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

Conflict of interest

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

Acknowledgements

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ORIGINAL ARTICLE

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

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

Introduction

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

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

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

Patients and methods

Patients

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

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Table 1 Patient characteristics ($n = 111$)

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

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

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

Clinical definitions

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

Sample preparation

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

Assay

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

Statistical analysis

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

Results

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

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

Table 2 Characteristics of the 12 patients who developed CNS dysfunction

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

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

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

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

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

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

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

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

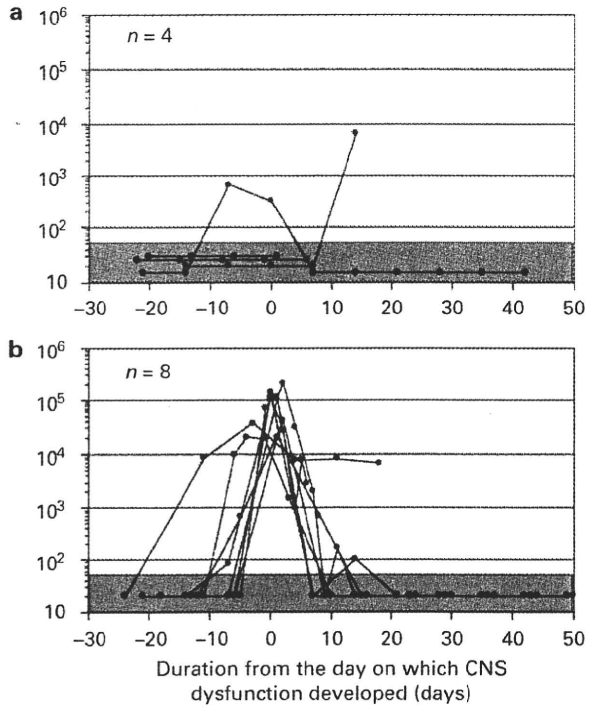


Figure 1 (**a**) Kinetics of plasma human herpesvirus (HHV)-6 DNA among patients in whom central nervous system (CNS) dysfunction showed no association with HHV-6 ($n = 4$). (**b**) Kinetics of plasma HHV-6 DNA for patients who developed HHV-6 encephalitis or CNS dysfunction potentially associated with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

Discussion

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

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

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

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

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

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

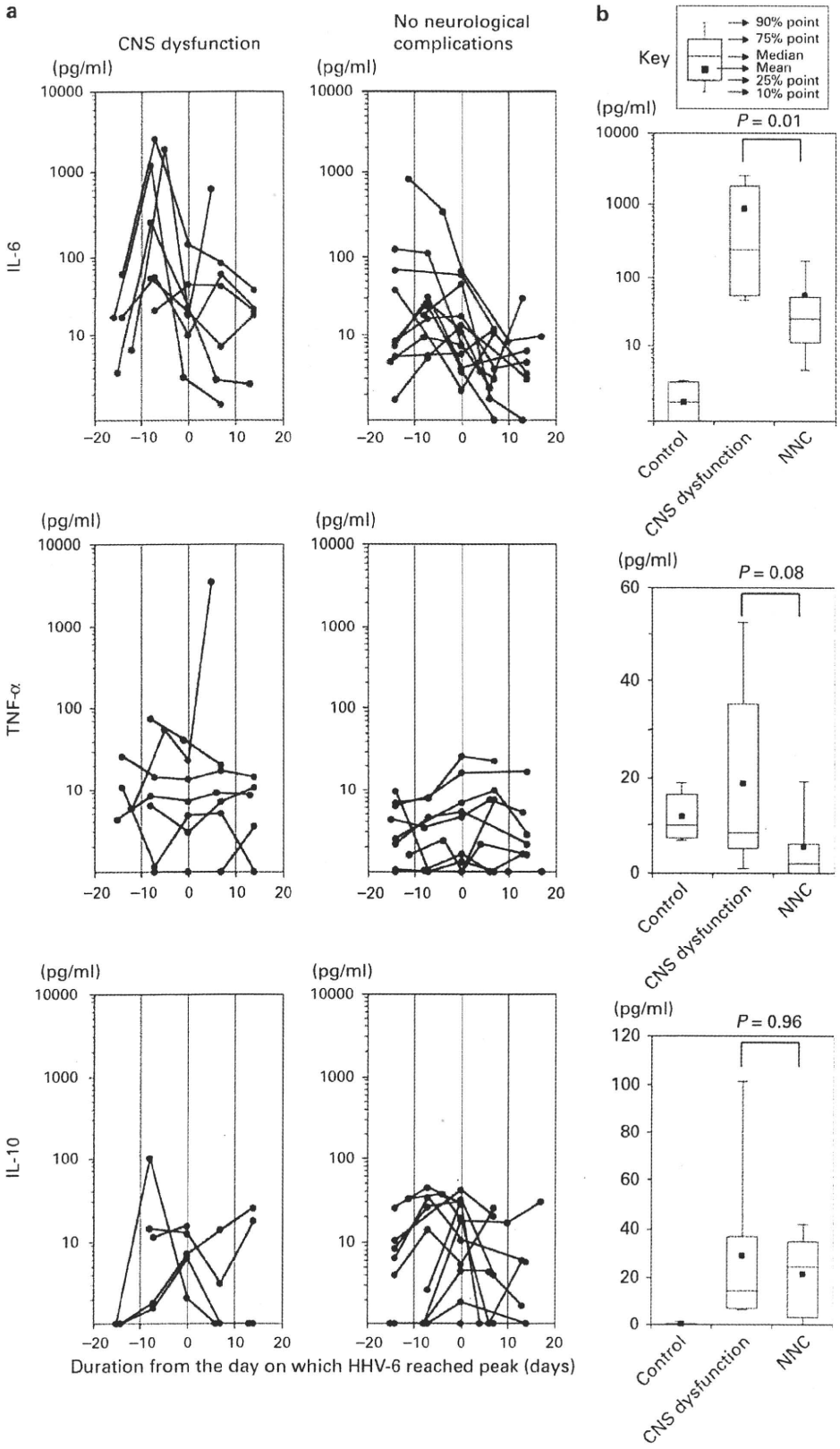
Table 4 Comparison of characteristics and laboratory data between two groups of patients displaying high-level HHV-6 DNA (plasma HHV-6 DNA $\geq 1 \times 10^4$ copies/ml)

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

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

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

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


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

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

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

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

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

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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

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