Recently, cord blood (CB) has been used as an alternative source of hematopoietic stem cells in unrelated allogeneic transplantation for adult patients without HLA-matched related or unrelated donors [10–12]. In the setting of cord blood transplantation (CBT), the lower risk of GVHD may be one of the most attractive advantages for older patients, despite there being less stringent criteria for human leukocyte antigen (HLA) matching in donor-recipient selection without compromising graft-versus-leukemia effects [13]. In addition, the absence of risk for donors may also be advantageous for older patients who do not have a family donor or a healthy sibling donor.

We previously reported that unrelated CBT after myeloablative conditioning is feasible in patients over the age of 45 and patients aged between 50 and 55 years [14, 15]. However, there have been no comparisons made to date in terms of outcome in older and younger patients following CBT after myeloablative conditioning. To evaluate the impact of older recipient age on the outcome of CBT after myeloablative conditioning in patients with acute leukemia, we retrospectively compared the results in patients aged between 50 and 55 years (n=19) with those aged less than 50 years (n=81) in a single institute setting.

Materials and methods

Study patients

We retrospectively reviewed the medical records of 100 consecutive patients with acute leukemia who received an unrelated CBT after a myeloablative conditioning regimen including 12 Gy total-body irradiation (TBI) at the Institute of Medical Science, University of Tokyo, from August 1998 to March 2007. Either myeloablative or non-myeloablative conditioning is offered to patients aged between 50 and 55 years according to individual institutions' preferences. In our institute, the upper age limit for myeloablative conditioning is 55 years old. To evaluate the impact of recipient age, patients were divided into two cohorts: 81 patients who were younger than 50 years old and 19 patients who were 50 years of age or older. Patients 49 years of age and younger were assigned to the younger group and patients aged between 50 and 55 years to the older group. Of the 100 acute leukemia patients, 55 had de novo acute myeloid leukemia (AML), 18 had myelodysplastic syndrome (MDS)-related secondary AML, and 27 had acute lymphoblastic leukemia (ALL). All patients were required to be 16 years or older and have no marked organ dysfunction or active infection at the time of transplantation. Patients were excluded if they had hematological malignancy other than acute leukemia, had received a reduced-intensity conditioning regimen, been transplanted double cord blood units, or had received a previous allogeneic transplantation. The clinical protocol was approved by the institutional review board of the Institute of Medical Science, University of Tokyo, and written informed consent was obtained from all patients.

Transplantation procedures

We previously reported the transplantation procedures [12–18]. All cord blood units were obtained from cord blood banks in the Japan Cord Blood Bank Network. Cord blood units were selected as reported previously [12]. All patients received 12 Gy TBI and chemotherapy as myeloablative conditioning. Seventy-five patients received four fractionated 12 Gy TBI, cytosine arabinoside (Ara-C, total dose, 12 g/m^2) combined with or without granulocyte-colony stimulating factor (G-CSF) (lenograstim) and cyclophosphamide (CY, total dose, 120 mg/kg). Fifteen patients received four fractionated 12 Gy TBI and CY at 120 mg/kg at standard dose. Ten patients received four fractionated 12 Gy TBI and other combination. Ninety-six patients received standard cyclosporin and short-term methotrexate, and four patients received cyclosporin as GVHD prophylaxis (Table 1). All patients received the same supportive care, such as antibacterial, antifungal, and antiviral agents, and blood component support, as previously reported [12, 16–18]. In addition, all patients received G-CSF by intravenous infusion starting on day 1 until durable granulocyte recovery was achieved.

End points and definitions

We focused on neutrophil and platelet recovery, the incidences of acute and chronic GVHD, TRM, disease relapse, overall survival (OS), and disease-free survival (DFS) within each subgroup. Neutrophil recovery time was defined as the first day an absolute neutrophil count of 0.5×10^9/L was achieved for three consecutive days. Platelet recovery time was defined as the first day an absolute platelet count of 50×10^9/L was achieved without the support of transfusions for three consecutive days. Assessment and grading of acute and chronic GVHD were made using the system of Glucksberg et al. [19] and Shulman [20], respectively. TRM was defined as death in continuous complete remission after transplantation, OS was calculated from transplantation to death from any cause, DFS was defined as the time from transplantation to either relapse or death in continuous complete remission, and disease relapse was defined as hematological recurrence in bone marrow or extramedullary sites.

Statistical analysis

Baseline patient and graft characteristics were compared using the chi-square test for categorical variables and the Kruskal–Wallis test for continuous variables. Cumulative incidence of acute and chronic GVHD was estimated using TRM and relapse as competing events [21]. Cumulative incidence of TRM and disease relapse was estimated using each event as a competing risk factor for the other. The probability of DFS and OS was estimated by the Kaplan–Meier method and compared using the log-rank test. The Cox proportional hazards model was used to model potential predictors of acute and chronic GVHD, TRM, OS, and DFS [22]. The following variables were considered: age, diagnosis, disease status at transplantation, and cord blood nuclear cell dose infused. All p values were two-tailed, and p values less than 0.05 were considered significant. Analysis was performed using computer software JMP version 6.0.3 (JMP, SAS Institute, Cary, NC, USA), SAS version 8.2 (SAS Institute), and S Plus 2000 (Mathsoft, Seattle, WA, USA). Analysis of data was performed on October 1st, 2007.

Results

Characteristics of patients and cord blood grafts

Among the 100 patients, 81 patients were below 49 years old and 19 patients were between 50 and 55 years of age. The characteristics of patients, cord blood grafts, and transplantation procedures are summarized in Table 1. Comparisons of characteristics in the two populations showed similar distributions for weight, gender ratio,

Table 1 Characteristics of patients, cord blood grafts, and transplantation

Characteristics	Younger group, n=81	Older group, n=19	P value
Age, years			
Median (range)	36 (16–49)	52 (50–55)	<0.0001
Weight, kg			
Median (range)	55 (36–76)	56 (42–67)	0.58
Sex, Male, no. (%)	47 (58%)	7 (37%)	0.09
CMV serological status, Positive, no. (%)	67 (83%)	19 (100%)	0.01
Diagnosis			
De novo AML, no. (%)	42 (52%)	13 (68%)	0.36
Secondary AML, no. (%)	15 (18%)	3 (16%)	
ALL, no. (%)	24 (30%)	3 (16%)	
Disease status at transplantation[a]	39 (49%)	6 (32%)	0.18
Standard risk, no. (%)	42 (51%)	13 (68%)	
High risk, no. (%)			
Conditioning			
TBI+CY+Ara-C/G-CSF, no. (%)	59 (73%)	16 (84%)	0.16
TBI+CY, no. (%)	12 (15%)	3 (16%)	
TBI+other combination, no. (%)	10 (12%)	0 (0%)	
GVHD prophylaxis			
CSP, no. (%)	4 (5%)	0 (0%)	0.18
CSP+sMTX, no. (%)	77 (95%)	19 (100%)	
Total nucleated cell dose/kg of body weight			
Median (range)	2.40 (1.16–5.29)	2.33 (1.95–3.53)	0.26
CD34+cell dose/kg of body weight			
Median (range)	0.92 (0.15–8.97)	0.81 (0.32–3.61)	0.74
HLA incompatibility[b]			
1, no. (%)	12 (15%)	3 (16%)	0.88
2, no. (%)	42 (52%)	11 (58%)	
3, no. (%)	24 (29%)	4 (21%)	
4, no. (%)	3 (4%)	1 (5%)	
Donor-recipient sex match			
M→M, no. (%)	26 (32%)	6 (32%)	0.13
F→F, no. (%)	15 (18%)	6 (32%)	
F→M, no. (%)	21 (26%)	1 (4%)	
M→F, no. (%)	19 (24%)	6 (32%)	
Follow-up for survivors, days			
Median (range)	1,408 (33–3274)	951 (253–2,715)	26

CMV cytomegalovirus, *AML* acute myelogenous leukaemia, *ALL* acute lymphoblastic leukaemia, *TBI* total body irradiation, *CY* cyclophosphamide, *Ara-C* cytosine arabinoside, *G-CSF* granulocyte-colony stimulating factor, *GVHD* graft-versus-host disease, *CSP* cyclosporine, *sMTX*, short-term methotrexate, *M* male, *F* female

[a] Disease status at transplantation defined as standard risk for CR1 or CR2 without poor prognostic karyotype and high risk for otherwise.

[b] Compatibility number defined as low resolution for HLA - A and - B and high resolution for HLA-DRB1

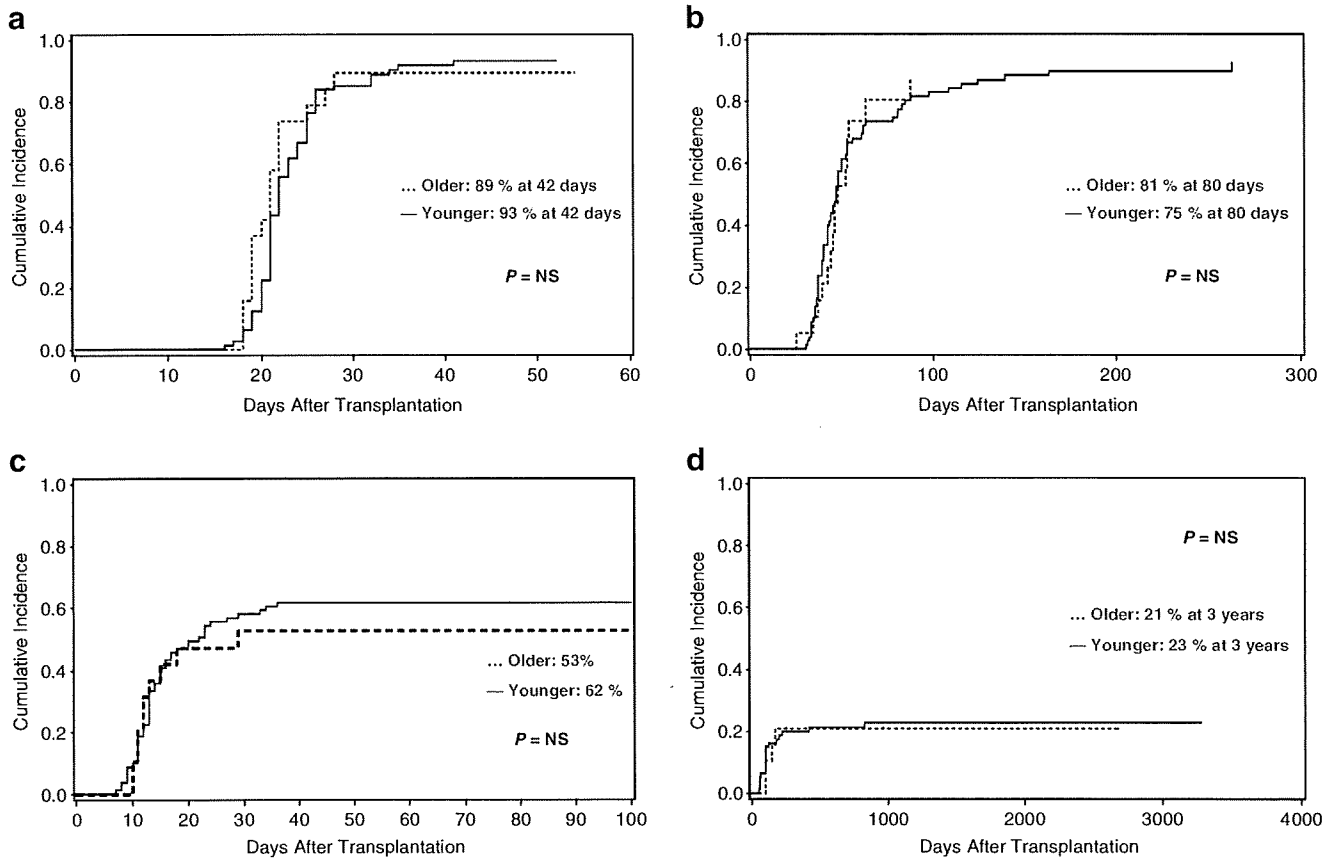


Fig. 1 Cumulative incidence of **a** neutrophil recovery, **b** platelet recovery greater than 50×10^9/L without the support of transfusions, **c** grades II to IV acute GVHD at 100 days, and **d** extensive-type chronic GVHD. There was no difference between the older and younger groups. The *solid* and *dashed lines* represent the younger and older groups, respectively, in all figures. *NS* not significant

diagnosis, disease status at transplantation, conditioning regimen, GVHD prophylaxis, cryopreserved total nucleated cell dose and CD34-positive cell dose infused, and proportions of HLA and sex incompatibility between donors and recipients. The two populations differed in age and CMV serological status. The proportion of positive CMV serological status was higher among the older group than the younger group. The median period of follow-up for survivors after CBT was 951 days (range, 253–2715 days) for the older group and 1,408 days (range, 33–3274 days) for the younger group.

Hematopoietic recovery

Among all patients, hematopoietic recovery could not be evaluated in three younger patients and one older patient due to death before day 28. For the entire group, the median interval between transplantation and neutrophil recovery was 21 days (range, 16–41) and that from transplantation to platelet recovery was 45 days (range, 25–253). The cumulative incidence of neutrophil recovery at 42 days was 93% (95% confidence intervals [CI], 88–99%) for the younger group and 89% (95% CI, 76–100%) for the older group (Fig. 1a). The cumulative incidence of platelet recovery at 80 days was 75% (95% CI, 65–85%) for the younger group and 81% (95% CI, 69–100%) for the older group (Fig. 1b).

Graft-versus-host disease

Six younger and two older patients could not be evaluated for acute GVHD because of death before day 28 or graft failure. Grades II to IV acute GVHD developed in ten of 17 patients in the older group and in 49 of 75 patients in the younger group. In contrast, grades III to IV acute GVHD did not develop in the older group but did in six patients in the younger group. The cumulative incidence of grades II to IV acute GVHD at 100 days was 62% (95% CI, 51–72%) for the younger group and 53% (95% CI, 30–75%) for the older group (Fig. 1c). There was no difference between the younger and older groups in the incidence of grades II to IV acute GVHD. Extensive-type chronic GVHD occurred in 4 of 15 evaluable older patients and in 18 of 69 evaluable younger patients. The cumulative incidence of extensive-

Table 2 Multivariate analysis of outcomes after CBT

Outcome/variable	Hazard ratio (95% CI)	*P* value
Grades II to IV acute GVHD		
Older group	0.83 (0.42–1.63)	0.59
Extensive-type chronic GVHD		
Older group	0.85 (0.29–2.53)	0.77
Treatment-related mortality		
Older group	0.66 (0.08–5.41)	0.70
Overall survival		
Older group	1.05 (0.21–5.03)	0.95
High-risk disease status at CBT	5.36 (1.15–24.91)	0.03
Disease-free survival		
Older group	1.11 (0.41–3.00)	0.82
High-risk disease status at CBT	2.84 (1.20–6.72)	0.02

CBT cord blood transplantation, *CI* confidence intervals, *GVHD* graft-versus-host disease

type chronic GVHD at 3 years was 23% (95% CI, 14–32%) for the younger group and 21% (95% CI, 3–39%) for the older group (Fig. 1d). In Cox regression analysis, the older group was not an independent predictor of increased incidence of grades II to IV acute GVHD and extensive-type chronic GVHD (Table 2).

Treatment-related mortality

Eight of 100 patients died of TRM. The causes of treatment-related death were pneumonia (n=2), encephalitis (n=1), hepatic venoocclusive disease (n=1), multiple organ failure (n=2), and chronic GVHD (n=2). TRM occurred in seven of 81 patients in the younger group and in one of 19 patients in the older group. The cumulative incidence of TRM at 3 years was 9% (95% CI, 3–16%) for the younger group and 5% (95% CI, 0–15%) for the older group (Fig. 2a).

Disease relapse, survival, and cause of death

Disease relapse was the primary cause of death among both the younger and older groups. Of 81 younger patients, 16 relapsed at a median of 247 days (range, 29–1,550 days) after CBT. Of 19 older patients, four relapsed at a median

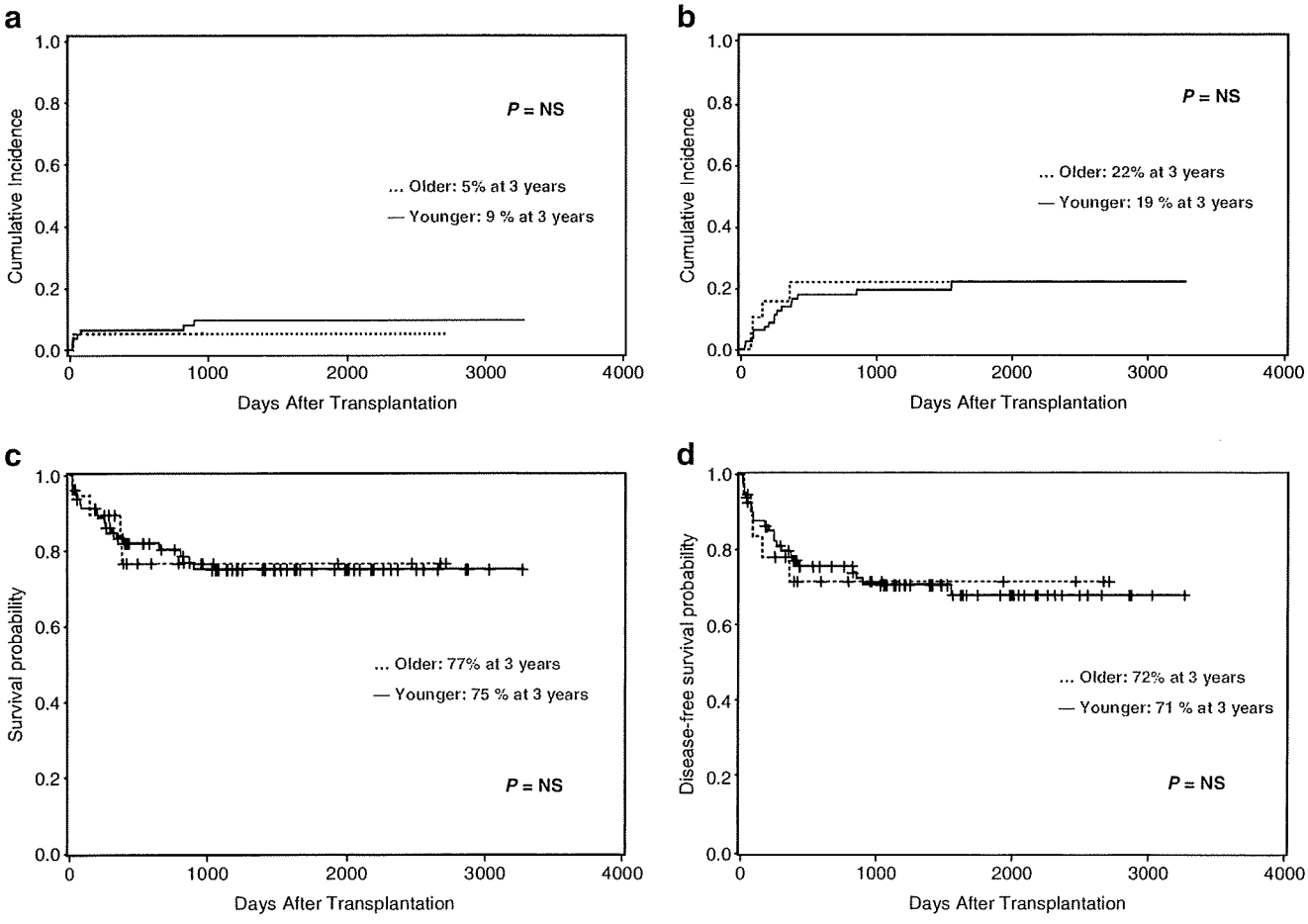


Fig. 2 Cumulative incidence of **a** treatment-related mortality and **b** disease relapse. Probability of **c** OS and **d** DFS 3 years after transplantation. There was no difference between the older and younger groups. The *solid* and *dashed lines* represent the younger and older groups, respectively, in all figures. *NS* not significant

of 124 days (range, 76–357 days) after CBT. The cumulative incidences of relapse at 3 years were not significantly different between the two groups: 19% for the younger group and 22% for the older group (Fig. 2b).

The probabilities of OS at 3 years were 75% (95% CI, 65–85%) for the younger group and 77% (95% CI, 56–97%) for the older group (Fig. 2c). The probability of DFS at 3 years was 71% (95% CI, 60–81%) for the younger group and 72% (95% CI, 50–83%) for the older group (Fig. 2d). In Cox regression analysis, high risk of disease was the only independent predictor of decreased OS and DFS, but not in the older group (Table 2). The causes of death in the entire cohort were disease relapse (n=16), GVHD (n=2), infection (n=3), organ failure (n=3), and secondary malignancy (n=1; Table 3).

Discussion

In the present study, we retrospectively compared the results in patients aged between 50 and 55 years (n=19) with those younger than 50 years (n=81) in the setting of CBT after myeloablative conditioning for acute leukemia. The incidence of grades II to IV acute GVHD and extensive-type chronic GVHD was similar in both the younger and older groups. The older group was not an independent predictor of TRM, OS, and DFS in multivariate analysis. However, the older group had a lower hazard ratio regarding the development of grades II to IV acute GVHD and extensive-type chronic GVHD, but a higher hazard ratio in OS and DFS (Table 2). Although this was a paradoxical finding, it was not statistically significant. In our study, the results for CB transplant recipients were better than previously reported in occidental patients [10, 11]. As the Japanese body size is relatively small, the availability of grafts containing sufficient numbers of cells might explain the favorable results in our study. On the other hand, a lower degree of diversity for HLA and minor histocompatibility antigen in the Japanese population might explain the relatively lower incidence of GVHD in our study.

Table 3 Cause of death

Cause	Younger group, n=21	Older group, n=4
Primary disease, no. (%)	13 (61%)	3 (75%)
GVHD, no. (%)	2 (10%)	0
Infection, no. (%)	2 (10%)	1 (25%)
Organ failure, no. (%)	3 (14%)	0
Secondary malignancy, no. (%)	1 (5%)	0

GVHD graft-versus-host disease

Advanced recipient age has been reported to be associated with an increased incidence of morbidity and mortality following allogeneic HSCT after myeloablative conditioning [1–7]. Older patients are more likely to have organ dysfunction or comorbidity and to develop severe acute and chronic GVHD, which may increase TRM following allogeneic HSCT after myeloablative conditioning. Moreover, older patients rarely have a matched sibling donor because of their age. Some of these problems could be overcome with the advantages of CBT, such as a lower risk of severe GVHD and quicker availability without risk to the donor. In general, approximately 50 to 55 years of age is the upper limit for allogeneic HSCT following myeloablative conditioning using adult stem cell grafts from a related or unrelated donor. Nonetheless, there have been no reports detailing results in relatively older patients after CBT using myeloablative conditioning.

Myeloablative conditioning for allogeneic HSCT has been restricted to younger patients because TRM occurs more frequently among older patients. In recent years, reduced-intensity or non-myeloablative conditioning followed by allogeneic HSCT has been developed, especially for older patients [23–26]. Recently, there have also been reports of CBT following reduced-intensity or non-myeloablative conditioning for older patients or those with comorbid disease [27–31]. Although this approach has the potential to reduce the risk of TRM, the risk of graft failure might have remained higher compared with myeloablative conditioning when CB is used as the stem cell source [30, 32]. It is important to note that a high risk of relapse is still the most important problem to address, especially in reduced-intensity conditioning.

There have been some clinical comparisons made between CBT and bone marrow transplantation (BMT) from unrelated donors in adults [10–12]. These studies demonstrated that the incidence of severe acute and chronic GVHD was significantly lower after CBT than after BMT. However, the DFS rate and relapse incidence in CB recipients were not inferior to those in bone marrow recipients [10, 11]. In older patients, the lower risk of GVHD without compromised graft-versus-leukemia effects is one of the most important advantages of CBT despite there being less stringent criteria for HLA matching in donor-recipient selection. In our study, none of the older patients developed grades III and IV acute GVHD, and the incidence of extensive-type chronic GVHD was also quite low in the older patients group. Although there is no comparative result between CBT and transplants using bone marrow or mobilized peripheral blood from adult stem cell grafts after myeloablative conditioning, we believe that the use of CB instead of bone marrow or mobilized peripheral blood as a stem cell source might offer the possibility of decreasing severe acute and chronic GVHD in older

patients. Several studies have reported that the incidence and severity of GVHD can be reduced or prevented by T cell depletion (TCD) from adult stem cell grafts, especially for older patients [33–35]. On the other hand, there are some disadvantages in transplants using TCD, such as a higher incidence of infection and relapse rate. As for the reduction of severe GVHD to achieve better outcomes of allogeneic HSCT in older patients, a comparative study seems to be warranted between CBT and transplants using TCD from adult stem cell grafts.

In summary, although the number of patients was small in our study and the observation period was limited, we conclude that, in patients with acute leukemia, myeloablative CBT might be as safe and effective in patients aged between 50 and 55 years as in younger patients.

Acknowledgments The authors are indebted to the medical and nursing staff for taking care of the patients in this study. We also thank the Kobayashi Foundation for financial support and Maki Monna-Oiwa for her secretarial assistance.

References

1. Klingemann HG, Storb R, Fefer A, Deeg HJ, Appelbaum FR, Buckner CD, Cheever MA, Greenberg PD, Stewart PS, Sullivan KM (1986) Bone marrow transplantation in patients aged 45 years and older. Blood 67:770–776
2. Yanada M, Emi N, Naoe T, Sakamaki H, Iseki T, Hirabayashi N, Karasuno T, Chiba S, Atsuta Y, Hamajima N, Takahashi S, Kato S (2004) Allogeneic myeloablative transplantation for patients aged 50 years and over. Bone Marrow Transplant 34:29–35 doi:10.1038/sj.bmt.1704518
3. Farag SS, Elder PJ, Marcucci G, Penza S, Mrozek E, Molina A, Lin T, Avalos BR, Copelan E (2003) Radiation-free regimens result in similar outcomes of allogeneic hematopoietic progenitor cell transplantation in patients aged >or=50 years compared to younger adults with low-risk disease. Bone Marrow Transplant 31:87–93 doi:10.1038/sj.bmt.1703785
4. Du W, Dansey R, Abella EM, Baynes R, Peters WP, Klein J, Akhtar A, Cherednikova L, Karanes C (1998) Successful allogeneic bone marrow transplantation in selected patients over 50 years of age—a single institution's experience. Bone Marrow Transplant 21:1043–1047 doi:10.1038/sj.bmt.1701210
5. Cahn JY, Labopin M, Schattenberg A, Reiffers J, Willemze R, Zittoun R, Bacigalupo A, Prentice G, Gluckman E, Hervé P, Gratwohl A, Gorin NC (1997) Allogeneic bone marrow transplantation for acute leukemia in patients over the age of 40 years. Acute Leukemia Working Party of the European Group for Bone Marrow Transplantation (EBMT). Leukemia 11:416–419 doi:10.1038/sj.leu.2400573
6. Ringdén O, Horowitz MM, Gale RP, Biggs JC, Gajewski J, Rimm AA, Speck B, Veum-Stone JA, de Witte T, Bortin MM (1993) Outcome after allogeneic bone marrow transplant for leukemia in older adults. JAMA 270:57–60 doi:10.1001/jama.270.1.57
7. Hansen JA, Gooley TA, Martin PJ, Appelbaum F, Chauncey TR, Clift RA, Petersdorf EW, Radich J, Sanders JE, Storb RF, Sullivan KM, Anasetti C (1998) Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med 338:962–968 doi:10.1056/NEJM199804023381405
8. Nash RA, Pepe MS, Storb R, Longton G, Pettinger M, Anasetti C, Appelbaum FR, Bowden RA, Deeg HJ, Doney K (1992) Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis cyclosporine and methotrexate. Blood 80:1838–1845
9. Carlens S, Ringdén O, Remberger M, Lönnqvist B, Hägglund H, Klaesson S, Mattsson J, Svahn BM, Winiarski J, Ljungman P, Aschan J (1998) Risk factors for chronic graft-versus-host disease after bone marrow transplantation: a retrospective single centre analysis. Bone Marrow Transplant 22:755–761 doi:10.1038/sj.bmt.1701423
10. Laughlin MJ, Eapen M, Rubinstein P, Wagner JE, Zhang MJ, Champlin RE, Stevens C, Barker JN, Gale RP, Lazarus HM, Marks DI, van Rood JJ, Scaradavou A, Horowitz MM (2004) Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Engl J Med 351:2265–2275 doi:10.1056/NEJMoa041276
11. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, Jacobsen N, Ruutu T, de Lima M, Finke J, Frassoni F, Gluckman E, Acute Leukemia Working Party of European Blood and Marrow Transplant Group Eurocord-Netcord Registry (2004) Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med 351:2276–2285 doi:10.1056/NEJMoa041469
12. Takahashi S, Iseki T, Ooi J, Tomonari A, Takasugi K, Shimohakamada Y, Yamada T, Uchimaru K, Tojo A, Shirafuji N, Kodo H, Tani K, Takahashi T, Yamaguchi T, Asano S (2004) Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood 104:3813–3820 doi:10.1182/blood-2004-03-1001
13. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S (2007) Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood 109:1322–1330 doi:10.1182/blood-2006-04-020172
14. Ooi J, Iseki T, Takahashi S, Tomonari A, Takasugi K, Uchiyama M, Konuma T, Futami M, Nomura A, Nakayama S, Soda Y, Ohno N, Nagamura F, Uchimaru K, Tojo A, Tani K, Asano S (2004) Unrelated cord blood transplantation after myeloablative conditioning in patients over the age of 45 years. Br J Haematol 126:711–714 doi:10.1111/j.1365-2141.2004.05130.x
15. Konuma T, Ooi J, Takahashi S, Tomonari A, Uchiyama M, Fukuno K, Tsukada N, Iseki T, Tojo A, Asano S (2006) Unrelated cord blood transplantation after myeloablative conditioning in patients with acute leukemia aged between 50 and 55 years. Bone Marrow Transplant 37:803–804 doi:10.1038/sj.bmt.1705334
16. Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Sato A, Takasugi K, Iseki T, Tojo A, Asano S (2007) Bacterial bloodstream infection in neutropenic adult patients after myeloablative cord blood transplantation: experience of a single institution in Japan. Int J Hematol 85:238–241 doi:10.1532/IJH97.06179
17. Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Takasugi K, Iseki T, Tojo A, Asano S (2008) Preemptive therapy with ganciclovir 5 mg/kg once daily for cytomegalovirus infection after unrelated cord blood transplantation. Bone Marrow Transplant 41:371–376 doi:10.1038/sj.bmt.1705910
18. Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Sato A, Iseki T, Yamaguchi T, Tojo A, Asano S (2007) Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation:

a single institute experience in Japan. Bone Marrow Transplant 40:523–528 doi:10.1038/sj.bmt.1705765
19. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG, Thomas ED (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 18:295–304 doi:10.1097/00007890-197410000-00001
20. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED (1980) Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 69:204–217 doi:10.1016/0002-9343(80)90380-0
21. Gooley TA, Leisenring W, Crowley J, Storer BE (1999) Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med 18:695–706 doi:10.1002/(SICI)1097-0258(19990330)18:6<695::AID-SIM60>3.0.CO;2-O
22. Cox DR (1972) Regression models and life tables. J R Stat Soc B 34:187–220
23. Alyea EP, Kim HT, Ho V, Cutler C, Gribben J, DeAngelo DJ, Lee SJ, Windawi S, Ritz J, Stone RM, Antin JH, Soiffer RJ (2005) Comparative outcome of nonmyeloablative and myeloablative cell transplantation for patients older than 50 years of age. Blood 105:1810–1814 doi:10.1182/blood-2004-05-1947
24. Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ, Frassoni F, Boiron JM, Yin JL, Finke J, Shouten H, Blaise D, Falda M, Fauser AA, Esteve J, Polge E, Slavin S, Niederwieser D, Nagler A, Rocha V, Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT) (2005) Comparative outcome of reduced intensity and myeloablative conditining regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group of Blood and Marrow Transplantation (EBMT). Leukemia 19:2304–2312 doi:10.1038/sj.leu.2403967
25. Shimoni A, Hardan I, Shem-Tov N, Yeshurun M, Yerushalmi R, Avigdor A, Ben-Bassat I, Nagler A (2006) Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. Leukemia 20:322–328 doi:10.1038/sj.leu.2404037
26. Kojima R, Kami M, Kanda Y, Kusumi E, Kishi Y, Tanaka Y, Yoshioka S, Morishima S, Fujisawa S, Mori SI, Kasai M, Hatanaka K, Tajima K, Kasai M, Mitani K, Ichinohe T, Hirai H, Taniguchi S, Sakamaki H, Harada M, Takaue Y (2005) Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years. Bone Marrow Transplant 36:667–674 doi:10.1038/sj.bmt.1705122
27. Uchida N, Wake A, Takagi S, Yamamoto H, Kato D, Matsuhashi Y, Matsumura T, Seo S, Matsuno N, Masuoka K, Kusumi E, Yuji K, Miyakoshi S, Matsuzaki M, Yoneyama A, Taniguchi S (2008) Umbilical cord blood transplantation after reduced-intensity conditioning for elderly patients with hematologic diseases. Biol Blood Marrow Transplant 14:583–590 doi:10.1016/j.bbmt.2008.03.003
28. Majhail NS, Brunstein CG, Tomblyn M, Thomas AJ, Miller JS, Arora M, Kaufman DS, Burns LJ, Slungaard A, McGlave PB, Wagner JE, Weisdorf DJ (2008) Reduced-intensity allogeneic transplant in patients older than 55 years: unrelated umbilical cord blood is safe and effective for patients without a matched related donor. Biol Blood Marrow Transplant 14:282–289 doi:10.1016/j.bbmt.2007.12.488
29. Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, McGlave PB, Wagner JE (2007) Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood 110:3064–3070 doi:10.1182/blood-2007-04-067215
30. Chao NJ, Koh LP, Long GD, Gasparetto C, Horwitz M, Morris A, Lassiter M, Sullivan KM, Rizzieri DA (2004) Adult recipients of umbilical cord blood transplants after nonmyeloablative preparative regimens. Biol Blood Marrow Transplant 10:569–575 doi:10.1016/j.bbmt.2004.05.001
31. Miyakoshi S, Yuji K, Kami M, Kusumi E, Kishi Y, Kobayashi K, Murashige N, Hamaki T, Kim SW, Ueyama J, Mori S, Morinaga S, Muto Y, Masuo S, Kanemaru M, Hayashi T, Takaue Y, Taniguchi S (2004) Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. Clin Cancer Res 10:3586–3592 doi:10.1158/1078-0432.CCR-03-0754
32. Narimatsu H, Watanabe M, Kohno A, Sugimoto K, Kuwatsuka Y, Uchida T, Murata M, Miyamura K, Morishita Y, Nagoya Blood and Marrow Transplantation Group (NBMTG) (2008) High incidence of graft failure in unrelated cord blood transplantation using a reduced-intensity preparative regimen consisting of fludarabine and melphalan. Bone Marrow Transplant 41:753–756 doi:10.1038/sj.bmt.1705978
33. Bär BM, De Witte T, Schattenberg A, Boezeman J, Hoogenhout J (1990) Favourable outcome of patients older than 40 years of age after transplantation with marrow grafts depleted of lymphocytes by counterflow centrifugation. Br J Haematol 74:53–60 doi:10.1111/j.1365-2141.1990.tb02537.x
34. Novitzky N, Thomas V, Hale G, Waldmann H (2005) Myeloablative conditioning is well tolerated by older patients receiving T-cell-depleted grafts. Bone Marrow Transplant 36:675–682 doi:10.1038/sj.bmt.1705119
35. Jakubowski AA, Small TN, Young JW, Kernan NA, Castro-Malaspina H, Hsu KC, Perales MA, Collins N, Cisek C, Chiu M, van den Brink MR, O'Reilly RJ, Papadopoulos EB (2007) T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin. Blood 110:4552–4559 doi:10.1182/blood-2007-06-093880

Bone Marrow Transplantation (2009) 44, 371–374

www.nature.com/bmt

ORIGINAL ARTICLE

Drug interaction between voriconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplant recipients

T Mori[1], Y Aisa[1], J Kato[1,2], Y Nakamura[1], Y Ikeda[1] and S Okamoto[1]

[1]*Division of Hematology, Department of Medicine, Keio University School of Medicine, Tokyo, Japan and* [2]*Novartis Pharma Program for the Clinical Therapeutics of Hematologic Malignancies, Keio University School of Medicine, Tokyo, Japan*

Although voriconazole has been shown to interact with calcineurin inhibitors, this interaction has not been thoroughly examined. The purpose of this study was to evaluate the drug interaction between voriconazole and calcineurin inhibitors among recipients of allogeneic hematopoietic stem cell transplantation (HSCT). Twenty-one recipients of allogeneic HSCT were evaluated. Those recipients had been on CsA ($n=10$) or tacrolimus ($n=11$) when voriconazole (400 mg per day orally, or 8 mg/kg per day, i.v.) was initiated. Trough concentrations of calcineurin inhibitors were measured before and periodically after initiating voriconazole to determine the concentration/dose (C/D) ratio of calcineurin inhibitors. Median C/D ratio significantly increased by initiating voriconazole: from 86.0 (range, 43.5–178.8) to 120.2 (range, 86.1–379.4) in CsA ($P<0.05$), and from 595.9 (range, 51.3–1643.3) to 890.7 (range, 94.1–4658.3) (ng/ml)/(mg/kg) in tacrolimus ($P<0.01$). Median increases in the C/D ratio did not differ significantly between CsA and tacrolimus (82.1%, ranging from −9.4 to 266.9% vs 115.6%, ranging from 25.4 to 307.6%). These results indicate that voriconazole alters the blood concentration of calcineurin inhibitors with a wide range of interindividual variability after allogeneic HSCT. Dose adjustment of calcineurin inhibitors on initiating voriconazole should not be decided uniformly, but determined on an individual basis by close monitoring of their blood concentrations.

Bone Marrow Transplantation (2009) **44,** 371–374; doi:10.1038/bmt.2009.38; published online 9 March 2009
Keywords: voriconazole; calcineurin inhibitors; CsA; tacrolimus; drug interaction; hematopoietic stem cell transplantation

Introduction

Patients with hematological malignancies receiving chemotherapy, particularly those undergoing hematopoietic stem cell transplantation (HSCT), are at high risk of developing invasive fungal disease (IFD). Fluconazole has been routinely recommended for the prophylaxis and treatment of IFD after HSCT.[1] However, because of the increasing incidence of mold infection, mainly *Aspergillus* species, effective prophylaxis and treatment of *Aspergillus* species has been required.[2,3] Voriconazole, a novel triazole antifungal agent, has a potent activity against a broad spectrum of fungi, including yeasts and molds. Voriconazole has been reported to improve the survival of patients with invasive aspergillosis as compared with amphotericin B, and has become the first option for the treatment of invasive aspergillosis.[4,5]

Voriconazole is metabolized by cytochrome P-450 (CYP) enzymes, namely CYP 2C9, 2C19 and 3A4.[5] *In vitro* studies have demonstrated that voriconazole could be a substrate as well as an inhibitor of these enzymes.[6] Therefore, its drug interaction with a variety of agents metabolized by these enzymes, including immunosuppressive agents (CsA, tacrolimus and sirolimus), has been recognized. Its drug interaction with CsA and tacrolimus is especially problematic, because calcineurin inhibitors have a narrow therapeutic window.[5–8] However, the drug interaction between voriconazole and calcineurin inhibitors has been systematically examined only in a limited number of renal transplant recipients (CsA) and in healthy subjects (tacrolimus), and documented sporadically in a few case reports.[7–12] In particular, the drug interaction between voriconazole and tacrolimus has not been systematically evaluated in patients to this date. In spite of such limited data, a uniform dose reduction of calcineurin inhibitors on initiating voriconazole (1/2 for CsA, 1/3 for tacrolimus) has been recommended by the manufacturer.[13] Although the evaluation of drug interaction between voriconazole and calcineurin inhibitors is highly relevant for clinical practice because of the increase in the use of voriconazole after allogeneic HSCT, there has been no such evaluation in recipients of allogeneic HSCT except for one case report.[12] This prompted us to study the drug interaction between voriconazole and calcineurin inhibitors in 21 recipients of allogeneic HSCT to confirm the appropriateness of uniform

Correspondence: Dr T Mori, Division of Hematology, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: tmori@sc.itc.keio.ac.jp
Received 29 September 2008; revised 29 December 2008; accepted 12 January 2009; published online 9 March 2009

dose modification of calcineurin inhibitors on initiating voriconazole in this patient population.

Patients and methods

Patient selection and drug administration

Recipients of allogeneic HSCT who had already been on a steady dose of calcineurin inhibitors (CsA or tacrolimus), and were started on oral or i.v. voriconazole for the treatment or prophylaxis of aspergillosis, were included in this study. Patient's blood levels of CsA or tacrolimus were stable when voriconazole was initiated. Oral voriconazole was administered under fasting conditions at a maintenance dose of 200 mg per body every 12 h after two initial doses of 300 mg per body 12 h apart. The median maintenance dose of voriconazole was 4.0 mg/kg per day (range, 3.1–4.6) every 12 h in patients who received voriconazole orally. Patients who received voriconazole orally did not have gastrointestinal symptoms. Voriconazole (i.v.) was administered at a dose of 4 mg/kg every 12 h after two initial doses of 6 mg/kg 12 h apart.

Determination of concentration/dose ratio of calcineurin inhibitors

Whole blood levels of CsA and tacrolimus were measured using standard fluorescence polarization immunoassay and microparticle enzyme immunoassay, respectively. Blood levels of calcineurin inhibitors were measured just before and every 1–2 days after initiating voriconazole for 7–10 days. Each physician decreased doses of the calcineurin inhibitors in response to rising levels every 1–2 days. The concentration/dose (C/D; (ng/ml)/(mg/kg)) ratio of calcineurin inhibitors was calculated 7–10 days after initiating voriconazole when the increased blood levels of calcineurin inhibitors had stabilized. The increase in the C/D ratio after initiating voriconazole was determined in comparison with that just before initiating the drug.

Statistical analysis

The Wilcoxon signed-rank test was used to compare the difference in the C/D ratio before and after initiating voriconazole. The Mann–Whitney *U*-test was used to compare the difference in percentage of the increase in C/D ratio between CsA- and tacrolimus-administered patients. *P*-values less than 0.05 were accepted as statistically significant.

Results

Patients

A total of 21 patients were evaluated, and their characteristics are shown in Table 1. Of them, 10 patients had been on CsA, and 11 patients had been on tacrolimus. The median post transplant day when voriconazole was initiated was 106 (range, 10–580). Voriconazole was given orally in 11 patients, and i.v. in 10 patients. All patients had a stable renal and hepatic function during the administration of voriconazole.

Table 1 Patient characteristics

	Total (n = 21)	*CsA (n = 10)*	*Tacrolimus (n = 11)*
Median age (range)	51 (23–59)	50 (41–59)	46 (23–57)
Gender			
Male/female	13/8	7/3	6/5
Median body weight, kg (range)	51.3 (42.0–80.3)	56.9 (45.4–67.6)	46.5 (42.0–80.3)
Underlying diseases			
Myclodysplastic syndrome	10	7	3
Acute leukemia	9	3	6
Malignant lymphoma	1	0	1
Myeloproliferative disease	1	0	1
Stem cell donor			
Related	9	8	1
Unrelated	12	2	10
Conditioning regimen			
Myeloablative	16	7	9
Reduced intensity	5	3	2
AcuteGVHD			
Grades 0–I	5	2	3
Grades II–IV	16	8	8
Route of voriconazole administration			
Oral	11	7	4
I.v.	10	3	7

Effect of voriconazole administration on C/D ratio of calcineurin inhibitors

Blood levels of CsA and tacrolimus increased steadily after initiating voriconazole in all patients except for one patient in whom voriconazole did not affect the levels of CsA. The median C/D ratio of CsA after initiating voriconazole was 120.2 (ng/ml)/(mg/kg) with a range of 86.1–379.4, which was significantly higher than that before initiating voriconazole (86.0 (ng/ml)/(mg/kg) with a range of 43.5–178.8; $P<0.05$; Table 2). The median C/D ratio of tacrolimus after initiating voriconazole was 890.7 (ng/ml)/(mg/kg) with a range of 94.1–4658.3, which was significantly higher than that before initiating voriconazole (595.9 with a range of 51.3–1643.3; $P<0.01$; Table 2). Median increases were 82.1% (range, −9.4–266.9) and 115.6% (range, 25.4–307.6) in CsA- and tacrolimus-administered patients, respectively. The difference in increases between CsA and tacrolimus was not significant ($P=0.14$). Neither the route of voriconazole administration (i.v. or oral) nor the gender significantly affected the increase in C/D ratio ($P=0.12$ and 0.60, respectively). No significant adverse effects associated with increased level of calcineurin inhibitors were observed.

Discussion

In this study, we demonstrated that orally or i.v. administered voriconazole exerts a clinically significant drug interaction with calcineurin inhibitors in allogeneic HSCT recipients, resulting in a significant increase in the blood concentration of calcineurin inhibitors. The results were consistent with those of two previous reports showing its drug interaction with CsA in 7 renal transplant recipients and with tacrolimus in 14 healthy individuals.[7,8] In spite of the limited number of subjects in the previous studies, uniform dose reduction of calcineurin inhibitors has been recommended for patients on these drugs who are initiating voriconazole; the purpose is to prevent the toxicity of calcineurin inhibitors from reaching the toxic threshold.[13] In decreasing the dose of calcineurin inhibitors according to the drug interaction, physicians should always weigh the risks of the toxicity of calcineurin inhibitors and the development of GVHD or graft rejection in solid organ transplantation. However, our results showed that there was considerable interpatient variability in the magnitude of drug interaction in terms of increases in the C/D ratio of calcineurin inhibitors. Therefore, we think that the dose reduction of calcineurin inhibitors should not be decided uniformly, but should instead be determined on an individual basis by careful and periodic monitoring of their blood concentrations.

Previous studies have assessed the magnitude of drug interaction between voriconazole and calcineurin inhibitors by comparing the concentrations of calcineurin inhibitors before and after initiating voriconazole, in some cases using the area under the concentration–time curve, or by presenting the dose reduction rate of calcineurin inhibitors determined by each physician.[7–12] However, such approaches are unable to evaluate the exact drug interaction quantitatively, because they focused either on the concentration or the dose of calcineurin inhibitors. In contrast, we used the C/D ratio ((ng/ml)/(mg/kg)) for the quantitative evaluation of drug interaction, which reflects both the concentration and dose of calcineurin inhibitors. We believe that the present results using this method could provide data of more clinical relevance.

The manufacturer's recommendation sets uniform dose reduction rates for calcineurin inhibitors on initiating voriconazole. This recommendation is based on the results of two small studies performed separately.[7,8] As opposed to the two previous studies, we found that the impact of voriconazole on the concentration of CsA and tacrolimus did not differ significantly. Thus, the manufacturer's recommendation gave the physicians the misleading impression that voriconazole had a greater effect on tacrolimus than on CsA.

The reasons accounting for the notable interindividual difference in the drug interaction between voriconazole and calcineurin inhibitors remains to be elucidated. One possible explanation is the difference in the activity of CYP among patients. Voriconazole is metabolized by three separate enzymes, CYP 2C9, 2C19 and 3A4.[5] The CYP 2C19, the major enzyme responsible for the metabolism of voriconazole, exhibits genetic polymorphisms, so that voriconazole could be metabolized to a greater or lesser extent among different individuals, resulting in significant differences in its concentration of voriconazole. Because voriconazole acts as an inhibitor as well as a substrate of CYP3A4, it is also plausible that the higher the concentration of voriconazole, the more the activity of CYP 3A4 is reduced. Together with the interindividual difference in the blood concentration of voriconazole documented in allogeneic HSCT recipients,[14] it is suggested that the interindividual difference in the metabolism of voriconazole is critical in its drug interaction with calcineurin inhibitors.

Table 2 Effect of voriconazole administration on the blood levels of calcineurin inhibitors

	Median C/D ratio of calcineurin inhibitors[a]		*Median increase of C/D ratio*
	Before voriconazole	*After voriconazole*	(%)
CsA, $n=10$ (range)	86.0 (43.5–178.8)	120.2[b] (86.1–379.4)	82.1 (−9.4–266.9)
Tacrolimus, $n=11$ (range)	595.9 (51.3–1634.3)	890.7[b] (94.1–4658.3)	115.6[c] (25.4–307.6)

[a]C/D indicates concentration/dose (ng/ml)/(mg/kg).
[b]Significantly higher than that before voriconazole ($P<0.05$, <0.01, respectively).
[c]Not significantly different as compared with CsA.

We conclude that the drug interaction between voriconazole and calcineurin inhibitors varies significantly among patients; thus, the dose adjustment of calcineurin inhibitors on initiating or discontinuing voriconazole should not be decided uniformly. Rather, close monitoring of the concentration in each individual is necessary to guide dosage adjustments with the goal of minimizing dose-related toxicity and maximizing efficacy of calcineurin inhibitors. The relationship between the blood concentration of voriconazole and its drug interaction with calcineurin inhibitors should be examined in a future study.

Conflict of interest

The author(s) declare no financial conflict of interest.

Acknowledgements

This study was supported in part by grants from Nagao Memorial Fund.

References

1 Dykewicz CA, Jaffe HW. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant* 2006; **6**: 670–672.
2 Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002; **34**: 909–917.
3 Kami M, Machida U, Okuzumi K, Matsumura T, Mori S, Hori A *et al.* Effect of fluconazole prophylaxis on fungal blood cultures: an autopsy-based study involving 720 patients with haematological malignancy. *Br J Haematol* 2002; **117**: 40–46.
4 Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW *et al.* Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002; **347**: 408–415.
5 Jeu L, Piacenti FJ, Lyakhovetskiy AG, Fung HB. Voriconazole. *Clin Ther* 2003; **25**: 1321–1381.
6 Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. *Pharmacotherapy* 2006; **26**: 1730–1744.
7 Romero AJ, Le Pogamp P, Nilsson LG, Wood N. Effect of voriconazole on the pharmacokinetics of cyclosporine in renal transplant patients. *Clin Pharmacol Ther* 2002; **71**: 226–234.
8 Wood N, Tank K, Allan R, Fielding A. Effect of voriconazole on the pharmacokinetics of tacrolimus. In: *Program and Abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy*, Chicago, Illinois, 22–25 September 2001 (abstract A-20). American Society of Microbiology: Washington, DC, 2001.
9 Tintillier M, Kirch L, Goffin E, Cuvelier C, Pochet JM. Interaction between voriconazole and tacrolimus in a kidney-transplanted patient. *Nephrol Dial Transplant* 2005; **20**: 664–665.
10 Venkataramanan R, Zang S, Gayowski T, Singh N. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. *Antimicrob Agents Chemother* 2002; **46**: 3091–3093.
11 Pai MP, Allen S. Voriconazole inhibition of tacrolimus metabolism. *Clin Infect Dis* 2003; **36**: 1089–1091.
12 Groll AH, Kolve H, Ehlert K, Paulussen M, Vormoor J. Pharmacokinetic interaction between voriconazole and ciclosporin A following allogeneic bone marrow transplantation. *J Antimicrob Chemother* 2004; **53**: 113–114.
13 Pfizer Inc. Vfend (voriconazole) package insert. New York, NY, 2008.
14 Trifilio S, Ortiz R, Pennick G, Verma A, Pi J, Stosor V *et al.* Voriconazole therapeutic drug monitoring in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2005; **35**: 509–513.

Bone Marrow Transplantation (2009), 1–8

www.nature.com/bmt

ORIGINAL ARTICLE

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

M Ogata[1,2], T Satou[2], R Kawano[2], S Takakura[2], K Goto[2], J Ikewaki[2], K Kohno[2], T Ikebe[3], T Ando[3], Y Miyazaki[3], E Ohtsuka[3], Y Saburi[3], T Saikawa[1] and J Kadota[2]

[1]*Blood Transfusion Center, Oita University Hospital, Oita, Japan;* [2]*Department of Hematology, Oita University Faculty of Medicine, Oita, Japan and* [3]*Department of Hematology, Oita Prefectural Hospital, Oita, Japan*

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.
Bone Marrow Transplantation advance online publication, 25 May 2009; doi:10.1038/bmt.2009.116
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

Correspondence: Dr M Ogata, Blood Transfusion Center, Oita University Faculty of Medicine, Hasama-machi, Yufu-city 879-5593, Japan.
E-mail: mogata@med.oita-u.ac.jp
Received 5 January 2009; revised 7 April 2009; accepted 8 April 2009

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, $P = 0.01$).

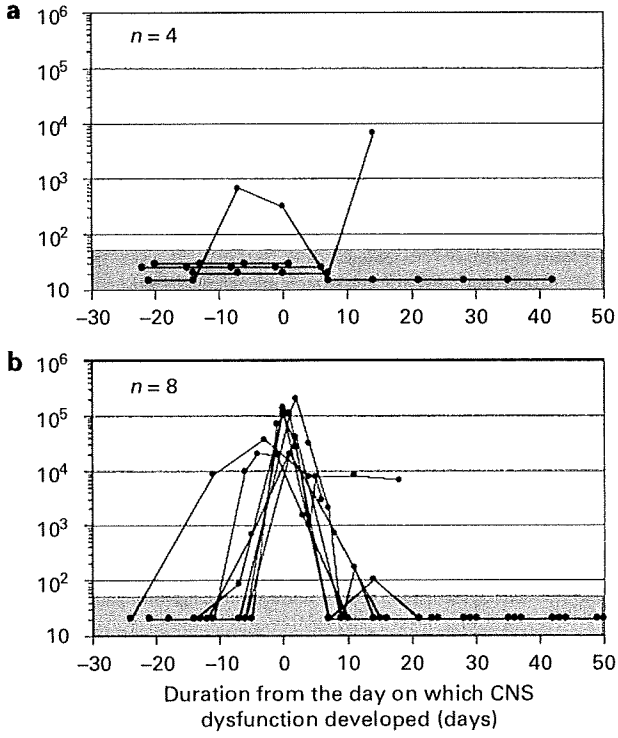


Figure 1 (a) Kinetics of plasma human herpesvirus (HHV)-6 DNA among patients in whom central nervous system (CNS) dysfunction showed no association with HHV-6 ($n = 4$). (b) Kinetics of plasma HHV-6 DNA for patients who developed HHV-6 encephalitis or CNS dysfunction potentially associated with HHV-6 ($n = 8$). 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.

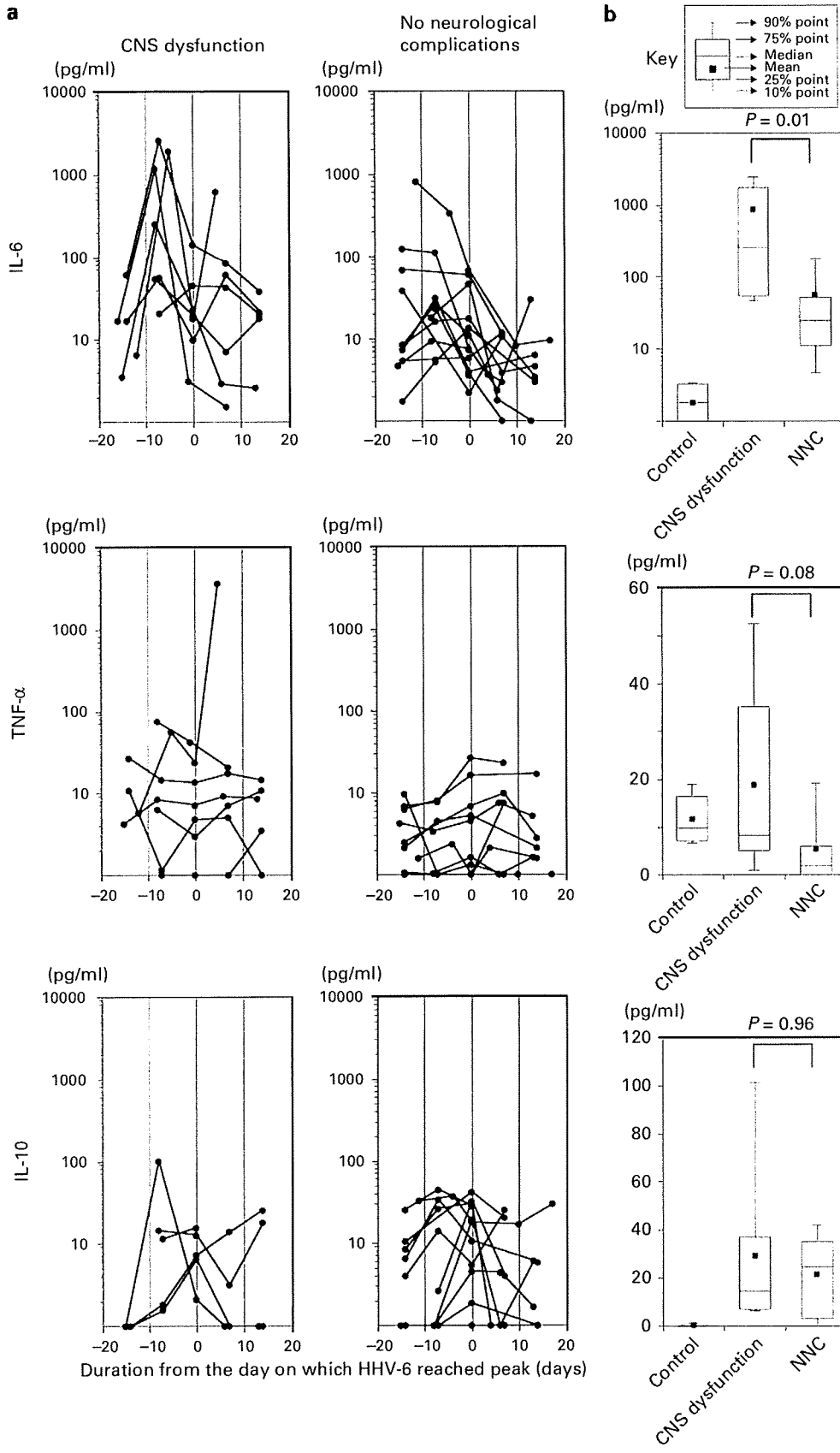
Table 4 Comparison of characteristics and laboratory data between two groups of patients displaying high-level HHV-6 DNA (plasma HHV-6 DNA $\geqslant 1 \times 10^4$ copies/ml)

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 $\geqslant$ 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
60
40
20
0
120
100
80
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

This study was presented in part at the Sixth International Conference on HHV-6&7 in Baltimore, MD, USA on 19–22 June 2008. The financial support was provided by Oita University Faculty of Medicine; Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant-in-Aid for Scientific Research 17015047).

References

1 Yoshikawa T, Asano Y, Ihira M, Suzuki K, Ohashi M, Suga S *et al.* Human herpesvirus 6 viremia in bone marrow transplant recipients: clinical features and risk factors. *J Infect Dis* 2002; **185**: 847–853.
2 Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clin Infect Dis* 2005; **40**: 932–940.
3 Ogata M, Kikuchi H, Satou T, Kawano R, Ikewaki J, Kohno K *et al.* Human herpesvirus 6 DNA load in plasma after allogeneic stem cell transplantation: incidence and clinical significance. *J Infect Dis* 2006; **193**: 68–79.
4 Yamane A, Mori T, Suzuki S, Mihara A, Yamazaki R, Aisa Y *et al.* Risk factors for developing human herpesvirus 6 (HHV-6) reactivation after allogeneic hematopoietic stem cell transplantation and its association with central nervous system disorders. *Biol Blood Marrow Transplant* 2007; **13**: 100–106.

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.

5 Zerr DM. Human herpesvirus 6 and central nervous system disease in hematopoietic cell transplantation. *J Clin Virol* 2006; **37**: 52–56.
6 Zerr DM. Human herpesvirus 6: a clinical update. *Herpes* 2006; **13**: 20–24.
7 Ogata M, Kadota J-i. Human herpesvirus-6 infections and infection-preventative measures in transplant recipients. *Future Virol* 2008; **3**: 567–578.
8 Ljungman P, de la Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P *et al.* Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with hematological malignancies and after SCT. *Bone Marrow Transplant* 2008; **42**: 227–240.
9 Yoshikawa T, Ihira M, Suzuki K, Suga S, Iida K, Saito Y *et al.* Human herpesvirus 6 infection after living related liver transplantation. *J Med Virol* 2000; **62**: 52–59.
10 Ihira M, Yoshikawa T, Suzuki K, Ohashi M, Suga S, Horibe K *et al.* Monitoring of active HHV-6 infection in bone marrow transplant recipients by real time PCR; comparison to detection of viral DNA in plasma by qualitative PCR. *Microbiol Immunol* 2002; **46**: 701–705.
11 Tomoiu A, Flamand L. Biological and clinical advances in human herpesvirus-6 and -7 research. *Future Virol* 2006; **1**: 623–635.
12 Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringden O *et al.* High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J Haematol* 2000; **111**: 774–781.
13 Ogata M, Satou T, Kawano R, Goto K, Ikewaki J, Kohno K *et al.* Plasma HHV-6 viral load-guided preemptive therapy against HHV-6 encephalopathy after allogeneic stem cell transplantation: a prospective evaluation. *Bone Marrow Transplant* 2008; **41**: 279–285.
14 Fujimaki K, Mori T, Kida A, Tanaka M, Kawai N, Matsushima T *et al.* Human herpesvirus 6 meningoencephalitis in allogeneic hematopoietic stem cell transplant recipients. *Int J Hematol* 2006; **84**: 432–437.
15 Vu T, Carrum G, Hutton G, Heslop HE, Brenner MK, Kamble R. Human herpesvirus-6 encephalitis following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2007; **39**: 705–709.
16 Muta T, Fukuda T, Harada M. Human herpesvirus-6 encephalitis in hematopoietic SCT recipients in Japan: a retrospective multicenter study. *Bone Marrow Transplant* 2009; **43**: 583–585.
17 Ito Y, Ichiyama T, Kimura H, Shibata M, Ishiwada N, Kuroki H *et al.* Detection of influenza virus RNA by reverse transcription-PCR and proinflammatory cytokines in influenza-virus-associated encephalopathy. *J Med Virol* 1999; **58**: 420–425.
18 Aiba H, Mochizuki M, Kimura M, Hojo H. Predictive value of serum interleukin-6 level in influenza virus-associated encephalopathy. *Neurology* 2001; **57**: 295–299.
19 Kawada J, Kimura H, Ito Y, Hara S, Iriyama M, Yoshikawa T *et al.* Systemic cytokine responses in patients with influenza-associated encephalopathy. *J Infect Dis* 2003; **188**: 690–698.
20 Otake Y, Yamagata T, Morimoto Y, Imi M, Mori M, Aihara T *et al.* Elevated CSF IL-6 in a patient with respiratory syncytial virus encephalopathy. *Brain Dev* 2007; **29**: 117–120.
21 Ichiyama T, Ito Y, Kubota M, Yamazaki T, Nakamura K, Furukawa S. Serum and cerebrospinal fluid levels of cytokines in acute encephalopathy associated with human herpesvirus-6 infection. *Brain Dev* 2008 (e-pub ahead of print).
22 Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **27**: 893–898.
23 Vogelsang GB, Hess AD. Graft-versus-host disease: new directions for a persistent problem. *Blood* 1994; **84**: 2061–2067.
24 Seeley WW, Marty FM, Holmes TM, Upchurch K, Soiffer RJ, Antin JH *et al.* Post-transplant acute limbic encephalitis: clinical features and relationship to HHV-6. *Neurology* 2007; **69**: 156–165.
25 Thomas ED, Storb R, Clift RA, Fefer A, Jhonson L, Neiman PE *et al.* Bone-marrow transplantation (second of two parts). *N Engl J Med* 1975; **292**: 895–902.
26 Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. *N Engl J Med* 1994; **330**: 1356–1360.
27 Wainwright MS, Martin PL, Morse RP, Lacaze M, Provenzale JM, Coleman RE *et al.* Human herpesvirus 6 limbic encephalitis after stem cell transplantation. *Ann Neurol* 2001; **50**: 612–619.
28 Fotheringham J, Akhyani N, Vortmeyer A, Donati D, Williams E, Oh U *et al.* Detection of active human herpesvirus-6 infection in the brain: correlation with polymerase chain reaction detection in cerebrospinal fluid. *J Infect Dis* 2007; **195**: 450–454.
29 Takatsuka H, Takemoto Y, Yamada S, Wada H, Tamura S, Fujimori Y *et al.* Complications after bone marrow transplantation are manifestations of systemic inflammatory response syndrome. *Bone Marrow Transplant* 2000; **26**: 419–426.
30 Nunoi H, Mercado MR, Mizukami T, Okajima K, Morishima T, Sakata H *et al.* Apoptosis under hypercytokinemia is a possible pathogenesis in influenza-associated encephalopathy. *Pediatr Int* 2005; **47**: 175–179.
31 Fujita A, Ihira M, Suzuki R, Enomoto Y, Sugiyama H, Sugata K *et al.* Elevated serum cytokine levels are associated with human herpesvirus 6 reactivation in hematopoietic stem cell transplantation recipients. *J Infect* 2008; **57**: 241–248.
32 Achour A, Boutolleau D, Gautheret-Dejean A, Agut H. Is human herpesvirus-6 DNA in plasma the right marker for active infection? *J Infect Dis* 2006; **194**: 1795–1796.
33 Achour A, Boutolleau D, Slim A, Agut H, Gautheret-Dejean A. Human herpesvirus-6 (HHV-6) DNA in plasma reflects the presence of infected blood cells rather than circulating viral particles. *J Clin Virol* 2007; **38**: 280–285.

J Clin Exp Hematopathol
Vol. 49, No. 2, Nov. 2009

Review Article

Human Herpesvirus 6 in Hematological Malignancies

Masao Ogata[1,2)]

Pathogenetic roles of human herpesvirus (HHV)-6 in lymphoproliferative diseases have been of continued interest. Many molecular studies have tried to establish a pathogenic role for HHV-6 in lymphoid malignancies. However, whether HHV-6 plays a role in these pathologies remains unclear, as positive polymerase chain reaction results for HHV-6 in those studies may reflect latent infection or reactivation rather than presence of HHV-6 in neoplastic cells. A small number of studies have investigated HHV-6 antigen expression in pathologic specimens. As a result, the lack of HHV-6 antigen expression on neoplastic cells argues against any major pathogenic role of HHV-6. The role of HHV-6 in childhood acute lymphoblastic leukemia (ALL) has also been of interest but remains controversial, with 2 studies documenting higher levels of HHV-6 antibody in ALL patients, and another 2 large-scale studies finding no significant differences in HHV-6 seroprevalences between ALL patients and controls. Alternatively, HHV-6 is increasingly recognized as an important opportunistic pathogen. HHV-6 reactivation is common among recipients of allogeneic stem cell transplantation (SCT), and is linked to various clinical manifestations. In particular, HHV-6 encephalitis appears to be significant, life-threatening complication. Most HHV-6 encephalitis develops in patients receiving transplant from an unrelated donor, particularly cord blood, typically around the time of engraftment. Symptoms are characterized by short-term memory loss and seizures. Magnetic resonance imaging typically shows limbic encephalitis. Prognosis for HHV-6 encephalitis is poor, but appropriate prophylactic measures have not been established. Establishment of preventive strategies against HHV-6 encephalitis represents an important challenge for physicians involved with SCT. [*J Clin Exp Hematopathol 49(2) : 57-67, 2009*]

Keywords: human herpesvirus 6, pathogenesis, lymphoproliferative disease, stem cell transplantation, encephalitis

INTRODUCTION

Human herpesvirus (HHV)-6 was isolated in 1986 from the peripheral blood mononuclear cells of 6 patients affected with various lymphoproliferative disorders.[1] This enveloped virion contains about 160 kb of linear double-stranded DNA,[2] and is now classified as a member of the *Roseolovirus* genus in the *Betaherpesvirinae* subfamily of human herpesviruses. Type A and type B variants of HHV-6 have been identified, exhibiting different epidemiological and biological characteristics and disease associations.[3] HHV-6B is highly prevalent in the human population, infecting virtually all children within the first few years of life.[4,5] Like the other herpesviruses, HHV-6 is capable of persisting in the host after primary infection. Under conditions of immunosuppression, HHV-6 can reactivate from latency.

Received : August 25, 2009
Accepted : September 1, 2009
[1)]Department of Hematology, Oita University Faculty of Medicine and [2)]Blood Transfusion Center, Oita University Hospital, Oita, Japan
Address correspondence and reprint request to Masao Ogata
Department of Hematology, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu-city, Oita 879-5593, Japan
E-mail : mogata@med.oita-u.ac.jp

Both HHV-6A and -6B replicate most efficiently *in vitro* in CD 4^+ T cells.[6] The host tissue range of HHV-6 *in vivo* is broad and includes peripheral blood mononuclear cells,[7] salivary glands, brain tissue, liver cells, lymph node, and endothelial cells.[8] Candidate sites for latency are salivary glands,[9,10] brain tissue,[11,12] monocytes,[13] and early bone marrow progenitor cells.[14]

Primary HHV-6 infection commonly causes exanthem subitum.[4,5] Associations between HHV-6 infection (reactivation) and development of many diseases have been investigated, including multiple sclerosis,[15] mesial temporal lobe epilepsy,[12,16] encephalitis in immunocompetent patients,[17] chronic fatigue syndrome,[18] drug-induced hypersensitivity syndrome,[19,20] Kikuchi's disease,[21] hematological malignancies, and complications following stem cell or organ transplantation.

To date, huge numbers of investigations have examined the roles of HHV-6 in the development of hematological malignancies (as an oncogenic agent), and the significance of HHV-6 infection during the course of treatment (as an opportunistic pathogen). However, careful interpretation of published data is required. The present work offers an overview of experimental and clinical observations supporting the involvement of HHV-6 in hematological malignancies.