San Diego, CA). Values of $P < 0.05$ were considered significant in all analyses.

RESULTS

Patients (n = 34) with acute/lymphoma-type ATL participated in this study. The characteristics of these patients are listed in Table I. One patient received two courses of pre-emptive ganciclovir treatment because positive CMV antigenemia was noticed. No other patient received any antiviral treatments. For the six patients who received hematopoietic stem cell transplantation, observation of viral reactivation for this study was stopped upon starting conditioning for transplantation. Median survival, based on Kaplan–Meier analysis, from the start of chemotherapy and 3-year overall survival rate with censoring transplantation patients were 244 days and 22.3%, respectively. A total of 22 patients died during the observation period: causes of death were ATL deterioration in 14 patients and infectious diseases in 8 patients. All patients who died of infectious diseases had concomitant, uncontrollable ATL.

Sequentially collected plasma samples were used to evaluate viral reactivation. Generally, plasma samples were collected from patients once every 7–14 days, but the interval was greater than 20 days for 6.5% of the samplings (28 of 434 intervals) because sampling was not performed during periods in which patients were not hospitalized. The median observation period for viral reactivation in individual patients was 113 days (range, 45–318 days).

Cumulative incidence of reactivation for each virus according to Kaplan–Meier analysis are shown in Figure 1, and the kinetics of plasma viral DNA load in patients who displayed positive viral DNA are shown in Figure 2. The characteristics of reactivation are shown in Table II. A Kaplan–Meier plot of the probability of survival according to viral reactivation is shown in Figure 3.

CMV Reactivation

The overall cumulative rate of a positive result for plasma CMV DNA by 100 days after the start of chemotherapy was 50.6% (Fig. 1). Univariate analysis revealed that higher white blood cell count and abnormal cell count at diagnosis were associated with CMV reactivation (Table I). As shown in Figure 2A,B, most CMV reactivations were self-limited. However, plasma CMV DNA levels had a tendency to persist or increase if and when the CMV DNA load reached a level of $\geq 10^4$ copies/ml (n = 5) (Fig. 2A). For the five patients whose plasma CMV DNA reached $\geq 10^4$ copies/ml, the median period from the detection of the first CMV-DNA-positive plasma sample to the attainment levels of $\geq 10^4$ copies/ml was 42 days (range, 21–98 days), and the period from first detection to peak levels was 98 days (range, 28–133 days). Median period from the first detection of a level of $\geq 10^4$ copies/ml CMV DNA to the death was 9 days (range, 1–152 days). One patient developed complications that were probably associated with CMV. A 72-year-old female developed fatal interstitial pneumonia at the time when the CMV DNA load peaked at 1.6×10^6 copies/ml. In this patient, the period from first detection of plasma CMV DNA to development of interstitial pneumonia was 98 days. The arrow in Figure 2A indicates the day on which the interstitial pneumonia developed. No other patient developed a clinical disease that was likely to be related to CMV reactivation. Kaplan–Meier analysis (Fig. 3) revealed median survival time from start of chemotherapy was 188 days in patients who experience CMV reactivation and 683 days in patients without CMV reactivation. CMV reactivation was negatively associated with survival, but the P-value for this association was at the borderline of statistical significance ($P = 0.052$, log-rank test).

HHV-6 Reactivations

The overall cumulative rate of a positive result for HHV-6 DNA in a plasma sample was 52.3% by 100 days after the start of chemotherapy (Fig. 1). No variables at diagnosis were identified as a risk factor associated with HHV-6 reactivation (Table I). Most HHV-6 reactivations were self-limited (Fig. 2C,D). The plasma HHV-6 DNA levels in the patient whose plasma HHV-6 DNA reached the highest value observed in this study suddenly climbed and dropped to an undetectable level three times (blue line in Fig. 2C). The duration in weeks of positive HHV-6 DNA tests in individual patients tended to be shorter compared with that of positive CMV DNA (Table II), but the difference was not statistical significance ($P = 0.11$, Mann–Whitney test). No patient developed complications, such as encephalitis or interstitial pneumonia, that were likely to be related to HHV-6 reactivation. There was no association between HHV-6 reactivation and survival (Fig. 3; $P = 0.35$, log-rank test).

EBV Reactivations

The overall cumulative rate of a positive result for EBV DNA in a plasma sample was 21.6% by 100 days of the start of chemotherapy (Fig. 1). No variables at diagnosis were identified as a risk factor associated with EBV reactivation (Table I). Most EBV reactivations were self-limited (Fig. 2E,F). Five patients had EBV DNA in the plasma sample taken within the first 7 days of treatment, and probability of incidence of EBV reactivation within the first 7 days of treatment was 14.7%. The duration, in weeks, of positive EBV-DNA tests in individual patients (Table II) was significantly shorter than that of positive CMV DNA tests ($P = 0.02$, Mann–Whitney test) but was not significantly different from that of positive HHV-6 DNA tests ($P = 0.18$). No patient developed EBV-associated lymphoproliferative disorder. There was no association between EBV reactivation and survival (Fig. 3; $P = 0.11$, log-rank test).

TABLE I. Patient Characteristics at Diagnosis and Association of These Variables With Herpesvirus Reactivation in Patients With Adult T Cell Leukemia (ATL) (n = 34)

		CMV reactivation			HHV-6 reactivation			EBV reactivation		
Characteristics	Total (n = 34)	Yes[a] (n = 22)	No[b] (n = 12)	*P*	Yes[a] (n = 20)	No[b] (n = 14)	*P*	Yes[a] (n = 11)	No[b] (n = 23)	*P*
Age in years, median (range)	65 (36–82)	69.5 (36–82)	56 (31–80)	0.03[f]	66.5 (36–82)	62 (38–80)	0.66[f]	68 (36–82)	61 (31–80)	0.85[f]
Sex										
Male	13 (38.2)	10 (45.5)	3 (25)	0.29[g]	9 (45)	4 (28.6)	0.48[g]	6 (54.5)	7 (30.4)	0.26[g]
ATL subtype										
Acute	22 (64.7)	17 (77.3)	5 (41.7)	0.06[g]	12 (60)	10 (71.4)	0.72[g]	8 (72.7)	14 (60.9)	0.70[g]
Lymphoma	12 (35.3)	5 (22.7)	7 (58.3)		8 (40)	4 (28.6)		3 (27.3)	9 (39.1)	
WBC count, /μL, median (range)	8,310 (2,500–64,550)	10,095 (3,930–64,550)	6,320 (2,500–43,800)	0.04[f]	10,375 (3,600–64,550)	7,370 (2,500–43,800)	0.16[f]	10,700 (4,200–10,700)	7,900 (2,500–64,550)	0.28[f]
Abnormal lymphocyte count, /μL, median (range)[c]	235 (0–56,804)	1,430 (0–56,804)	22.5 (0–31,098)	0.02[f]	201 (0–56,804)	387 (0–31,098)	0.98[f]	351 (0–12,527)	222 (0–56,804)	0.80[f]
LDH, more than twice the upper limit	18 (52.9)	14 (63.6)	4 (33.3)	0.15[g]	10 (50)	8 (57.1)	0.74[g]	7 (63.6)	11 (47.8)	0.48[g]
Hypercalcemia	6/33 (18.2)	5 (22.7)	1 (9.1)	0.64[g]	4 (20)	2 (6.5)	>0.99[g]	1 (9.1)	5 (22.7)	0.64[g]
sIL2R, U/L, median (range)[d]	14,200 (598–96,700)	18,750 (3,750–96,700)	8,350 (598–73,800)	0.14[f]	17,600 (879–96,700)	9,650 (598–73,800)	0.38[f]	20,750 (6,040–96,700)	11,300 (598–73,800)	0.17[f]
Initial treatment										
Regimen[e]										
THP-CEP	15	10	5		8	7		5	10	
THP-COP	10	6	4		5	5		3	7	
mLSG15	8	5	3		6	2		3	5	
CHOP	1	1	0		1	0		0	1	

Data represent number (%) of patients, unless otherwise indicated.
WBC, white blood cell; sIL2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase.
[a]Patients with positive DNA of each viral genome in plasma at any time after start of chemotherapy.
[b]Patients without positive DNA of each viral genome in plasma throughout the clinical course after start of chemotherapy.
[c]Data were missing for two patients.
[d]Normal range, 145–519 U/ml. Data were missing for two patients.
[e]Agents which constitutes a regimen were indicated in the Materials and Methods Section.
[f]Mann–Whitney *U* test.
[g]Fisher's exact test.

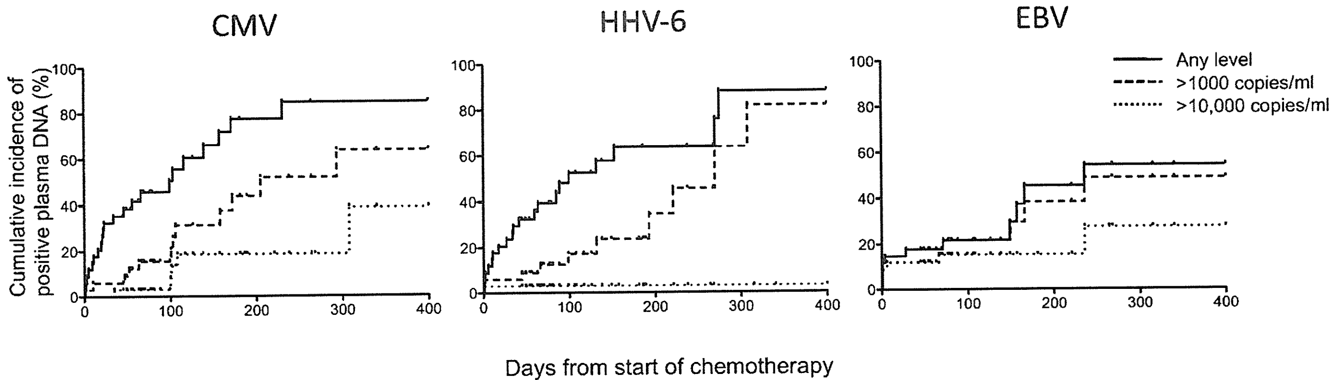

Fig. 1. Kaplan–Meier plot of the cumulative incidence of reactivation of CMV, HHV-6, or EBV, by number of copies of each viral DNA per ml in the day after start of chemotherapy.

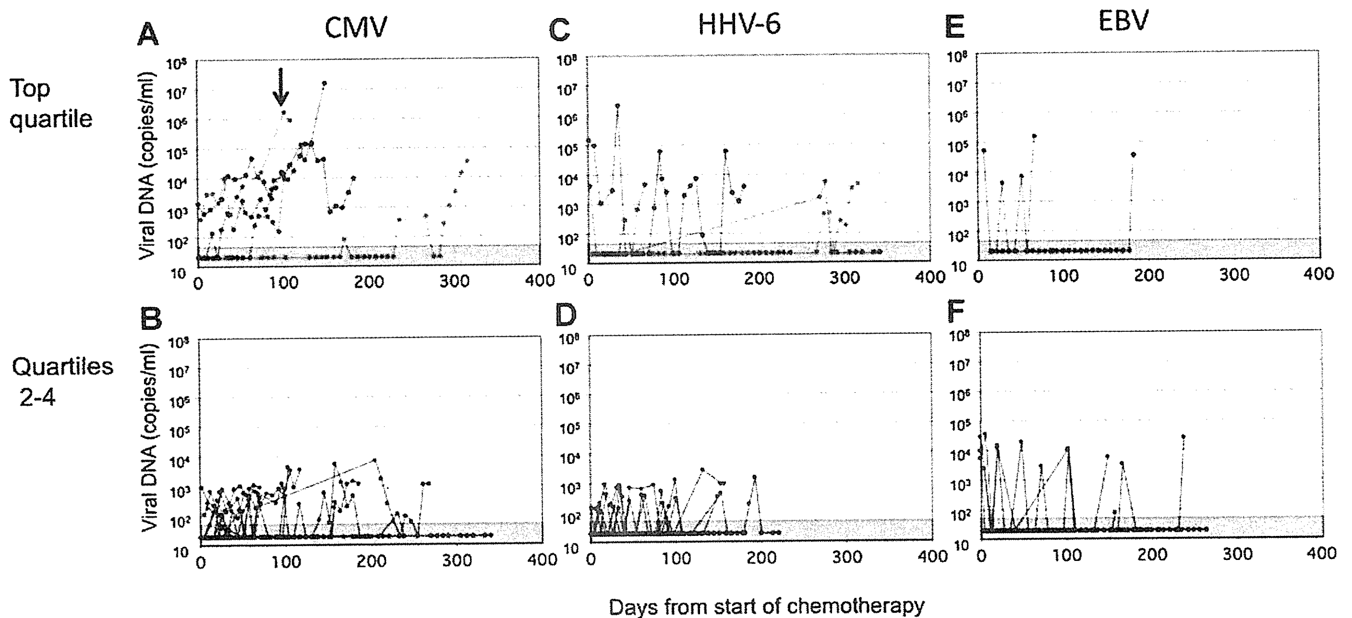

Fig. 2. Kinetics of the course of reactivation of CMV, HHV-6, and EBV. Each line represents an individual patient. The shaded area indicates values below the threshold for detection (<50 copies/ml of plasma). The top graphs (A,C, and E) show the kinetics of viral load among patients whose peak DNA load exceeded the upper one-fourth and the bottom graphs (B,D, and F) show those among patients whose peak DNA load were less than the upper one-fourth. The arrow in the (A) depicting the kinetics of CMV DNA reactivation indicates the day on which interstitial pneumonia developed in one patient.

TABLE II. Characteristics of Each Virus Reactivation Among Positive Cases

	CMV	HHV-6	EBV
Time to onset from start of chemotherapy			
Median, days (range)	28.5 (1–157)	38.5 (1–275)	28 (1–236)
Mean, days (SD)	61.4 (65.7)	70.8 (81.9)	73.7 (87.3)
Time to peak viral DNA from start of chemotherapy			
Median, days (range)	104.5 (2–315)	84 (1–315)	71 (1–238)
Mean, days (SD)	115.8 (74.9)	101.9 (91.8)	87.4 (81.8)
Duration of positive viral DNA in each patient			
Median, weeks (range)	5.5 (1–26)	2 (1–16)	1 (1–4)
Mean, weeks (SD)	7.1 (6.9)	3.8 (4.0)	1.9 (1.1)
Peak viral load			
Median, copies/ml (range)	1,216 (35–14,743,520)	1,117 (69–2,041,969)	10,810 (80–343,140)

SD, standard deviation.

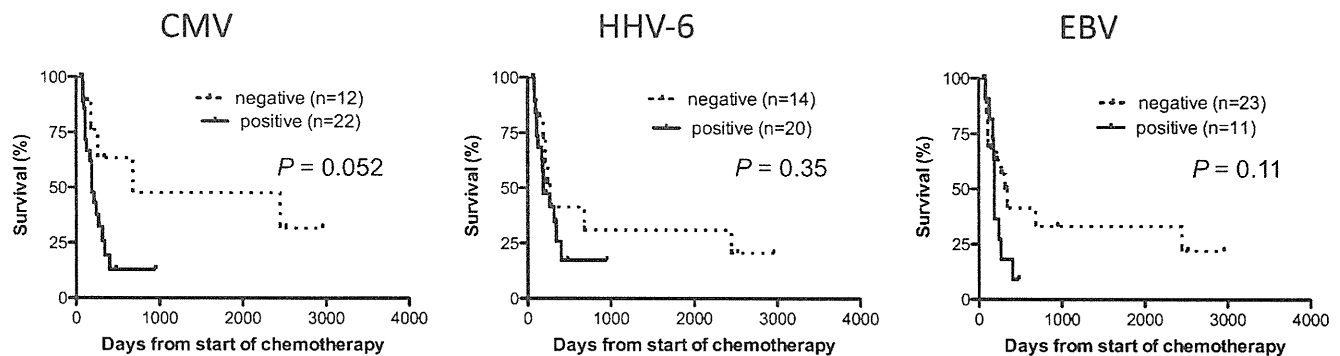

Fig. 3. Kaplan–Meier plot of the probability of survival according to viral reactivation. In each figure, "positive" indicates that tested positive for DNA of a viral genome in plasma at any time after start of chemotherapy, and "negative" indicates patients without positive DNA for a particular viral genome at any point after start of chemotherapy. Comparisons of survival were made using the log-rank test.

DISCUSSION

Immunosurveillance by virus-specific T cells prevents reactivation of herpesviruses. Compromised cellular immune responses are associated with reactivation of herpesviruses. In patients who have received solid-organ or allogeneic stem cell transplantation, reactivation of CMV, HHV-6, and EBV is closely related to serious complications—including CMV pneumonia [Boeckh and Ljungman, 2009], HHV-6 encephalitis [Zerr, 2006a; Ogata et al., 2010], and EBV-related post-transplant lymphoproliferative disorder [Shapiro et al., 1999; Omar et al., 2009]. Interestingly, reactivations of EBV and CMV are also common in patients with severe aplastic anemia who received immunosuppressive therapies but are rarely associated with clinical disease even in patients with high-level reactivation [Scheinberg et al., 2007]. These results indicate that clinical syndromes accompanied by herpesvirus reactivation depend on not only on viral reactivation but also on the patient's background or the cause of immunosuppression.

This study showed that CMV, HHV-6, or EBV reactivation was surprisingly frequent in ATL patients receiving chemotherapy. Some ATL patients developed profound reactivation. For example, plasma CMV viral load exceeded 10^6 copies/ml in two ATL patients. Such high levels of reactivation had not been observed in a previous study investigating CMV reactivation in patients who received stem cell transplantation at Oita University Hospital [Ikewaki et al., 2005]. A recent report demonstrated that plasma HHV-6 DNA levels of $\geq 10^4$ copies/ml are associated with the development of HHV-6 encephalitis in stem cell transplant recipients [Ogata et al., 2010]. In this study, one ATL patient repeatedly experienced such a high-level of HHV-6 reactivation throughout the clinical course.

Each virus had distinct reactivation kinetics. In patients with high levels of CMV DNA (i.e., $\geq 10^4$ copies/ml plasma), plasma CMV DNA levels increased gradually and needed at least 4 weeks to reach a peak CMV viral load. Furthermore, plasma CMV DNA did not disappear if the CMV DNA load reached a level of $\geq 10^4$ copies/ml of plasma. Subsequent prognosis of patients whose CMV DNA load reached this level was very poor. These findings suggest that there is a threshold level of CMV reactivation after which spontaneous improvement of the reactivation cannot be expected. A CMV load $\geq 10^4$ copies/ml plasma is indicative of subsequent exacerbation of CMV reactivation and development of serious clinical course. In contrast, most HHV-6 reactivation peaked within 1 week, and high-level reactivations could disappear and reappear suddenly. The dynamic reactivation pattern of HHV-6 in ATL patients may be similar to that of recipients of stem cell transplant: in stem cell transplantation patients, plasma HHV-6 DNA can elevate to peak within 1 week but do not persist in most cases [Ogata et al., 2008]. Most EBV reactivation did not persist. Interestingly, the rate of EBV-DNA-positive samples near the initiation of chemotherapy was relatively high. The viral DNA observed in the early phase of chemotherapy disappeared rapidly. Polyclonal EBV-infected cells were frequently observed in the lymph nodes of patients with incipient ATL [Ohshima et al., 1997], and this observation may be associated with the EBV-DNA-positive samples observed in the early phase of chemotherapy. The characteristics of the reactivation kinetics of each herpesvirus are probably affected by the doubling-time of each virus, the ability of virus-specific T cells to control each virus in the host, and the responsiveness of each virus to the host immune response.

This study has several limitations—including the retrospective design, the small number of patients enrolled, research at only two institutes, and the irregularity of the sampling intervals. This study may underestimate the incidence of viral reactivation because some plasma-sampling intervals were long.

The results of this study raised the possibility that routine prospective monitoring for CMV DNA in plasma may be useful in preventing CMV-related disease in ATL patients. CMV pneumonia is major cause of death in ATL patients [Suzumiya et al., 1993]. In fact, one patient in this study developed fatal interstitial

pneumonia when CMV DNA reached peak levels. Pre-emptive anti-viral therapy using active agents, such as ganciclovir or foscarnet, when CMV DNA levels reach $\geq 10^4$ copies/ml may be justified. Because at least 3 weeks were needed to reach CMV DNA $\geq 10^4$ copies/ml, routine monitoring of CMV DNA once every 7–14 days is proposed. In contrast, the levels of HHV-6 and EBV DNA in plasma increased and decreased suddenly in patients experiencing high-level reactivations, and these dynamic kinetics make prediction of subsequent viral reactivation or disease development based on the plasma HHV-6 or EBV DNA load difficult. Although HHV-6 encephalitis or EBV-associated lymphoproliferative disorder in ATL patients has been reported, these complications are very rare. Therefore practical routine monitoring for HHV-6 and EBV to prevent disease from HHV-6 or EBV is not justified in patients with ATL.

In conclusion, this study showed that CMV, HHV-6, and EBV reactivation was common in ATL patients who were receiving cytotoxic chemotherapy. Routine monitoring of CMV reactivation may be useful for the early detection of CMV-related diseases. Clinical trials of plasma-CMV-DNA-guided, preemptive approaches against CMV-related diseases are warranted. In contrast, the practical usefulness of monitoring for HHV-6 or EBV was not evident based on the results of this study.

REFERENCES

Boeckh M, Ljungman P. 2009. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood 113:5711–5719.

Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. 1996. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: A randomized double-blind study. Blood 88:4063–5719.

Chen S, Ishii N, Ine S, Ikeda S, Fujimura T, Ndhlovu LC, Soroosh P, Tada K, Harigae H, Kameoka J, Kasai N, Sakaki T, Sugamura K. 2006. Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. Int Immunol 18:269–277.

Fujiwara H, Matsumoto T, Eizuru Y, Matsushita K, Ohtsubo H, Kukita T, Imaizumi R, Matumoto M, Hidaka S, Arima N, Tei C. 2000. Cytomegalovirus infection is not necessarily a poor prognostic factor in adult T-cell leukemia/lymphoma. J Med Virol 62:140–143.

Fujiwara H, Eizuru Y, Matsumoto T, Kukita T, Imaizumi R, Kawada H, Ohtsubo H, Matsushita K, Arima N, Tei C. 2001. The significance of cytomegalovirus infection over the clinical course of adult T-cell leukemia/lymphoma. Microbiol Immunol 45:97–100.

Han XY. 2007. Epidemiologic analysis of reactivated cytomegalovirus antigenemia in patients with cancer. J Clin Micrbiol 45:1126–1132.

Idutsu K, Abe Y, Otonari J, Tachikawa Y, Ohtsuka R, Choi I, Muta K, Takayanagi R. 2007. Human herpesvirus 6 encephalitis in a patient with adult T-cell leukemia/lymphoma. Rinsho Ketsueki 48:664–666 [article in Japanese].

Ikewaki J, Ohtsuka E, Satou T, Kawano R, Ogata M, Kikuchi H, Nasu M. 2005. Real-time PCR assays based on distinct genomic regions for cytomegalovirus reactivation following hematopoietic stem cell transplantation. Bone Marrow Transplant 35:403–410.

Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, Matsuyama T, Morishima T. 1999. Quantitative analysis of Epstein–Barr virus by using a real-time PCR assay. J Clin Microbiol 37:132–136.

Ljungman P. 2010. Molecular monitoring of viral infections after hematopoietic stem cell transplantation. Int J Hematol 91:596–601.

Locatelli G, Santoro F, Veglia F, Gobbi A, Lusso P, Malnati MS. 2000. Real-time quantitative PCR for human herpesvirus 6 DNA. Clin Microbiol 38:4042–4048.

Meerbach A, Wutzler P, Hafer R, Zintl F, Gruhn B. 2008. Monitoring of Epstein–Barr virus load after hematopoietic stem cell transplantation for early intervention in post-transplant lymphoproliferative disease. J Med Virol 80:441–454.

Nguyen Q, Estey E, Raad I, Rolston K, Kantarjian H, Jacobson K, Konoplev S, Ghosh S, Luna M, Tarrand J, Whimbey E. 2001. Cytomegalovirus pneumonia in adults with leukemia: An emerging problem. Clin Infect Dis 21:539–545.

Ogata M, Kikuchi H, Satou T, Kawano R, Ikewaki J, Kohno K, Kashima K, Ohtsuka E, Kadota J. 2006. Human herpesvirus 6 DNA load in plasma after allogeneic stem cell transplantation: Incidence and clinical significance. J Infect Dis 193:68–79.

Ogata M, Satou T, Kawano R, Goto K, Ikewaki J, Kohno K, Ando T, Miyazaki Y, Ohtsuka E, Saburi Y, Saikawa T, Kadota J. 2008. Plasma HHV-6 viral load-guided preemptive therapy against HHV-6 encephalopathy after allogeneic stem cell transplantation: A prospective evaluation. Bone Marrow Transplant 41:279–285.

Ogata M, Satou T, Kawano R, Takakura S, Goto K, Ikewaki J, Kohno K, Ikebe T, Ando T, Miyazaki Y, Ohtsuka E, Saburi Y, Saikawa T, Kadota J. 2010. Correlations of HHV-6 viral load and plasma IL-6 concentration with HHV-6 encephalitis in allogeneic stem cell transplant recipients. Bone Marrow Transplant 45:129–136.

Ohshima K, Suzumiya J, Kato A, Tashiro K, Kikuchi M. 1997. Clonal HTLV-1-infected CD4+ T-lymphocytes and non-clonal non-HTLV-1-infected giant cells in incipient ATLL with Hodgkin-like histologic features. Int J Cancer 72:592–598.

Omar H, Hagglund H, Gustafsson-Jernberg A, LeBlanc K, Mattsson J, Remberger M, Ringden O, Sparelid E, Sudnin M, Winiarski J, Yun Z, Ljungman P. 2009. Target monitoring of patients at high risk of post-transplant lymphoproliferative disease by quantitative Epstein–Barr virus polymerase chain reaction. Transpl Infect Dis 11:393–399.

Scheinberg P, Fischer SH, Li L, Nunez O, Wu CO, Sloand EM, Cohen JI, Young NS, John Barrett A. 2007. Distinct EBV and CMV reactivation patterns following antibody-based immunosuppressive regimens in patients with severe aplastic anemia. Blood 109:3219–3224.

Shapiro R, Nalesnik M, McCauley J, Fedorek S, Jordan ML, Scantlebury VP, Jain A, Vivas C, Ellis D, Lombardozzi-Lane S, Randhawa P, Johnston J, Hakala TR, Simmons RL, Fung JJ, Starzl TE. 1999. Posttransplant lymphoproliferative disorders in adult and pediatric renal transplant patients receiving tacrolimus-based immunosuppression. Transplantation 68:1851–1854.

Shimoyama M. 1991. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984–87). Br J Haematol 79:428–437.

Suzumiya J, Marutsuka K, Nabeshima K, Nawa Y, Koono M, Tamura K, Kimura N, Hisano S, Tachibana N, Inoue S, Sumiyoshi A. 1993. Autopsy findings in 47 cases of adult T-cell leukemia/lymphoma in Miyazaki prefecture, Japan. Leuk Lymphoma 11:281–286.

Takeda N, Isonuma H, Sekiya S, Ebe T, Matsumoto T, Watanabe K. 2001. Studies of anti-cytomegalovirus IgG antibody positive rate and cytomegalovirus mononucleosis in adults. Kansenshogaku Zasshi 75:775–779 [article in Japanese].

Tanaka Y, Kondo T, Kishimoto W, Kanda J, Tashima M, Mizumoto C, Icinohe T, Ishikawa T, Uchiyama T, Haga H, Manabe T. 2008. Epstein–Barr virus-associated B cell lymphoproliferative disorder complicated in adult T cell leukemia. Rinsho Ketsueki 49:263–269 [article in Japanese].

Tobinai K, Ohtsu T, Hayashi M, Kinoshita T, Matsuno Y, Mukai K, Shimoyama M. 1991. Epstein–Barr virus (EBV) genome carrying monoclonal B-cell lymphoma in a patient with adult T cell leukemia-lymphoma. Leuk Res 15:837–846.

Tsukasaki K, Utsunomiya A, Fukuda H, Shibata T, Fukushima T, Takatsuka Y, Ikeda S, Masuda M, Nagoshi H, Ueda R, Tamura K, Sano M, Momita S, Yamaguchi K, Kawano F, Hanada S, Tobinai K, Shimoyama M, Hotta T, Tomonaga M. 2007. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. J Clin Oncol 25:5458–5464.

Uchiyama T. 1997. Human T cell leukemia virus type 1 (HTLV-1) and human diseases. Annu Rev Immunol 15:15–37.

van Brussel M, Salemi M, Liu HF, Goubau P, Desmyter J, Vandamme AM. 1999. The discovery of two new divergent STLVs has

implications for the evolution and epidemiology of HTLVs. Rev Med Virol 9:155–170.

Yasunaga J, Sakai T, Nosaka K, Etoh Ki, Tamiya S, Koga S, Mita S, Uchino M, Mitsuya H, Matsuoka M. 2001. Impaired production of naive T lymphocytes in human T-cell leukemia virus type I-infected individuals: Its implications in the immunodeficient state. Blood 97:3177–3183.

Yoshikawa T, Asano Y, Ihira M, Suzuki K, Ohashi M, Suga S, Kudo K, Horibe K, Kojima S, Kato K, Matsuyama T, Nishiyama Y. 2002. Human herpesvirus 6 viremia in bone marrow transplant recipients: Clinical features and risk factors. J Infect Dis 185:847–853.

Zerr DM. 2006a. Human herpesvirus 6 and central nervous system disease in hematopoietic cell transplantation. J Clin Virol 37:52–56.

Zerr DM. 2006b. Human herpesvirus 6: A clinical update. Herpes 13:20–24.

Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA, Corey L. 2005a. A population-based study of primary human herpesvirus 6 infection. N Engl J Med 352:768–776.

Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. 2005b. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis 40:932–940.

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Analysis of rotavirus antigenemia in hematopoietic stem cell transplant recipients

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Abstract : Systemic rotavirus infection, such as rotavirus antigenemia, has been found in immunocompetent rotavirus gastroenteritis patients. However, the pathogenesis of rotavirus infection in immunocompromised transplant recipients remains unclear. Enzyme-linked immunosorbent assay was used to measure rotavirus antigen levels in serially collected serum samples obtained from 62 pediatric patients receiving allogeneic hematopoietic stem cell transplants (HSCT). Rotavirus antigen was detected in 43 (6.8%) of 633 serum samples (8 of 62 patients). The duration of rotavirus antigenemia ranged between 1 and 10 weeks, and diarrhea was concurrent with rotavirus antigenemia in Cases 3, 6, 7, and 8. The level of viral antigen in the transplant recipients (0.19 ± 0.20) was significantly lower than that observed in serum samples collected from immunocompetent patients on either day 1 (0.49 ± 0.18, P = 0.0011) or day 3 (0.63 ± 0.09, P = 0.0005). A patient who received a graft from a human leukocyte antigen (HLA)-mismatched donor was at significant risk for rotavirus antigenemia (P = 0.024; odds ratio = 9.44) in comparison to patients who received grafts from HLA-matched donors. Although the duration of antigenemia was clearly longer in HSCT patients than in immunocompetent rotavirus gastroenteritis patients, the levels of viral antigen were not as high. Therefore, mismatched HLA may be a risk factor for rotavirus antigenemia after HSCT.

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Key words: rotavirus; antigenemia; hematopoietic stem cell transplantation; pediatric

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Rotavirus is the major cause of gastroenteritis in young children worldwide. Severe dehydration caused by rotavirus-induced diarrhea and vomiting can be fatal in developing countries. In addition, the gastroenteritis induced by rotavirus infection causes a large economic burden in developed countries. Rotaviral infection is generally benign and self-limited in immunocompetent children. In contrast, it has been reported that rotavirus can cause severe diarrhea resulting in fatal outcomes for immunocompromised transplant recipients (1–5). Thus, the pathogenesis of rotaviral infection may differ between immunocompetent and immunocompromised individuals.

Initially, rotavirus replication was thought to be limited to the gastrointestinal tract in patients with rotavirus gastroenteritis. However, recently, rotavirus antigen and RNA were detected in the sera of rotavirus-infected children (6–9). In addition, rotavirus antigen was detected not only in the serum but also in several organs, including the stomach, intestine, liver, lung, spleen, kidney, pancreas, thymus, and bladder in rotavirus-infected animals (10). These findings suggest that rotavirus spreads beyond the intestine in children with rotavirus gastroenteritis, resulting in systemic viral infection. Recently, we found that rotavirus antigenemia was frequently observed during the acute phase of rotavirus

gastroenteritis (11). Rotavirus antigen peaked on day 2 of the illness, with the amount of viral antigen gradually decreasing to nearly undetectable levels by day 6. We also found that cytokines were involved in controlling antigenemia levels. The results of this study, together with those from previous studies (7, 8, 12, 13), suggested that host immune responses have important roles in regulating viral replication. Therefore, the kinetics of rotavirus antigenemia in transplant recipients may be different from those in immunocompetent rotavirus gastroenteritis patients. In this study, we sought to elucidate the kinetics of rotavirus antigenemia in hematopoietic stem cell transplant (HSCT) recipients. We measured rotavirus antigen levels in serum samples serially collected from pediatric HSCT recipients and analyzed the associations between antigenemia and clinical features.

Patients and methods

Patient characteristics

Between September 2004 and February 2007, 62 patients received allogeneic HSCT (17 with allogeneic bone marrow transplant [BMT] from human leukocyte antigen [HLA]-matched siblings, 9 with allogeneic BMT from HLA-mismatched siblings, 22 with allogeneic BMT from unrelated donors, 2 with peripheral blood stem cell transplants, and 12 with umbilical cord blood transplants) at the Division of Hematology-Oncology at the Children's Medical Center, the Japanese Red Cross Nagoya First Hospital, or the Department of Pediatrics at the Nagoya University Graduate School of Medicine. The patients' guardians provided written consent for their participation in this study. This study was approved by the review boards of the 3 institutes. Patient characteristics are summarized in Table 1 and include age, gender, underlying diseases, type of graft, HLA matching, having received total body irradiation (TBI) or anti-thymocyte globulin (ATG) in conditioning regimen, and occurrence of acute graft-versus-host disease (GVHD).

Patient management

Details of the conditioning regimen and GVHD prophylaxis have been previously described (14, 15). In brief, patients with hematologic malignancies were conditioned with high-dose chemotherapy consisting of melphalan (180 mg/m^2) plus busulfan (16 mg/kg) or TBI (12 Gy). Patients with severe aplastic anemia were conditioned with 200 mg/kg cyclophosphamide and 10 mg/kg rabbit ATG for transplantation from a matched sibling donor. For patients transplanted with an unrelated bone marrow donor, TBI (5–10 Gy) was added (16). GVHD prophylaxis consisted of cyclosporine or tacrolimus with short-term methotrexate. All patients received trimethoprim–sulfamethoxazole orally or inhaled pentamidine as prophylaxis against *Pneumocystis jirovecii*. The standard doses of oral amphotericin B and acyclovir were administered as prophylaxis for fungal and herpes simplex virus infections. Intravenous γ-globulin preparations were administered weekly during the first 3 months as prophylaxis for cytomegalovirus (CMV) infection. In addition, ganciclovir was given as preemptive therapy against CMV infection following a positive result from a CMV antigenemia assay. Acute and chronic GVHD was diagnosed and graded according to established criteria.

Experimental design

Serum samples were collected from 62 recipients at the time of HSCT, weekly for 3 or 4 months post transplant. Ultimately, 633 serum samples were analyzed in this study. In addition to these samples, 15 serum samples were collected from rotavirus gastroenteritis patients on days 1, 3, and 5 of illness and used as controls. Clinical data were collected retrospectively and assessed to determine associations with rotavirus antigenemia.

Rotavirus antigen detection

Rotavirus antigen was measured using a previously described enzyme-linked immunosorbent assay (ELISA) for the detection of VP6 antigen of the virus (11). Diluted serum (1:16, 50 μL) was used to detect rotavirus antigen. Ninety-six–well well plates (Nalgen Nunc, Rochester, New York, USA) coated with a monoclonal antibody against the VP6 antigen of rotavirus (YO-156) (17) were used for the ELISA (18). The plates were incubated with 50 μL of diluted serum at 4°C overnight. Then, 50 μL of anti-human rotavirus hyperimmune rabbit serum (diluted 1:5000 with phosphate buffered saline [PBS] containing 0.05% Tween-20 [PBST] and 2.5% skim milk) was added to each well. Peroxidase-conjugated donkey anti-rabbit immunoglobulin G (IgG) (diluted

Patient characteristics and risk factors for rotavirus antigenemia after hematopoietic stem cell transplantation

Categories	Rotavirus antigenemia Yes (n = 8)	No (n = 54)	Odds ratio (95% CI)	P
Age (years)	7.4 ± 5.6	8.3 ± 5.1	...	0.652
Sex				
Male	4	37	0.46 (0.10–2.06)	0.312
Female	4	17		
Underlying disease*				
Malignancy	4	36	2.0 (0.44–8.93)	0.368
Non-malignancy	4	18		
Total body irradiation				
Yes	6	41	1.05 (0.18–5.85)	0.955
No	2	13		
Anti-thymocyte globulin				
Yes	4	11	0.25 (0.05–1.19)	0.080
No	4	43		
Acute graft-versus-host disease (grade 2–4)				
Yes	3	17	0.77 (0.16–3.58)	0.705
No	5	37		
Source of the graft				
Related donor	3	23	1.0	
Unrelated donor	3	19	1.21	0.827
CBT	1	11	0.70	0.760
PBSCT	1	1	7.67	0.206
HLA matching				
Match	1	31	9.44 (1.09–82.11)	0.024
Mismatch	7	23		

*Acute lymphoblastic leukemia, 23; aplastic anemia, 12; acute myeloid leukemia 11; myelodysplastic syndrome, 4; rhabdomyosarcoma, 1; malignant lymphoma, 2; neuroblastoma, 1; others, 8.
CI, confidence interval; CBT, cord blood transplant; PBSCT, peripheral blood stem cell transplant; HLA, human leukocyte antigen.

Table 1

1:5000, Jackson ImmunoResearch Laboratory Inc., West Grove, Pennsylvania, USA) was used as a secondary antibody. Finally, the amount of rotavirus VP6 antigen bound to specific monoclonal antibody was assessed by adding substrate. The optical density (OD) was read using spectrophotometry at 492 nm, and an appropriate cut-off value was established based on data from 20 serum samples collected from control subjects. As the mean OD of the control samples was 0.084 ± 0.014, we defined 0.13 (mean + 3 standard deviations [SD]) as the baseline value in this study.

Measurement of rotavirus-specific IgG

Serum anti-rotavirus IgG antibody titer was determined using sandwich ELISA. Briefly, 96-well plates were coated with rabbit anti-rotavirus serum diluted 1:10,000 in 10 mM PBS overnight at 4°C. After the plates were washed twice with 10 mM PBS containing 0.05% PBST, then 1% bovine serum albumin in PBST was added, and the plates were incubated for 4 h at 4°C. The plates were washed twice with PBST. SA-11-infected culture fluid was then added to the plates and incubated for 1 h at 37°C. Serum samples diluted with 2.5% skim milk in

PBST were allowed to react for 1 h at 37°C. After washing 4 times with PBST, donkey anti-human IgG (H+L) conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratory Inc.) diluted 1:1000 in 2.5% skim milk in PBST was added. The plates were incubated for 1 h at 37°C, then washed 4 times with PBST, and o-phenylenediamine-2HCl substrate was added. The OD was read by spectrophotometry at 492 nm. As the mean OD of the control samples was 0.047 ± 0.026, we defined 0.124 (mean + 3 SD) as the baseline value in this study.

Statistical analyses

Statistical analyses were performed using JMP7 (SAS Institute, Cary, North Carolina, USA). A Mann–Whitney *U*-test was used to compare the levels of rotavirus antigenemia between sera collected from transplant recipients and immunocompetent rotavirus gastroenteritis patients (days 1, 3, and 5). The antigen levels in 16-fold and 4-fold diluted serum samples were compared using a Wilcoxon's signed-ranks test. The anti-rotavirus IgG antibody levels with and without rotavirus antigenemia were compared using a Student's *t*-test.

To elucidate risk factors for rotavirus antigenemia in transplant recipients, pre-transplant variables and transplant variables were compared between recipients with and without antigenemia. Pre-transplant variables included age, gender, and underlying diseases. Transplant variables included TBI, ATG, HLA matching, type of graft source, and acute GVHD. The ages of the recipients with and without rotavirus antigenemia were compared using a Student's *t*-test. Odds ratios (and 95% confidence intervals) were based on 2 × 2 contingency tables and were calculated to assess the association between rotavirus antigenemia and demographics. The significance of measurement was determined by chi-square and Fisher's exact tests.

Results

Rotavirus antigen was detected in 43 (6.8%) of 633 serum samples (8 of 62 patients). The kinetics and season of rotavirus antigenemia are shown in Figure 1. Rotavirus antigenemia lasted between 1 and 10 weeks. Rotavirus antigenemia started within 4 weeks of the transplant in all 8 recipients. Although the endemic seasons for rotavirus gastroenteritis are generally in the winter and spring in Japan, rotavirus antigenemia was observed in non-endemic periods in Cases 1, 2, 3, 4, and 7. Figure 1 also shows a temporal relationship between rotavirus antigenemia and diarrhea. Diarrhea was concurrent with rotavirus antigenemia in Cases 3, 6, 7, and 8. Meanwhile, rotavirus antigenemia persisted after the cessation of diarrhea in Cases 6 and 7. Moreover, diarrhea was not observed during rotavirus antigenemia in Cases 1, 2, and 5.

To determine whether the amount of serum rotavirus antigen was higher in HSCT recipients than in immunocompetent rotavirus gastroenteritis patients, the antigen levels were compared between the 2 groups (Fig. 2). As expected, rotavirus antigen peaked on day 3 after illness onset in the serum samples collected from immunocompetent rotavirus gastroenteritis patients. The levels of rotavirus antigenemia in the transplant recipients (0.22 ± 0.19) and day 5 serum samples collected from immunocompetent rotavirus gastroenteritis patients (0.19 ± 0.20) were similar (P = 0.9060). The level of viral antigen in the transplant recipients was significantly lower than that observed in either day 1 (0.49 ± 0.18, P = 0.0011) or day 3 (0.63 ± 0.09, P = 0.0005) of serum samples collected from immunocompetent rotavirus gastroenteritis patients. Although a remarkable peak in rotavirus antigen levels was observed in immunocompetent rotavirus gastroenteritis patients (11), no such peak was seen in the kinetics of rotavirus antigenemia in HSCT recipients (data not shown).

As rotavirus antigenemia levels were low in HSCT recipients, antigen level was measured using less dilute

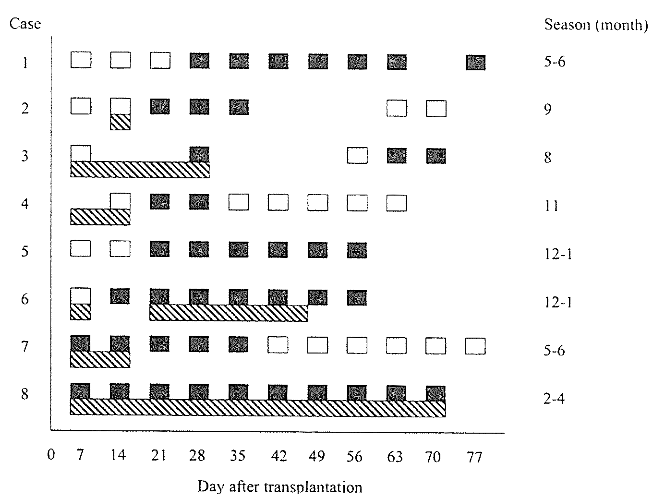

Fig. 1. Associations between rotavirus antigenemia (black boxes) and diarrhea (shaded bars) are shown. White boxes indicate antigenemia-negative serum samples.

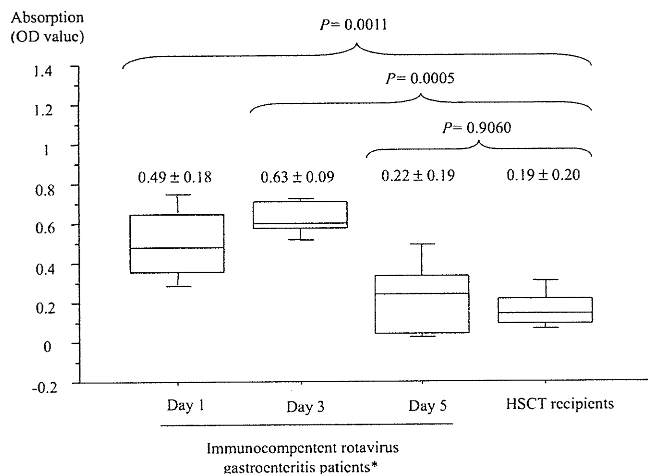

Fig. 2. Comparison of rotavirus antigen levels between immunocompetent rotavirus gastroenteritis patients and hematopoietic stem cell transplant (HSCT) recipients. *Days after onset of the illness. OD, optical density.

sera (1:4) to determine whether these positive samples contained low levels of rotavirus antigen. Forty-three antigen-positive serum samples and 40 randomly selected antigen-negative samples were used in this experiment (Fig. 3). The lower dilutions of antigen-positive serum samples (1:4) demonstrated significantly higher rotavirus antigen levels than the 1:16 diluted sera ($P < 0.0001$). However, no statistical difference was observed between 1:4 and 1:16 dilutions of antigen-negative samples ($P = 0.2733$). In addition, the immune response against rotavirus was also examined to confirm rotavirus infection; only one recipient with rotavirus antigenemia demonstrated a marked increase in rotavirus IgG antibody titers (Fig. 4).

Table 1 summarizes the results of statistical analyses identifying risk factors for rotavirus antigenemia in HSCT recipients. No statistical difference was seen between the ages of recipients with (7.4 ± 5.6 years) and without (8.3 ± 5.1 years) rotavirus antigenemia ($P = 0.652$). Moreover, neither gender ($P = 0.312$) nor underlying disease ($P = 0.368$) correlated with occurrence of rotavirus antigenemia. Of the 4 transplant-related variables, neither having received TBI ($P = 0.955$), having received ATG ($P = 0.080$), complications from acute GVHD ($P = 0.705$), nor type of graft source (related vs. unrelated; $P = 0.827$, related vs. cord blood; $P = 0.760$, related vs. peripheral blood stem cell transplant; $P = 0.206$) were associated with occurrence of rotavirus antigenemia. However, a patient who received a graft from an HLA-mismatched donor was at significant risk for rotavirus antigenemia ($P = 0.024$; odds ratio = 9.44) in comparison to patients who received a graft from an HLA-matched donor.

Discussion

Although it has been reported that rotavirus can cause severe clinical manifestations in immunocompromised transplant recipients (1–4), few studies have been conducted to elucidate the full spectrum of

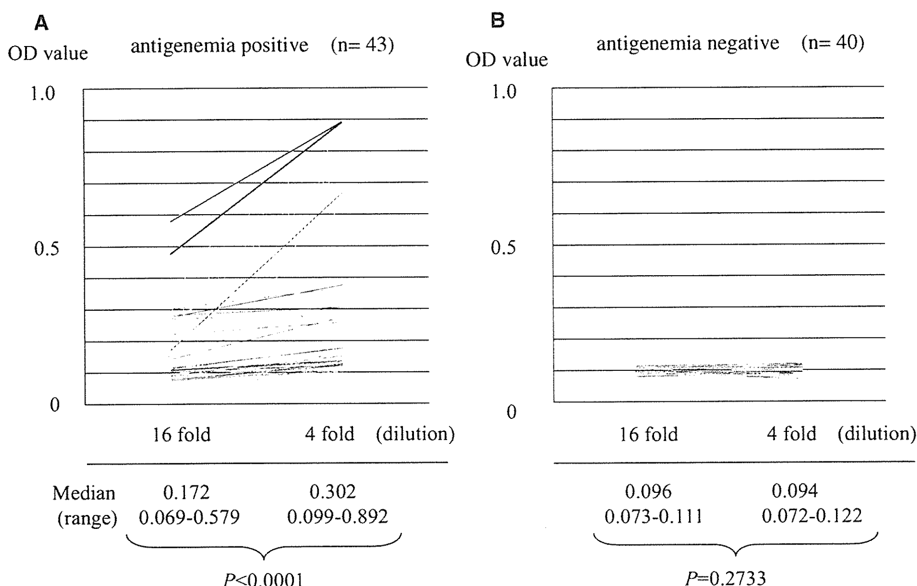

Fig. 3. Comparison of rotavirus antigen levels between 16-fold and 4-fold diluted serum samples to determine rotavirus antigenemia. OD, optical density. (A) Antigenemia positive (n = 43). (B) Antigenemia negative (n = 40).

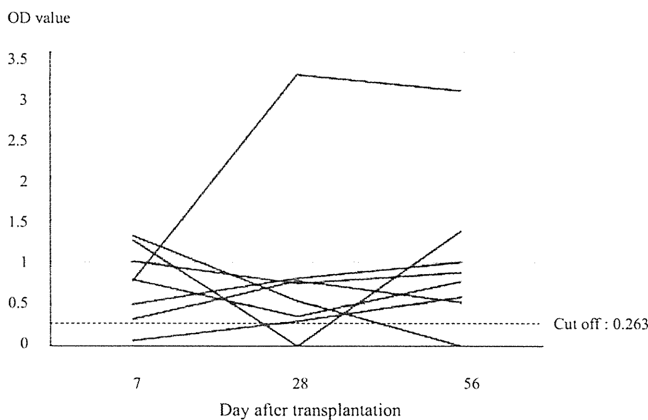

Fig. 4. Kinetics of anti-rotavirus immunoglobulin G (IgG) antibody titers in rotavirus antigenemia-positive patients (n = 8). IgG antibody titers were determined using enzyme-linked immunosorbent assay. OD, optical density.

rotavirus infection in transplant recipients (4, 19–21). In this study, rotavirus antigen was detected in 43/633 (6.8%) serum samples, and rotavirus antigenemia was observed in 8/62 (12.9%) transplant recipients. To the best of our knowledge, this is the first study that demonstrates rotavirus antigenemia in transplant recipients. Stelzmueller et al. (4) demonstrated that rotavirus infection was observed in 1.5% of solid organ transplant (SOT) recipients, and the highest frequency of rotavirus infections was observed in pediatric liver transplant recipients (52%) based on conventional rotavirus antigen detection analysis of stool samples. In addition, previous reports have identified rotavirus infection in 10–12% of pediatric BMT recipients (1, 20). Thus, although the clinical specimens used to detect rotavirus antigen were different, the frequency of antigenemia in our pediatric HSCT recipients was similar to previous studies (1, 20).

Both rotavirus-specific neutralizing antibodies and $CD8^+$ cytotoxic T cells have been shown to play important roles in terminating rotavirus infection (22–25). In addition to adaptive immunity, it has been suggested that the innate immune response is also important for protecting the host from rotaviral infection (13, 26). Thus, it is likely that rotaviral infection would be more severe in transplant recipients with severe immunosuppression than in immunocompetent children. Persistent rotavirus excretion in the stool has been documented in children with a congenital T-cell deficiency (27) and BMT recipients (5). We previously reported that the duration of rotavirus antigenemia in immunocompetent rotavirus gastroenteritis patients was short (11). Our present study suggests that rotavirus antigenemia persists for a longer period in transplant recipients, and this corresponds to rotavirus excretion in the stool (5).

To confirm specificity of the low levels of antigenemia in transplant recipients, antigen levels were measured in less diluted serum samples (Fig. 3). As less dilute (1:4) sera demonstrated statistically higher levels of antigen than that of a higher dilution (1:16), we considered that serum demonstrating low level of antigen really contained low levels of rotavirus antigen. Ray et al. (8) and Blutt et al. (9) reported that serum rotavirus antigen levels were negatively associated with rotavirus antibody levels in children with rotavirus antigenemia. Moreover, serum rotavirus antigen levels were significantly lower in patients who had a subsequent infection than in those with primary infection (7). The ages of the patients in this study ranged between 8 months and 23 years old. Thus, most of the recipients in this study could have previously had a primary rotavirus infection, which may have caused their lower levels of rotavirus antigen. Further detailed immunologic analysis of rotavirus infection is needed to clarify the pathogenesis of the characteristic kinetics of rotavirus antigenemia (low levels and long duration) observed in HSCT recipients.

Seven of 8 recipients with rotavirus antigenemia failed to demonstrate an antibody response against rotavirus. There are 2 possible explanations for such a low immune response rate. One is low immunogenicity of the low levels of antigenemia, and another is severe immunosuppression in transplant recipients. Although it is not clear whether positive antigenemia indicates active rotaviral infection, a serological assay is insufficient for monitoring rotavirus antigenemia in HSCT recipients.

It has been suggested that rotavirus can cause severe diarrhea (1, 3–5) and toxic megacolon (28), and may be confused with enteric GVHD (29), which results in significant morbidity in transplant recipients. As shown in Figure 1, persistent diarrhea and rotavirus antigenemia were concurrent in Cases 3, 6, 7, and 8. Meanwhile, rotavirus antigenemia persisted for a few weeks after the resolution of diarrhea in Cases 3, 6, and 7. Diarrhea was not observed in the 2 cases with persistent rotavirus antigenemia. Although it would be difficult to prove an association between rotavirus antigenemia and persistent diarrhea, because no complete examinations were carried out to exclude all other pathogens that would cause diarrhea, the current findings suggest that rotavirus antigenemia may be involved in the persistent diarrhea in HSCT recipients in some recipients. It was difficult to determine how many patients without rotavirus antigenemia had

diarrhea during the observation period following transplantation, because this study was a retrospective study using stored serum samples. Therefore, future prospective study is needed to elucidate the precise association between rotavirus antigenemia and diarrhea.

Moreover, asymptomatic rotavirus antigenemia was demonstrated in these patients. Asymptomatic rotavirus excretion in stool has also been reported in HSCT recipients, which could potentially make them index cases for nosocomial rotavirus infections (19). Thus, it is important to elucidate the ability of virus to be transmitted from asymptomatic rotavirus antigenemia patients. A prospective study that concurrently monitors rotavirus antigenemia and viral excretion in the stool is currently underway.

Notably, the timing after transplantation and occurrence of rotavirus antigenemia in non-endemic seasons in HSCT recipients were quite different from expected. According to a previous study based on detecting rotavirus antigen in stool, the median duration of rotavirus infection is 20 days after SOT (4). In our study, rotavirus antigenemia started within 4 weeks after transplant in all 6 cases except for Cases 7 and 8. Six of the 8 recipients were cared for in laminar airflow rooms at the beginning of rotavirus antigenemia. Although medical personnel may have caused nosocomial transmission, the likelihood of this possibility is very low, because standard precautions were thoroughly followed. Kang et al. (20) detected rotavirus antigen in the stool of an HSCT recipient at the time of pre-transplant screening. From a clinical standpoint, a large-scale molecular epidemiological study is needed to elucidate the route of viral transmission in HSCT recipients. In addition to the timing of rotavirus antigenemia, the seasons in which the rotavirus antigenemia occurred are another remarkable finding in this study. Although the endemic seasons in Japan for rotavirus infection are the winter and spring, rotavirus antigenemia was observed in the summer and fall in several cases (Fig. 1). Recently, a similar finding was demonstrated in SOT recipients (4). One possible mechanism for rotavirus antigenemia outside of its endemic season is the persistence of rotavirus infections in immunocompromised patients. Further human or animal studies are necessary to determine whether rotavirus can persistently infect a host.

We believe that this is the first report to demonstrate HLA mismatches as a significant risk factor for rotavirus antigenemia after HSCT, which is similar to Epstein–Barr virus (EBV) infection (30). ATG administration, which is another well-known risk factor for EBV infection after HSCT (30), tended to be a risk for rotavirus antigenemia, though it did not reach the level of statistical significance. Several characteristic factors have been suggested to pose significant risks for each viral infection in HSCT recipients. The present study suggests that the risk factors for rotavirus antigenemia are similar to those for EBV infection after HSCT. If the clinical significance of rotavirus antigenemia in HSCT recipients is confirmed, predictions about patients at high risk for rotavirus antigenemia would be important for improving their prognosis. Further clinical analysis of rotavirus antigenemia should be continued to determine the significance of rotavirus antigenemia on the morbidity or mortality of HSCT recipients.

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References

1. Yolken RH, Bishop CA, Townsend TR, et al. Infectious gastroenteritis in bone-marrow-transplant recipients. N Engl J Med 1982; 306: 1010–1012.
2. Willoughby RE, Wee SB, Yolken RH. Non-group A rotavirus infection associated with severe gastroenteritis in a bone marrow transplant patient. Pediatr Infect Dis J 1988; 7: 133–135.
3. Kanfer EJ, Abrahamson G, Taylor J, Coleman JC, Samson DM. Severe rotavirus-associated diarrhoea following bone marrow transplantation: treatment with oral immunoglobulin. Bone Marrow Transplant 1994; 14: 651–652.
4. Stelzmueller I, Wiesmayr S, Swenson BR, et al. Rotavirus enteritis in solid organ transplant recipients: an underestimated problem? Transpl Infect Dis 2007; 9: 281–285.
5. Liakopoulou E, Mutton K, Carrington D, et al. Rotavirus as a significant cause of prolonged diarrhoeal illness and morbidity following allogeneic bone marrow transplantation. Bone Marrow Transplant 2005; 36: 691–694.
6. Blutt SE, Kirkwood CD, Parreno V, et al. Rotavirus antigenaemia and viraemia: a common event? Lancet 2003; 362: 1445–1449.
7. Fischer TK, Ashley D, Kerin T, et al. Rotavirus antigenemia in patients with acute gastroenteritis. J Infect Dis 2005; 192: 913–919.
8. Ray P, Fenaux M, Sharma S, et al. Quantitative evaluation of rotaviral antigenemia in children with acute rotaviral diarrhea. J Infect Dis 2006; 194: 588–593.
9. Blutt SE, Matson DO, Crawford SE, et al. Rotavirus antigenemia in children is associated with viremia. PLoS Med 2007; 4: e121.
10. Crawford SE, Patel DG, Cheng E, et al. Rotavirus viremia and extraintestinal viral infection in the neonatal rat model. J Virol 2006; 80: 4820–4832.

11. Sugata K, Taniguchi K, Yui A, et al. Analysis of rotavirus antigenemia and extraintestinal manifestations in children with rotavirus gastroenteritis. Pediatrics 2008; 122: 392–397.
12. Feng N, Kim B, Fenaux M, et al. Role of interferon in homologous and heterologous rotavirus infection in the intestines and extraintestinal organs of suckling mice. J Virol 2008; 82: 7578–7590.
13. Aich P, Wilson HL, Kaushik RS, Potter AA, Babiuk LA, Griebel P. Comparative analysis of innate immune responses following infection of newborn calves with bovine rotavirus and bovine coronavirus. J Gen Virol 2007; 88: 2749–2761.
14. Azuma E, Kojima S, Kato K, et al. Conditioning with cyclophosphamide/antithymocyte globulin for allogeneic bone marrow transplantation from HLA-matched siblings in children with severe aplastic anemia. Bone Marrow Transplant 1997; 19: 1085–1087.
15. Matsuyama T, Horibe K, Kato K, Kojima SS. Bone marrow transplantation for children with acute myelogenous leukaemia in the first complete remission. Eur J Cancer 2000; 36: 368–375.
16. Kojima S, Inaba J, Yoshimi A, et al. Unrelated donor marrow transplantation in children with severe aplastic anaemia using cyclophosphamide, anti-thymocyte globulin and total body irradiation. Br J Haematol 2001; 114: 706–711.
17. Taniguchi K, Urasawa T, Urasawa S, Yasuhara T. Production of subgroup-specific monoclonal antibodies against human rotaviruses and their application to an enzyme-linked immunosorbent assay for subgroup determination. J Med Virol 1984; 14: 115–125.
18. Taniguchi K, Urasawa T, Morita Y, Greenberg HB, Urasawa S. Direct serotyping of human rotavirus in stools by an enzyme-linked immunosorbent assay using serotype 1-, 2-, 3-, and 4-specific monoclonal antibodies to VP7. J Infect Dis 1987; 155: 1159–1166.
19. van Kraaij MG, Dekker AW, Verdonck LF, et al. Infectious gastro-enteritis: an uncommon cause of diarrhoea in adult allogeneic and autologous stem cell transplant recipients. Bone Marrow Transplant 2000; 26: 299–303.
20. Kang G, Srivastava A, Pulimood AB, Dennison D, Chandy M. Etiology of diarrhea in patients undergoing allogeneic bone marrow transplantation in South India. Transplantation 2002; 73: 1247–1251.
21. Kamboj M, Mihu CN, Sepkowitz K, Kernan NA, Papanicolaou GA. Work-up for infectious diarrhea after allogeneic hematopoietic stem cell transplantation: single specimen testing results in cost savings without compromising diagnostic yield. Transpl Infect Dis 2007; 9: 265–269.
22. Franco MA, Greenberg HB. Immunity to rotavirus infection in mice. J Infect Dis 1999; 179 (Suppl 3): S466–S469.
23. Franco MA, Tin C, Rott LS, VanCott JL, McGhee JR, Greenberg HB. Evidence for CD8 + T-cell immunity to murine rotavirus in the absence of perforin, fas, and gamma interferon. J Virol 1997; 71: 479–486.
24. Green KY, Kapikian AZ. Identification of VP7 epitopes associated with protection against human rotavirus illness or shedding in volunteers. J Virol 1992; 66: 548–553.
25. Ward RL. Mechanisms of protection against rotavirus in humans and mice. J Infect Dis 1996; 174(Suppl 1): S51–S58.
26. Barro M, Patton JT. Rotavirus nonstructural protein 1 subverts innate immune response by inducing degradation of IFN regulatory factor 3. Proc Natl Acad Sci U S A 2005; 102: 4114–4119.
27. Wood DJ, David TJ, Chrystie IL, Totterdell B. Chronic enteric virus infection in two T-cell immunodeficient children. J Med Virol 1988; 24: 435–444.
28. Stelzmueller I, Dunst KM, Hengster P, et al. A cluster of rotavirus enteritis in adult transplant recipients. Transpl Int 2005; 18: 470–474.
29. Troussard X, Bauduer F, Gallet E, et al. Virus recovery from stools of patients undergoing bone marrow transplantation. Bone Marrow Transplant 1993; 12: 573–576.
30. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood 1999; 94: 2208–2216.

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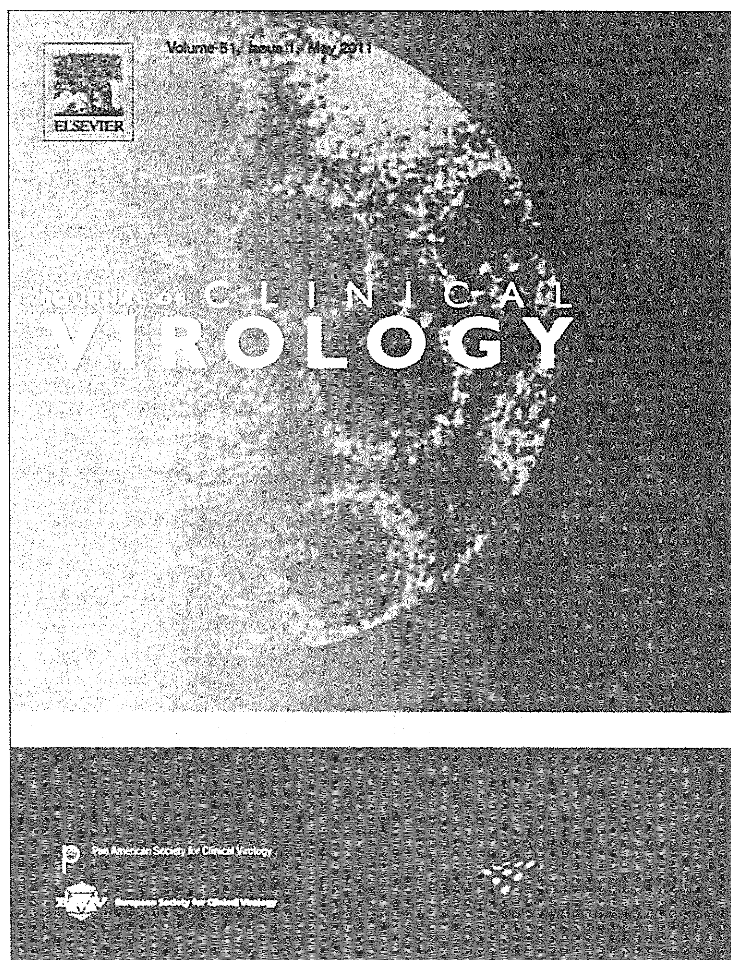

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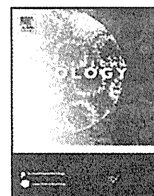

Different characteristics of human herpesvirus 6 encephalitis between primary infection and viral reactivation

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ABSTRACT

Background: Pathogenesis of human herpesvirus 6 (HHV-6) encephalitis, in particular difference between HHV-6 encephalitis at the time of primary infection and reactivation remains unclear.
Objectives: To elucidate the mechanism of HHV-6 encephalitis at the time of primary infection and reactivation.
Study design: Twenty-two HHV-6 encephalitis patients at the time of primary infection, 6 febrile convulsion (FC) patients caused by HHV-6 infection, and 14 FC patients without HHV-6 infection (non HHV-6 FC) were enrolled. Additionally, 7 stem cell transplant recipients with HHV-6 encephalitis and eight adult controls were also enrolled in this study. Cerebrospinal fluid (CSF) HHV-6 DNA copy numbers and biomarkers levels were compared.
Results: Low copy number of CSF HHV-6 DNA was detected in 7 of the 22 patients with HHV-6 encephalitis in primary infection, whereas all seven CSF samples collected from post-transplant HHV-6 encephalitis patients contained high viral DNA copy numbers ($P<0.001$). CSF concentrations of IL-6 ($P=0.032$), IL-8 ($P=0.014$), MMP-9 ($P=0.004$), and TIMP-1 ($P=0.002$) were significantly higher in patients with HHV-6 encephalitis in primary infection than non-HHV-6 FC. CSF IL-6 ($P=0.008$), IL-8 ($P=0.015$), and IL-10 ($P=0.019$) concentrations were significantly higher in patients with post-transplant HHV-6 encephalitis than adult controls.
Conclusion: The present study suggests that the characteristics of HHV-6 encephalitis are different between HHV-6 encephalitis at the time of primary infection and reactivation in transplant recipients.
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1. Background

Primary human herpesvirus 6 (HHV-6) B infection (exanthem subitum)[1,2] is considered a benign febrile illness that rarely causes neurological complications such as febrile convulsion and encephalitis. According to a recent survey,[3] an annual incidence of exanthem subitum-associated encephalitis/encephalopathy was estimated at 60 cases per year in Japan, and the prognosis of the disease was unexpectedly poor. In addition to primary infection, it has been suggested that HHV-6 reactivation can cause encephalitis in transplant recipients[4–8] and patients with drug-induced hypersensitivity syndrome.[9–11]

Highly sensitive nested polymerase chain reaction (PCR) detected HHV-6 DNA in cerebrospinal fluid (CSF) collected from HHV-6 encephalitis patients[12–14] and exanthem subitum patients with febrile convulsion,[15] suggesting direct invasion of the virus into central nervous system (CNS). Furthermore, both viral DNA and viral antigens were detected in the postmortem brain tissues[16] and brain tissues collected from mesial temporal lobe epilepsy patients.[17] In addition, HHV-6 also infects neuroglial cell lines and modulates cytokine synthesis in HHV-6-infected an astrocytoma cell line.[18] Collectively, these findings highlight the neurovirulence of HHV-6. However, the pathogenesis of HHV-6 encephalitis, in particular the difference between HHV-6 encephalitis at the time of primary infection and viral reactivation remains unclear.

2. Objective

In the present study, we assessed viral copy number and expression of several biomarkers in CSF samples collected from HHV-6 encephalitis patients to elucidate the mechanism of

Abbreviations: HHV-6, human herpesvirus 6; PCR, polymerase chain reaction; CSF, cerebrospinal fluid; CNS, central nervous system; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

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Table 1
Characteristics of the patients.

	HHV-6 encephalitis at primary inf. (n=22)	FC with HHV-6 inf. (n=6)	FC w/o HHV-6 inf. (n=14)	Post-transplant HHV-6 encephalitis (n=7)	Adult controls (n=8)
Mean age ± SD (years old)	0.7 ± 0.8	0.8 ± 0.8	1.1 ± 0.6	43.9 ± 15.2	66.9 ± 11.0
Gender (M/F)	11/11	2/4	4/10	5/2	4/4

FC; febrile convulsion, inf.; infection.
Adults controls: 2 patients with involuntary movement, 2 patients with spinocerebellar degeneration, 1 patient with frontotemporal dementia, 1 patient with normal pressure hydrocephalus, 1 patient with progressive supranuclear palsy, 1 patient with Parkinson disease.

HHV-6 encephalitis at the time of primary infection and reactivation.

3. Study design

3.1. Patients

Twenty-two patients with HHV-6 encephalitis at the time of primary infection (HHV-6 encephalitis at primary infection) were enrolled. Thirteen of the 22 patients had convulsions and became comatose within a few days after the onset of fever, and 9 of the 22 patients had cluster convulsions at the eruptive phase. As control groups, six patients with febrile convulsion due to primary HHV-6 infection (HHV-6 FC) and 14 patients with febrile convulsion without HHV-6 infection (non HHV-6 FC) were also examined. The viral reactivation group included seven allogeneic hematopoietic stem cell transplant recipients with HHV-6 encephalitis. Eight adult control patients were enrolled. Patient demographics are summarized in Table 1. This study was approved by the review boards of Fujita Health University. Guardians or the patients consented to their participation in this study.

Primary HHV-6 infection was defined on the basis of viral isolation or detection of viral DNA in serum and serological analysis. HHV-6 encephalitis in primary infection was defined as the patients with stupor and/or convulsion at the time of primary HHV-6 infection. To exclude patients with a severe form of febrile convulsion, abnormal radiological examinations were required for diagnosis of HHV-6 encephalitis in this study. Reactivation of HHV-6 was confirmed by a presence of HHV-6 IgG antibody and either detection of viral DNA in serum or viral isolation from peripheral blood at the time of disease onset in transplant recipients. All seven post-transplant HHV-6 encephalitis patients had typical clinical symptoms and radiological findings for limbic encephalitis.

3.2. Measurement of CSF cytokines, MMP-9 and TIMP-1

CSF samples were collected from patients within 48 h of the onset of neurological symptoms and stored at −70 °C. The concentrations of interleukin (IL)-8, IL-1β, IL-6, IL-10, TNF-α, and IL-12p70 in CSF were determined using the cytometric bead array kit according to the manufacturer's instructions (BD Biosciences, CA, USA). Matrix metalloproteinase (MMP)-9 (pro- and active forms) concentrations in the CSF (1:5 dilution) were determined by enzyme-linked immunosorbent assay (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions. Tissue inhibitor of metalloproteinase (TIMP)-1 concentration in CSF (1:8 dilution) was also determined by enzyme-linked immunosorbent assay (Invitrogen, CA, USA).

3.3. DNA extraction and real-time PCR

DNA was extracted from 200 μl of CSF or serum using QIAamp Blood Kit (QIAGEN, Chatsworth, CA) and eluted in 50 μl of elution buffer, then stored at −20 °C. The details of the real-time PCR methods for measuring viral DNA copy numbers have been described elsewhere.[19] Detection limit of the real-time PCR was 125 copies/ml.

3.4. Statistical analysis

CSF viral DNA copy number or concentration of the biomarkers between the two different groups of the patients were compared: (1) HHV-6 encephalitis in primary infection vs HHV-6 FC; (2) HHV-6 encephalitis in primary infection vs non-HHV-6 FC; (3) HHV-6 FC vs non-HHV-6 FC; (4) HHV-6 encephalitis in primary infection without neurological sequelae vs HHV-6 encephalitis in primary infection with neurological sequelae; (5) HHV-6 encepalitis in primary infection vs post-transplant HHV-6 encephalitis; and (6) post-transplant HHV-6 encephalitis vs adult control. The statistical comparison of age and gender was evaluated by Student's t-test and Fisher's exact test, respectively. The statistical comparison of CSF biomarkers levels was evaluated by Mann–Whitney U-test. $P < .05$ was considered to be statistically significant. The statistical analysis was performed with StatView software, version J-5.0.

4. Results

4.1. Patients

As shown in Table 1, there was no significant age and gender difference among 22 patients with HHV-6 encephalitis in primary infection, 6 patients of HHV-6 FC, and 14 patients of non-HHV-6 FC. Although only one encephalitis patient had mild pleocytosis (13 cell/μl), CSF cell counts were normal in the remaining 19 patients. Seven of the 22 HHV-6 encephalitis patients with primary infection had severe neurological sequelae such as hemiplegia (2 cases), quadriplegia (2 cases), and mental retardation (3 cases). The 13 patients revealed no severe neurological sequelae. No detail information about sequelae was available from remaining the two cases. All seven post-transplant HHV-6 encephalitis patients were adult. Although no statistical gender difference was demonstrated between post-transplant encephalitis patients and adult control (P = 0.608), mean age of the controls (66.9 ± 11.0 years) was significantly higher than post-transplant HHV-6 encephalitis patients (43.9 ± 15.2 years) (P = 0.008). In order to exclude possibility for chromosomal integration of the HHV-6 genome in post-transplant HHV-6 encephalitis patients, copy numbers of HHV-6 DNA was measured in serum or CSF samples collected after antiviral treatment. CSF HHV-6 DNA decreased to undetectable level in 3 of the 7 patients. No viral DNA in serum was demonstrated in the remaining 4 recipients (data not shown). These results suggested that the 7 post-transplant HHV-6 encephalitis patients were not chromosomal integrated HHV-6 patients.

4.2. HHV-6 DNA copy numbers in CSF

Detection rate and copy numbers of HHV-6 DNA in CSF samples are shown in Table 2. Small amounts of HHV-6 DNA was detected in CSF from 7 of the 22 patients (31.8%) with HHV-6 encephalitis in primary infection (median, interquartile range

Table 2
Copy numbers and detection rate of HHV-6 DNA in cerebrospinal fluid.

	Number of positive cases/ number of tested (%)	Copy numbers of HHV-6 DNA; mean ± SD (copies/ml)	P-value
HHV-6 encephalitis at primary infection	7/22 (31.8)	13.22 ± 39.16	a, c, d
HHV-6 FC	1/6 (16.7)	48.75 ± 119.41	a, b
Non HHV-6 FC	0/14 (0)	0 ± 0	b, c
Post-transplant HHV-6 encephalitis	7/7 (100)	464090.4 ± 1185622.0	d, e
Adult controls	0/8 (0)	0 ± 0	e

(a) Comparison between patients with HHV-6 encephalitis at primary infection and those with HHV-6 FC (P=0.648). (b) Comparison between patients with HHV-6 FC and those with non-HHV-6 FC (P=0.127). (c) Comparison between patients with HHV-6 encephalitis at primary infection and those with non-HHV-6 FC (P=0.021). (d) Comparison between patients with HHV-6 encephalitis at primary infection and those with post-transplant HHV-6 encephalitis (P<0.001). (e) Comparison between patients with post-transplant HHV-6 encephalitis and adult controls (P<0.001).

(IQR); 0, 0–3.8 copies/ml), and in 1 of the 6 patients (16.7%) with HHV-6 FC (292.5 copies/ml). However, no HHV-6 DNA was detected from CSF in any patients with non HHV-6 FC (control group). In contrast to HHV-6 encephalitis in primary infection, all seven CSF samples collected from post-transplant HHV-6 encephalitis contained high copy of HHV-6 DNA (median, IQR; 1820.0, 848.3–46120.8 copies/ml), which was significantly higher than the patients with HHV-6 encephalitis at primary infection (P<0.001).

4.3. CSF concentrations of cytokines, MMP-9, and TIMP-1

Comparison of the biomarkers among HHV-6 encephalitis at primary infection, HHV-6 FC, and non-HHV-6 FC is presented in Fig. 1. CSF IL-8 concentrations were significantly higher in patients with HHV-6 encephalitis at primary infection (median, IQR; 291.0, 115.1–801.6 pg/ml) than those with HHV-6 FC (median, IQR; 63.5, 56.2–173.2 pg/ml, P=0.025) or those with non-HHV-6 FC (median, IQR; 105.5, 77.6–196.8 pg/ml, P=0.014). CSF IL-6 concentrations were also significantly higher in patients with HHV-6 encephalitis at primary infection (median, IQR; 22.6, 9.2–64.0 pg/ml) than those with non-HHV-6 FC (median, IQR; 10.7, 7.9–16.5 pg/ml, P=0.032). Moreover, the concentration of MMP-9 (median, IQR; 7.9, 7.1–8.3 pg/ml) and TIMP-1 (median, IQR; 110.2, 87.0–153.3 pg/ml) was significantly higher in patients with HHV-6 encephalitis at primary infection than those with non-HHV-6 FC (MMP-9, median, IQR; 6.3, 4.7–7.3 pg/ml, P=0.004; TIMP-1, median, IQR; 55.2, 47.9–77.5 pg/ml, P=0.002). Additionally, HHV-6 encephalitis in primary infection patients had a significantly higher concentration of TIMP-1 (median, IQR; 110.2, 87.0–153.3 pg/ml) than patients with HHV-6 FC (median, IQR; 71.6, 65.9–82.1 pg/ml, P=0.025).

Interestingly, CSF IL-6 concentrations were significantly higher in patients with HHV-6 encephalitis at primary infection with neurological sequelae (median, IQR; 29.7, 22.6–127.4 pg/ml) than those without neurological sequelae (median, IQR; 13.3, 5.5–25.6 pg/ml, P=0.017) (Fig. 2). No other biomarkers were significantly different between the two groups.

Next, concentrations of CSF biomarkers were compared between post-transplant HHV-6 encephalitis patients and adult controls or patients with HHV-6 encephalitis at primary infection (Fig. 3). CSF IL-8 (median, IQR; 200.0, 107.1–316.5 pg/ml vs 60.3, 41.7–74.6 pg/ml; P=0.015), IL-6 (median, IQR; 23.8, 7.8–143.3 pg/ml vs 2.7, 2.3–4.1 pg/ml; P=0.008), and IL-10 (median, IQR; 2.5, 0.9–5.1 pg/ml vs 0.0, 0.0–0.0 pg/ml; P=0.019) concentrations were significantly higher in post-transplant HHV-6 encephalitis than adult controls. No statistical difference was observed in all of the biomarkers between post-transplant HHV-6 encephalitis patients and HHV-6 encephalitis at primary infection patients. Although CSF MMP-9 concentrations were not significantly different between post-transplant HHV-6 encephalitis patients and adult controls, MMP-9 concentrations were significantly lower in post-transplant HHV-6 encephalitis (median, IQR; 4.2, 3.6–6.0 pg/ml) than HHV-6 encephalitis at primary infection (median, IQR; 7.9, 7.1–8.3 pg/ml; P=0.01). In contrast to MMP-9, its inhibitor, CSF TIMP-1 was significantly higher in post-transplant HHV-6 encephalitis patients (median, IQR; 207.4, 180.1–221.8 pg/ml) than either adult controls (median, IQR; 77.7, 66.8–95.0 pg/ml; P=0.001) or HHV-6 encephalitis at primary infection (median, IQR; 110.2, 87.0–153.3 pg/ml; P=0.001).

5. Discussion

Quantification of viral DNA copy number in CSF using real-time PCR provided valuable information regarding the pathogenesis of HHV-6 encephalitis. Although high copy numbers of HHV-6 DNA have been detected in CSF collected from post-transplant HHV-6 encephalitis,[8,17,20,21] to our knowledge, this is the first report demonstrating a difference in CSF HHV-6 DNA copy numbers between HHV-6 encephalitis in primary infection and post-transplant HHV-6 encephalitis patients using same real-time PCR method. These data suggest that replication of HHV-6 in the CNS plays an important role in the pathogenesis of post-transplant HHV-6 encephalitis. However, it seems that direct viral replication in the brain tissue is not main pathological mechanism for HHV-6 encephalitis in primary infection. Thus, the pathogenesis of HHV-6 encephalitis would be different between primary infection and reactivation in transplant recipients.

Elevation of two (IL-6 and IL-8) of the six cytokines in CSF was striking in patients with HHV-6 encephalitis in primary infec-

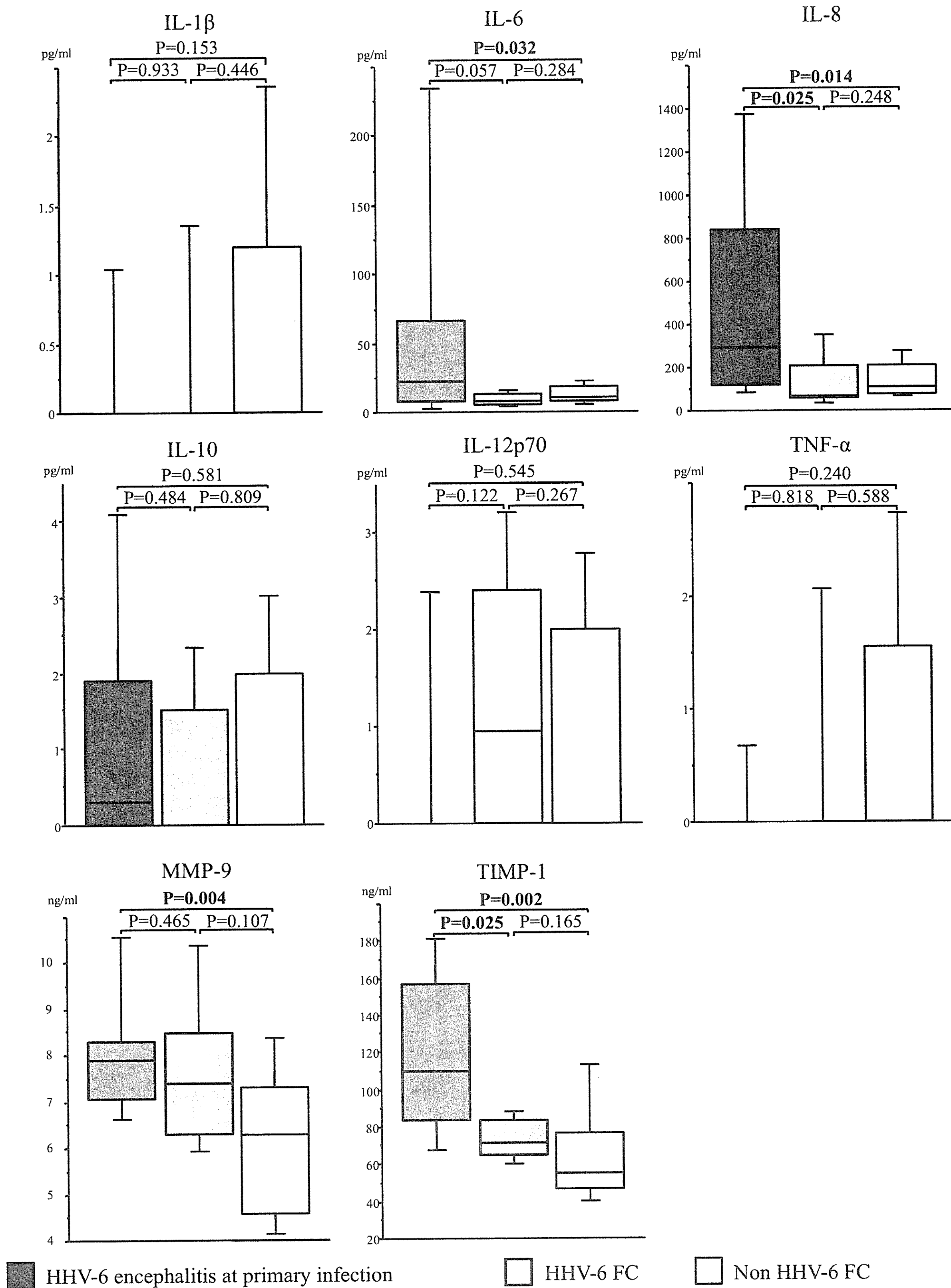

Fig. 1. Comparison of the eight cerebrospinal fluid (CSF) biomarkers (IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, MMP-9, and TIMP-1) levels among patients with HHV-6 encephalitis in primary infection (n = 22), febrile convulsion due to primary HHV-6 infection (HHV-6 FC) (n = 6), and febrile convulsion without HHV-6 infection (non HHV-6 FC) (n = 19). Data are shown as box plots, where the boxes represent the first through third quartiles, the lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values.

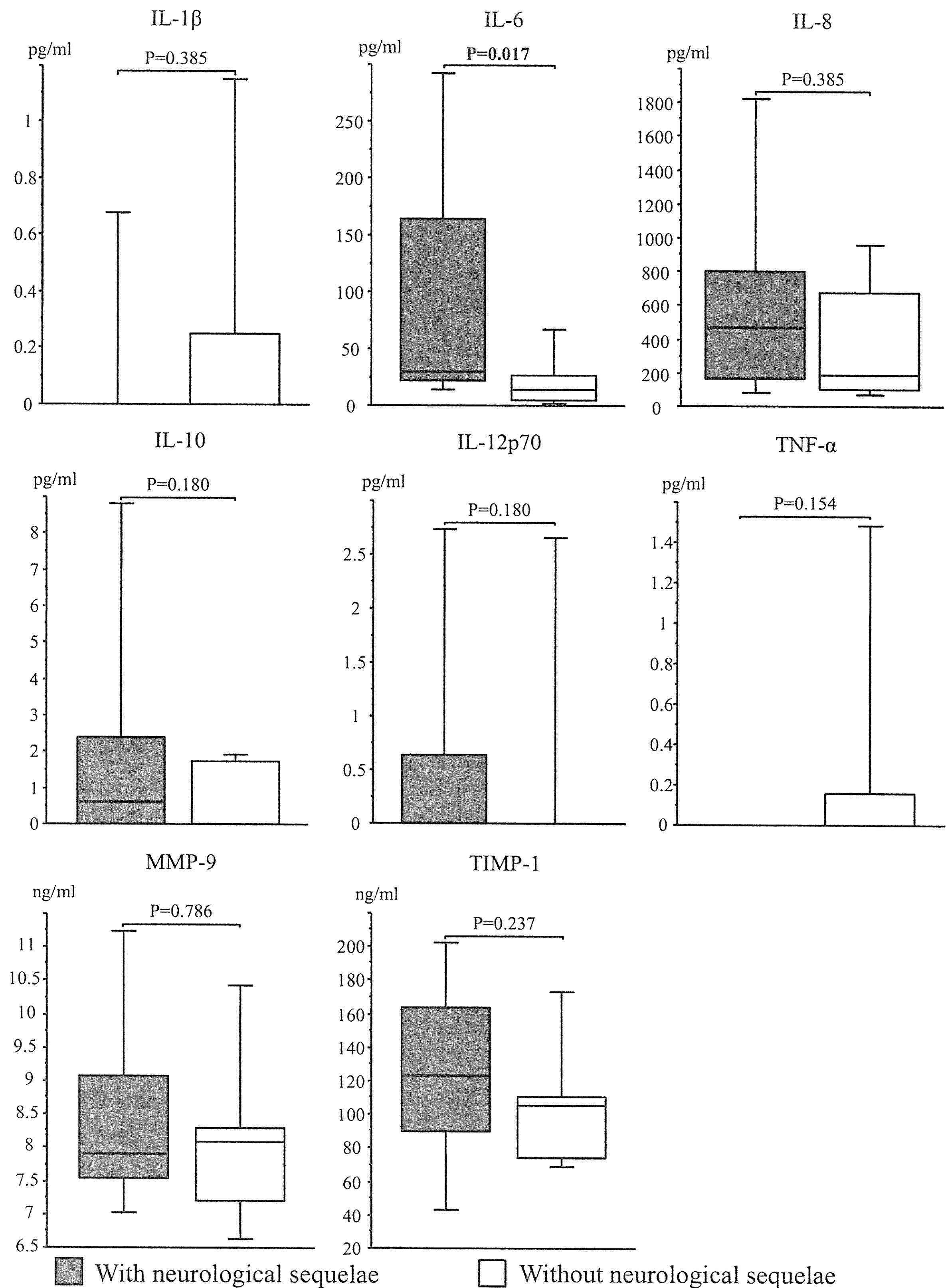

Fig. 2. Comparison of the eight cerebrospinal fluid (CSF) biomarkers (IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, MMP-9, and TIMP-1) levels between HHV-6 encephalitis patients (at primary infection) with neurological sequelae ($n = 7$) and those without neurological sequelae ($n = 13$). Data are shown as box plots, where the boxes represent the first through third quartiles, the lines within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values.

tion. Recently, Ichiyama et al.[22] reported similar findings in HHV-6 encephalitis in primary infection. These findings suggest that IL-6 may play an important role in the pathogenesis of HHV-6 encephalitis in primary infection. Furthermore, significantly higher concentration of CSF IL-6 was observed in HHV-6 encephalitis in primary infection with severe neurological sequelae. Similar observations have been demonstrated in herpes simplex virus encephalitis patients[23] and Japanese encephalitis patients.[24]