survival	62 (62.2)	64 (78.0)	126 (69.2)	0.02
At 12 weeks				
Favorable Response	41 (41.0)	41 (50.0)	82 (45.0)	ns
Survival	42 (42.0)	49 (59.8)	91 (50.0)	0.017

*Group A vs B
† Number of complete or partial responses obtained at any time throughout the 12 weeks of the study

P736

The impact of cytomegalovirus reactivation after cord blood transplantation in adults

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Study purpose: We have shown cord blood naive T cells could obtain memory and effector function in vivo with antigen-specific manner during early phase of post-CBT without effect of HLA disparity (EBMT, 2009). In this study, we investigated to find graft- and patient-related factors affecting to CMV reactivation after HLA-mismatched CBT and the impact of CMV reactivation to clinical outcome of CBT in adults.

Patients and methods: We studied the clinical outcomes of 152 consecutive adult patients who received CBT between 1998 and 2009. All patients received myeloablative regimens including 12 Gy of TBI, CsA plus short term MTX for GVHD prophylaxis and almost the same supportive care. In graft-versus-host direction, 7 were antigen-matched, 53 were 1 antigen- and 92 were 2 antigens-mismatched in HLA-A, -B and -DR loci by low-resolution method. After engraftment, all patients were monitored using a CMV antigenemia assay with C10/C11 monoclonal antibodies twice a week during hospitalization. Twenty-eight (18%) were suffered from 10 times or more positive results defended as the higher-frequent positivity (H) and 124 (82%) were categorized as lower-frequent positivity or negative (L). If antigenemia assay was positive, 5 mg/kg ganciclovir was started as preemptive therapy. We evaluated clinical factors correlated with CMV antigenemia positivity by the Chi-square test and the impact of positive antigenemia on clinical results using the Pepe and Mori's test. OS rate was calculated using the Kaplan-Meier method and analyzed by the log-rank test. Multivariate analysis has performed using the Competing risk regression and the Cox regression.

Results: OS and DFS rates were 73% and 69% at 3 years, respectively. One patient (14%) in HLA-matched, 7 (13%) in 1 antigen-mismatched and 20 (22%) in 2 antigens-mismatched patients showed H (ns). CMV antibody positivity ($P=0.032$) and use of steroid after transplant ($P<0.001$) significantly affected to H. Patients with H stayed in hospital significantly longer (median: 161.5 days) than L (105 days) in multivariate analysis ($P=0.037$). The incidence of chronic GVHD in patients with H (65% at 1 year) tended to be higher than L (22%, $P=0.083$). OS rate in patients with H was lower (61% at 3 years) than with L (74%), but the difference was not significant.

Conclusion: HLA disparities in CBT were not affected to CMV antigenemia results. Longer stay in hospital was needed in high-frequent CMV reactivated patients after CBT.

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Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study

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Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study

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Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used as a curative option for adult T-cell leukemia (ATL), an intractable mature T-cell neoplasm causally linked with human T-cell leukemia virus type I (HTLV-I). We compared outcomes of 386 patients with ATL who underwent allogeneic HSCT using different graft sources: 154 received human leukocyte antigen (HLA)–matched related marrow or peripheral blood; 43 received HLA-mismatched related marrow or peripheral blood; 99 received unrelated marrow; 90 received single unit unrelated cord blood. After a median follow-up of 41 months (range, 1.5-102), 3-year overall survival for entire cohort was 33% (95% confidence interval, 28%-38%). Multivariable analysis revealed 4 recipient factors significantly associated with lower survival rates: older age (> 50 years), male sex, status other than complete remission, and use of unrelated cord blood compared with use of HLA-matched related grafts. Treatment-related mortality rate was higher among patients given cord blood transplants; disease-associated mortality was higher among male recipients or those given transplants not in remission. Among patients who received related transplants, donor HTLV-I seropositivity adversely affected disease-associated mortality. In conclusion, allogeneic HSCT using currently available graft source is an effective treatment in selected patients with ATL, although greater effort is warranted to reduce treatment-related mortality. (*Blood.* 2010;116(8):1369-1376)

Introduction

Adult T-cell leukemia (ATL) is a mature T-cell neoplasm developing in a minority of persons infected with human T-cell leukemia virus type I (HTLV-I), the first retrovirus isolated from a human malignant disease.[1-4] HTLV-I is estimated to infect 10 to 20 million people worldwide and is endemic in some areas of Japan, sub-Saharan Africa, the Caribbean Basin, and South America.[5,6] The area with the highest HTLV-I prevalence is the Kyushu district in southwestern Japan, where more than 10% of the general population is infected and the cumulative incidence of developing ATL among adult virus carriers is estimated at approximately 6.6% for males and 2.1% for females.[7] The onset of ATL after HTLV-I infection appears to require a long latency period because the median age at diagnosis ranges from 40 to 60 years in most endemic regions where mother-to-child viral transmission had been previously common.[4-6]

Clinical manifestation of ATL is heterogeneous and characterized by various degrees of lymphadenopathy, abnormal lymphocytosis, hepatosplenomegaly, skin lesions, and hypercalcemia, dividing the disease into 4 subtypes: acute, lymphomatous, chronic, and smoldering.[8] Patients with acute or lymphomatous type had extremely poor prognosis, mainly because of resistance to a variety of cytotoxic agents and susceptibility to opportunistic infections. Chronic and smoldering forms have relatively indolent clinical courses but can transform into more aggressive subtypes. During the past 3 decades since the clinical discovery of ATL,[1] the results of conventional cytotoxic chemotherapy remain dismal because of low response rates and lack of long-term efficacy. The

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median survival time that followed the best clinical results to date is approximately 13 months[9,10]; complete response can only be achieved in 25%-40% of treated cases and most of them eventually relapsed with the median progression-free survival time of 5 to 7 months, whereas available treatment options are extremely limited in those who failed initial chemotherapy.[11-14]

Although the early experience of ablative chemoradiotherapy with autologous hematopoietic stem cell rescue for ATL resulted in a high incidence of relapse and fatal toxicities,[15] allogeneic hematopoietic stem cell transplantation (HSCT) has been explored as a promising alternative that can provide long-term remission in a proportion of patients with ATL.[16-19] Although the mechanisms by which allografting can eradicate HTLV-I–infected neoplastic T cells are not fully elucidated, several reports have suggested the role of graft-versus-HTLV-I or graft-versus-ATL effects.[20-23] Over the past decade, improved access to alternative stem cell sources and the development of less toxic conditioning regimens have led to a rapid increase in the number of cases of ATL treated with allogeneic HSCT, albeit without consistent efficacy.[24-30] Therefore, we conducted a nationwide retrospective cohort study to identify pretransplantation factors that affect survival after allografting for ATL, with special emphasis on the effect of graft source: we compared the outcomes of human leukocyte antigen (HLA)–mismatched related bone marrow or peripheral blood transplantation, unrelated bone marrow transplantation, and unrelated cord blood transplantation with those of HLA-matched related bone marrow or peripheral blood transplantation as treatment for ATL. We also evaluated the effect of donor HTLV-I serostatus on outcomes among patients who received transplants from related donors.

Methods

Collection of data

Data on 417 patients with acute or lymphomatous type ATL who had received T-cell–replete allogeneic bone marrow, peripheral blood, or cord blood transplantation between January 1, 1996, and December 31, 2005, were collected through the 3 largest hematopoietic cell transplant registries in our country: the Japan Society for Hematopoietic Cell Transplantation (JSHCT), the Japan Marrow Donor Program (JMDP), and the Japan Cord Blood Bank Network (JCBBN). The patients were included from 102 transplant centers; the data were updated as of December 2008. To evaluate the effect of HTLV-I infection in donors on transplantation outcomes, additional questionnaires were sent to 77 centers in January 2010 to retrieve data on donor HTLV-I serostatus in 217 related transplants registered with the JSHCT. Our analysis included patients for whom there was data on age at transplantation, sex, donor type, stem cell source, and agents used in the conditioning regimen and graft-versus-host disease (GVHD) prophylaxis. Twenty-two patients who missed any of these data, and 8 patients who had a history of prior autologous or allogeneic stem cell transplantation were excluded from the analysis. One patient who had received an ex vivo T-cell–depleted graft was also excluded. Two independent physicians reviewed the quality of collected data, and a total of 386 patients (209 males and 177 females), with a median age of 51 years (range, 18-79 years), were found to fulfill the inclusion criteria: 197 patients from JSHCT, 99 from JMDP, and 90 from JCBBN. No overlapping cases were identified. Data on engraftment or graft failure were missing in 23 patients. Data on acute GVHD were not available in 53 patients because of early death or missing data.

The JSHCT registry currently includes more than 390 transplant centers variously located in Japan and collects data on transplantation by use of autologous or related stem cell grafts. The JMDP includes more than 190 centers and collects data on unrelated bone marrow transplantation. The JCBBN, a national network of 11 cord blood banks, collects data on unrelated cord blood transplantations reported individually from more than 220 transplant centers to each bank. Participating centers to these registries are requested to report each type of transplantation consecutively and longitudinally. Until 2005, the 3 registries were operated separately from one another: however, a project attempting to unify them has been launched via development of the Transplant Registry Unified Management Program, which enables participating centers to use a shared format for data submission to each registry.[31] All unrelated donor transplants in Japan were facilitated through the JMDP and JCBBN, although peripheral blood donation from unrelated volunteers has not yet been instituted as of March 2010. The study was approved by the data management committees of the JSHCT, JMDP, and JCBBN, as well as by the institutional review boards of Kyoto University, Graduate School of Medicine, where this study was organized.

End points

The primary end point of the study was overall survival, defined as the time from the date of transplantation until date of death from any cause. Patients who remained alive at the time of last follow-up were censored. Reported causes of death were reviewed and categorized into disease-associated or treatment-associated deaths. Disease-associated deaths were defined as deaths from relapse or progression of ATL among patients who survived for at least 30 days after transplantation. Treatment-related deaths were defined as any death other than disease-associated deaths. Neutrophil recovery was considered to have occurred when an absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation. Primary graft failure was evaluated in patients who survived at least 30 days and was defined as no evidence of neutrophil recovery after transplantation. Acute and chronic GVHD were diagnosed and graded using traditional criteria by the physicians who performed transplantations at each center.[32,33] The incidence of acute GVHD was evaluated in patients who survived for at least 7 days, and that of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used for summarizing variables related to patient demographics and transplant characteristics. Comparisons among the groups were performed by use of the χ^2 statistic or extended Fisher exact test as appropriate for categorical variables, and the Kruskal-Wallis test for continuous variables. The probability of overall survival was estimated according to the Kaplan-Meier method, and univariable comparisons among the groups were made using the log-rank test. Probabilities of acute and chronic GVHD, treatment-related mortality, and disease-associated mortality were estimated with the use of cumulative incidence curves to accommodate the following competing events[34]: death without GVHD for acute and chronic GVHD, disease-associated death for treatment-related mortality, and treatment-related death for disease-associated mortality. Data on patients who were alive at the time of last follow-up were censored. Cox proportional-hazards regression was used to evaluate variables potentially affecting overall survival, whereas Fine and Gray proportional-hazard model was used to evaluate variables affecting other outcomes.[35] The variables considered were recipient age group ($\leq$ 50 years or $>$ 50 years at transplantation); recipient sex; disease status before transplantation; type of conditioning regimen; type of GVHD prophylaxis; type of graft source; time from diagnosis to transplantation (within 6 months or longer than 6 months); and year of transplantation. Only factors differing in distribution among the graft source groups and factors associated with outcomes by univariable comparison were included in the final models. The effect of donor HTLV-I seropositivity on outcomes after related donor transplantation was also evaluated by univariable and multivariable analysis with the use of data on 156 patients given transplants from siblings or other related family members for whom data on the HTLV-I serostatus were available. Results were expressed as hazard ratios and their 95% confidence interval (CI). All tests were 2-sided, and a P value of less than .05 was considered to indicate statistical significance. All statistical analyses were performed with STATA software (Version 11; Stata Corporation).

Results

Patients

Table 1 shows characteristics of the patients and transplantation procedures. Compared with HLA-matched related bone marrow or

Table 1. Characteristics of allografted patients with ATL

Patient variables	No. of recipients by graft source type (%)				
	HLA-matched related bone marrow or peripheral blood (N = 154)	HLA-mismatched related bone marrow or peripheral blood (N = 43)	Unrelated bone marrow (N = 99)	Unrelated cord blood (N = 90)	*P*
Age range at transplantation, y					.001
30 or younger	4 (3)	1 (2)	2 (2)	1 (1)	
30-40	21 (14)	4 (9)	8 (8)	3 (3)	
40-50	56 (36)	12 (28)	44 (44)	21 (23)	
50-60	57 (37)	22 (51)	43 (43)	47 (52)	
Older than 60	16 (10)	4 (9)	2 (2)	18 (20)	
Sex					.257
Male	76 (49)	21 (49)	60 (61)	52 (58)	
Female	78 (51)	22 (51)	39 (39)	38 (42)	
Disease status					.001
Complete remission	50 (32)	7 (16)	35 (35)	26 (29)	
Not in complete remission	102 (66)	35 (81)	52 (53)	57 (63)	
Unknown	2 (1)	1 (2)	12 (12)	7 (8)	
Conditioning regimen					< .001
CY-TBI or BU-CY	51 (33)	6 (14)	43 (43)	14 (16)	
Purine analog–containing	72 (47)	23 (53)	37 (37)	64 (71)	
Others	31 (20)	14 (33)	19 (19)	12 (13)	
GVHD prophylaxis					< .001
Cyclosporine-based	146 (95)	11 (26)	29 (29)	60 (67)	
Tacrolimus-based	6 (4)	31 (72)	68 (69)	25 (28)	
Others	2 (1)	1 (2)	2 (2)	5 (6)	
Source of stem cells					< .001
Bone marrow	46 (30)	12 (28)	99 (100)	-	
Peripheral blood	106 (69)	31 (72)	-	-	
Bone marrow + peripheral blood	2 (1)	0 (0)	-	-	
Cord blood	-	-	-	90 (100)	
HLA compatibility*					
Matched	154 (100)	-	83 (84)	3 (3)	< .001
One-antigen mismatch		19 (44)	12 (12)	29 (32)	
Two-antigen mismatch		13 (30)	0 (0)	57 (63)	
Three-antigen mismatch		7 (16)	0 (0)	1 (1)	
Uncertain/missing		4 (9)	4 (4)	0 (0)	
Time from diagnosis to transplantation					< .001
6 months or less	92 (60)	26 (60)	22 (22)	49 (54)	
More than 6 months	52 (34)	16 (37)	75 (76)	41 (46)	
Uncertain/missing	10 (6)	1 (2)	2 (2)	0 (0)	
Year of transplantation					< .001
1995-1999	18 (12)	1 (2)	5 (5)	0 (0)	
2000-2002	66 (43)	15 (35)	26 (26)	12 (13)	
2003-2005	70 (45)	27 (63)	68 (69)	78 (87)	
Follow-up of survivors†					
Median mo (range)	40.5 (1.5-102.3)	36.7 (8.8-85.1)	40.2 (16.0-81.2)	48.9 (1.6-73.5)	.847

ATL indicates adult T-cell leukemia; HLA, human leukocyte antigen; GVHD, graft-versus-host disease; CY-TBI, cyclophosphamide with total-body irradiation; BU-CY, busulfan and cyclophosphamide; purine analog–containing, conditioning regimens containing fludarabine, cladribine, or pentostatin; cyclosporine-based, cyclosporine with or without other agents; and tacrolimus-based, tacrolimus with or without other agents.

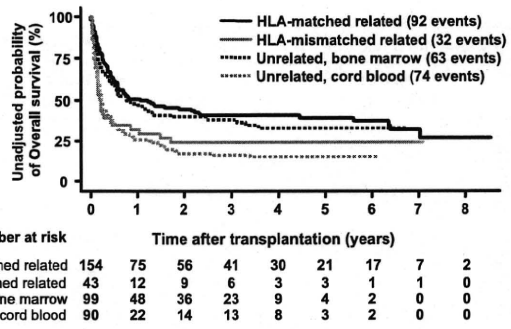
*HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, HLA-B, and HLA-DR antigens.

†Data are time interval in months.

peripheral blood recipients, HLA-mismatched bone marrow or peripheral blood recipients were more likely to receive tacrolimus for GVHD prophylaxis; unrelated bone marrow recipients took a longer time from diagnosis to transplantation, were more likely to have attained complete remission at transplantation, and were more likely to receive tacrolimus for GVHD prophylaxis; unrelated cord blood recipients were older, underwent transplantation more recently, and were more likely to receive purine analog–containing conditioning regimens. All unrelated cord blood recipients received a single cord blood unit that was not manipulated ex vivo. The median weight of unrelated cord blood recipients was 52.0 kg (range, 31.0-90.2 kg); the median dose of nucleated cells and $CD34^+$ progenitor cells in the grafts, measured before freezing, was 2.55×10^7 (range, $1.39\text{-}5.34 \times 10^7$) and 0.79×10^5 (range, $0.07\text{-}3.15 \times 10^5$) per kg of recipient body weight, respectively.

Engraftment and GVHD

Of 310 patients who survived 30 days after transplantation and were evaluable for engraftment, primary graft failure was reported in 2 (6%) of 35 recipients of HLA-mismatched related grafts and in 12 (17%) of 70 recipients of unrelated cord blood, whereas the remaining 296 patients had evidence of initial engraftment. Acute GVHD of grades II, III, or IV occurred in 158 (47%) of 333



Number at risk									
HLA-matched related	154	75	56	41	30	21	17	7	2
HLA-mismatched related	43	12	9	6	3	3	1	1	0
Unrelated, bone marrow	99	48	36	23	9	4	2	0	0
Unrelated, cord blood	90	22	14	13	8	3	2	0	0

Figure 1. Unadjusted probability of overall survival according to type of graft source. The unadjusted Kaplan-Meier estimates of overall survival stratified according to type of graft source are shown.

evaluable patients; 69 (49%) of 140 HLA-matched related bone marrow or peripheral blood recipients, 20 (56%) of 36 HLA-mismatched related bone marrow or peripheral blood recipients, 40 (44%) of 91 unrelated bone marrow recipients, and 29 (44%) of 66 unrelated cord blood recipients. In a multivariable analysis, rates of grades II to IV acute GVHD did not significantly differ among the 4 groups (supplemental Table 1; available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Chronic GVHD occurred in 94 (48%) of 195 evaluable patients at a significantly lower rate among the unrelated cord blood recipients than among HLA-matched graft recipients (hazard ratio, 0.25; 95% CI, 0.10-0.61, $P = .002$).

Relapse and disease progression

Of 333 patients who survived 30 days after transplantation, 136 patients experienced relapse or progression of ATL at a median of 76 days (range, 1-1964 days) after transplantation. ATL recurred or progressed in 52 (37%) of 141 recipients of HLA-matched related grafts, in 19 (51%) of 37 recipients of HLA-mismatched related grafts, in 27 (32%) of 85 recipients of unrelated bone marrow, and 38 (54%) of 70 recipients of unrelated cord blood. Of 113 patients who were evaluable for the date of relapse or disease progression, the median time from transplantation to relapse or progression of ATL was 65.5 days (range, 1-1964 days) for HLA-matched related bone marrow or peripheral blood recipients, 63 days (range, 22-269 days) for HLA-mismatched related bone marrow or peripheral blood recipients, 152 days (range, 42-819 days) for unrelated bone marrow recipients, and 83 days (range, 7-596 days) for unrelated cord blood recipients.

Overall survival

Of 386 patients included in the study, a total of 125 patients were alive and 101 patients were alive in continuous complete remission after a median follow-up of 41 months (range, 1.5-102 months). The unadjusted 3-year probability of overall survival was 33% (95% CI, 28%-38%) for the whole cohort; 41% (95% CI, 33%-49%) in HLA-matched related graft recipients; 24% (95% CI, 12%-38%) in HLA-mismatched related graft recipients; 39% (95% CI, 29%-49%) in unrelated bone marrow recipients; and 17% (95% CI, 9%-25%) in unrelated cord blood recipients (Figure 1). The median overall survival time after transplantation was 9.8 months for HLA-matched related bone marrow or peripheral blood recipients, 2.5 months for HLA-mismatched related bone marrow or peripheral blood recipients, 9.6 months for unrelated bone marrow recipients, and 2.6 months for unrelated cord blood recipients. Patients who received transplants in complete remission had a higher probability of survival than those who received transplants not in complete remission (51% [95% CI, 41%-60%] vs 26% [95% CI, 20%-31%], $P < .001$). Multivariable analyses revealed 4 factors that adversely affected overall survival: older recipient age (> 50 years; hazard ratio, 1.56; 95% CI, 1.14-2.12, $P = .005$), male recipient (hazard ratio, 1.37; 95% CI, 1.07-1.77, $P = .014$), lack of complete remission at transplantation (hazard ratio, 2.01; 95% CI, 1.50-2.71, $P < .001$), and transplantation of unrelated cord blood. Hazard ratios for death among recipients of HLA-mismatched related transplants, unrelated bone marrow transplants, and unrelated cord blood transplants, compared with that among recipients of HLA-matched related transplants, were 1.55 (95% CI, 0.98-2.45, $P = .063$), 1.24 (95% CI, 0.82-1.88, $P = .312$), and 2.08 (95% CI, 1.43-3.02, $P < .001$), respectively (Table 2).

Treatment-related mortality and disease-associated mortality

Overall, 161 (43%) of 376 evaluable patients succumbed to treatment-related complications. Cumulative incidence of treatment-related mortality at 3 years after transplantation was 37% (95% CI, 29%-45%) in HLA-matched related bone marrow or peripheral blood recipients, 43% (95% CI, 28%-57%) in HLA-mismatched related bone marrow or peripheral blood recipients, 42% (95% CI, 32%-51%) in unrelated bone marrow recipients, and 52% (95% CI, 41%-62%) in unrelated cord blood recipients (Figure 2A). When adjusted by multivariable analysis, patients given unrelated cord blood (hazard ratio, 1.77; 95% CI, 1.10-2.86, $P = .019$) had higher treatment-related mortality rates (Table 2).

Deaths from progression of ATL occurred in 90 (24%) patients. Cumulative incidence of disease-associated mortality at 3 years after transplantation was 21% (95% CI, 14%-28%) in HLA-matched related bone marrow or peripheral blood recipients, 32% (95% CI, 19%-47%) in HLA-mismatched related bone marrow or peripheral blood recipients, 19% (95% CI, 12%-28%) in unrelated bone marrow recipients, and 30% (95% CI, 21%-40%) in unrelated cord blood recipients (Figure 2B). In multivariable analysis, patients given transplants not in remission (hazard ratio, 2.55; 95% CI 1.50-4.33, $P = .001$) or male recipients (hazard ratio, 1.86; 95% CI, 1.17-2.95, $P = .008$) had higher rates of disease-associated mortality (Table 2).

Causes of death after transplantation are summarized in Table 3. Of the 161 patients who died of treatment-related complications, 51 (32%) succumbed to infection and 53 (33%) to organ failure. Treatment-related events were principal causes of early death, whereas death from relapse or progression of ATL was more common later than 100 days after transplantation, irrespective of types of graft source.

Effect of donor HTLV-I serostatus on outcomes

Data on donor HTLV-I serostatus were available for analysis in 156 of 197 patients given related transplants; 68 received transplants from an HTLV-I–seropositive donor and 88 from an HTLV-I–seronegative donor. Patients who received transplants from HTLV-I–seropositive donors and those from HTLV-I–seronegative donors had similar background characteristics (supplemental Table 2). Among 113 patients who had data on donor HTLV-I serostatus and maintained or attained complete remission after transplantation, relapse of ATL was observed in 18 (38%) of 48 patients who received transplants from an HTLV-I–seropositive donor, and 16 (25%) of 65 patients who received transplants from an HTLV-I–seronegative donor with a median follow-up time for survivors of 40 months (range, 7.3-102 months). In univariable and

Table 2. Multivariable analysis of transplantation outcomes

Variables	Overall survival			Treatment-related mortality			Disease-associated mortality		
	Number*	Hazard ratio (95% CI)	P	Number*	Hazard ratio (95% CI)	P	Number*	Hazard ratio (95% CI)	P
Age group, y									
50 or younger	109/177	1.00	Reference	70/173	1.00	Reference	35/173	1.00	Reference
Older than 50	152/209	1.56 (1.14-2.12)	.005	91/203	1.40 (0.96-2.05)	.084	55/203	1.22 (0.71-2.10)	.465
Sex of recipient†									
Female	105/177	1.00	Reference	68/171	-	-	31/171	1.00	Reference
Male	156/209	1.37 (1.07-1.77)	.014	93/205	-	-	59/205	1.86 (1.17-2.95)	.008
Disease status									
Complete remission	60/118	1.00	Reference	43/117	1.00	Reference	16/117	1.00	Reference
Not in complete remission	184/246	2.01 (1.50-2.71)	< .001	106/238	1.30 (0.92-1.84)	.137	70/238	2.55 (1.50-4.33)	.001
Unknown	17/22	2.01 (1.15-3.50)	.014	12/21	1.74 (0.89-3.40)	.105	4/21	1.42 (0.45-4.52)	.554
Conditioning regimen									
CY-TBI or BU-CY	68/114	1.00	Reference	45/112	1.00	Reference	21/112	1.00	Reference
Purine analog–containing	136/196	1.05 (0.75-1.48)	.777	79/191	0.86 (0.56-1.32)	.487	52/191	1.34 (0.72-2.48)	.360
Others	57/76	1.26 (0.86-1.84)	.240	37/73	1.23 (0.78-1.95)	.377	17/73	1.10 (0.56-2.13)	.784
GVHD prophylaxis‡									
Cyclosporine-based	160/246	1.00	Reference	99/241	1.00	Reference	56/241	1.00	Reference
Tacrolimus-based	91/130	1.09 (0.78-1.51)	.614	55/127	1.13 (0.72-1.75)	.599	33/127	1.05 (0.57-1.93)	.887
Others	10/10	1.74 (0.89-3.42)	.105	7/8	2.29 (1.14-4.62)	.020	1/8	0.32 (0.04-2.42)	.268
Type of graft source									
Matched related bone marrow or peripheral blood	92/154	1.00	Reference	57/149	1.00	Reference	30/149	1.00	Reference
Mismatched related bone marrow or peripheral blood	32/43	1.55 (0.98-2.45)	.063	18/42	1.12 (0.59-2.12)	.722	13/42	1.50 (0.67-3.37)	.329
Unrelated bone marrow	63/99	1.24 (0.82-1.88)	.312	41/99	1.19 (0.71-1.98)	.512	22/99	1.06 (0.46-2.48)	.888
Unrelated cord blood	74/90	2.08 (1.43-3.02)	< .001	45/86	1.77 (1.10-2.86)	.019	25/86	1.49 (0.80-2.80)	.211
Time from diagnosis to transplantation									
6 months or less	128/189	1.00	Reference	81/183	1.00	Reference	41/183	1.00	Reference
More than 6 months	125/184	1.03 (0.78-1.35)	.834	76/180	0.86 (0.61-1.22)	.395	45/180	1.32 (0.82-2.12)	.258
Uncertain/missing	8/13	1.01 (0.49-2.09)	.971	4/13	0.64 (0.25-1.60)	.340	4/13	1.93 (0.77-4.87)	.163
Year of transplantation									
1995-1999	18/24	1.00	Reference	11/24	1.00	Reference	7/24	1.00	Reference
2000-2002	85/119	1.01 (0.58-1.74)	.979	56/113	1.13 (0.59-2.13)	.716	23/113	0.61 (0.26-1.46)	.269
2003-2005	158/243	0.73 (0.41-1.32)	.296	94/239	0.75 (0.37-1.51)	.416	60/239	0.70 (0.29-1.73)	.442

CI indicates confidence interval; GVHD, graft-versus-host disease; CY-TBI, cyclophosphamide with total-body irradiation; BU-CY, busulfan and cyclophosphamide; purine analog–containing, regimens containing fludarabine, cladribine or pentostatin; cyclosporine-based, cyclosporine with or without other agents; tacrolimus-based, tacrolimus with or without other agents; and Reference, reference category in regression models.

*Number of events/number of evaluable patients.

†Sex of recipient was not included as a confounder in the multivariable final model for treatment-related mortality because it was not found to be a significant factor in univariable comparison.

‡GVHD prophylaxis other than cyclosporine- or tacrolimus-based regimen was not considered as a significant variable associated with treatment-related mortality because of the small number of patients in this group.

multivariable analysis, patients who received transplants from an HTLV-I–seropositive donor had a higher risk of disease-associated mortality compared with those who received transplants from an HTLV-I–seronegative donor, whereas they had similar overall survival and treatment-related mortality rates (Table 4).

Discussion

The aim of this nationwide registry-based study was to compare overall survival after allogeneic HSCT with the use of various graft sources as treatment for ATL, and to identify factors that may influence transplantation outcomes. Despite the retrospective nature of the study, the validity of our analysis is strengthened by the fact that our cohort included most of the related transplants and nearly all unrelated transplants for ATL performed over a decade in our country.

We found that a substantial proportion of patients with ATL, including those who did not achieve complete remission, could enjoy long-term survival after allogeneic HSCT, validating the results of earlier observations.[18,19] However, our analysis in this cohort also revealed a high rate of treatment-related mortality. In particular, frequent incidence of fatal infectious complications may reflect preexisting profound immunodeficiency observed in patients with ATL.[4,5] Improved supportive care for opportunistic infection might be especially important for reducing treatment-related mortality in allografting for ATL.

Multivariable analysis revealed 4 factors that affected survival: recipient age, recipient sex, disease status before transplantation, and type of graft source. Although higher age of the recipient was associated with lower posttransplantation survival, most of the patients with ATL were older than age 50 years and were less likely to be candidates for fully ablative conditioning. Recently, 2 small prospective trials have demonstrated the feasibility and efficacy of allogeneic stem cell transplantations using reduced-intensity conditioning.[26,29] Although we observed no significant differences in overall survival between patients who received conventional conditioning regimens and those who received purine analog–

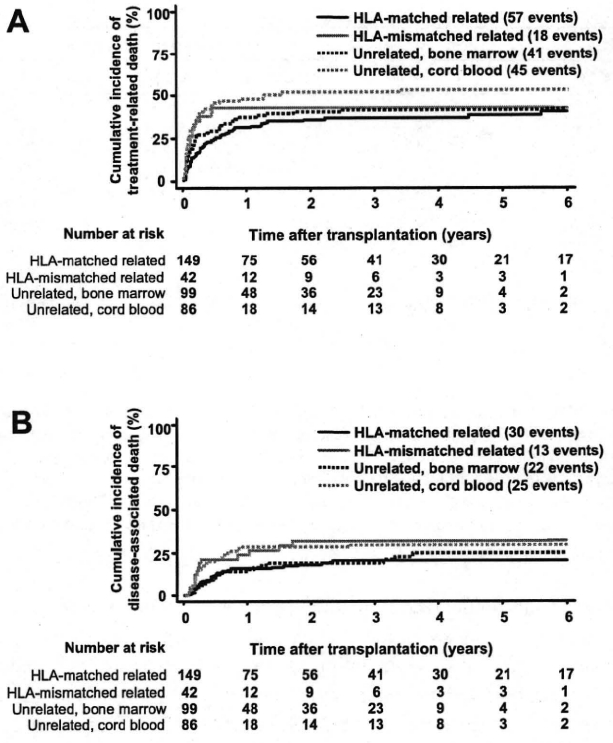


Figure 2. Cumulative incidence of treatment-related mortality and disease-associated mortality according to type of graft source. The unadjusted cumulative incidence curves for treatment-related mortality (A) and disease-associated mortality (B) stratified according to type of graft source are shown after allogeneic hematopoietic stem cell transplantation in patients with adult T-cell leukemia.

based regimens in the present study, it was difficult to evaluate the effect of conditioning dose intensity because data on doses of agents or total-body irradiation used in these regimens were not fully available in our cohort. Further studies are warranted to identify unfit or elderly ATL patients who can benefit from allogeneic stem cell transplantation with the use of less toxic conditioning.

A further novel finding in this study was that female patients with ATL had a more favorable outcome after allogeneic stem cell transplantation compared with male patients. Incidence of ATL in Japan is generally higher in male than in female populations, which was partly explained by the difference in routes of HTLV-I transmission between males and females. Sexual transmission of the virus can also occur, predominantly from males to females in adult life, thereby lowering the apparent incidence of ATL among female HTLV-I carriers.[7] However, the estimated ATL mortality among a prospective cohort of perinatally infected HTLV-I carriers was still higher for male patients,[36] suggesting that female sex itself might have a protective role against ATL development. Although much of the underlying mechanism for male predominance in ATL remains to be elucidated,[37] unidentified biologic or immunologic aspects of sex difference may contribute not only to development of ATL in HTLV-I carriers, but also to outcomes in allografted patients with ATL.

Despite the high risk for relapse after transplantation, survival rates observed in patients who received transplants not in complete remission were encouraging. Intriguingly, withdrawal of immunosuppressive agents or donor lymphocyte infusion can induce remission in relapse of ATL after allogeneic HSCT, implying the presence of a graft-versus-ATL effect.[19-23] Because several antigens have recently been identified as putative targets for cytotoxic T-cell responses against ATL,[38,39] future development of cellular immunotherapy targeting these molecules would reduce the incidence of relapse and improve survival in patients with residual ATL after allogeneic transplantation. Further investigations are warranted to elucidate the association between the occurrence of GVHD and disease response among allografted patients with ATL because our preliminary analysis using a similar cohort[40] suggested that patients who developed mild acute GVHD had a better posttransplantation survival compared with those who did not develop acute GVHD (J.K., M. Hishizawa, A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, Y.A., R.S., T.K., K.M., T.N.-I., S. Kato, H.S., Y. Morishima, J.O., T.I., and T.U., manuscript in preparation).

Finally, the use of unrelated cord blood was associated with lower survival, most likely a result of higher treatment-related mortality. Two major causes of early treatment-related death were infection and organ failure. Because the development of ATL usually worsens preceding immunodeficiency associated with HTLV-I infection, it is imperative to establish effective measures to manage posttransplantation infections in allografted patients with ATL. In addition, the use of more intense conditioning for refractory disease in relatively elderly recipients may increase the risk of regimen-related toxicities especially in the setting of unrelated donor transplantation. However, direct comparison of

Table 3. Cause of death according to type of graft source

	Deaths within 100 days per graft source (%)				Deaths later than 100 days per graft source (%)			
Cause of death	HLA-matched related bone marrow or peripheral blood	HLA-mismatched related bone marrow or peripheral blood	Unrelated bone marrow	Unrelated cord blood	HLA-matched related bone marrow or peripheral blood	HLA-mismatched related bone marrow or peripheral blood	Unrelated bone marrow	Unrelated cord blood
Primary disease	11 (28)	9 (35)	6 (18)	15 (30)	19 (37)	4	16 (53)	10 (42)
Treatment-related								
GVHD	3 (8)	1 (4)	2 (6)	2 (4)	4 (8)	1	2 (7)	1 (4)
Infection	7 (18)	5 (19)	9 (27)	12 (24)	9 (17)	0	4 (13)	5 (21)
Organ failure	12 (30)	3 (12)	13 (39)	11 (22)	9 (17)	1	4 (13)	0 (0)
Others	6 (15)	7 (27)	3 (9)	10 (20)	7 (13)	0	4 (13)	4 (17)
Undetermined	1 (3)	1 (4)	0 (0)	0 (0)	4 (8)	0	0 (0)	4 (17)
Total no. of deaths	40 (100)	26 (100)	33 (100)	50 (100)	52 (100)	6	30 (100)	24 (100)
Patients at risk	154	43	99	90	113	17	66	39

HLA indicates human leukocyte antigen; GVHD, graft-versus-host disease.

Data are number of deaths to total deaths (%) after transplantation in the group according to type of graft source. Percentages are not provided for groups having fewer than 10 patients in total.