[16-18]. The results of the present study clinically support this observation. However, it should be considered that tacrolimus also causes side effects, such as nephrotoxicity, when its blood concentration remains within the toxic range (presently considered to be above 20 ng/mL) for a prolonged period [6,7]. Once its toxicity arises, physicians need to discontinue the drug temporarily or decrease the target range. Such an unexpected and unplanned dose adjustment of tacrolimus is likely to increase the risk of developing aGVHD, and this could be 1 of the plausible explanations for the failure of the previous reports to demonstrate the dose-dependent efficacy of tacrolimus, because these reports applied wider and higher (>20 ng/mL) target ranges [6,7]. Therefore, our results suggest that the in vivo efficacy of tacrolimus in preventing aGVHD is dose dependent as long as its concentration is strictly maintained within a less toxic therapeutic range.

In addition to the blood concentration of tacrolimus, we found that the donor age was also a significant factor affecting the development of aGVHD. Older donor age (35 years old or older) increased the incidence of grades II-IV aGVHD with an odds ratio (OR) of 4.28 (95% confidence interval [CI]: 1.15-15.92). There have been a series of reports evaluating the risk factors for aGVHD, and diverse factors have been identified [19-27]. However, the effect of donor age on aGVHD remains controversial. Among those studies, only a few studies identified donor age as a significant factor affecting the incidence of aGVHD; in these studies, older donor age significantly increased the incidence of aGVHD [26,27].

Renal toxicity is 1 of the most common adverse effects of tacrolimus. In the present study, in which the target level of tacrolimus was set at 10 to 20 ng/mL, only 5% of the patients experienced renal impairment defined by doubled serum creatinine levels compared with those before transplantation. In addition, there was no significant correlation between the concentration of tacrolimus and an increase in the serum creatinine level. In this study, the dose of tacrolimus was adjusted on a daily basis, not only according to its steady-state blood concentration of tacrolimus but also the serum creatinine level. In addition, efforts, such as hydration and dose adjustment of other nephrotoxic drugs concurrently given, were made to correct renal impairments. Therefore, our experience strongly suggested that tacrolimus could be safely administered at a concentration of 10 to 20 ng/mL if patients were optimally managed.

In conclusion, physicians should recognize the significance of early posttransplantation blood concentration of tacrolimus in preventing aGVHD, and should maintain the concentration between 15 and 20 ng/mL. Further prospective studies are warranted to evaluate the efficacy and safety of this target range of blood tacrolimus concentration in allogeneic HSCT recipients.

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ORIGINAL ARTICLE

Foscarnet against human herpesvirus (HHV)-6 reactivation after allo-SCT: breakthrough HHV-6 encephalitis following antiviral prophylaxis

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High incidences of human herpesvirus (HHV)-6 encephalitis have recently been reported from several Japanese SCT centers. To evaluate the effect of low-dose foscarnet (PFA) in preventing HHV-6 infection among recipients of unrelated BM or cord blood (CB), we examined consecutive cohorts without prophylaxis against HHV-6 (Cohort 1, $n=51$) and with PFA prophylaxis (Cohort 2, PFA 50 mg/kg/day for 10 days after engraftment, $n=67$). Plasma real-time PCR assay was performed weekly. High-level reactivation defined as HHV-6 DNA $\geqslant 10^4$ copies/mL by day 70 was the primary endpoint. No significant reduction of high-level reactivation was seen in Cohort 2 (19.4%) compared with Cohort 1 (33.8%, $P=0.095$). A trend was identified toward fewer high-level HHV-6 reactivations in Cohort 2 among recipients of unrelated BM ($P=0.067$), but no difference in incidence was observed among CB recipients ($P=0.75$). Breakthrough HHV-6 encephalitis occurred following PFA prophylaxis in three patients, and incidence of HHV-6 encephalitis did not differ between Cohort 1 (9.9%) and Cohort 2 (4.5%, $P=0.24$). In conclusion, 50 mg/kg/day of PFA does not effectively suppress HHV-6 reactivation and cannot prevent all cases of HHV-6 encephalitis. To effectively prevent HHV-6 encephalitis, alternative approaches based on the pathogenesis of HHV-6 encephalitis will probably be required.

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Keywords: human herpesvirus-6; HHV-6 encephalitis; foscarnet; prophylaxis; allo-SCT

INTRODUCTION

Human herpesvirus (HHV)-6 reactivates early after hematopoietic SCT and is found in the blood in about half of allo-SCT recipients.[1–4] HHV-6 reactivation is potentially associated with various post-transplant complications, including those affecting the skin, lung, gastrointestinal tract and central nervous system (CNS).[5,6] In particular, HHV-6 encephalitis has been recognized as a life-threatening complication associated with HHV-6 reactivation.

HHV-6 encephalitis typically developed 3 weeks after SCT.[7,8] Memory disturbance is a typical initial symptom, and consciousness loss, disorientation and seizures can subsequently develop.[3,7–9] The limbic system is a target of HHV-6.[3,6–10] Recipients of unrelated transplants and cord blood (CB) display a high risk of HHV-6 encephalitis.[3,4,7,8] HHV-6 encephalitis develops concomitant to the appearance of a high-HHV-6 DNA load in plasma.[3,11,12] This suggests that systemic high-level HHV-6 replication is a key factor in the development of HHV-6 encephalitis and that HHV-6 encephalitis is preventable as long as high-level HHV-6 reactivation can be suppressed.

Foscarnet (PFA), ganciclovir (GCV) and cidofovir (CDV) have been shown to display *in vitro* inhibitory effects against HHV-6,[5] and most patients with reported HHV-6 encephalitis are treated with GCV and/or PFA.[7,8] However, more than half of the patients who developed HHV-6 encephalitis developed neurological sequelae such as memory disturbance, or died of encephalitis.[3,7,8,13–15] The efficacy of antiviral treatment after development of HHV-6 encephalitis thus appears insufficient.

The European Conference on Infections in Leukemia does not recommend antiviral prophylaxis against HHV-6, because of the low risk of HHV-6 disease and the toxicity of the available antiviral drugs.[16] However, a high incidence of HHV-6 encephalitis has recently been reported from several Japanese SCT centers[3,4,12–15,17–19] (Table 1). Several investigators have asserted that the establishment of methods to prevent HHV-6 encephalitis is needed for patients at high risk of this complication.[6,11,14,15,20,21]

To prevent the development of HHV-6 encephalitis, we have attempted pre-emptive GCV therapy guided by plasma HHV-6 DNA load.[11] However, dynamic kinetics of plasma HHV-6 viral load make pre-emptive therapy difficult. Prophylactic administration of active agents against HHV-6 may be useful to prevent HHV-6 encephalitis. The important issue is whether the benefits of preventing HHV-6 encephalitis outweigh the toxicities associated with the antiviral drug. HHV-6 reactivation concentrates during the first 10 days after engraftment,[3] and prophylaxis using GCV during this period may be associated with prolonged neutropenia.[22] While PFA may be more appropriate than GCV as a prophylactic agent, renal toxicity remains problematic for prophylactic use.[21] The effects of PFA in preventing CMV infection have been studied previously,[23,24] but this agent is not currently in common used for prophylaxis due to its toxicity.

Although two studies evaluating the effects of prophylaxis to prevent HHV-6 reactivation have been reported,[25,26] those investigations were very small. The present study investigated whether prophylactic measures are effective for preventing high-level

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HHV-6 reactivation and HHV-6 encephalitis. Given the myelosuppressive effects of GCV, we chose PFA as a prophylactic agent. As PFA shows dose-dependent renal toxicity, a low dosage (50 mg/kg) and short-dosing period (10 days) were applied. Kinetics of plasma HHV-6 DNA among patients who received prophylactic PFA were compared with those of our historic controls.

PATIENTS AND METHODS

All study protocols were approved by the ethics committees of both the Oita University Faculty of Medicine and Oita Prefectural Hospital, and written informed consent was obtained from each patient before participation.

Patients

Cohort 1. This cohort comprised patients who received BMT from unrelated donor or CB transplantation (CBT) at Oita University Hospital or Oita Prefectural Hospital between November 1995 and February 2007. Eligibility criteria were as follows: age between 15 and 65 years; no severe concomitant disease; creatinine clearance >1 mL/min/kg bodyweight; and no prophylactic administration of GCV, PFA or CDV. Patients who received pre-emptive administration of GCV based on positive results for CMV

Table 1. Summary of reported incidence for HHV-6 encephalitis/myelitis in Japanese institutes

No. of subjects	N *(%) of patients who developed HHV-6 encephalitis/myelitis*	*Institute/group*	*Study type*	*Year*	*Reference*
1148	11 (0.96)	Kanto Group for Cell Therapy	RS	2006	13
50	4 (8.0)	Oita University Hospital	RS	2006	3
46	3 (6.5)	Keio University Hospital	RS	2007	4
362[a]	25 (7.3[b])	Tranomon Hospital	RS	2009	17
111	8 (7.2)	Oita SCT Group	RS	2010	12
228	13 (5.7[c])	Kyushu University Hospital	RS	2010	14
44[a]	5 (11.4)	Nagoya Red Cross Hospital	RS	2010	18
197	8 (4.0)	Yokohama City University Medical Center	RS	2011	15
130	8 (6.2)	Kanagawa group	RS	2011	19

Abbreviations: HHV-6 = human herpesvirus-6; RS = retrospective study. [a]All subjects were recipients of cord blood. [b]Cumulative incidence on 50 days after transplantation. [c]Incidence of HHV-6 encephalitis in recipients of cord blood was 15.7%.

Table 2. Clinical characteristics of the study population

Variables	*Cohort 1: control (November 1995–Feburary 2007)*	*Cohort 2: prophylaxis with PFA (Feburary 2007–August 2011)*	P
Number	51	67	
Age, years, median (range)	46 (17–57)	52 (17–69)	0.0004[a]
Male	32 (62.7)	37 (55.2)	0.45[b]
Underlying disease			
AML	12 (23.5)	22 (32.8)	
ALL	6 (11.8)	4 (6.0)	
Mixed phenotype acute leukemia	1 (2.0)	1 (1.5)	
Malignant lymphoma	7 (13.7)	10 (14.9)	
Adult T-cell leukemia	12 (23.5)	16 (23.9)	
CML	5 (9.8)	0 (0.0)	
Myelodysplatic syndrome	8 (15.7)	11 (16.4)	
Multiple myeloma	0 (0.0)	3 (4.5)	
Disease stage at transplantation			
Early	16 (31.4)	19 (28.4)	0.84[b]
Non-early	35 (68.6)	48 (71.6)	
Type of transplanted cells			
Unrelated BM	40 (78.4)	57 (85.1)	0.47[b]
Cord blood	11 (21.6)	10 (14.9)	
Matching of HLA			
Allele match	25 (49.0)	41 (61.2)	0.20[b]
Allele mismatch	26 (51.0)	26 (38.8)	
Ag match	36 (70.6)	45 (67.2)	
Ag mismatch	15 (29.4)	22 (32.8)	0.84[b]
Conditioning regimen			
Myeloablative	39 (76.5)	24 (35.8)	<0.0001[b]
Non-myeloablative	12 (23.5)	43 (64.2)	
Observation period for blood HHV-6 DNA, days after transplantation, median (range)	66 (31–97)	76 (36–98)	0.021[a]
Number of blood samples per patient, median (range)	10 (5–12)	11 (5–17)	0.0037[a]

Abbreviations: HHV-6 = human herpesvirus-6; PFA = foscarnet. Unless otherwise stated, all observations are given as the number and the percentage in parentheses. [a]Mann–Whitney *U*-test. [b]Fisher's exact test.

antigenemia were not excluded. Patients in Cohort 1 were used as historical controls to evaluate the effects of PFA prophylaxis in Cohort 2. Cohort 1 included 31 patients who had participated in an earlier retrospective study linking plasma HHV-6 DNA with clinical manifestations[3] and 20 patients treated subsequently.

Cohort 2. This cohort comprised patients who received BMT from unrelated donors or CBT at Oita University Hospital or Oita Prefectural Hospital between February 2007 and August 2011. Eligibility criteria were as follows: age between 15 and 65 years; no severe concomitant disease; and creatinine clearance >1 mL/min/kg bodyweight.

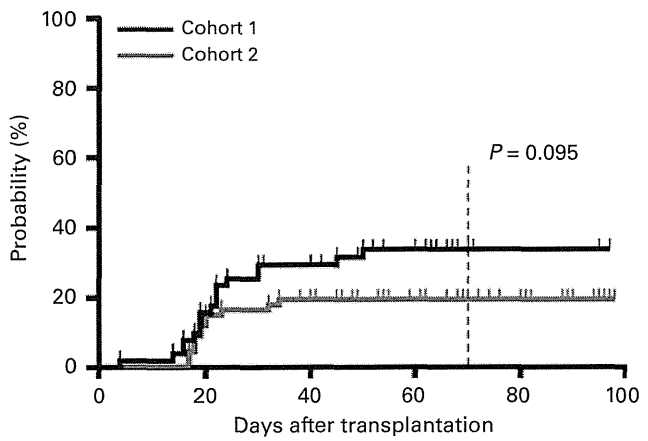

Figure 1. Kaplan–Meier estimates of the *P* of high-level HHV-6 reactivation (plasma HHV-6 DNA $\geqslant 10^4$ copies/mL) in Cohort 1 (without prophylaxis against HHV-6, $n=51$) and Cohort 2 (with PFA prophylaxis, $n=67$). At day 70 after transplantation, the rate of plasma HHV-6 DNA $\geqslant 10^4$ copies/mL was 33.8% in Cohort 1 and 19.4% in Cohort 2 ($P=0.095$).

Patients in whom chromosomally integrated HHV-6[27] was suspected based on the findings of persistent-positive results for plasma HHV-6 DNA ($\geqslant$80% of plasma samples) were excluded from analysis.

Prophylactic PFA in Cohort 2

Patients in Cohort 2 were prospectively enrolled for this study and given i.v. PFA at 50 mg/kg/day, for 10 days from the day of neutrophil engraftment. If engraftment had not been achieved by 18 days after transplantation, PFA was started on the 18th day after SCT and continued for 10 days. Any patient showing $\geqslant$grade 2 renal dysfunction (serum creatinine $\geqslant$1.6 mg/dL) or severe concomitant organ dysfunction on the date of starting PFA was excluded from this study. During the dosing period, if a patient showed serum creatinine level of at least grade 2, non-hematological toxicity of at least grade 3 other than nausea or vomiting, or refusal to continue administration, PFA administration was interrupted. Patients who start to receive prophylactic PFA but interrupted administration at any time were still included in the analyses.

Assay

In both cohorts, collection of EDTA-treated peripheral blood was started within 1 week after SCT, and performed weekly during hospitalization. Samples collected until 100 days after SCT were used for analysis for both of cohort groups. HHV-6 DNA copy numbers in plasma samples were measured using real-time PCR methods, as described previously.[3]

Definitions

The primary endpoint was the incidence of high-level HHV-6 reactivation by day 70 after SCT. High-level HV-6 reactivation was defined as a plasma HHV-6 DNA $\geqslant 10^4$ copies/mL. The primary endpoint was set based on our findings that the threshold level for the development of HHV-6 encephalitis was 10^4 copies/mL plasma under our real-time PCR assay system.[12] Secondary endpoints were the incidence of HHV-6 reactivation, adverse events during the PFA-dosing period and development of HHV-6 encephalitis or possible HHV-6 encephalitis. HHV-6 reactivation was defined as detection of HHV-6 DNA in plasma at any level. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.0 (National Cancer Institute, Bethesda, MD, USA). HHV-6

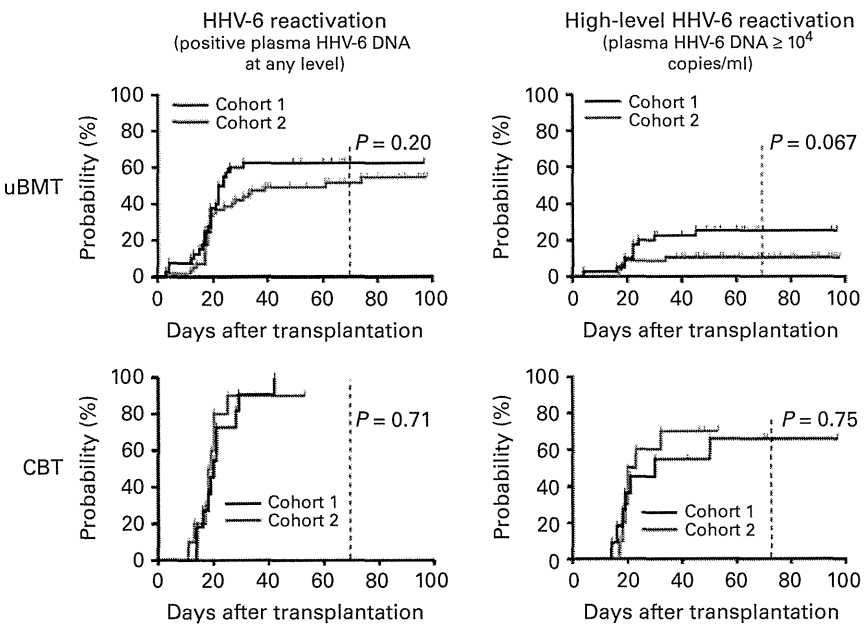

Figure 2. Kaplan–Meier estimates of the *P* of HHV-6 reactivation (positive plasma HHV-6 DNA at any level, left side of the figure) and high-level HHV-6 reactivation (plasma HHV-6 DNA $\geqslant 10^4$ copies/mL, right side of the figure), stratified by stem cell source (top side, unrelated BM; bottom, CB). For recipients of unrelated BM ($n=97$), the rate of HHV-6 reactivation at day 70 was 62.5% in Cohort 1 and 51.6% in Cohort 2 ($P=0.20$) and the rate of high-level HHV-6 reactivation was 25.2% and 10.5% in the two cohorts ($P=0.067$), respectively. For recipients of CB ($n=21$), all patients in Cohort 1 and 90% of patients in Cohort 2 experienced HHV-6 reactivation by day 42 and day 53, respectively ($P=0.71$). Rate of HHV-6 DNA $\geqslant 10^4$ copies/mL was 65.9% (at day 70) and 70% (at day 53) in Cohorts 1 and 2, respectively ($P=0.75$).

encephalitis was defined when patients satisfied all of the following criteria: (1) presence of CNS dysfunction; (2) a positive PCR result for HHV-6 in cerebrospinal fluid (CSF); and (3) the absence of other identified causes of CNS dysfunction. If no testing was performed to test for the presence of HHV-6 DNA in CSF, possible HHV-6 encephalitis was defined when patients satisfied all of the following criteria: (1) presence of CNS dysfunction; (2) demonstration of limbic encephalitis on magnetic resonance imaging (MRI); (3) positive plasma HHV-6 DNA; and (4) the absence of other identified causes of CNS dysfunction. Early stage was defined as acute leukemia during the first or second remission and CML during the first chronic phase, and myelodysplastic syndrome classified as refractory cytopenia with unilineage dysplasia, refractory cytopenia with multilineage dysplasia or refractory anemia with ringed sideroblasts; all other combinations were considered to represent non-early stage. Neutrophil engraftment was defined as an ANC of 0.5×10^9 neutrophils/L.

Statistical analysis

Comparisons of baseline characteristics between the two groups were made using Fisher's exact test or the Mann–Whitney *U*-test. The *P* of first incidence of HHV-6 reactivation, high-level HHV-6 reactivation and HHV-6 encephalitis were estimated with Kaplan and Meier curves. The log-rank test was used to compare incidences between groups. Values of $P<0.05$ were considered statistically significant in all analyses. Prism for Macintosh version 5 software (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses.

RESULTS

Patient characteristics

Cohort 1 included a total of 51 consecutive patients who underwent BMT from unrelated donor or CBT. For Cohort 2, 73 patients were preliminarily enrolled before the start of preconditioning. Among these, a total of six patients withdrew or were judged as ineligible before starting PFA. The reasons for not receiving PFA were as follows: renal dysfunction ($n=2$); liver dysfunction ($n=1$); CNS complications of unknown origin ($n=1$); and death in the early phase because of sepsis ($n=1$) or heart failure ($n=1$). As a result, 67 patients received prophylactic PFA. Among these 67 patients who started to receive PFA, 11 patients were unable to complete 10 days of PFA administration. Reasons for not completing the full 10-day PFA administration were as follows: serum creatinine level reaching ⩾grade 2 (study termination rule) ($n=3$, all were grade (2); patient refusal to continue administration because of nausea ($n=3$, all were grade (3) or headache ($n=1$, grade 2); and discretion of the attending physician because of electrolyte loss ($n=1$, grade 2), graft failure ($n=1$) or liver dysfunction due to GVHD ($n=2$). Median duration of PFA administration in these 11 patients was 6 days (range, 2–9 days). These patients who received interrupted administration of PFA were included in the analyses.

Characteristics of the patients are listed in Table 2. Median age was significantly higher ($P=0.0004$) and *P* of receiving myeloablative conditioning was significantly lower ($P<0.0001$) in Cohort 2 than in Cohort 1.

HHV-6 reactivation

In both cohorts, no patient showed persisting positive plasma HHV-6 DNA suggestive of HHV-6 chromosomal integration. The *P* of first high-level HHV-6 reactivation (HHV-6 DNA $\geqslant 10^4$ copies/mL plasma) by day 70 after transplantation was the primary endpoint of the study. No significant reduction in high-level HHV-6 reactivation was seen in patients who received prophylactic PFA (33.8% and 19.4% in Cohorts 1 and 2, respectively; $P=0.095$) (Figure 1). Figure 2 shows the incidence of HHV-6 reactivation (left) and high-level HHV-6 reactivation (right) stratified by stem cell sources. Among unrelated BMT recipients (upper), a trend was seen toward fewer high-level HHV-6 reactivations in Cohort 2 (25.2 and 10.5% at day 70 after transplantation, in Cohorts 1, and 2, respectively, $P=0.067$). Among CBT recipients (bottom), no differences were identified in the incidence of either HHV-6 reactivation or high-level HHV-6 reactivation.

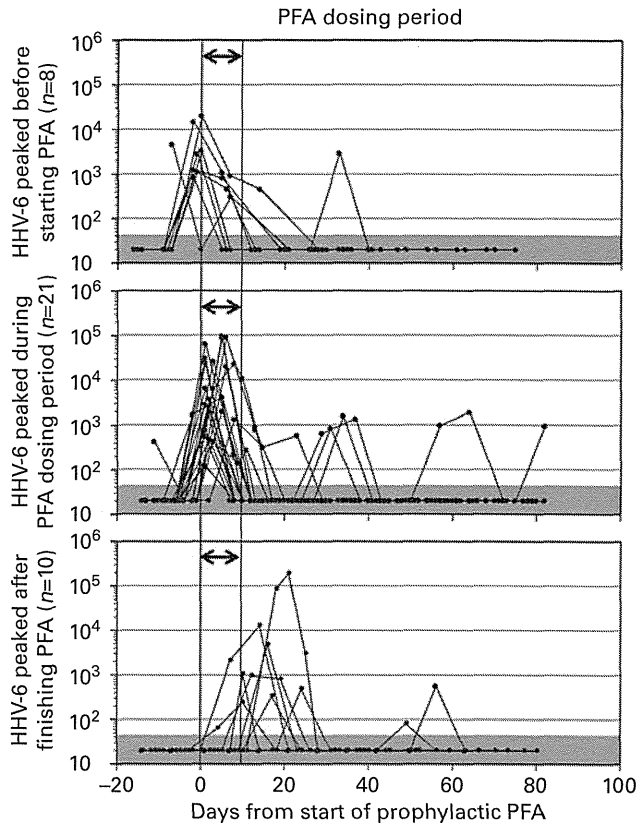

Figure 3. Kinetics of plasma HHV-6 DNA among patients who displayed positive HHV-6 DNA despite receiving PFA prophylaxis ($n=39$). The shaded area indicates values below the threshold for detection (<50 copies/mL of plasma). Periods indicated by arrows represent the PFA-loading period. The upper figure shows kinetics of plasma HHV-6 DNA in patients for whom HHV-6 DNA peaked before starting PFA ($n=8$), the middle figure shows those in patients for whom HHV-6 DNA peaked during the PFA-dosing period ($n=21$) and the bottom figure shows those in patients for whom HHV-6 DNA peaked after finishing PFA prophylaxis ($n=10$).

Analysis of the kinetics of plasma HHV-6 DNA in patients who displayed positive results in Cohort 2 (Figure 3) revealed that HHV-6 DNA peaked outside of the dosing period for PFA in 18 patients (before the dosing period in 8 patients; after the dosing period in 10 patients) and during the dosing period in 21 patients.

HHV-6 encephalitis

A total of five patients developed HHV-6 encephalitis ($n=2$) or possible HHV-6 encephalitis ($n=3$) in Cohort 1, while three patients developed HHV-6 encephalitis in Cohort 2. No significant difference in the incidence of HHV-6 encephalitis/possible HHV-6 encephalitis by day 70 was observed between cohorts (Kaplan and Meier analysis, 9.9% and 4.5% in Cohorts 1 and 2, respectively, $P=0.24$). Clinical courses and brain MRI findings of three patients who developed HHV-6 encephalitis despite receiving PFA prophylaxis are shown in Figures 4 and 5, respectively, and details are described below.

Patient 1 was a 31-year-old man who received CBT for the treatment of refractory EBV-positive lymphoma. Plasma HHV-6 DNA level continued increasing despite prophylactic administration of 50 mg/kg PFA. Short-term memory loss developed concomitant to peak plasma HHV-6 DNA (23 080 copies/mL), and neurological status changed with loss of consciousness and

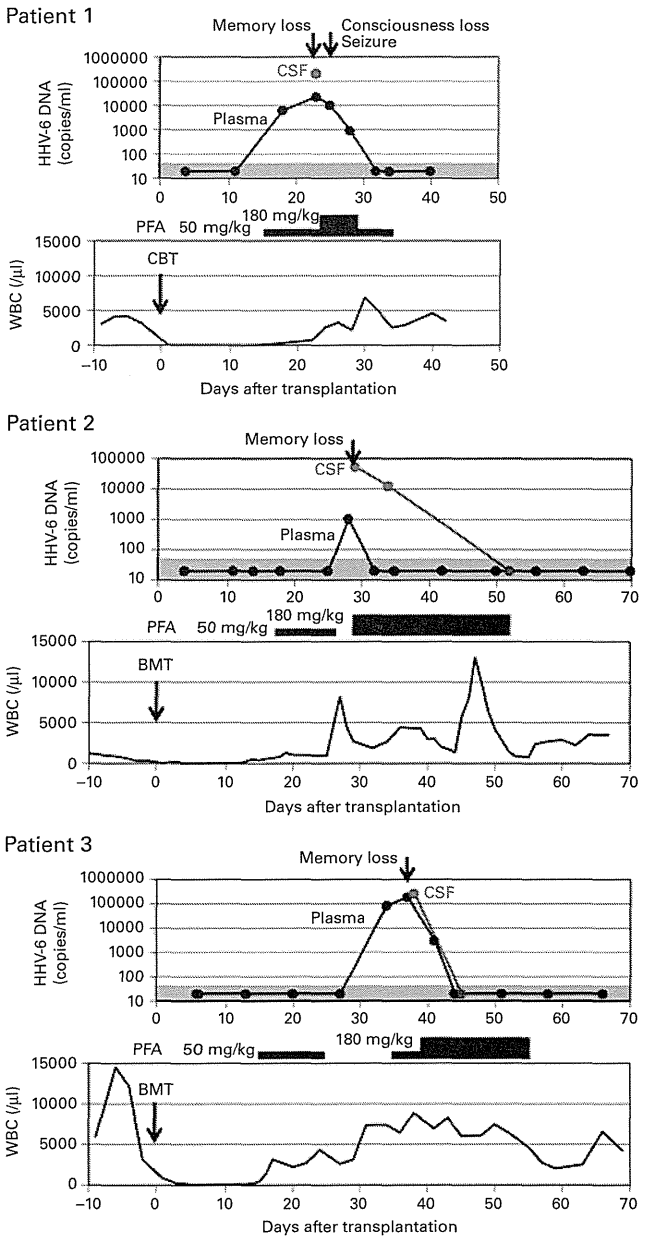

Figure 4. Clinical courses of the three patients who developed HHV-6 encephalitis in Cohort 2 (PFA prophylaxis). CBT = cord blood transplantation; CSF = cerebrospinal fluid; PFA = foscarnet. Dots and lines in red indicate kinetics of HHV-6 DNA in CSF.

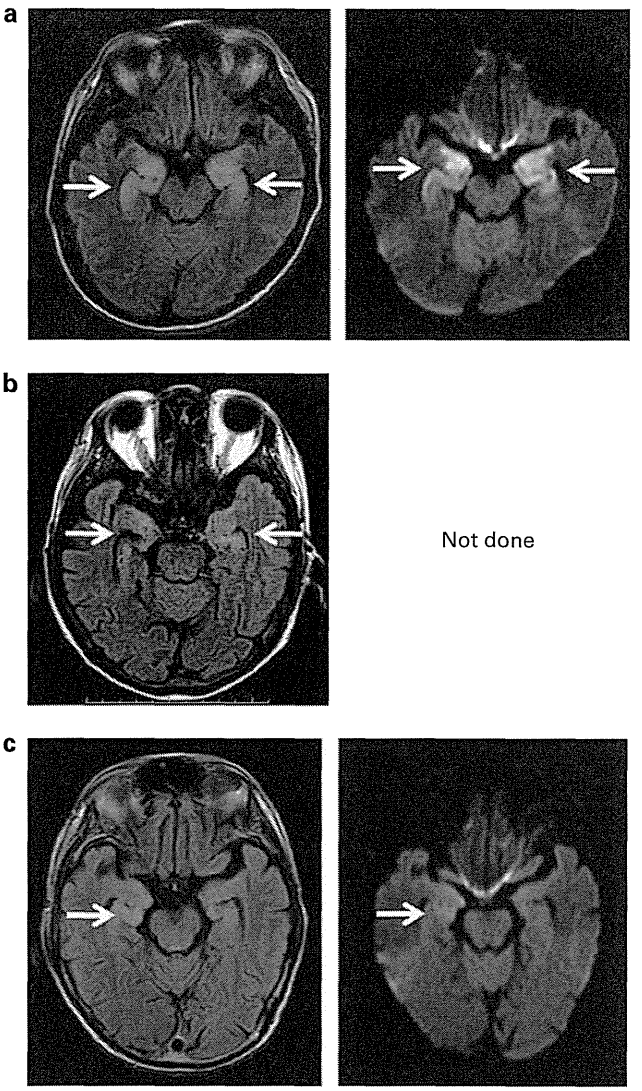

Figure 5. MRI of the brain for the three patients who developed HHV-6 encephalitis in Cohort 2, showing axial images at the level of the basal ganglia. Left side of figure shows T2-weighted fluid-attenuated inversion recovery imaging and right side shows diffusion-weighted imaging. Arrows indicate signal hyperintensities in limbic system. (**a**) For Patient 1 at 26 days after transplantation, 3 days after onset of neurological symptoms; (**b**) for Patient 2 at 28 days after SCT, on the day of onset of neurological symptoms; and (**c**) for Patient 3 at 43 days after SCT, 6 days after onset of neurological symptoms.

seizures. The patient died of pneumonia 43 days after SCT. Patient 2 was a 61-year-old man who received BMT from an HLA Ag-mismatched unrelated donor for the treatment of myelodysplastic syndrome. Plasma HHV-6 DNA turned positive 2 days after finishing prophylactic PFA, and disturbance of memory appeared. After treatment with 180 mg/kg of PFA, neurological symptoms gradually improved but memory difficulties remained until his death (due to GVHD) on day 152 after transplantation. Patient 3 was a 42-year-old man who received BMT from an HLA-matched unrelated donor for the treatment of refractory adult T-cell leukemia. Plasma HHV-6 DNA became positive (85 033 copies/mL) 9 days after finishing PFA. Although 50 mg/kg PFA was restarted, plasma HHV-6 DNA copy number increased to 190 236 copies/mL, and memory loss and mental confusion developed. After treatment with 180 mg/kg of PFA, the patient showed good cognitive recovery. The patient did not have neurological sequelae but died of GVHD 6 months after SCT.

In each patient with HHV-6 encephalitis, positive HHV-6 DNA in CSF was demonstrated at the time of developing HHV-6 encephalitis (54 305 copies/mL, 209 678 copies/mL and 262 447 copies/mL in Patients 1, 2 and 3, respectively). MRI showed signal abnormalities in the region of the hippocampus in each patient (Figure 5). HHV-6 DNA copy number in both plasma and CSF decreased rapidly after initiating administration of PFA at 180 mg/kg.

Risk factor analysis for high-level HHV-6 reactivation and HHV-6 encephalitis

The important risk factors for high-level HHV-6 reactivation were CBT and HLA-mismatched donor status (Table 3). Among

Table 3. Potential risk factors for high-level HHV-6 reactivation and HHV-6 encephalitis by 70 days after transplantation

Variables	*No. of patients*	*HHV-6 DNA ⩾ 10 000* copies/mL		*HHV-6 encephalitis/possible HHV-6 encephalitis*	
		P *by day 70 (%)*[a]	P[b]	P *by day 70 (%)*[a]	P[b]
Age, years					
≤49 years	59	30.5	0.15	6.8	0.97
>49 years	59	20.8		6.8	
Sex					
Male	69	32.3	0.07	10.3	0.09
Female	49	16.3		2.0	
Disease stage at transplantation					
Early	35	20.2	0.38	5.8	0.76
Non-early	83	27.9		7.3	
Type of transplanted cells					
Unrelated BM	97	16.6	<0.0001	7.3	0.69
Cord blood	21	69.5		4.8	
Matching of HLA					
Allele match	66	13.8	0.0008	4.6	0.27
Allele mismatch	52	40.9		9.7	
Ag match	81	14.9	<0.0001	6.2	0.73
Ag mismatch	37	49.3		8.1	
Conditioning regimen					
Myeloablative	63	28.6	0.39	8.0	0.60
Non-myeloablative	55	22.3		5.5	
Peak plasma HHV-6 DNA					
<10 000 copies/mL	88	NA	NA	1.1	<0.0001
⩾10 000 copies/mL	30	NA	NA	23.6	
Study group					
Cohort 1 (control)	51	33.8	0.09	9.9	0.24
Cohort 2 (PFA prophylaxis)	67	19.4		4.5	

Abbreviations: HHV-6 = human herpesvirus-6; NA = not applicable; PFA = foscarnet. [a]*P* of incidence was estimated by Kaplan–Meier analysis. [b]Log-rank test.

recipients of unrelated BMT, HLA mismatch was not significantly associated with high-level HHV-6 reactivation (HLA allelic match vs mismatch, $P=0.30$; HLA Ag match vs mismatch, $P=0.39$). For HHV-6 encephalitis, high-level HHV-6 reactivation was the only significant risk factor. The treatment group (Cohort 1 vs Cohort 2) was not significantly associated with incidence of either high-level HHV-6 reactivation or HHV-6 encephalitis.

Toxicity

Adverse events during the period of PFA administration in Cohort 2 are shown in Table 4. Nausea and vomiting were common adverse events. No patients developed grade 3–5 adverse events of electrolyte (magnesium, calcium or potassium) loss or increased serum creatinine levels.

DISCUSSION

This study showed that in the setting of allo-SCT from unrelated donors or CB, PFA given at 50 mg/kg once daily for 10 days in the early phase after allo-SCT is a safe but relatively ineffective strategy for preventing either high-level HHV-6 reactivation or HHV-6 encephalitis compared with a historical control cohort. PFA was suggested to suppress high-level HHV-6 reactivation in recipients of unrelated BM, but no benefit was observed among CBT recipients. Unexpectedly, breakthrough HHV-6 encephalitis developed in 3 of the 67 patients receiving prophylactic PFA.

One possible reason for this disappointing result is that the dose of PFA was insufficient. Analysis of the timing of peak plasma HHV-6 DNA in relation to the dosing period for PFA (Figure 3) showed that plasma HHV-6 DNA peaked during the PFA-dosing period in more than half of the patients. Among the three patients who developed HHV-6 encephalitis in Cohort 2, plasma HHV-6 DNA in two patients increased during the period in which PFA was given at 50 mg/kg/day (Patient 1, during dosing period of planned prophylactic PFA; Patient 3, during dosing period of repeated PFA) and HHV-6 encephalitis developed concomitant to peak plasma HHV-6 DNA. The effect of PFA at 50 mg/kg/day appears, at least with a short-dosing period before the detection of positive plasma HHV-6 DNA, insufficient to suppress the replication of HHV-6.

What is an appropriate strategy to prevent HHV-6 encephalitis? The kinetics of HHV-6 DNA in patients who developed HHV-6 encephalitis following PFA prophylaxis (Figure 3) showed that HHV-6 DNA from either plasma or CSF disappeared rapidly and CNS symptoms in two of these three patients started to improve immediately after starting PFA at 180 mg/kg/day. These observations suggest that PFA at 180 mg/kg is sufficient to suppress HHV-6 replication. PFA at higher doses may be recommended not only for the treatment of HHV-6 encephalitis, but also for prophylaxis against HHV-6 reactivation. However, adverse effects of PFA including dose-dependent renal toxicity, electrolyte loss and frequent gastrointestinal toxicities make it difficult to recommend prophylactic administration of high-dose PFA in the wider SCT recipient population. Clinical trials using an increased dose of prophylactic PFA for a longer period may be warranted in patients at higher risk of HHV-6 encephalitis. Furthermore, efforts must be made to establish measures other than PFA prophylaxis to prevent HHV-6 encephalitis.

Table 4. Adverse events during prophylactic foscarnet administration in Cohort 2 ($n = 67$)

Adverse event	*No. of patients (%)*
Nausea	
Grade 2	8 (11.9)
Grade 3	13 (19.4)
Grade 4–5	0 (0)
Vomiting	
Grade 2	7 (10.4)
Grade 3	2 (3.0)
Grade 4–5	0 (0)
Creatinine, serum-high	
Grade 2	3 (4.5)
Grade 3–5	0 (0)
Magnesium, serum-low	
Grade 2	4 (6.0)
Grade 3–5	0 (0)
Calcium, serum-low	
Grade 2	3 (4.5)
Grade 3–5	0 (0)
Potassium, serum-low	
Grade 2	4 (6.0)
Grade 3–5	0 (0)

Several questions remain regarding the pathogenic mechanisms underlying HHV-6 encephalitis. Elucidation of these questions may contribute to the establishment of preventative strategies for HHV-6 encephalitis. First, many cases of HHV-6 encephalitis appear to develop after episodes of pre-engraftment immune reaction, engraftment syndrome or GVHD.[3,12,14,18] In patients who developed HHV-6 encephalitis, an IL 6 surge was observed 1 week before development of CNS dysfunction.[12] If a hypercytokinemic state around the time of engraftment has a causative role in the development of HHV-6 encephalitis, intensive control of immune reactions around this period may reduce the incidence of HHV-6 encephalitis. Second, little is known about the pathogenesis of HHV-6 encephalitis after SCT. Whether HHV-6 reactivates within the CNS,[21] and the primary region of HHV-6 reactivation in patients who progress to HHV-6 encephalitis have yet to be clarified. If HHV-6 reactivates primarily in peripheral blood or parotid gland and enters the CNS, CDV, the drug with the strongest in vitro activity against HHV-6,[28] but poor CNS penetration, may be effective for preventing HHV-6 encephalitis. A lipid-ester derivative of CDV, hexadecyloxypropylcidofovir, may represent an alternative drug for HHV-6 prophylaxis.[21] Third, the role of HHV-6 in the development of HHV-6 encephalitis has not been defined fully. MRI of patients with HHV-6 encephalitis commonly shows bilateral findings in the region of the limbic system. Such symmetrical findings suggest the existence of systemic processes rather than local infection. Furthermore, HHV-6-negative post-transplant acute limbic encephalitis has been reported.[10] Such findings suggest the existence of multiple pathogenic mechanisms, including immune attack, underlying the CNS manifestations of HHV-6 encephalitis.

One could ask whether the incidence of HHV-6 encephalitis is really high among Japanese SCT recipients. In fact, all reports on the incidence of HHV-6 encephalitis (Table 1) have been retrospective studies. To clarify this issue, we are now undertaking a prospective multicenter study to evaluate the incidence of and risk factors underlying HHV-6 reactivation and HHV-6 encephalitis in Japan.

In conclusion, these data indicate that PFA given at 50 mg/kg in the early phase after allo-SCT is relatively ineffective strategy for preventing HHV-6 encephalitis. The pathogenesis of HHV-6 encephalitis deserves future investigation to establish safe and effective prophylactic measures against HHV-6 encephalitis. We hope the present results will be useful for future medical practice to prevent HHV-6 encephalitis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Correlations of HHV-6 viral load and plasma IL-6 concentration with HHV-6 encephalitis in allogeneic stem cell transplant recipients

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This study investigated factors associated with the development of human herpesvirus (HHV)-6 encephalitis. Among 111 enrolled subjects, 12 patients developed central nervous system (CNS) dysfunction. CNS dysfunction in four patients was found to have no association with HHV-6. The remaining eight patients displayed HHV-6 encephalitis ($n=3$), limbic encephalitis (HHV-6 DNA in cerebrospinal fluid was not examined; $n=3$) or CNS dysfunction because of an unidentified cause ($n=2$). Real-time PCR showed CNS dysfunction in the latter eight patients, which developed concomitant with the appearance of high plasma levels of HHV-6 DNA ($\geqslant 10^4$ copies/ml). Overall, eight of the 24 patients with high-level HHV-6 DNA developed CNS dysfunction, whereas no patients developed CNS dysfunction potentially associated with HHV-6 infection if peak HHV-6 DNA was $<10^4$ copies/ml. We next analyzed plasma concentrations of IL-6, IL-10 and tumor necrosis factor-α among patients who displayed high-level plasma HHV-6 DNA and found elevated IL-6 concentrations preceding HHV-6 infection in patients who developed CNS dysfunction. (Mean ± s.d.: 865.7 ± 1036.3 pg/ml in patients with CNS dysfunction; 56.5 ± 192.9 pg/ml in others; $P=0.01$). These results suggest that high-level HHV-6 load is necessary for the development of HHV-6 encephalitis, and systemic inflammatory conditions before HHV-6 infection form the preparatory conditions for progression to encephalopathy.
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Keywords: Allo-SCT; human herpesvirus 6; central nervous system dysfunction; encephalitis; cytokines; IL-6

Introduction

Human herpesvirus (HHV)-6 infection is relatively common among SCT recipients,[1–4] and a minority of patients with HHV-6 infection develop HHV-6-associated complications. Encephalitis (encephalopathy) has been recognized as a life-threatening complication associated with HHV-6 infection in SCT recipients.[5–7]

Quantification of the level of HHV-6 DNA in peripheral blood is useful to detect active HHV-6 infection in SCT recipients.[2–4,6–11] To date, five longitudinal studies have shown a correlation between the detection of HHV-6 DNA in peripheral blood and the development of central nervous system (CNS) dysfunction, with incidences ranging from 3.6 to 8.0%.[2–4,12,13] Retrospective surveillance by a Japanese group has shown that 0.96% of patients were identified with HHV-6 encephalitis.[14] A high incidence (11.6%) of HHV-6 encephalitis in patients receiving alemtuzumab-supported conditioning has been reported.[15] Mortality rate for HHV-6 encephalitis is high, and surviving patients often display lingering neurological compromise.[3,5,16] Factors associated with the development of encephalopathy, however, have yet to be clarified well. Each of these epidemiological studies[2–4,12,13] has indicated that higher levels of HHV-6 DNA in peripheral blood are associated with the development of CNS dysfunction. However, not all patients with high HHV-6 load develop CNS dysfunction, suggesting that other factors are required for progression to encephalopathy.

Increased proinflammatory cytokines are considered to play pathogenic roles in the development of CNS manifestations in various viral infections, including influenza virus,[17–19] respiratory syncytial virus infection[20] and primary HHV-6 infection.[21] SCT recipients are considered to have a tendency to display hypercytokinemia in the early phase of SCT because of engraftment syndrome (ES),[22] GVHD[23] or infectious diseases. Hypercytokinemia may thus be involved in the development of HHV-6 encephalitis in SCT. This study measured HHV-6 DNA loads and cytokine concentrations in plasma among the SCT recipients, and evaluated associations between development of HHV-6 encephalitis and these factors.

Patients and methods

Patients

This study involved consecutive patients who received hematopoietic SCT at Oita University Hospital between

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Table 1 Patient characteristics ($n=111$)

Characteristic	*Value*
Age in years, median (range)	46.0 (12–63)
Sex, male	67 (60)
Underlying diagnosis	
Hematological malignancies	
AML	25 (23)
ALL	14 (13)
Biphenotypic acute leukemia	1 (1)
CML	10 (9)
MDS	11 (10)
ATL	24 (22)
ML	18 (16)
MM	6 (5)
Renal cell carcinoma	1 (1)
Aplastic anemia	1 (1)
Disease phase at transplant[a]	
Early	36 (33)
Non-early	74 (67)
Pre-transplant conditioning	
Myeloablative	70 (63)
Non-myeloablative	41 (37)
Transplant type	
Related BM or PB	40 (36)
Unrelated BM	52 (47)
CB	19 (17)
Matching of HLA	
Allele match	67 (60)
Allele mismatch, antigen match	12 (11)
Antigen mismatch	32 (29)

Abbreviations: ATL = adult T-cell leukemia; CB = cord blood; MDS = myelodysplastic syndrome; ML = malignant lymphoma; MM = multiple myeloma; PB = peripheral blood.
[a]Disease phase was evaluated against patients with hematological malignancies. Early stage was defined as: acute leukemia in first or second remission; chronic myelogenous leukemia in first chronic phase; or myelodysplastic syndrome classified as refractory anemia or refractory anemia with ringed sideroblasts. All others were considered non-early stage.
Data represent no. (%) unless otherwise indicated.

January 1995 and September 2008 or Oita Prefectural Hospital between September 2005 and September 2008. Patients who died from any cause within 21 days of SCT, and patients who received a second transplant were excluded, resulting in a final total of 111 subjects. This included 79 patients who had participated in earlier reported studies: one as a retrospective study linking plasma HHV-6 DNA with clinical manifestations, particularly encephalitis ($n=50$);[3] and the other as a study evaluating the efficacy of pre-emptive approaches to prevent HHV-6 encephalitis ($n=29$).[13] Patient characteristics are summarized in Table 1. All study protocols were approved by the ethics committee of the Oita University Faculty of Medicine, and the written informed consent was obtained from each patient before participation.

Clinical definitions

The diagnosis of CNS dysfunction was based on a careful neuropsychiatric evaluation by a neurologist. CNS dysfunction was defined as the presence of lethargy or apathy, disorientation regarding time or place, personality change, systemic convulsions, loss of consciousness or memory loss (could not remember daily events and failed memory testing on routine neurological examination) that persisted for >24 h. HHV-6 encephalitis was defined as the presence of CNS dysfunction, a positive PCR result for HHV-6 in cerebrospinal fluid (CSF), and the absence of other identified cause of CNS dysfunction.[14] If no testing was performed to test for the presence of HHV-6 DNA in CSF, possible HHV-6 encephalitis was defined as the presence of CNS dysfunction, demonstration of limbic encephalitis[24] on magnetic resonance imaging, and the absence of other identified cause of CNS dysfunction. Diagnosis and grading of acute GVHD was on the basis of the standard clinical criteria.[25] Presence of ES was defined according to the criteria proposed by Spitzer.[22]

Sample preparation

The EDTA-treated peripheral blood was collected weekly. Blood samples collected 1–70 days after transplantation were evaluated. A median of 10.0 samples (range: 3–12) was obtained from each patient. Total number of plasma samples was 970.

Assay

Plasma HHV-6 DNA copy numbers were measured using real-time PCR methods, as described earlier.[3] Plasma HHV-6 load was quantified retrospectively for the first 50 patients, and prospectively for the last 61 patients. Plasma concentrations of IL-6, IL-10 and tumor necrosis factor-α were determined using sandwich-type ELISA kits (R&D Systems, Minneapolis, MN, USA) in accordance with the instructions from the manufacturer.

Statistical analysis

Statistical tests were performed using Statview for Macintosh software (version 5; Abacus Concepts) and Prism for Macintosh (version 5; GraphPad Software, San Diego, CA, USA). Univariate analyses were performed using Fisher's exact test or Mann–Whitney *U*-test. Values of $P<0.05$ were considered statistically significant in all analyses.

Results

Among the enrolled 111 patients, 60 patients (54%) displayed positive HHV-6 DNA in plasma. Onset of positive HHV-6 DNA results occurred at a median of 18 days (range: 3–61 days) after SCT. Median maximum plasma HHV-6 DNA load among positive cases was 3717.25 copies/ml plasma (range: 63.5–372, 696.0 copies/ml plasma).

By day 70 after transplantation, 12 patients had developed CNS dysfunction (Table 2). CNS dysfunctions in four cases (Cases 1–4) were found to have no association with HHV-6 (calcineurin inhibitor-associated encephalopathy, $n=1$; CNS dysfunction because of hypercalcemia, $n=1$; CNS leukemia, $n=1$ and CNS dysfunction because of unidentified cause but negative results for HHV-6 DNA in CSF, $n=1$). In these four patients, plasma HHV-6 DNA

Table 2 Characteristics of the 12 patients who developed CNS dysfunction

Case	*Age, years (sex)*	*Disease*	*Lesion on MRI (Performed day after onset of CNS dysfunction)*
1	37 (M)	ALL	Posterior horn of lateral ventricle (3) Multiple white matter lesions (11)
2	49 (M)	ML	NE
3	45 (F)	ATL	Negative (2) Negative (22)
4	50 (M)	ATL	Negative (0) Negative (11)
5	43 (M)	ATL	Negative (3) Bilateral limbic area (6)
6	46 (M)	ATL	Multiple white matter lesions including limbic area (5)
7	44 (M)	ALL	Bilateral limbic area (7)
8	53 (M)	AML	Bilateral limbic area (0)
9	54 (F)	AML	Negative (2) Bilateral limbic area (20)
10	34 (M)	CML	Bilateral limbic area (10)
11	56 (M)	ATL	Negative (0)
12	32 (F)	AML	Bilateral basal ganglia (2)

Case	*CT findings (performed day after onset of CNS dysfunction)*	*HHV-6 DNA in CSF*	*Definitions*	*References*
1	Negative (1)	NE	Calcinurin inhibitor-associated encephalopathy	
2	Negative (7)	NE	Hypercalcemia	
3	Negative (6)	NE	CNS leukemia	
4	Negative (3)	Negative	Unknown (other than HHV-6)	
5	Negative (3)	Positive	HHV-6 encephalitis	Ogata *et al.*[3]
6	NE	Positive	HHV-6 encephalitis	Ogata *et al.*[13]
7	NE	Positive	HHV-6 encephalitis	
8	NE	NE	Possible HHV-6 encephalitis	Ogata *et al.*[3]
9	NE	NE	Possible HHV-6 encephalitis	Ogata *et al.*[3]
10	Negative (4)	NE	Possible HHV-6 encephalitis	Ogata *et al.*[3]
11	NE	NE	Unknown	Ogata *et al.*[13]
12	NE	NE	Unknown	

Abbreviations: CNS = central nervous system; CT = computed tomography; HHV = human herpesvirus; MRI = magnetic resonance imaging; NE = not evaluated.

did not peak at the time of developing CNS dysfunction (Figure 1a). Although HHV-6 DNA peaked 7 days before developing CNS dysfunction and was also detectable on the day of CNS dysfunction in Case 3, the cause of CNS dysfunction in this case was revealed as CNS leukemia. Among the remaining eight patients (Table 2), three patients were defined as having HHV-6 encephalitis (Cases 5–7) and three patients were defined as having possible HHV-6 encephalitis (Cases 8–10). Etiologies for CNS dysfunction in the remaining two cases (Cases 11 and 12) could not be shown. CNS symptoms for Case 11 were systemic convulsions and coma after short-term memory loss, whereas those for Case 12 were systemic convulsions and coma. Details of the characteristics and clinical courses for six of these eight patients have been described in earlier reports.[3,13] Examination of plasma HHV-6 DNA levels showed that HHV-6 DNA peaked concomitant to the development of CNS dysfunction in all eight patients (Figure 1b). The range of peak HHV-6 DNA among each of the eight patients was 20 647–208 614 copies/ml plasma (median, 72 403 copies/ml plasma).

Table 3 shows the association between peak HHV-6 load in each recipient and development of CNS dysfunction. None of the 87 patients developed HHV-6 encephalitis or possible HHV-6 encephalitis if peak HHV-6 DNA in plasma was $<10^4$ copies/ml, whereas six of 24 patients who developed HHV-6 encephalitis or possible HHV-6 encephalitis showed HHV-6 DNA exceeding 10^4 copies/ml ($P<0.0001$, Fisher's exact test). None of the 36 patients with peak HHV-6 DNA at 50–9999 copies/ml plasma developed CNS dysfunction concomitant to presenting with peak HHV-6 DNA, whereas eight of the 24 patients with peak HHV-6 DNA $\geqslant 10^4$ copies/ml developed CNS dysfunction concomitant to presenting with peak HHV-6 DNA ($P=0.0003$, Fisher's exact test). Thus, we defined plasma HHV-6 DNA level of $\geqslant 10^4$ copies/ml as high-level HHV-6 DNA.

Eight of 24 patients (33.3%) who displayed high-level HHV-6 DNA developed CNS dysfunction at the time of presenting with peak HHV-6 DNA, whereas the remaining 16 patients with high-level HHV-6 DNA did not develop CNS dysfunctions. Characteristics were compared between recipients who developed CNS dysfunctions and those who developed high-level HHV-6 DNA but no CNS dysfunction (Table 4). Episodes of ES or GVHD $\geqslant$grade II at 0–10 days before the day of peak HHV-6 load were significantly associated with progression to CNS dysfunction ($P=0.03$, Fisher's exact test). ES cannot be separated from GVHD in some cases, because ES was likely to represent an early manifestation of GVHD in these cases. C-reactive protein levels at 0–10 days before the day of peak HHV-6 load were significantly higher in patients

who developed CNS dysfunction than in patients who displayed high-level HHV-6 DNA without CNS dysfunction ($P=0.03$).

We next analyzed plasma concentrations of IL-6, IL-10 and tumor necrosis factor-α among recipients who displayed high-level HHV-6 DNA. This examination was available for 17 patients (CNS dysfunction, $n=7$; no neurological complications, $n=10$) for whom plasma samples had been cryopreserved. Figure 2a shows the sequential analysis of plasma cytokine concentrations. In most patients who developed CNS dysfunction, plasma IL-6 levels sharply elevated around 1 week (5–8 days) before the day of peak HHV-6 load. Maximum cytokine concentrations from samples collected 0–10 days before the day of peak HHV-6 load in each patient were compared (Figure 2b). Mean (±s.d.) IL-6 concentrations were significantly higher in recipients who developed CNS dysfunction (865.7 ± 1036.3 pg/ml) than in recipients without neurological complications (56.5 ± 192.9 pg/ml, $P=0.01$).

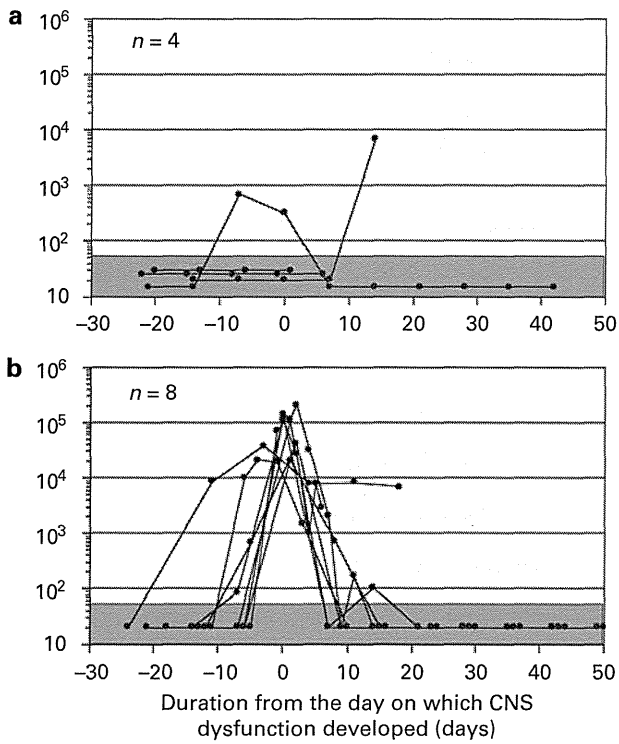


Figure 1 (**a**) Kinetics of plasma human herpesvirus (HHV)-6 DNA among patients in whom central nervous system (CNS) dysfunction showed no association with HHV-6 ($n=4$). (**b**) Kinetics of plasma HHV-6 DNA for patients who developed HHV-6 encephalitis or CNS dysfunction potentially associated with HHV-6 ($n=8$). The day on which CNS dysfunction developed was plotted as day 0. The shaded area indicated values below the threshold for detection (<50 copies/ml).

Discussion

Although HHV-6 reactivation is relatively common among SCT recipients, the incidence of HHV-6 encephalitis is low. Little is known, however, about factors associated with the development of HHV-6 encephalitis.

Twelve patients in this study developed CNS dysfunction. Of these, four patients showed no association with HHV-6. Clinical definition of the remaining eight patients was HHV-6 encephalitis ($n=3$), possible HHV-6 encephalitis based on magnetic resonance imaging findings (limbic encephalitis) ($n=3$), and CNS dysfunctions because of unidentified cause ($n=2$), because of a the lack of testing for HHV-6 DNA in CSF for five patients. In all of the eight patients, however, real-time PCR showed CNS dysfunction developed concomitant to the presence of high-level HHV-6 DNA in plasma. This observation strongly suggests an association between HHV-6 and the CNS dysfunction (HHV-6 encephalitis).

The present findings suggest that high-level plasma HHV-6 DNA is associated with the development of HHV-6 encephalitis. Under our real-time PCR assay system, threshold level for the development of HHV-6 encephalitis is thought to be around 10^4 copies/ml plasma. However, even in patients with high-level HHV-6 DNA, less than half of the patients developed CNS dysfunction. Comparison of characteristics between recipients who developed CNS dysfunction and those who developed high-level HHV-6 DNA without CNS dysfunction showed that episodes of ES or GVHD ⩾grade II and levels of C-reactive protein at 0–10 days before the day of peak

Table 3 Association between plasma HHV-6 load and development of CNS dysfunction

Peak HHV-6 DNA in plasma of each patient (copies/ml)	*CNS dysfunction (%)*	*HHV-6 encephalitis, or possible HHV-6 encephalitis (%)*	*CNS dysfunction developing concomitant to peak HHV-6 DNA (%)*[a]
<50 ($n=51$)	2 (3.9)[b]	0 (0)	NA
50–9999 ($n=36$)	2 (5.6)[c]	0 (0)	0 (0)
⩾10 000 ($n=24$)	8 (33.3)[d]	6 (25.0)	8 (33.3)
10 000–99 999 ($n=18$)	4 (22.2)	3 (16.6)	4 (22.2)
⩾100 000 ($n=6$)	4 (66.7)	3 (50.0)	4 (66.7)

Abbreviations: CNS = central nervous system; HHV = human herpesvirus; NA = not available.
[a]CNS dysfunction developed around 3 days before or after the day of peak HHV-6 load.
[b]Etiologies were CNS dysfunction because of hypercalcemia and unidentified causes, but negative results were obtained for HHV-6 DNA in cerebrospinal fluid.
[c]Etiologies were calcineurin inhibitor-associated encephalopathy and CNS leukemia. Kinetics of plasma HHV-6 DNA are shown in Figure 1a.
[d]Etiologies were HHV-6 encephalitis ($n=3$), possible HHV-6 encephalitis ($n=3$) and CNS dysfunction because of unidentified cause ($n=2$). Kinetics of plasma HHV-6 DNA are shown in Figure 1b.

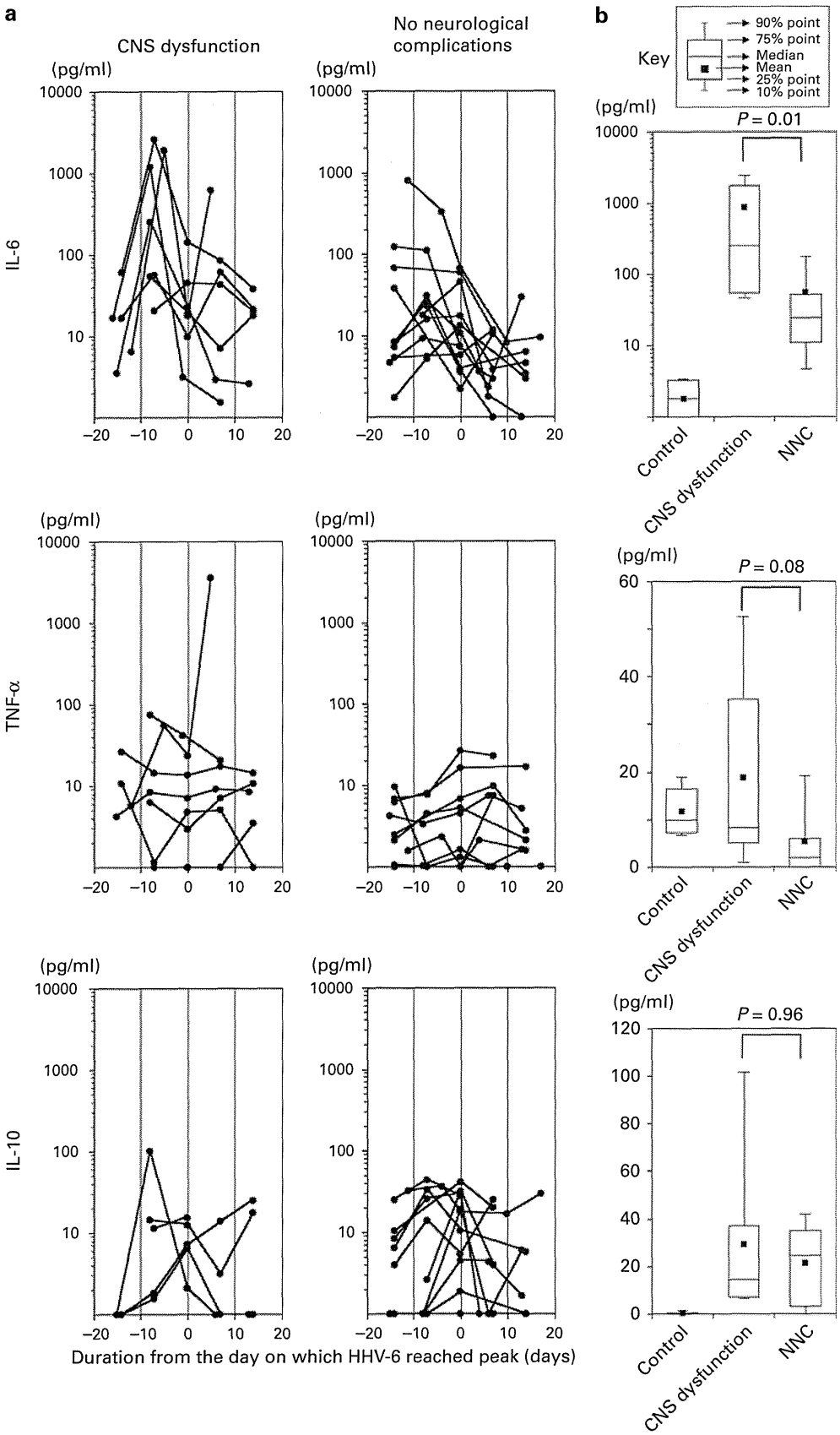
Table 4 Comparison of characteristics and laboratory data between two groups of patients displaying high-level HHV-6 DNA (plasma HHV-6 DNA $\geqslant 1 \times 10^4$ copies/ml)

Characteristic	*CNS dysfunction (n = 8)*	*No neurological complication (n = 16)*	
Age in years, median (range)	46.5 (32–56)	48.5 (21–61)	0.74
Sex, male	6 (75)	11 (69)	>0.99
Underlying diagnosis			
AML	3	1	
ALL	1	2	
CML	1	1	
MDS		3	
ATL	3	1	
ML		7	
MM		1	
Disease phase at transplant			
Early	4	3	0.17
Non-early	4	13	
Pre-transplant conditioning			
Myeloablative	6	7	0.21
Non-myeloablative	2	9	
Transplant type			
Related BM/PB	1	4	0.63[a]
Unrelated BM	5	4	0.10[a]
Cord blood	2	8	0.39[a]
Matching of HLA			
Allele match	3	3	0.36
Allele mismatch	5	13	
Ag match	5	4	0.10
Ag mismatch	3	12	
Conditions during 10 days before the day of peak HHV-6 load			
GVHD ⩾grade II or ES			
Yes	6	4	0.03
No	2	12	
Steroid therapy			
Yes	6	9	0.66
No	2	7	
Bacteremia[b]			
Yes	1[c]	1[d]	>0.99
No	7	15	
Maximum CRP, mean ± s.d.	17.6 ± 6.1	9.6 ± 8.9	0.03
Maximum HHV-6 DNA load in plasma (copies/ml), median (range)	72 403 (20 647–208 614)	30 337.5 (10 081–372 696)	0.12

Abbreviations: CRP = C-reactive protein; ES = engraftment syndrome; PB = peripheral blood.
[a]Versus other two types.
[b]Blood culture was performed during this period for seven of eight patients in the 'CNS dysfunction' group and 11 of 16 patients in the 'No neurological complication' group.
[c]Infection with the *α-hemolytic streptococci.*
[d]Infection with *Staphylococcus epidermidis.*

HHV-6 load were associated with development of CNS dysfunction. Pre-transplant conditioning and transplant type were not identified as significant factors in the development of CNS dysfunction. Longitudinal analysis of cytokine concentrations in plasma indicated that increased IL-6 preceding HHV-6 infection was associated with the development of CNS dysfunction. In most patients who developed CNS dysfunction concomitant to HHV-6 infection, IL-6 was suddenly and transiently elevated about 1 week before the development of CNS dysfunction. Such dynamic kinetics were not seen in patients who displayed high-level HHV-6 DNA without CNS dysfunction.

Several investigators have shown HHV-6 infection in the brain on samples from recipients who died of encephalopathy after SCT, showing that HHV-6 displays tropism for hyppocampal astrocytes.[26–28] These studies have suggested direct destruction of the CNS by HHV-6. This study showed a significant association between level of HHV-6 DNA and development of CNS dysfunction. CNS dysfunction developed around the day on which plasma HHV-6 DNA peaked at high level. These findings support the direct mechanism of HHV-6 establishing encephalopathy in SCT recipients and suggest that high-level HHV-6 DNA in plasma offers a marker for HHV-6 encephalitis.


a
CNS dysfunction
No neurological complications
(pg/ml)
IL-6
TNF-α
IL-10
10000
1000
100
10
–20 –10 0 10 20
Duration from the day on which HHV-6 reached peak (days)
b
Key
90% point
75% point
Median
Mean
25% point
10% point
P = 0.01
P = 0.08
P = 0.96
Control
CNS dysfunction
NNC

We also showed the possibility that increased IL-6 before HHV-6 infection is predictive of the development of CNS dysfunction among patients displaying high-level HHV-6 DNA. Mechanisms potentially explaining the role of IL-6 are diverse and complex. Several reports have shown that increased proinflammatory cytokine levels, particularly IL-6, are correlated with the development of encephalopathy in various viral infections.[17–21] Influenza-associated encephalopathy is thought to be a consequence of systemic immune responses,[18,19] and high plasma concentrations of IL-6 can predict the development of influenza-associated encephalopathy.[18] Infants with encephalopathy associated with primary HHV-6 infection show higher serum and CSF levels of IL-6.[21] As for HHV-6 encephalitis in SCT recipients, cytokines themselves may mediate the pathogenesis of CNS dysfunction. Possible mechanisms include epithelial and endothelial injury,[29] increased permeability of the blood–brain barrier or apoptosis in the cerebrum[30] resulting from hypercytokinemia. Interaction of hypercytokinemia and HHV-6 reactivation[31] may likewise be associated with progression to encephalopathy. Other possibilities can also be proposed. Therapy against hypercytokinemia-associated conditions may be linked to the development of CNS dysfunction. In this study, hypercytokinemia seems to have been caused by ES or GVHD in most patients who developed CNS dysfunction, and these recipients therefore received intensive steroid therapy. Steroid therapy is considered a strong risk factor for the development of HHV-6 encephalitis.[3,5,16] Impaired T-cell responses against HHV-6 because of steroid therapy may contribute to the development of CNS dysfunction. Whether the inflammatory reaction itself plays a causative role, or whether therapy against conditions showing an inflammatory reaction (steroid therapy for ES or GVHD) is associated with the development of HHV-6 encephalitis remains unclear. This issue needs to be clarified in the future.

The significance of plasma HHV-6 DNA for the detection of active HHV-6 infection may be controversial.[32,33] The argument might be made that reverse transcription PCR for leukocytes to detect viral RNA offers a more reliable indicator of active HHV-6 replication. However, reliability of the procedure has not been evaluated well in SCT recipients, negative results may be difficult to interpret in leukopenic patients[6] and a specialized technique is required to quantify viral load. Quantification of viral DNA in peripheral blood by PCR is thought to be useful for monitoring active HHV-6 infection,[2–4,6–11] and such testing is recommended for the diagnosis of HHV-6 infection.[8] Therefore, we quantified plasma HHV-6 DNA copy number to evaluate active HHV-6 infection in this study.

This study displays some limitations. We evaluated only three types of cytokines, because only very small quantities of plasma had been saved in most cases. CSF samples were unavailable for analysis. Extensive analysis of cytokines and other humoral factors in plasma and CSF may provide a better understanding of the pathophysiological mechanisms underlying the development of HHV-6 encephalitis.

In conclusion, our analyses showed that HHV-6 encephalitis developed among patients who displayed high-level systemic HHV-6 infection, and increased levels of plasma IL-6 concentration before high-level HHV-6 DNA seem to predict progression to CNS dysfunction. The precise pathogenetic role of increased IL-6 remains obscure, but increased IL-6 may form a preparatory state for the development of CNS dysfunction. Countermeasures to the development of systemic inflammatory response in the early phase of SCT may be useful in preventing the development of HHV-6 encephalitis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Figure 2 (**a**) Kinetics of plasma cytokine concentration among patients displaying high-level HHV-6 load in plasma (peak HHV-6 load, $\geqslant 10^4$ copies/ml plasma). The left graph (CNS dysfunction) shows the kinetics of patients who developed CNS dysfunction ($n=7$ for IL-6 and TNF-α; $n=5$ for IL-10), whereas the right graph (no neurological complications) shows the kinetics of recipients who did not develop neurological complications ($n=12$). If cytokine concentration of the sample was $\leqslant 1$ pg/ml, cytokine concentration was plotted as 1 pg/ml. The day on which plasma HHV-6 DNA load peaked is expressed as day 0. In patients who developed CNS dysfunction (left graph), onset of encephalopathy began around day −3 to day 3 in all cases. (**b**) Maximum cytokine concentration among plasma samples collected 0–10 days before the day of peak HHV-6 load in each patient were compared between recipients with encephalopathy and those who displayed high-level HHV-6 DNA in the absence of encephalopathy. Cytokine concentrations in plasma from normal subjects ($n=5$) were used as controls. NNC = no neurological complications.

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LETTER TO THE EDITOR

Comparison of outcomes between autologous and allogeneic hematopoietic stem cell transplantation for peripheral T-cell lymphomas with central review of pathology

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High-dose therapy with autologous hematopoietic stem cell transplantation (auto-SCT) for peripheral T-cell lymphomas (PTCLs) has been evaluated as a consolidation of the first remission and salvage therapy for relapse.[1–7] However, no randomized trial comparing conventional chemotherapy followed by auto-SCT and conventional chemotherapy alone has been performed. Although data for allogeneic (allo-) SCT in selected patients with PTCLs have been reported to be promising,[8–13] no large-scale prospective study has been performed thus far. To clarify the roles of auto- and allo-SCT in the treatment of PTCLs, the Japan Study Group for Cell Therapy and Transplantation conducted a retrospective study of SCT for patients with PTCLs with central review of pathology. The study protocol was approved by the institutional review board at each center.

We collected the clinical data of 336 patients with PTCLs diagnosed at 52 Japanese and 8 Korean centers between September 1991 and December 2008, obtained the pathological samples of 307 patients, and conducted central review of pathology on the basis of the World Health Organization classification (Supplementary Figure S1).[14] Patients with adult T-cell leukemia/lymphoma (ATLL) and natural killer (NK) cell tumors, aged less than 15 years at SCT and who received a planned tandem SCT were excluded. Following the review, we analyzed the data of 231 patients (135 auto-SCTs and 96 allo-SCTs).

Patient characteristics are summarized in Table 1. The clinical characteristics at SCT in the auto-SCT group were more favorable than those in the allo-SCT group except for median age (52 years in the auto-SCT group vs 45 years in the allo-SCT group). The proportion of patients previously treated with three or more chemotherapy regimens in the auto-SCT group was lower than that in the allo-SCT group (20% vs 60%, $P<0.0001$). The proportion of patients with stage III or IV disease at SCT in the auto-SCT group was lower than that in the allo-SCT group (29% vs 70%, $P<0.0001$). The transplantation procedures are summarized in Supplementary Table S1.

The median follow-up period for surviving patients was 46 months (range, 2.3–136 months). The 5-year overall survival (OS) and progression-free survival rates after allo-SCT for PTCLs were comparable to those after auto-SCT (48% vs 46%, $P=0.34$, Figure 1a; 40% vs 37%, $P=0.54$, Figure 1b) even though the allo-SCT group had more unfavorable factors than the auto-SCT group. The 5-year non-relapse mortality and relapse/disease progression (PD) rates in the allo- and auto-SCT groups were 33% and 15% ($P=0.0003$, Figure 1c) and 40% and 57% ($P=0.12$, Figure 1d), respectively. According to the disease status at SCT, the 5-year OS rates in the allo-SCT group were 69% in first complete remission or partial remission (CR1/PR1, $n=16$), 28% in second CR or PR (CR2/PR2, $n=16$), 43% in the resistant relapse ($n=25$) and

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Table 1. Patient characteristics

	Auto-SCT (n = *135*)	*MAC allo-SCT* (n = *34*)	*RIC allo-SCT* (n = *62*)
Age at SCT (years), median (range)	52 (15–72)	39 (18–57)	55 (18–69)
Patient sex, male	97 (72%)	18 (53%)	41 (66%)
Histopathologic subtype			
PTCL-NOS	55 (41%)	16 (47%)	32 (52%)
AITL	47 (35%)	5 (15%)	15 (24%)
ALCL	20 (15%)	4 (12%)	4 (7%)
ALK, positive	12	4	2
ALK, negative	5	0	2
ALK, not tested	3	0	0
EATL	7 (5%)	0	4 (7%)
Others	6[a] (4%)	9[b] (27%)	7[c] (11%)
No. of prior chemotherapy regimens			
1	48 (36%)	6 (18%)	6 (10%)
2	60 (44%)	7 (21%)	19 (31%)
>2	27 (20%)	21 (62%)	37 (60%)
Prior radiotherapy	24 (18%)	8 (24%)	11 (18%)
Prior auto-SCT before allo-SCT	—	6 (18%)	19 (31%)
Disease status			
CR1/PR1	72 (53%)	7 (21%)	9 (15%)
CR2/PR2	32 (24%)	6 (18%)	10 (16%)
Resistant relapse	5 (4%)	8 (24%)	17 (27%)
Primary refractory	10 (7%)	9 (27%)	11 (18%)
Performance status, 2–4	10 (7%)	9 (27%)	18 (29%)
Elevated LDH	34 (25%)	20 (59%)	26 (42%)
Bulky mass	2 (2%)	1 (3%)	0
Stage III–IV	39 (29%)	28 (82%)	39 (63%)
No. of extranodal localization sites			
0	107 (79%)	9 (27%)	30 (48%)
1	21 (16%)	12 (35%)	19 (31%)
>1	7 (5%)	13 (38%)	13 (21%)
Bone marrow invasion	7 (5%)	14 (41%)	21 (34%)
IPI >2	14 (10%)	11 (37%)	19 (31%)
PIT >1	14 (10%)	14 (41%)	20 (32%)
Median follow-up period, months (range)	50 (2.3–136)	51 (2.5–98)	41 (2.3–97)

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; Allo-SCT, allogeneic hematopoietic stem cell transplantation; Auto-SCT, autologous hematopoietic stem cell transplantation; CR1, first complete remission; CR2, second CR; EATL, enteropathy-associated T-cell lymphoma; IPI, international prognostic index; LDH, lactate dehydrogenase; MAC, myeloablative conditioning; PIT, prognostic index for PTCL-unspecified; PR1, first partial remission; PR2, second PR; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; RIC, reduced-intensity conditioning; —, not applicable. [a]Diagnoses of others were subcutaneous panniculitis-like T-cell lymphoma (SPTCL) ($n=3$), primary cutaneous ALCL, Epstein-Barr virus-associated T-cell lymphoproliferative disorder (EBV-T-LPD) and unclassifiable PTCL ($n=1$ for each diagnosis). [b]Diagnoses of others were SPTCL, hepatosplenic T-cell lymphoma (HSTCL), T-cell prolymphocytic leukemia (T-PLL) ($n=2$ for each diagnosis), Sézary syndrome (SS), EBV-T-LPD, and unclassifiable PTCL ($n=1$ for each diagnosis). [c]Diagnoses of others were SPTCL ($n=2$), HSTCL, mycosis fungoides (MF), SS, T-PLL and unclassifiable PTCL ($n=1$ for each diagnosis).

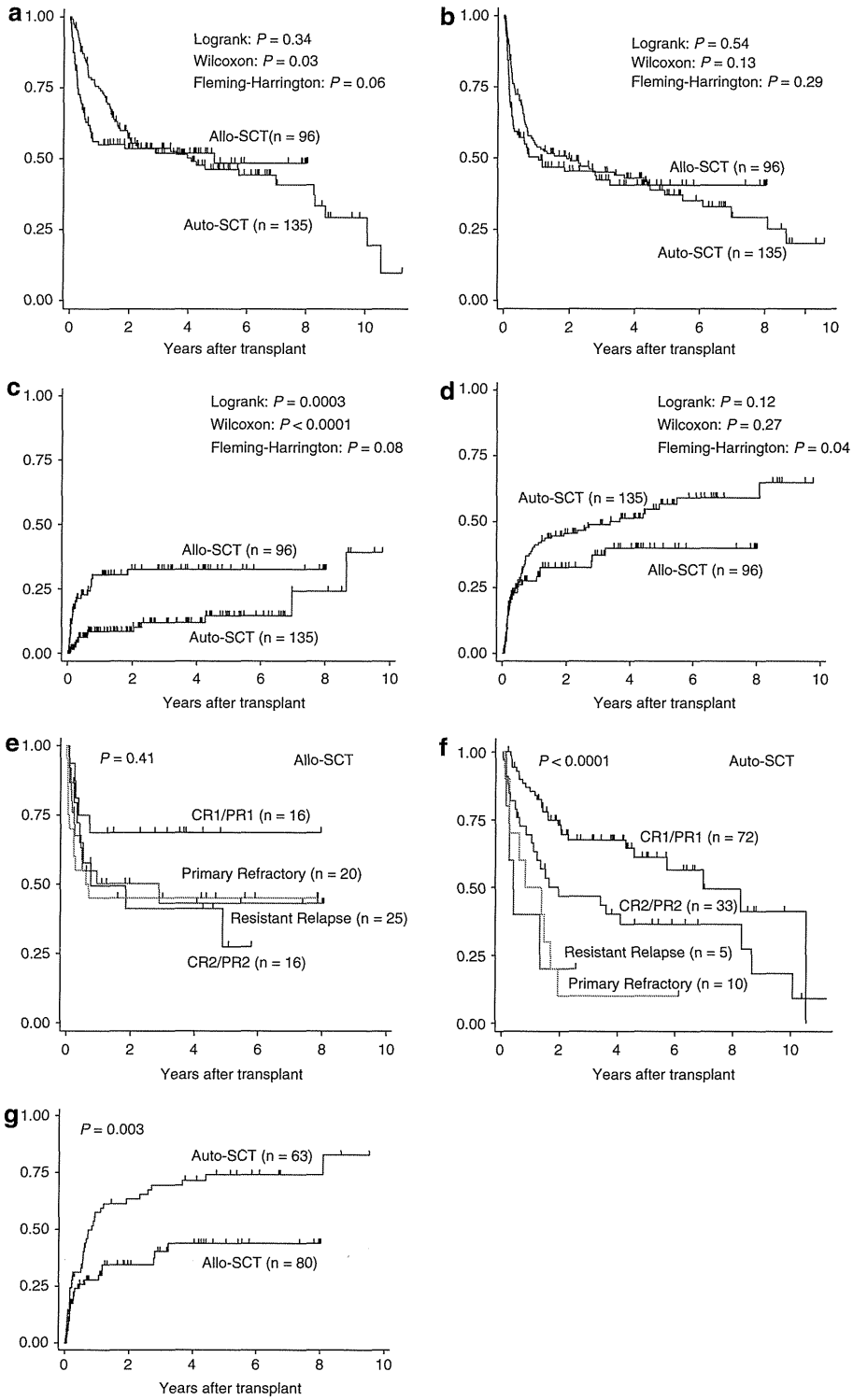

Figure 1. Clinical outcomes. (**a**) OS in the allo- and auto-SCT groups. (**b**) Progression-free survival in the allo- and auto-SCT groups. (**c**) Non-relapse mortality in the allo- and auto-SCT groups. (**d**) Relapse/PD in the allo- and auto-SCT groups. (**e**) OS stratified according to the disease status at transplantation in the allo-SCT group. (**f**) OS stratified according to the disease status at transplantation in the auto-SCT group. (**g**) Relapse/PD in non-CR1/PR1 in the allo- and auto-SCT groups.

45% in primary refractory disease ($n=20$) ($P=0.41$, Figure 1e). The 5-year OS rates in the auto-SCT group were 62% in CR1/PR1 ($n=72$), 36% in CR2/PR2 ($n=33$) and 10% in primary refractory disease ($n=10$) ($P<0.0001$, Figure 1f). In patients with non-CR1/PR1 at SCT, the 5-year relapse/PD rate in the allo-SCT group was significantly lower than that in the auto-SCT group (44% vs 74%, $P=0.003$, Figure 1g), which may have contributed to the longer OS in patients who received allo-SCT. According to the three main histologic subtypes, the 5-year OS rates in the allo-SCT group were 41% in PTCL-not otherwise specified (NOS, $n=48$), 55% in angioimmunoblastic T-cell lymphoma (AITL, $n=20$) and 38% in anaplastic large cell lymphoma (ALCL, $n=8$) ($P=0.24$,