Journal of Medical Virology 83:702–709 (2011)

High Incidence of Cytomegalovirus, Human Herpesvirus-6, and Epstein–Barr Virus Reactivation in Patients Receiving Cytotoxic Chemotherapy for Adult T Cell Leukemia

Masao Ogata,[1,2]* Takako Satou,[2] Rie Kawano,[2] Tetsushi Yoshikawa,[3] Junji Ikewaki,[2] Kazuhiro Kohno,[2] Takeaki Ando,[2] Yasuhiko Miyazaki,[4] Eiichi Ohtsuka,[4] Yoshio Saburi,[4] Hiroshi Kikuchi,[5] Tetsunori Saikawa,[1] and Jun-ichi Kadota[2]

[1]*Blood Transfusion Center, Oita University Hospital, Oita, Japan*
[2]*Department of Hematology, Oita University Faculty of Medicine, Oita, Japan*
[3]*Department of Pediatrics, Fujita Health University School of Medicine, Aichi, Japan*
[4]*Department of Hematology, Oita Prefectural Hospital, Oita, Japan*
[5]*Nishibeppu National Hospital, Oita, Japan*

The etiology of cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), and Epstein–Barr virus (EBV) reactivation and the potential for complications following cytotoxic chemotherapy in the absence of allogeneic transplantation are not clearly understood. Patients with adult T cell leukemia (ATL) are susceptible to opportunistic infections. In this study, the incidence, kinetics and clinical significance of reactivation of CMV, HHV-6, and EBV in ATL patients were investigated. Viral DNA in a total of 468 plasma samples from 34 patients was quantified using real-time PCR. The probability of CMV, HHV-6, and EBV reactivation by 100 days after the start of chemotherapy was 50.6%, 52.3%, and 21.6%, respectively. Although most CMV reactivations were self-limited, plasma CMV DNA tended to persist or increase if the CMV DNA levels in plasma reached $\geq 10^4$ copies/ml. CMV reactivation was negatively associated with survival, but the *P*-value for this association was near the borderline of statistical significance ($P = 0.052$). One patient developed fatal interstitial pneumonia concomitant with peak CMV DNA accumulation (1.6×10^6 copies/ml plasma). Most HHV-6 and EBV reactivations were self-limited, and no disease resulting from HHV-6 or EBV was confirmed. HHV-6 and EBV reactivation were not associated with reduced survival ($P = 0.35$ and 0.11, respectively). These findings demonstrated that subclinical reactivation of CMV, HHV-6, and EBV were common in ATL patients receiving chemotherapy. There were differences in the viral reactivation patterns among the three viruses. A CMV load $\geq 10^4$ copies/ml plasma was indicative of subsequent exacerbation of CMV reactivation and developing serious clinical course. ***J. Med. Virol. 83:702–709, 2011.***

KEY WORDS: ATL; CMV; HHV-6; EBV; real-time PCR

INTRODUCTION

Cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), and Epstein–Barr virus (EBV) are ubiquitous in the human population. More that 90% of Japanese individuals over the age of 50 have antibodies against CMV or EBV [Takeda et al., 2001]. HHV-6 infects virtually all individuals during childhood [Zerr et al., 2005a]. These herpesviruses establish latency after primary infection and can reactivate under immunosuppressive condition. Reactivation of CMV, HHV-6, and/or EBV is common after solid-organ or hematopoietic stem cell transplantation and is linked to various serious clinical diseases [Boeckh et al., 1996; Shapiro et al., 1999; Yoshikawa et al., 2002; Zerr et al., 2005b; Ogata et al., 2006], and it is common practice to treat CMV or EBV reactivation in recipients of stem cell transplants [Meerbach et al., 2008; Boeckh and

Grant sponsor: Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant-in-aid for scientific research); Grant number: 17015047.

*Correspondence to: Masao Ogata, Blood Transfusion Center, Oita University Hospital, Hasama-machi, Oita 879-5593, Japan. E-mail: mogata@med.oita-u.ac.jp

Accepted 10 November 2010
DOI 10.1002/jmv.22013
Published online in Wiley Online Library (wileyonlinelibrary.com).

Ljungman, 2009; Omar et al., 2009]. CMV is increasingly recognized as a significant pathogen in patients receiving only chemotherapy without transplants. Han [2007] reported that a high portion of non-transplant patients with lymphoid malignancies (13.6%) were positive for CMV antigenemia. Nguyen et al. [2001] reported that the frequency of CMV pneumonia has been increasing in adults with leukemia who have not undergone transplantation.

Adult T cell leukemia (ATL) is an aggressive T cell malignancy caused by a retrovirus, human T-cell leukemia virus type I (HTLV-I), which is endemic in Japan, Melanesia/Australia, the Caribbean, parts of South America, and part of Africa [Van Brussel et al., 1999]. Prognosis for patients with ATL is extremely poor due to multidrug resistance of malignant cells and frequent complications due to opportunist infections. ATL patients are susceptible to various opportunistic infections, including pneumocyctis pneumonia, fungal infections, and herpesvirus disease due to defective cellular immunity [Uchiyama, 1997; Yasunaga et al., 2001; Chen et al., 2006]. Suzumiya et al. [1993] reported that CMV was involved in 35 of 47 (74.5%) autopsied cases of ATL and that CMV pneumonia is a significant cause of death in ATL patients. EBV-associated B cell lymphoproliferative disorder [Tobinai et al., 1991; Tanaka et al., 2008] and HHV-6 encephalitis [Idutsu et al., 2007] has also been reported in patients with ATL. Development of these herpesvirus diseases may indicate that subclinical reactivations of herpesviruses are common in ATL patients. To date, two studies evaluated CMV reactivation in ATL patients using an antigenemia assay [Fujiwara et al., 2000, 2001]. However, in both studies, antigenemia was assessed only on admission and when a patient exhibited a fever. Furthermore, less is known about incidence or significance of HHV-6 or EBV reactivation.

Understanding the dynamics of reactivation of these herpesviruses in ATL patients may facilitate the prevention of CMV-, HHV-6-, or EBV-related diseases. Presence of plasma viral DNA can be a good indicator of active CMV [Boeckh and Ljungman, 2009] or HHV-6 [Zerr, 2006b] infection. There is debate over which sample type, whole blood versus plasma or serum is most suitable for monitoring EBV reactivation; nevertheless, the detection of EBV DNA genomes in plasma that does not contain B-cells is more likely the result of a lytic EBV infection and indicates that the patients has an active EBV infection [Ljungman, 2010]. The present study used real-time polymerase chain reaction (PCR) to detect viral DNA genomes in plasma to specifically evaluate the epidemiology and kinetics of CMV, HHV-6, and EBV reactivation in ATL patients receiving chemotherapies.

MATERIALS AND METHODS

Patients

Thirty-four patients who were admitted to Oita University Hospital or Oita Prefectural Hospital and who had acute/lymphoma-type ATL that met the Japan Lymphoma Study Group criteria [Shimoyama, 1991] were enrolled in this study. The diagnosis of ATL was made based on seropositivity for HTLV-I and a histologically and/or cytologically proven peripheral T-cell malignancy. Study protocols were approved by the Ethical Committee of the Oita University Faculty of Medicine, and patients participating in this study signed an informed consent form for study protocols. Initial treatment regimens included cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP), cyclophosphamide, pirarubicin, vincristine, and prednisolone (THP-COP), cyclophosphamide, pirarubicin, etoposide, and prednisolone (THP-CEP), or mLSG15 (sequential therapy by VCAP-AMP-VECP) [Tsukasaki et al., 2007]. Salvage treatments were selected at the discretion of the physicians for management of refractory disease. No patients were treated with prophylactic anti-viral agents. Patients who received hematopoietic stem cell transplantation were censored from this study upon beginning preconditioning for transplantation.

Real-Time PCR

Blood sampling to monitor viral DNA was started after initiation of chemotherapy and performed during the period in which patients were hospitalized in order to receive chemotherapy. Interval of sampling was at baseline, once every 7–14 days. Plasma samples were separated from EDTA-treated whole blood by centrifugation (1,750*g* for 10 min) and filtration through a 0.22-μm pores filter. The design of the PCR primers (5′-TCACCAGTGTCGTGTATGCCA-3′ and 5′-CACACAGCGCTCGTTGTAATC-3′) and a TaqMan probe (5′-[FAM]CCCATGAACGTGCTCATCGAC GTGA[TAMRA]-3′) for CMV quantitation were based on the UL97 open reading frame of CMV, and quantitation was performed as previously described [Ikewaki et al., 2005]. Primers and probe for evaluation of HHV-6 DNA load were based on sequences from U67 according to the methods originally described by Locatelli et al. [2000] and performed as previously described [Ogata et al., 2006]. For EBV, PCR primers were complementary to sequences in the BALF5 gene and performed as previously described [Kimura et al., 1999].

Analyses

Viral reactivation was defined as the detection of viral DNA in a sample of plasma. Duration of the reactivation event was defined as the period of consecutive positive results. The statistical significance of differences between the groups was assessed by Fisher's exact test or the Mann–Whitney *U* test as appropriate. The cumulative incidence of viral reactivation and survival was calculated according to the Kaplan–Meier method, and comparisons of survival were made using the log-rank test. Statistical analyses were performed using Prism 5 for Macintosh software (GraphPad Software,

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)

Characteristics	Total (n = 34)	CMV reactivation			HHV-6 reactivation			EBV reactivation		
		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.

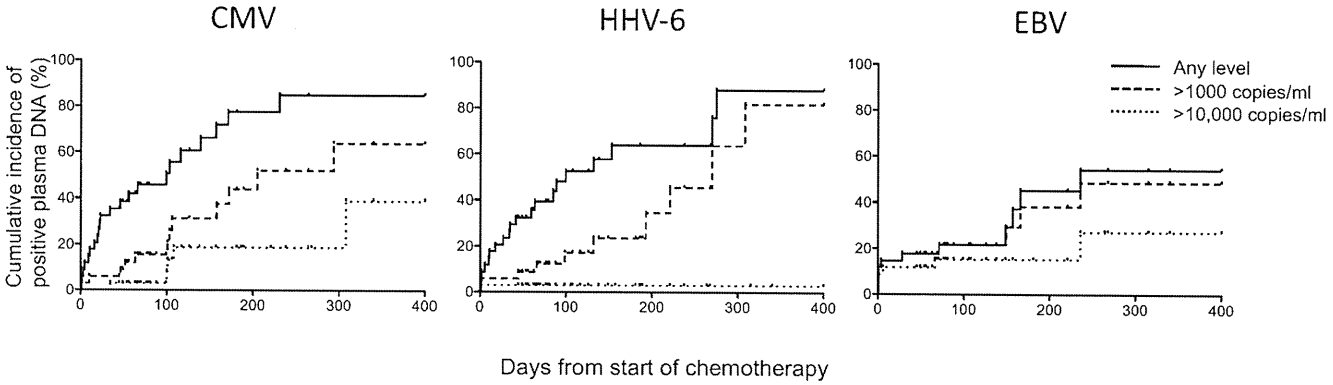

Fig. 1. Kaplan–Meier plot of the cumulative incidence of reactivation of CMV, HHV-6, or EBV, by number of copies of each viral DNA per ml in the day after start of chemotherapy.

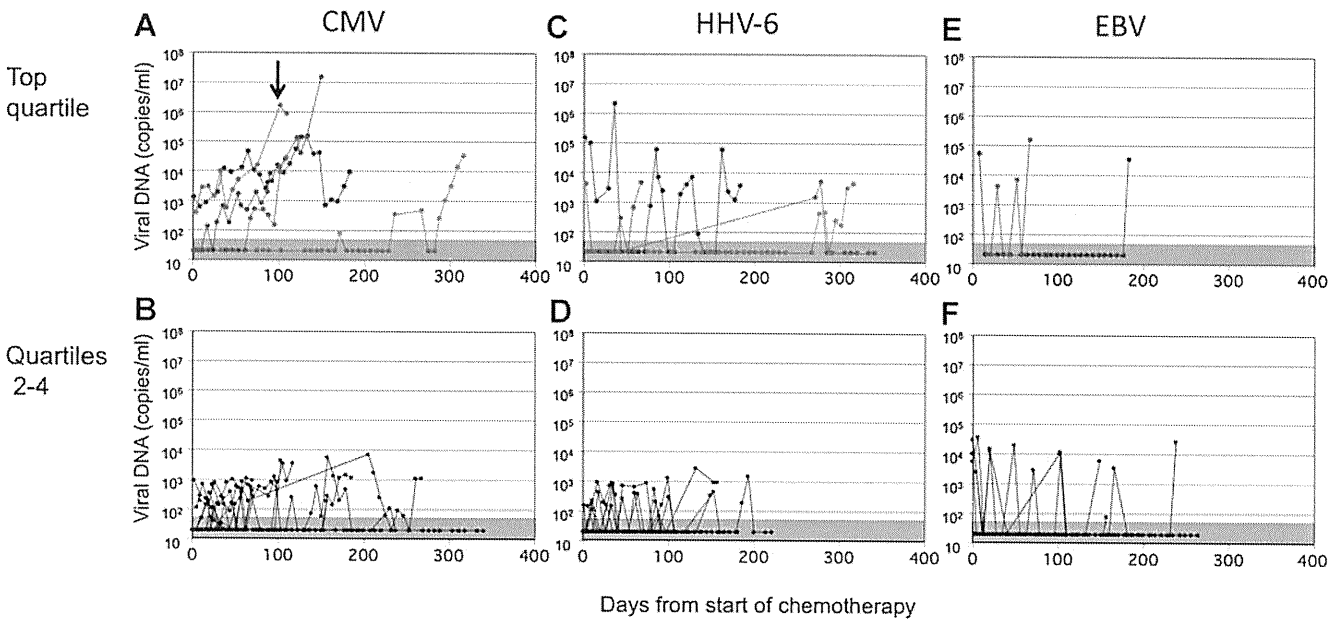

Fig. 2. Kinetics of the course of reactivation of CMV, HHV-6, and EBV. Each line represents an individual patient. The shaded area indicates values below the threshold for detection (<50 copies/ml of plasma). The top graphs (A,C, and E) show the kinetics of viral load among patients whose peak DNA load 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.

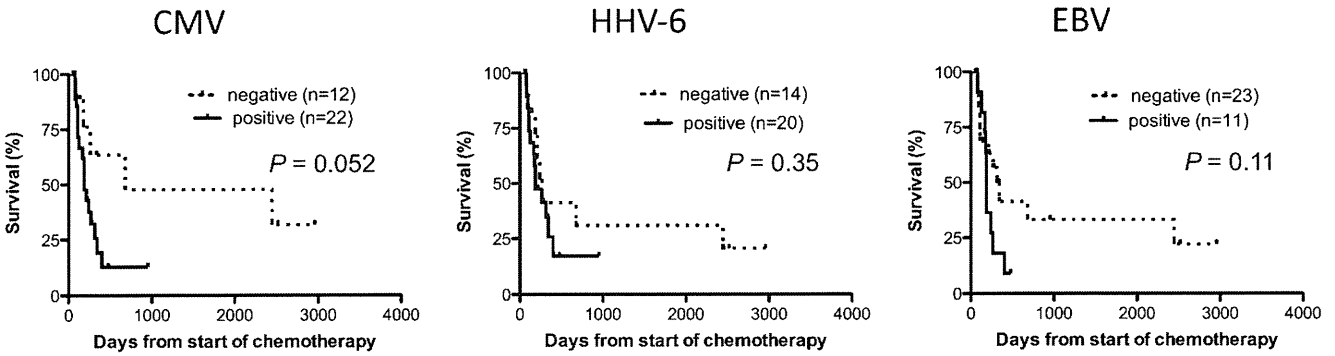

Fig. 3. Kaplan–Meier plot of the probability of survival according to viral reactivation. In each figure, "positive" indicates that tested positive for DNA of a viral genome in plasma at any time after start of chemotherapy, and "negative" indicates patients without positive DNA for a particular viral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