marrow (BM), liver, spleen, lymph nodes, skin, and central nervous system (CNS) [3,4]. FHL is a fatal disease if allogeneic hematopoietic stem cell transplantation (SCT) has not been successfully performed.

Epstein–Barr virus (EBV)-associated HLH (EBV-HLH) is a severe form of secondary HLH more frequently occurring in Asian children [5–7]. Activated EBV-infected $CD8^+$ T cells account for the disease process of EBV-HLH [8], however no predisposing factors have yet been clarified. EBV-HLH patients mostly respond to immunochemotherapy, but a small fraction of patients experience a fatal course without SCT. Therefore, although numbers were still small, SCT has been included in the salvage for refractory EBV-HLH cases [9–11]. The optimal timing of SCT, the source of donor cells and the conditioning are critical, particularly for young HLH patients. In this setting, the appropriate SCT for HLH patients needs to be established.

This study analyzed the outcomes of patients with FHL or EBV-HLH who underwent SCT in Japan over the past 10 years, in order to address the issues in the transplant-related problems including engraftment, late sequelae as well as to find out if there are distinct transplant strategies for FHL and EBV-HLH patients.

PATIENTS AND METHODS

Data Collection

The HLH/LCH Committee in the Japanese Society of Pediatric Hematology (JSPH) sent the first questionnaires to the hospitals administered by JSPH members based on the SCT registry in JSPH, asking if SCT was performed for any HLH patients between 1995 and 2005. The second questionnaires were sent to 57 hospitals with SCT cases, asking the patients' characteristics, treatment prior to SCT, donor sources, conditioning regimens, complications, and outcome. Of the 47 responses (recover rate 82%), 61 definite SCT cases from 33 hospitals were eligible for the study (mean 1.7 case/hospital, Supplemental Table). Forty-three FHL patients underwent 46 SCT, while 14 EBV-HLH patients underwent a total of 15 SCT. The majority of SCT (EBV-HLH 87%, FHL 89%) were performed between 2000 and 2005.

Diagnosis and Classification

All 57 patients fulfilled the diagnostic criteria of HLH [12]. FHL was diagnosed when the patient had a genetic abnormality, positive family history, and/or other evidence such as impaired natural killer cell activity [13]. The genetic study of FHL 2, 3, and 4, approved by the ethics committee of Kyushu University, Japan (No. 45), was partly completed postmortem according to our methods [14–17]. FHL2 and FHL3 determined by *PRF1* or *UNC13D* mutations accounted for 28% (n = 12), and 26% (n = 11), respectively, in this group. In addition, a total of eight patients were found with siblings diagnosed as having HLH. EBV infection might be associated with the development of HLH in four FHL patients (one FHL2, one FHL3, and two familial). These cases were classified as FHL, not as EBV-HLH. Other types of primary HLH such as XLP were excluded in this study.

EBV-HLH was diagnosed when a non-FHL patient had a primary infection or reactivation of EBV at the onset of HLH. EBV infection was assessed by the detection of EBV DNA and/or the pattern of serum EBV-specific antibody titers [18]. Cases with secondary HLH occurring in a chronic active EBV infection [19], and/or a histologically confirmed EBV-related lymphoma were excluded in this study. CNS involvement was determined when patients showed neurological manifestations, clinically as well as with any evidence of abnormality in the cerebrospinal fluids (CSF), neuroimagings (CT/MRI), and/or electroencephalography (EEG).

Prior Treatment to SCT

Treatment was based on the HLH-94 protocol using a combination of corticosteroid, cyclosporine-A (CSA), and etoposide (VP16) for both groups [20,21]. As the multidrug chemotherapy, CHOP-VP16-based regimen (VP16, vincristine, cyclophosphamide [CY], doxorubicin, and prednisolone) was chiefly employed. SCT was performed for all FHL patients, but limited for EBV-HLH patients who were resistant to any other treatments.

SCT

Allogeneic SCT was performed in 53 of the 57 patients (93%). Autologous SCT and identical-twin donor SCT were performed in three and one sporadic patients, respectively, because the molecular diagnosis was not available at the time of SCT. Donor sources, infused cell doses, conditioning regimens, and other SCT-related data are summarized in Table I. Allogeneic donor sources for EBV-HLH were HLA-matched sibling peripheral blood (PB) 1, haploidentical parent BM/PB 2, HLA-matched unrelated BM 1, HLA-matched unrelated cord blood (UCB) 2, and HLA-mismatched UCB 5, and those for FHL were HLA-matched related BM 7 (sibling 6), haploidentical parent BM/PB 2, HLA-matched unrelated BM 12, HLA-matched UCB 9, and HLA-mismatched UCB 12. All CBs were obtained from unrelated donors registered in the Japanese Cord Blood Bank Network. All unrelated donor BMs were obtained from the Japanese Marrow Donor Program. Myeloablative conditioning for EBV-HLH included VP16/busulfan (BU)/CY in 8 patients (4 in UCB transplantation [UCBT]) and other regimens in 3 patients, while those for FHL were VP16/BU/CY plus or minus anti-thymocyte globulin (ATG) in 23 patients (10 in UCBT) and others in 8 patients. Reduced intensity conditioning (RIC) for EBV-HLH included melphalan (MEL)/fludarabine (FLU) plus or minus thoracoabdominal irradiation in three patients (two in UCBT), and those for FHL were MEL/FLU plus or minus low-dose total body irradiation plus or minus ATG in eight patients (four in UCBT) and others in three patients. Donor chimerism was assessed by using short tandem repeats or sex chromosome analyses.

Evaluation of Late Sequelae

Long-term survivors were further questioned concerning their physical growth, endocrinological status, and neurological deficits. Neurological development including cognitive functions was assessed by Karnofsky score, developmental quotient and/or school performance.

Statistical Analysis

The 10-year overall survival (OS) rate with 95% confidence intervals were estimated by the Kaplan–Meier method. The OS was calculated for the period from the day of SCT until the death of any cause or the final observation. All results were updated to May 31,

TABLE I. Profiles of Patients Who Underwent Hematopoietic Stem Cell Transplantation

	EBV-HLH	FHL	*P*-value
Number, male:female	14, 4:10	43, 23:20	0.37
Age at onset (median, range)	5.5y, 6m–18y	0.5y, 6d–12y	<0.0001
Age at SCT (median, range)	5.9y, 1.4–18y	1.2y, 0.4–15y	0.0002
Observation period (median, range)	5.5y, 0.3–16y	4.8y, 0.2–19y	0.94
Manifestation at diagnosis (%)			
Fever	100	95	>0.99
Hepatosplenomegaly	86	86	>0.99
Lymphadenopathy	36	21	0.30
Skin eruption	7	14	0.67
Respiratory failure	36	14	0.12
DIC	50	33	0.26
Treatment prior to SCT (%)			
HLH94 only	36 (5/14)	60 (25/42)	0.14
Multidrug chemotherapy	57 (8/14)	19 (8/42)	0.017
Diagnosis to SCT (median, range)	5.8m, 1.8–24m	7.5m, 1.6–84m	0.18
SCT (n)			
Allogeneic	11	42	
Auto/Identical twin	3	1	
Nucleated cell doses ($\times 10^8$/kg)	1.3 (0.2–6.6)	2.5 (0.1–12.7)	0.14
Donor			
UCB	7	21	0.94
Others	7	22	
HLA disparity no	4	28	0.09
HLA disparity yes (>1 locus[a])	7	14	
Conditioning			
Myeloablative[b]	11	31	>0.99
RIC[c]	3	11	
Irradiation yes	4	11	0.73
Irradiation no	9	31	
ATG yes	0	8	0.18
ATG no	14	34	
CNS abnormality (%)			
At diagnosis	29[d] (4/14)	21[d] (9/42)	0.72
Before SCT	57 (8/14)	67 (28/42)	0.52
CSF pleocytosis	25 (2/8)	32 (7/22)	>0.99
MRI abnormality	36 (5/14)	51 (20/39)	0.36
Convulsion	43 (6/14)	41 (17/41)	0.93
Disturbed consciousness	36 (5/14)	24 (10/41)	0.49
Post-transplant state (n)			
Early death (<100 days)	2	7	0.48
Alive	12	29	0.31
Neurological deficit (%)	8[d] (1/12)	29[d] (7/24)	0.22
Late sequelae[e] (%)	8 (1/12)	52 (11/21)	0.022

ATG, anti-thymocyte globulin; BU, busulfan; CNS, central nervous system; CSF, cerebrospinal fluid; CY, cyclophosphamide; DIC, disseminated intravascular coagulopathy; EBV, Epstein–Barr virus; FHL, familial hemophagocytic lymphohistiocytosis; FLU, fludarabine; HLH, hemophagocytic lymphohistiocytosis; MEL, melphalan; MRI, magnetic resonance imaging; SCT, hematopoietic stem cell transplantation; TAI, thoracoabdominal irradiation; TBI, total body irradiation; UCBT, unrelated donor cord blood transplantation; VP16, etoposide. Parenthesis means the positive number of patients per the evaluable number of patients. The observation period means the time from the onset to the last visit or death. [a]Human leukocyte antigen (HLA) disparity was assessed by the serotyping data of HLA-A, -B, and -DR; [b]Myeloablative conditionings for EBV-HLH were VP16/BU/CY 8 (4 in UCBT) and others 3, and those for FHL were VP16/BU/CY + ATG 23 (10 in UCBT) and others 8; [c]Reduced intensity conditionings (RIC) for EBV-HLH were MEL/FLU + TAI 3 (2 in UCBT), and those for FHL were MEL/FLU + low dose TBI + ATG 8 (4 in UCBT) and others 3; [d]The proportion of patients having neurological abnormality was lower in survived patients with EBV-HLH ($P = 0.0015$). Survived patients were neurodevelopmentally assessed at the last visit to the hospital; [e]Late sequela(e) in EBV-HLH was hemiparesis (n = 1), and those in FHL were short stature (n = 5), endocrinological abnormality (n = 1), psychomotor retardation with or without seizure (n = 5), brain atrophy (n = 1), and hearing difficulty (n = 1).

2008. An analysis of the risk factors for SCT outcome was possible for FHL, but not for EBV-HLH because of the small number of subjects. Age at onset of HLH or at the SCT, duration from the onset to SCT, CNS disease before SCT, donor sources, and the type of conditioning were tested using the log-rank method. Cox proportional-hazard model was employed to examine the association between selected clinical variables and the risk for death. A logistic regression model was used to investigate factors associated with neurological sequelae. Chi-square test or Fisher's exact test were employed in other comparisons. P values less than 0.05 were considered to be significant.

RESULTS

Profiles of EBV-HLH and FHL Patients

A comparison of the clinical profiles (Table I) revealed that the ages at disease onset and at the time of SCT were each higher in EBV-HLH than in FHL patients ($P < 0.0001$, $P = 0.0002$, respectively). No clinical manifestations differed between the two groups during the disease course, including respiratory failure as well as CNS abnormalities at diagnosis. The proportion of patients who failed VP16 and CSA therapy including HLH94 protocol and needed combination chemotherapy such as CHOP-VP16 before planning SCT was higher in EBV-HLH patients than FHL patients (57% vs. 19%, $P = 0.0168$).

Outcomes of SCT

Engraftment and survival. Post-transplant outcomes of 43 FHL patients and 14 EBV-HLH patients are summarized in Figures 1 and 2. The 10-year OS rates (median ± SE%) of FHL and EBV-HLH patients were 65.0 ± 7.9% and 85.7 ± 9.4%, respectively ($P = 0.24$; Fig. 3). In the allogeneic SCT cases with FHL (Fig. 1), 29 attained engraftment, 6 had rejection or graft failure, and 7 were undetermined. On the other hand, in EBV-HLH (Fig. 2), seven were engrafted, three were rejected, and one was undetermined. Of all 29 FHL patients engrafted after the first SCT, 26 were alive with no HLH relapse, but 3 died of treatment-related mortality (TRM). Seven engrafted patients with EBV-HLH were alive and well at the final follow-up. Among the nine rejection/graft failure patients (six FHL, three EBV-HLH), a second UCBT was successful in three of the four patients (three FHL, one EBV-HLH). Twelve of the UCBT recipients for FHL that received a graft with the first UCBT and two that received a second UCBT were alive at the last follow-up; while seven died; six were due to TRM and one was due to active HLH disease. Six of the seven UCBT recipients for EBV-HLH were alive and well at the last follow-up, while only one died of active HLH disease on day 18 post-transplant. A total of 29 FHL survivors after allogeneic SCT(s) had 17 complete donor chimera (2 patients after second UCBTs), 3 mixed chimera (1 had 42% donor chimera in remission 18 months after SCT, 2 attained >90% donor chimera until 6 months after SCT), 8 undefined, and 1 graft failure with CNS disease. Ten EBV-HLH survivors after allogeneic SCT attained eight complete donor chimera (seven patients after the first SCT and one patient after second SCT [UCBT]), and two with autologous recovery. Two of three EBV-HLH patients who rejected allogeneic cells were alive and disease free more than 6 years post-transplant. One of two EBV-HLH patients who underwent autologous SCT was alive and well 13 years post-transplant [22]. One EBV-HLH patient was alive and well 10 years after the identical twin donor BMT.

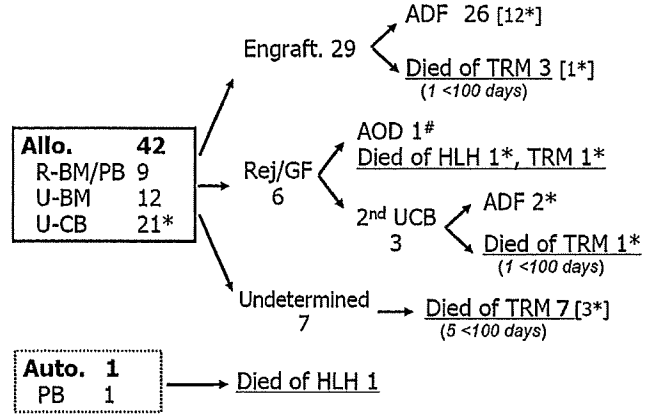

Fig. 1. Cohort diagram for the clinical outcome of 43 patients with familial hemophagocytic lymphohistiocytosis (FHL) who underwent stem cell transplantation (SCT). Of 42 patients after allogeneic SCT, 29 achieved engraftment (18 complete, 3 mixed) and 6 failed to engraft. One (#) with graft failure was alive with central nervous system disease 12 years after SCT. A total of 29 patients (67%) were alive after SCT. The underlined data indicate the number of deceased patients. Seven patients died within 100 days post-SCT (parenthesis). Asterisk (*) means UCB. R, related; U, unrelated; BM, bone marrow; PB, peripheral blood; CB, cord blood; ADF, alive with the disease free state; AOD, alive on disease; Rej/GF, rejection or graft failure; TRM, treatment-related mortality.

Causes of death. Of 14 deceased FHL patients, 12 died of TRM, including 3 chronic GVHD while 2 died of recurrent HLH. Seven patients experienced early death from TRM within 100 days after SCT (Fig. 1). One patient, later diagnosed with FHL2, died of CNS disease 5 years after autologous SCT [14]. Two EBV-HLH patients died of recurrent HLH within 50 days after SCT (Fig. 1). No TRM-related deaths were noted among the EBV-HLH patients.

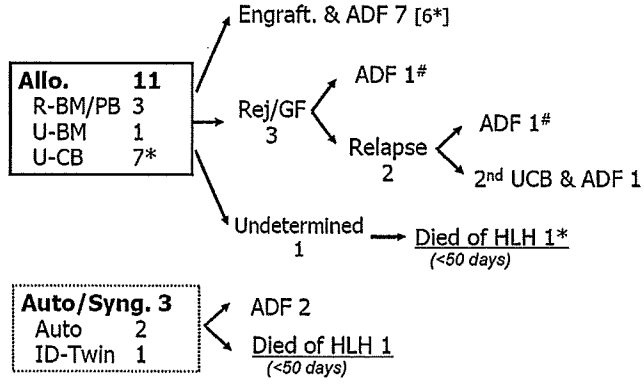

Fig. 2. Cohort diagram for the clinical outcome of 14 patients with Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) who underwent SCT. Among 11 patients after the first allogeneic SCT, 7 achieved successful engraftment and 3 failed to engraft. A total of 12 patients (86%) were alive after SCT. Two patients (#) were alive and well more than 6 years after SCT failure. The underlined data indicate the number of deceased patients. Two patients died within 50 days post-SCT (parenthesis). Asterisk (*) means UCB. Auto/Syng: autologous/syngeneic, ID: identical.

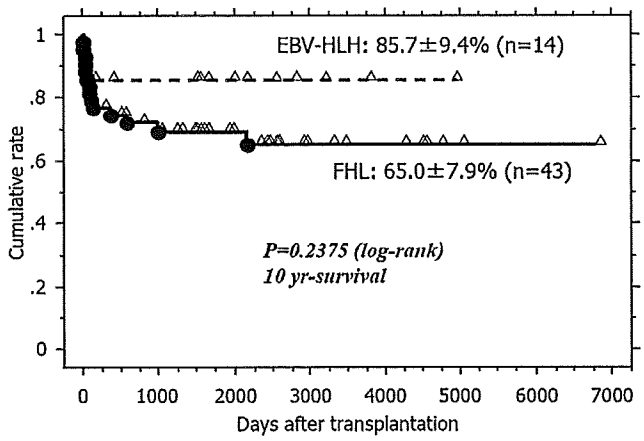

Fig. 3. Cumulative probability of post-transplant overall survival of FHL (*solid line*) and EBV-HLH patients (*dashed line*) who underwent SCT. Closed circle and open triangle represent deceased and alive patients, respectively. Each value indicates the 10-year overall survival rate plus or minus standard error assessed by the log-rank test.

Analysis of Prognostic Factors in FHL

A log-rank test on the OS rate did not show any significant difference in terms of age at SCT (<2 years vs. ≥2 years), time of SCT from HLH treatment (<6 months vs. ≥6 months), conditioning regimens (myeloablative vs. RIC) and various donor sources (R-PB/BM vs. UCBT vs. UBM; Table II). The Cox hazard model with adjustment for gender and age at engraftment indicated that the risk of death for UBM might be higher than that for R-PB/BM (adjusted hazard ratio = 0.07, 95% confidence interval [CI] = 0.01–1.02, $P = 0.05$) and that for UCB (0.27, 95% CI = 0.07–1.09, $P = 0.07$; Table II). No significant variables were found to predict the risk of early death within 100 days post-transplant, or the risk of neurological sequelae.

CNS Abnormalities and Late Sequelae

Table I shows that the frequency of CNS abnormalities at onset and the time of SCT did not differ between the EBV-HLH and FHL patients. Whereas, post-transplant CNS abnormalities were significantly higher in the FHL patients ($P = 0.0015$). Eleven FHL patients (52%) have had late sequelae including neurological as well as endocrinological problems, in comparison to only one EBV-HLH patient with left hemiparesis ($P = 0.022$). Late sequelae of FHL

TABLE II. Association Variables Influencing on the Risk of Mortality in FHL Patients

(A) Log-rank analysis Variables	No.	Survival (OS %)		*P*-value
Age				
<2 years	30	66.2 ± 8.7		0.56
≥2 years	12	75.0 ± 12.5		
Time from HLH treatment				
<6 months	14	62.9 ± 13.3		0.65
≥6 months	28	71.4 ± 8.5		
Conditioning				
Myeloablative	31	71.0 ± 8.2		0.50
RIC	11	60.6 ± 15.7		
Donor sources				
R-PB/BM, a	9	88.9 ± 10.5	a vs. b	0.22
UCB, b	21	65.6 ± 10.6	a vs c	0.15
UBM, c	12	58.3 ± 14.2	b vs c	0.61

(B) Cox's model analysis Variables	No.	Adjusted hazard ratio	95% CI lower–upper limit	*P*-value
Stem cell source				
Unrelated BM	12	1.00	Reference	
Unrelated CB	21	0.27	0.07–1.09	0.07
Related PB/BM	9	0.07	0.01–1.02	0.05
Conditioning				
Reduced intensity	11	1.00	Reference	
Myeloablative	31	0.48	0.09–2.47	0.38
Radiation				
No	31	1.00	Reference	
Yes	11	0.52	0.11–2.52	0.41
Use of ATG				
No	34	1.00	Reference	
Yes	8	0.91	0.18–4.70	0.91
HLA disparity				
No	28	1.00	Reference	
Yes (>1 locus)	14	2.79	0.75–10.38	0.13

Both analyses (A, B) were performed for 42 FHL patients who underwent the first allogeneic SCT. The Cox model analysis was performed with adjustment for selected variables including sex and age at engraftment.

included psychomotor retardation with or without seizures (n = 5), brain atrophy (n = 1), hearing difficulty (n = 1), short stature (n = 5), and impaired sexual development (n = 1).

DISCUSSION

No underlying immunodeficiency has yet been identified for idiopathic EBV-HLH, which has been recognized to be distinct from familial or inherited disease-related HLH like FHL. However, EBV also acts as a trigger in the development of HLH episodes in FHL patients. Therefore, caution must be exercised in the differentiation of the two types of HLH disease. Strict use of the renewed diagnostic criteria for the registered cases in Japan enabled an analysis of the SCT results of 43 FHL and 14 EBV-HLH patients. The data first revealed a high survival rate in UCBT recipients in either type of HLH, indicating that CB could be preferable BM as the unrelated donor source in SCT for pediatric patients with refractory HLH. In addition, SCT in FHL patients was more problematic than that in EBV-HLH, where it was associated with a high incidence of post-transplant early death rate as well as late sequelae including neurological deficits. The EBV-HLH patients showed no apparent sequelae even if they had CNS involvement at diagnosis.

Information concerning SCT for HLH patients has been accumulated mostly in FHL, but little has been published in EBV-HLH except for sporadic case reports [10,11]. Previously published major studies on SCT in FHL patients are summarized in Table III. Because of the historical changes in the available genetic analyses, supportive care practices, donor sources and conditioning, the pre-2000 studies [23–27] might not be comparable to the current data. Henter et al. [21] showed the improved survival of patients treated with HLH-94 followed by BMT, in which the 3-year post-BMT survival was 62%. Horne et al. [28] noted significant TRM due to venoocclusive disease (VOD) after myeloablative conditioning, and that an active disease status at SCT was associated with a poor prognosis. Ouachee-Chardin et al. [29] reported 59% of OS in a series of 48 patients including 60% of haploidentical SCT, and indicated a high TRM due to VOD associated with young age. Recently, Baker et al. [30] reported that BU/CY/VP16 plus or minus ATG-conditioning provided a cure in 53% of patients after unrelated donor BMT, but a high mortality rate at day 100 (32 of 50 [64%] deceased patients). The present study showed a comparably high OS rate (69%) and similarly high incidence of early death until day 100 (7 of 13 [54%] deaths after allogeneic SCT) in Japan. Probably, the major distinction of the current study from the other reports is a higher usage of UCBT (50%) and RIC (26%). Unfortunately, the combined usage of RIC-UCBT was applied only in eight cases (14%) in this study, which was insufficient to fully evaluate its effectiveness. With regard to RIC-SCT with or without UCBT for FHL, Cooper et al. [31] reported a high disease free survival (75%) in 12 HLH patients (including 5 FHL) who underwent RIC-SCT from matched family/unrelated or haploidentical donor, in which 3 of 9 survivors had mixed chimerism but remain free of disease. The most recent report by Cesaro et al. [32] analyzed 61 cases including an appreciable number of RIC (18%) and UCBT (10%), but did not document the superiority of RIC-UCBT. In the present study, UCBT had a tendency to yield a more favorable outcome than UBMT, although the difference was not statistically significant. FHL infants received SCT early; however the fact that survival of FHL patients who underwent SCT at <2 years of age was not better than later SCT might reflect the difficulty in determining the optimal timing of SCT

TABLE III. Reports on the Clinical Outcome of Patients With HLH Who Underwent Allogeneic Hematopoietic Stem Cell Transplantation

No. pts	Median age at SCT (months)	FH (%)	Major conditioning regimen	Donor	Source	OS (%)	Engraft. (%)	Causes of death	Refs.
9	13	45	Myeloab	VP16/BU/CY ±anti-LFA1	MRD/MMRD/haplo	BM	44.0	100	TR, HLH	[24]
29	NR	48	Myeloab	NR	MRD/MUD/haplo	BM	66.0	72	TR, HLH	[25]
20	9	30	Myeloab	VP16/BU/CY ±ATG	MSD/URD (80%)	BM	45.0	90	TR, HLH	[26]
14	14	36	Myeloab	VP16/BU/CY, ATG/BU/CY	MMRD/MUD	BM (T cell depleted)	64.3	65	TR, HLH	[27]
12	18	42	Myeloab	VP16/BU/CY	MSD/URD (67%)	BM	100	100	No	[33]
17	NR	NR	Myeloab	VP16/BU/CY ±ATG, TBI	MRD/URD/haplo	BM, CB (2), PB, CD34	58.0	94	TR, HLH, lymphoma	[8]
65[a]	13	31	Myeloab	VP16/BU/CY ±ATG	MRD/URD/haplo	BM, CB (5), PB, CD34	62.0	89	TR, HLH, AML	[21]
86[a]	13	34	Myeloab	VP16/BU/CY ±ATG, TBI	MRD/URD/haplo	BM, CB (7)	64.0	90	TR, HLH, 2nd AML	[28]
48	6	35	Myeloab	VP16/BU/CY, ATG/BU/CY	MSD/URD/haplo	BM, PB	58.5	78	HLH	[29]
12	14	17	RIC	FLU/MEL ± BUS, FLU/2GyTBI	MRD/URD/haplo	BM, CD34	75.0	100	TR	[31]
91	12	NR	Myeloab	VP16/BU/CY ±ATG	URD	BM, PB, CB (9)	45.0	83	TR, HLH	[30]
61	13	20	RIC (18%)	VP16 or MEL/BU/CY ±ATG	MRD/MMRD/URD	BM, PB, CB (6)	63.9	78	TR (68%), HLH (27%)	[32]
42	17	55	RIC (26%)	VP16/BU/CY ±ATG, TBI	MRD/MMRD/URD	BM, PB, CB (21)	69.0	78	TR (79%), HLH (21%)	Ours

AML, acute myelogeneous leukemia; BM, bone marrow; BU, busulfan; CB, cord blood; CY, cyclophosphamide; FHL, familial hemophagocytic lymphohistiocytosis; FH, family history; FLU, fludarabine; MEL, melphalan; MMRD, HLA-mismatched related donor; MRD, HLA-matched related donor; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; NR, not recorded; PB, peripheral blood; RIC, reduced intensity conditioning; TBI, total body irradiation; TR, transplantation-related events; URD, unrelated donor; VP16, etoposide. [a]Sixty four of 65 patients studied by Henter et al. [21] were included in 86 patients by Horne et al. [28].

or introducing appropriate RIC regimens in young infants. In UCBT, a major obstacle was thought to be early graft failure, but once engrafted no late graft failure could not be seen [29]. We confirmed this finding in our UCBT cases.

Dürken et al. [33] reported that six HLH patients with CNS disease underwent allogeneic BMT and three of them had no persistent neurological problems after transplant. More recently, SCT is thought to be preferable for FHL patients at the early stage of CNS disease with variable presentation [34,35]. Fludarabine-based RIC has been preferred in SCT for FHL patients in order to reduce late sequelae [36,37]. Since CNS disease itself had no impact on the OS in the current study, but nearly half of the long-term survivors of FHL had late sequelae associated with growth and development, further prospective studies should be focused on how to reduce late sequelae in SCT for FHL patients.

In the treatment of refractory EBV-HLH, no consensus has yet been reached concerning the treatment of patients who fail to respond to the HLH-2004 protocol type immunochemotherapy. Several reports documented that SCT led to a complete remission in such cases [8,10,11,28,38,39]. The present study revealed that use of pre-SCT combination chemotherapy might be associated with a better therapeutic impact on subsequent SCT in patients with EBV-HLH. Furthermore, long-term survival, that is, a probable cure, could be obtained even after autologous SCT [22] or identical twin donor BMT, suggesting that a reconstitution of allogeneic hematopoietic stem cells was not essential in the successful SCT for EBV-HLH patients as described in the autologous PBSCT success for lymphoma-associated HLH [40]. In addition, long-term survival even after graft failure or post-transplant relapse in EBV-HLH patients might suggest the possibility of resetting the adaptive immune response to the virus as postulated in autologous SCT for the treatment of autoimmune diseases [41,42]. Moreover, successful syngeneic SCT may imply that EBV-HLH is not a monogenic disease, since Chen et al. [43] observed that a primary infection of EBV incited HLH in a pair of the twins, but not in the identical twin counterpart. These observations implied that the genetic influence in patients with EBV-HLH might be distinct from that in patients with FHL on precipitating the excessive immune activation. Further prospective studies should therefore be directed toward not only the optimization of UCBT-RIC to improve survival of FHL patients, but to better understanding of the pathological interaction between cytotoxic granule disorders and EBV.

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ORIGINAL ARTICLE

Cardiovascular Complications Associated with Chronic Active Epstein–Barr Virus Infection

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Abstract This study aimed to assess the outcome of cardiovascular diseases for patients with chronic active Epstein–Barr virus infection (CAEBV). The study enrolled 15 patients (7 boys and 8 girls) who fulfilled the diagnostic criteria for CAEBV, including 10 patients with T-cell type and 3 patients with natural killer (NK)-cell type. The median age at the CAEBV onset was 6.3 years (range, 1.2–17.8 years). Regular cardiologic studies were performed during the median follow-up period of 8 years (range, 2–20 years). Nine patients (60%) had cardiac diseases including coronary artery lesion (CAL) ($n = 4$, 44%), decreased left ventricular ejection fraction and pericardial effusion in ($n = 3$, 33%), complete atrioventricular block ($n = 1$), and sudden arrest ($n = 1$). The frequency of fever (78%, $p = 0.04$) or cytopenias (100%, $p = 0.01$), as the major symptom among patients with cardiac complications, was higher than among those without complications. The median time from disease onset to detection of CAL was 3.4 years (range, 1.8–8.6 years). The mean z-score increased to 3.98. Seven patients (78%) with cardiac complications died of disease progression, hematopoietic stem cell transplantation–related events, or both. In two patients, CAL regressed after allogeneic cord blood transplantation. Among CAEBV patients, CAL was the most common cardiac complication and could not be controlled without the eradication of EBV-infected T- and NK-cells.

Keywords Cardiac complication · Coronary artery lesion · Epstein–Barr virus

Abbreviations

BMT	Bone marrow transplantation
CAL	Coronary artery lesion
CBT	Cord blood transplantation
CAEBV	Chronic active Epstein–Barr virus infection
HSCT	Hematopoietic stem cell transplantation
LVEF	Left ventricular ejection fraction

Introduction

Epstein–Barr virus (EBV) infects human B-cells and persists for the person's lifetime. Humans with primary EBV infection are asymptomatic or experience acute infectious mononucleosis. Chronic active EBV infection (CAEBV) is a rare mononucleosis syndrome characterized by fever, liver dysfunction, hepatosplenomegaly, and the unique presentation of coronary artery lesions (CALs), interstitial pneumonitis, chorioretinitis, sicca, hypersensitivity to mosquito bites, and hydroa vacciniforme [17, 27]. Occurring in previously healthy children with no underlying diseases, CAEBV has been recognized as an EBV-associated T-cell and natural killer (NK)-cell lymphoproliferative disease based on the detection of clonally proliferating EBV-infected T-cells (T-cell type) or NK-cells (NK-cell type).

Neither type of CAEBV is curable without successful allogeneic hematopoietic stem cell transplantation (HSCT) [29]. Cardiac, neurologic, and enteral complications may

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be poor prognostic factors. A rupture of coronary artery aneurysms is reported to be a cause of death, as seen in Kawasaki disease [4, 23]. Meanwhile, little information is available about the cardiac involvement during the prolonged disease course.

The current study evaluated cardiac diseases in CAEBV patients at a single institution over 20 years while also discussing the presentation and pathogenesis of EBV-associated cardiovasculopathy.

Patients and Methods

Patients

From 1987 to 2007, CAEBV was diagnosed in 15 patients (7 boys and 8 girls) at Kyushu University Hospital. All these patients fulfilled the diagnostic guidelines [26], showing abnormally high titers of anti-EBV antibodies, detection of the EBV genome, or both. Serologic or genetic studies excluded any other causative infections. Data were retrospectively reviewed for the clinical manifestations, laboratory findings, age at the onset, and time between the onset and initial detection of cardiac diseases and therapy. Step-up treatment was used according to disease severity, classified as stage 0 (observation), stage 1 (antiviral therapy and/or high-dose γ-globulin), stage 2 (immunotherapy using prednisolone, cyclosporine-A, interleukin-2 [IL], or interferon [IFN]-α), stage 3 (cytotoxic chemotherapy), or stage 4 (HSCT).

Determination of EBV Genome

For this study, DNA was extracted from peripheral blood, mononuclear cells, or biopsied samples. The copy number of EBV was measured by real-time polymerase chain reaction (PCR) using the primers from BNRF1 (National Institute of Infectious Diseases, Tokyo) [27]. The T-cell or NK-cell type of CAEBV was determined by the copy number of EBV-DNA in fractionated $CD3^+$ or $CD56^+$ cells. The clonality of the EBV-infected cells was assessed by Southern blotting using EBV terminal repeats or T-cell receptor genes. The EBV-encoded RNAs (EBERs), latent infection membrane protein (LMP)-1 positivity, or both were tested by immunohistochemistry [27, 29].

Assessment of Cardiac Diseases

Cardiologic studies including chest X-ray, echocardiography, and electrocardiography (ECG) were repeated every 6 months. Two-dimensional echocardiography was used to measure the internal lumen diameter of the left main coronary artery; the proximal and distal left anterior descending coronary arteries; and the circumflex, posterior descending, and proximal and distal right coronary arteries. CAL was defined according to the criteria of the American Heart Association guidelines for Kawasaki disease [25].

Coronary artery diameters were assessed using the z-score, calculated as follows: (observed diameter − mean normal diameter)/(standard deviation of the normal diameter) [19]. Measurements for the internal diameter of the coronary arteries by transthoracic echocardiography were interpreted as follows: dilated (z-score, 2–3), ectasia (z-score, >3 within the uniform dilation of the vessel), and aneurysm (a focally dilated segment with a z-score >3) [5]. Pericardial effusion was defined in cases with circumferential extent and more than 1 mm of fluid. Left ventricular ejection fraction (LVEF) was measured on the conventional M-mode. A decreased LVEF was defined as below 60% of the normal level. The ECG was recorded to assess arrhythmia (atrial, ventricular, and conduction disturbance) or ischemic changes (ST-T change, amplitude of R and T waves, and abnormal Q wave). One patient underwent multislice spiral computed tomography for evaluation of the CAL after HSCT.

Results

Disease Course

The clinical characteristics of the 15 study patients are shown in Table 1. The median age at onset of CAEBV was 6.3 years (range, 1.2–17.8 years). The clinical manifestations at diagnosis included persistent or recurrent fever in 11 patients (73%), liver dysfunction in 6 patients (40%), cytopenias in 5 patients (33%), skin or subcutaneous lesions in 5 patients (33%), cough and chest X-ray abnormalities in 3 patients (20%), lymphadenopathy in 2 patients (13%), hepatosplenomegaly in 2 patients (13%), tachycardia in 1 patient, and hematuria in 1 patient. The EBV-infected cell type was T-cell in 10 patients, NK-cell in 3 patients, and not determined in 2 patients. At the time of diagnosis, the copy number of EBV-DNA ranged from 200 to 50,000 in 1 ml of peripheral blood.

Allogenic HSCT was performed for eight patients, cord blood transplantation (CBT) for five patients, peripheral blood stem cell transplantation for two patients, and bone marrow transplantation (BMT) for one patient [9]. Patients 2, 5, and 11 obtained a complete remission and an EBV-free state after HSCT. The median follow-up period was 8 years (range, 2–20 years). Of the 15 patients, 9 (60%) patients died during the follow-up period.

Table 1 Clinical presentation and outcome of patients with chronic active Epstein–Barr virus (EBV) infection

Pt	Sex	Onset age (years)	EBV status at diagnosis				Major symptom at the detection of cardiac disease/during illness			Treatment before onset of cardiac complications/ during illness	Complications during the disease course				HSCT as the final treatment	Outcomes (age: years)	Causes of death
			Cell type	Clonality	EBV (copies/ ml PB)	Abnormal EBV Abs	Fever	Hepato-spleno-megaly	Cytopenias		GI	CNS	HMB/HV	Cardiac diseases (age at detection: years)			
1	M	1	T	Mono	QL1[a]	Yes	Yes	Yes	Yes	IVIG, PSL, IL–2, IFNα	No	Yes	No	Yes (8)	CBT	Dead (10)	TRE
2	F	2	NK	Di	3,000	No	Yes	Yes	Yes	PSL	Yes	No	Yes	Yes (4)	CBT	ADF (6)	No
3	F	3	T	Mono	5,000	Yes	Yes	Yes	Yes	PSL, CSA	No	No	No	Yes (7)	CBT	Dead (8)	TRE
4	M	5	T	Mono	3,000	Yes	Yes	Yes	Yes	ACV, AraC, PSL, IFNα	No	No	No	Yes (8)	CBT	Dead (14)	MOF
5	M	6	T	Mono	50,000	Yes	Yes	Yes	Yes	IVIG, ACV, AraA, PSL	No	No	No	Yes (10)	CBT	ADF (13)	No
6	F	9	γδT	Mono	900	Yes	Yes	Yes	Yes	IVIG, PSL, CSA, IFNα	No	No	No	Yes (18)	No	Dead (18)	MOF
7	F	11	NK	Mono	800	No	No	Yes	Yes	PSL, CSA, VP16	No	No	Yes	Yes (14)	No	Dead (19)	Sudden death
8	F	14	T	Mono	QL2[a]	Yes	Yes	Yes	Yes	IVIG, PSL, VP16/CHOP	No	No	No	Yes (16)	No	Dead (18)	MOF
9	F	16	T	Mono	900	No	No	No	Yes	PSL, VP16/ CHOP	No	No	Yes	Yes (18)	PBSCT	Dead (24)	TRE
10	F	1	ND	ND	ND	Yes	No	Yes	Yes	IVIG, ACV, PSL, splenectomy	No	No	No	No	No	Dead (2)	Sepsis
11	M	2	T	Mono	1,000	Yes	No	Yes	No	ACV, PSL, VP16/CHOP	No	No	No	No	BMT	ADF (6)	No
12	F	6	γδT	Mono	1,000	No	No	No	No	No	No	No	Yes	No	No	AOD (9)	No
13	M	6	T	Mono	200	Yes	No	Yes	No	IVIG, PSL, VP16/CHOP	No	No	No	No	PBSCT	Dead (18)	TRE
14	M	8	NK	Di	800	No	Yes	No	No	IVIG, ACV, PSL	No	No	Yes	No	No	AOD (14)	No
15	M	13	ND	ND	300	Yes	No	No	Yes	No	Yes	No	No	No	No	AOD (18)	No

Pt patient, *HSCT* hematopoietic stem cell transplantation, *PB* peripheral blood, *Abs* antibodies, *GI* gastrointestinal tract, *CNS* central nervous system, *HMB* hypersensitivity to mosquito bites, *HV* hydroa vacciniforme, *M* male, *F* female, *T* T-cell, *Mono* monoclonal, *QL* qualitative polymerase chain reaction, *QL1* spleen only but not in PB *IVIG* intravenous immunoglobulin, *PSL* prednisolone, *IL-2* interleukin-2, *IFN* interferon, *CBT* cord blood transplantation, *TRE* transplantation-related event, *NK* natural killer cell, *Di* diclonal, *ADF* alive on disease free, *CSA* cyclosporin-A, *ACV* acyclovir, *AraC* citarabine, *MOF* multiple organ failure, *QL2* systemic sites from PB, effusion, hypopyon etc., *VP16*, etoposide, *CHOP* combine chemotherapy with cyclophosphamide, hydroxydoxorubicin, vincristine, and prednisolone, *PBSCT* peripheral blood stem cell transplantation, *ND* not determined, *BMT* bone marrow transplantation, *AOD* alive on disease

Cardiac Complications

Of the 15 patients, 9 (3 boys and 6 girls, 60%) had cardiac complications including CAL ($n = 4$, 27%), pericardial effusion ($n = 3$, 20%), decreased LVEF ($n = 2$, 13%), complete atrioventricular block ($n = 1$), and sudden cardiac arrest ($n = 1$) (Table 2). The median age at detection of cardiac complications was 6.1 years (range, 0–12.5 years). The clinical profile was compared between the patients with and those without cardiac complications (Table 3). The EBV load tended to be higher in patients with cardiac complications (median, 3,000/ml) than in those without complications (median, 800/ml), but this difference did not reach statistical significance. When the major symptoms were compared between patients with and those without cardiac disease, the frequency of fever (77.8% vs. 16.7%; $p = 0.0406$) or cytopenia (100% vs. 33.3%; $p = 0.0110$) was higher in patient with cardiac involvement than in those without cardiac involvement.

Before the onset of cardiac diseases, all patients required stage 2 or 3 treatment. Meanwhile, two of six patients free of cardiac diseases received no medication (stage 0). Seven of nine patients with cardiac complication died of disease progression (patients 4, 6, 7, and 8) or transplantation-related events (patients 1, 3, and 9), whereas two patients underwent successful CBT. Six patients had no cardiac disease. Patient 10 died of an overwhelming postsplenectomy infection; patient 13 died of graft-versus-host disease and EBV reactivation; and patient 11 survived after BMT from an human leukocyte antigen-identical sibling donor. At this writing, three patients are alive with disease but no recurrent fever.

The four patients with CAL had a median age of 9 years (range, 4.8–17.7 years) at the detection of CAL (Table 2). The median time between onset of CAEBV and detection of CAL was 3.4 years (range, 1.8–8.6 years). The case of patient 6 involved both coronary arteries, whereas patients 2, 4, and 5 had left CAL only. The mean maximum diameter of the affected artery was 4.4 mm (range, 4–5 mm), and the mean z-score was 3.98 (range, 2.15–6.08). The patients with CAL receiving aspirin therapy had no ischemic events. For two patients with CALs who underwent successful CBT, the lumen of the coronary arteries regressed to normal size, and the echogenecity of the arterial walls reduced normally (Fig. 1, arrows; patient 2 A1→A2, patient 5 B1→B2). Multislice spiral computed tomography 2 years after CBT confirmed no lesion in the affected coronary arteries including dilation, stenosis, or calcification in patient 5 (Fig. 1 C2).

Echocardiography and chest X-ray showed pericardial effusion and decreased LVEF in two patients and isolated pericardial effusion in one patient. ECG showed abnormal ST-T voltage indicative of myocarditis. Patient 3 experienced pancarditis at the first presentation, showing massive pericardial effusion with prolonged PR (0.17 s) and QTc intervals (0.54 s) as well as subsequently high echogenecity of coronary arteries [35]. Patients 8 and 9 experienced massive pericardial effusion and decreased LVEF 2 or 7 years after the onset of CAEBV. Patient 1 had complete atrioventricular block 7 years after the onset of CAEBV. The arrhythmia was controlled after antiviral and steroid therapy, but patient 1 died of neurologic involvement. Patient 12 died of sudden arrest during the chemotherapy, which suggests fatal arrhythmias with myocardial damage, although no autopsy was obtained.

Table 2 Coronary artery lesions in patients with chronic active Epstein–Barr virus (EBV) infection

Pt	Cardiac diseases	Affected branch	Diameter (mm)	z-score	Shape of vessel[a]	Outcome of CAL
1	Complete AV block					
2	CAL	LCA[a]	4.0	3.91	Ectasia	Regressed after the eradication of EBV
3	Low LVEF, PE	LCA[b]	2.7	0.73	Not dilated	
4	CAL	LCA[a]	5.0	6.08	Aneurysm	Persisted until the death from MOF
5	CAL	LCA[a]	4.5	4.13	Ectasia	Regressed after the eradication of EBV
6	CAL	LCA[a]/RCA[a]	4.0/4.0	2.15/3.63	Ectasia	Persisted until the death from MOF
7	Sudden cardiac arrest					
8	Fatal PE					
9	Low LVEF, PE					

Pt patient, *CAL* coronary artery lesion, *AV* atrioventricular, *LCA* left coronary artery, *LVEF* left ventricular ejection fraction, *PE* pericardial effusion, *MOF* multiple organ failure, *RCA* right coronary artery

[a] All affected vessels as CAL in patients 2, 4, 5, and 6 showed high echogenicity with enlarged internal diameter, as assessed by z-score

[b] Coronary arteries of patient 3 showed high echogenicity without enlarged internal diameter, as assessed by z-score

Table 3 Chronic active Epstein–Barr virus (EBV) infection patients with or without cardiac complications

	Complication	No complication	*p* value
Patient no. (male:female)	9 (3:6)	6 (4:2)	NS
Age at onset[a] (years)	7 (1–17)	6 (1–14)	NS
Observation period[a] (years)	8 (1–9)	3 (1–12)	NS
T-cell type: NK-cell type	7:2	3:1	NS
EBV load (copies/ml PB)	3,000 (800–50,000)	800 (200–1,000)	0.0720
Age at start of therapy[a] (years)	11 (1–18)	6 (1–17)	NS
Cardiac disease			
Age at detection of cardiac disease[a] (years)	10 (4–18)		
From onset of illness to cardiac disease[a] (years)	4 (0–9)		
Coronary artery lesion: *n* (%)	4 (44)		
Pericardial effusion: *n* (%)	3 (33)		
Arrhythmia: *n* (%)	2 (22)		
Major symptom (%)	At the cardiac disease	During the illness	
Fever (38.5°C)	78 (CAL 100%)	17	0.0406
Hepatosplenomegaly	89	67	NS
Cytopenias	100	33	0.0110
Enteropathy	10	0	NS
CNS disease	10	0	NS
HMB and/or HV	33	33	NS
Evolution to lymphoma: *n* (%)	5 (63)	1 (17)	NS
Treatment			
No medication: *n* (%)	0 (0)	2 (33)	NS
HSCT: *n* (%)	6 (67)	2 (17)	NS
Outcome			
Dead: *n* (%)	7 (78)	2 (33)	0.0824
Alive on disease: *n* (%)	0 (0)	3 (50)	
Alive on disease-free[b]: *n* (%)	2 (22)	1 (17)	

NS not significant, *NK* natural killer, *PB* peripheral blood, *CAL* coronary artery lesion, *CNS* central nervous system, *HMB* hypersensitivity to mosquito bites, *HV* hydroa vacciniforme, *HSCT* hematopoietic stem cell transplantation

[a] Median value (range)

[b] Only the patients who underwent successful HSCT obtained a disease-free state

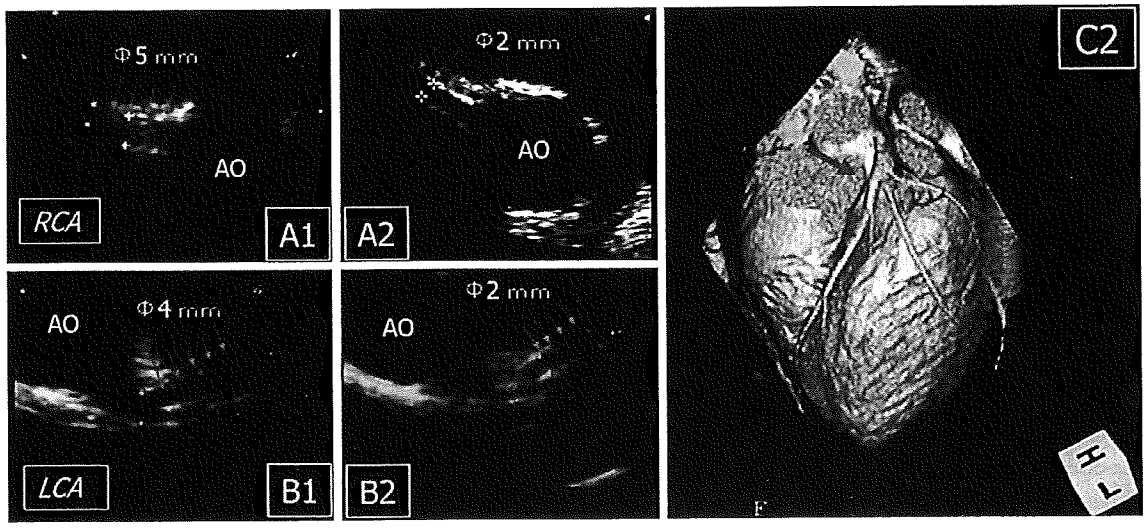

Fig. 1 Echocardiography (A1, A2, B1, B2) and multislice spiral computed tomography (C2) of coronary arteries. The dilated right coronary artery (RCA) in patient 2 (A1) regressed after an unrelated-donor cord blood transplantation (A2: *arrows*). The dilated left coronary artery (LCA) in patient 5 (B1) resulted in a normal size after an unrelated-donor cord blood transplantation (CBT) (B2: *arrows*). Computed tomography 2 years after a successful CBT showed an intact image of the coronary arteries (*arrow*) in patient 5 (C2). AO, aorta

Table 4 Reported cardiovascular complications among patients with chronic active Epstein–Barr virus (EBV)[a]

Pt	Age (years)	Sex	Cardiac complications	Major infiltrating cells positive for EBV genome (histopathologic samples)	Outcomes (causes of death)	Reports
1	2	M	CAL (rupture)	$CD4^+$T-cell (peripheral blood)	Dead	Kikuta et al. [15, 16]
2	6	F	CAL, pericarditis	ND	Dead	Kikuta et al. [15]
3	5	F	CAL, pericarditis	ND	Dead	Kikuta et al. [15]
4	23	M	Giant CAL	$CD4^+$T-cell (kidney)	Dead	Muso et al. [22]
5	4	M	CAL	$CD8^+$T-cell (systemic organ)	Dead (sepsis)	Nakagawa et al. [24]
6	5	F	CAL, abdominal aortic aneurysm	$CD8^+$T-cell (systemic organ)	Dead (respiratory failure)	Nakagawa et al. [23, 24]
7	9	M	CAL	$CD8^+$T-cell (systemic organ)	Dead (ICH)	Nakagawa et al. [24]
8	11	M	Carditis	CD45RO/$CD8^+$T-cell (heart)	Dead (heart failure)	Fujiwara et al. [6]
9	28	M	Carditis	CD45RO/$CD43^+$T-cell (heart)	Dead (heart failure)	Hauptmann et al. [8]
10	45	M	Carditis	$CD45RO^+$T-cell (heart)	Dead	Takano et al. [33]
11	10	F	Aortitis	$CD3^+$cell (aorta)	Dead	Murakami et al. [21]
12	11	F	Aortitis[b]	$CD56^+$cell (aorta)	Dead	Sato et al. [31]

M male, *CAL* coronary artery lesion, *F* female, *ND* not described about infiltrating cells but the presence of EBV genome, *ICH* intracranial hemorrhage

[a] All our reported patients were excluded

[b] Dilation of valsalva sinus and descending aorta

Discussion

The current study showed that cardiovascular disease occurred in 60% of CAEBV patients during the long-term follow-up period. Half of these patients experienced CAL at a median of 3.4 years after the onset of illness. Seven (78%) of nine deceased patients had cardiac complications, and all causes of death were disease progression or transplantation-related events.

On the other hand, CAL regressed in only two patients who achieved EBV eradication after CBT. These results suggest that EBV-infected T- and NK-cells play a central role in the onset and progression of cardiovasculopathy in CAEBV patients.

As reported by a nationwide survey in Japan, coronary artery aneurysms developed in 9% and myocarditis in 6% of CAEBV patients, and both were associated with death [18]. The higher occurrence of cardiac diseases among the current patients may be explained by the differing disease severity, follow-up period, and scrutiny procedures. Of 12 reported patients with cardiac complications (Table 4), CAL occurred in 7 (58%), carditis in 3 (25%), and aortitis in 2 patients. The diverse age distribution and equivocal sex difference were similar to those of the patients in the current study. Because all reported patients died, autopsies provided a pathologic clue of the cardiovasculopathy.

Based on the major infiltrating cells testing positive for the EBV gene [15, 16, 22, 23], both $CD4^+$T-cells and $CD8^+$T-cells could be associated with CAL (Table 4). Fujiwara et al. [6] demonstrated that EBER1-positive $CD45RO^+CD8^+$T-cells infiltrated into the myocardium of an 11-year-old boy with CAEBV who died of carditis. Cardiotropic infiltration of activated EBV^+T-cells is reported in adult patients with fatal carditis [8, 33]. Both EBER1-positive T- and NK-cells have been identified in the aortic tissues [21, 31].

In the current study, patient 3 showed prolonged intervals of PR and QTc and pericardial effusion. Patient 1 experienced complete atrioventricular block at exacerbation of disease. The sudden cardiac arrest in patient 7 could have originated from lethal arrhythmia associated with occult cardiac damage. Ottaviani et al. [30] reported a patient with EBV^+B-cell lymphoma who died suddenly of lymphomatous infiltrations into the cardiac conduction system. Cardiac complications tended to be associated with high EBV load but not with the type of CAEBV or the interval from disease onset to the development of cardiac disease. In CAEBV patients, heterogenous subsets of T- and NK-cells are the target of EBV infection [14]. Taken together, it is likely that the infiltration of EBV-infected lymphocytes primarily contributes to the development of cardiovasculopathy during the progressive disease course.

CAL was detected in CAEBV patients at a median 3.4 years from the onset of illness, whereas it usually occurs in Kawasaki disease patients after 7 to 10 days of illness [2]. The CAEBV patients with cardiac complications presented fever more frequently than the patients without complications (Table 3). High EBV load, fever,

and cytopenias may be risk factors for the development of CAL. Several reports [13, 32] addressed the microscopic findings of skin vascular lesions in CAEBV patients with hypersensitivity to mosquito bites. The histologic findings were characterized by an intimal thickening of the arterial wall, especially small-to-medium-sized muscular arteries; vast infiltration of mononuclear cells throughout the vessel walls; and destruction of internal elastic lamina, which are similarly observed in Kawasaki disease [1, 11]. According to the clinical and pathologic findings, CAEBV and Kawasaki disease may share the inflammatory process of vasculopathy. Proinflammatory cytokines such as IL-1β and IL-6 play a pivotal role in the pathophysiology of Kawasaki disease [2]. Findings show that EBV-specific genes such as LMP1 upregulate proinflammatory (TNFα and IL-16) and Th1 type cytokines (IL-2, IL-12, IL-18, and IFNγ), together with immunoregulatory cytokines (IL-10, TGFβ) that may induce the growth advantage or inhibition of apoptosis for EBV-infected T-cells [3, 20, 28]. Monokine induced by IFNγ (MIG) and IFNγ-inducible protein-10 (IP-10) are produced as chemotactic factors for stimulated T- and NK-cells, and contribute to the tissue necrosis and vascular damage in EBV$^+$ lymphoproliferative disorders by the enhanced release of granule-derived serine esterases, granzymes, and perforin [34]. In this line, EBV-associated vasculopathy may be a consequence of arteritis under the balance of pro- and antiinflammatory cytokines during the prolonged course [28].

In the current study, all four patients with CAL had already received steroid therapy at the time CAL was detected. Prolonged steroid therapy may be associated with the development of CAL in both CAEBV and Kawasaki disease.

The pathogenesis of cardio- and vasculotropic infiltration of EBV-infected T- and NK-cells remains elusive. As shown in Table 4, EBV-infected CD8$^+$T-cells were the major infiltrating cells in the cardiovascular lesion. The CD8$^+$T-cells are the target population of EBV infection in EBV-associated hemophagocytic lymphohistiocytosis [14]. However, there has been no report of CALs associated with the disease. In CAEBV patients, EBV-infected T- and NK-cells often lead to the lymphomatous lesions but not to leukemic expansion in the periphery.

Iwatsuki et al. [10] showed that hydroa vacciniforme lesions exclusively contain EBV-infected T-cells rather than NK-cells. Patient 14 had an increased number of circulating EBV$^+$NK-cells and a dominant infiltration of EBV$^+$T-cells in hydroa vacciniforme. Not only EBV-infected T- and NK-cells but also CD8$^+$ effector T-cells infiltrate the affected organs of CAEBV patients [7, 28]. Reportedly, EBER1 positive cells have accounted for approximately 20% of infiltrating cells in the myocardium, and the accompanying degenerative tissues may be a consequence of prolonged inflammation [8, 31]. This raises the possibility that the direct invasion of EBV-infected T- and NK-cells and the associated inflammatory changes contribute to the cardiovasculopathy in CAEBV patients. Further study should be directed toward the mechanisms of organ and tissue tropism of EBV-infected cells.

Notably, small coronary aneurysms regressed to normal size in two CAEBV patients after successful HSCT (respectively 2 and 4 years after the regression of the CAL). The majority of small-to-moderate-sized CALs associated with Kawasaki disease could regress to almost normal size within several years [2]. Multislice spiral computed tomography is a useful imaging tool for assessing CALs in patients with Kawasaki disease [12, 36]. It demonstrated no residual lesions from the CAL such as dilation, stenosis, and calcification in patient 5. This procedure also may document the sequelae of moderate-to-large-sized CAL in CAEBV patients. Cardiac complications could be a significant risk factor in the mortality of CAEBV patients, although these were not the primary causes of death. Early HSCT may therefore be recommended for CAEBV patients, at least before the detection of cardiovascular diseases.

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ORIGINAL PAPER

Fulminant sepsis/meningitis due to *Haemophilus influenzae* in a protein C-deficient heterozygote treated with activated protein C therapy

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Abstract A 13-month-old Japanese female with *Haemophilus influenzae* type b meningitis presented with unusually severe septic shock and cerebral infarction in half a day of fever. The initial therapy of plasma-derived activated protein C (Anact C®) led to an impressive effect on the aggressive condition. However, purpura fulminans and the consistent decline of plasma protein C activity (<20%) required prolonged activated protein C therapy and gene analysis. The patient carried a novel heterozygous mutation of *PROC* (exon 4; 335 GAC>TAC, Asp46Tyr). This is the first report of infectious purpura fulminans in a protein C-deficient heterozygote. The clinical onset and treatment course adequately corroborated the aggravated immune/hemostatic reactions and the cytoprotective effects of activated protein C replacement in human heterozygous protein C deficiency. The monitoring of plasma protein C activity and sufficient administration of activated protein C product could improve the outcome of severe sepsis in children.

Keywords Purpura fulminans · Sepsis · Heterozygous protein C deficiency · Activated protein C therapy

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Abbreviations

aPC	Activated protein C
APTT	Activated partial thromboplastin time
CSF	Cerebrospinal fluid
CRP	C-reactive protein
CT	Computed tomography
DIC	Disseminated intravascular coagulopathy
FVL	Factor V Leiden
FDP	Fibrinogen and fibrin degradation products
Hib	*Haemophilus influenzae* type b
PC	Protein C
PIC	Plasmin α2–antiplasmin complex
PS	Protein S
PT	Prothrombin time
SIRS	Systemic inflammatory response syndrome
TAT	Thrombin–antithrombin complex

Introduction

Sepsis/systemic inflammatory response syndrome (SIRS) involves the hemostatic system. Excessive activation of procoagulant and anti-fibrinolytic processes, along with endothelial damage, leads to the formation of microthrombi in the microvasculature and disseminated intravascular coagulopathy (DIC). Purpura fulminans is a life-threatening complication in sepsis children, presenting with petechiae, ecchymosis, hemorrhagic bullae, and acral necrosis that may require amputation [2, 3, 8]. It occurs spontaneously in the neonates with homozygous protein C (PC)/protein S (PS) deficiency. Young or middle-aged adults with the heterozygous PC/PS mutation of are at risk of stroke, pulmonary embolism, and deep vein thrombosis. Activated

PC (aPC) involves not only anticoagulant activity via the proteolytic inactivation of factors Va and VIIIa, and aPC resistance often caused by factor V Leiden (FVL), but also direct cytoprotective effects distinct from the anticoagulant activity [5, 9, 11, 12]. These beneficial effects include alterations in the gene expression profile, anti-inflammatory action, anti-apoptosis, and the stabilization of endothelium [13]. The aPC replacement is a sole anti-coagulant therapy used to reduce the mortality of sepsis patients. PC-deficient mice show the exacerbated hemostatic and inflammatory reactions in sepsis/endotoxemia models, according to the endogenous PC levels [5, 11, 12]. However, neither purpura fulminans nor accelerated septic response has been documented in human heterozygous PC deficiency.

We report a 13-month-old heterozygote of *PROC* mutation who presented with an aggressive onset of *Haemophilus influenzae* type b (Hib) meningitis. Severe sepsis and purpura fulminans were treated with prolonged administration of plasma-derived aPC (Anact C®). The potential risk of sepsis children with inherited PC deficiency and appropriate aPC therapy were discussed.

Case report

A 13-month-old Japanese female was hospitalized with left-sided paralysis after hemiconvulsion within a few hours of fever. Emergency studies revealed; WBC 7.200×10^9/L, Hb 10.7 g/dl, platelets 185×10^9/L, and C-reactive protein (CRP) 1.55 mg/dl. Computed tomography (CT) scanning revealed a right edematous hemisphere (Fig. 1a). The cerebrospinal fluid (CSF) showed pleocytosis (leukocyte counts 311/μl, 99% of neutrophils). Hib-antigen test was positive. Hib was then isolated from blood and CSF cultures. She was diagnosed as having purulent meningitis and was immediately treated with ampicillin and cefotaxime following dexamethasone injection. Just after the initial infusion of the antibiotics, the patient went into shock and was immediately transferred to us upon receiving cardiopulmonary resuscitation. The comatose infant showed; WBC 5.880×10^9/L, Hb 7.5 g/dl, platelets 35×10^9/L, creatine kinase 954 U/L, CRP 7.72 mg/dl, fibrinogen <50 mg/dl, prothrombin time (PT) 41.6 s (PT-% 16%), activated partial thromboplastin time (APTT) >120 s, and fibrinogen degradation products (FDP) 76.8 μg/ml. Laboratory data on day 2 of illness showed; CRP 9.19 mg/dl, creatine kinase 31,869 U/L, and platelets 9×10^9/L. The thrombin–antithrombin complex (TAT) was >80.0 ng/ml and the plasmin α2–antiplasmin complex (PIC) was 1.5 μg/ml. CSF became sterile, containing leukocytes 3,070/μl, red cells >1,500/μl, protein 546 mg/dl, and glucose 66 mg/dl. The serum endotoxin level was 6.3 pg/ml (normal <5 pg/ml). Brain CT scan showed expanded low-density areas, spotty bleedings in the edematous right hemisphere, and subarachnoid hemorrhage (Fig. 1b). Purpura, acral gangrene (Fig. 2a,b), and hemoglobinuria emerged with the rapid rise of FDP level and low PC activity (Fig. 3). Plasma-derived aPC (Anact C®) therapy led to the drastic improvement of clinical and laboratory findings (Figs. 2c and 3). Despite the initial severity, the subsequent treatment course was not complicated. The patient escaped amputation (Fig. 2d,e), but has been challenged by the cerebral damage.

During the treatment course, the end of aPC therapy lead to the drop of PC activity (31→19%) (Fig. 3). Her family history revealed that her maternal grandfather died of cerebral infarction at 44 years of age and his brother died of myocardial infarction at 41 years of age. These prompted us to conduct the genetic analysis by our established method [10]. This patient, but not 99 healthy control Japanese, carried an unreported heterozygous mutation in exon 4 of *PROC* (335 GAC>TAC, Asp46Tyr). There was

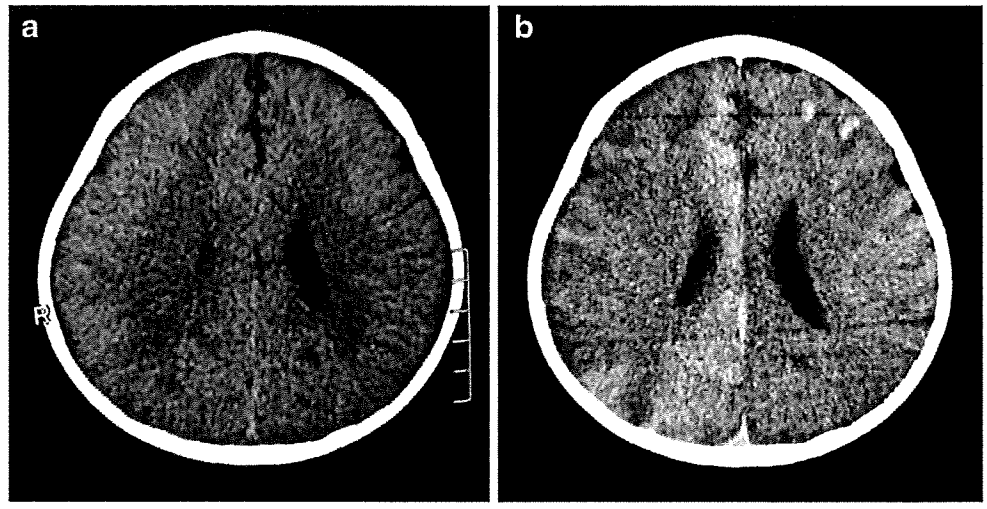

Fig. 1 Computed tomography (CT) scan of the brain at first admission in an affiliated hospital (**a** several hours after the onset of fever), showing a right edematous hemisphere, and in our hospital (**b** 48 h after the onset of fever), showing the expanded low-density and spotty parenchymal bleeding in the edematous right hemisphere, along with subarachnoid hemorrhage. Venous thrombosis was suggested as the underlying process of these findings. The patient survived with neurological sequelae

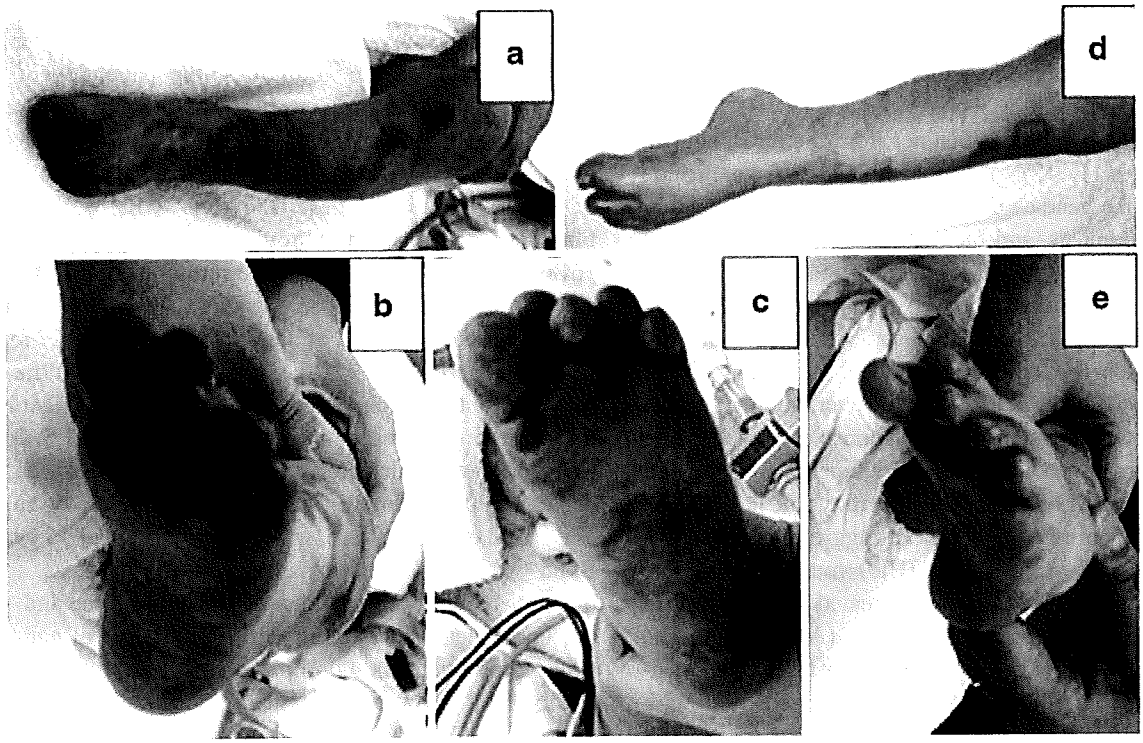

Fig. 2 Changes in the acral gangrene and purpura of the foot (**a**, **b**) before and (**c**) after the continuous infusion of plasma-derived activated protein C product (Anact C®). No amputation was required (**d**, **e**)

no protein S gene (*PROS*) mutations in the patient. Plasma PC activity was 50% in the mother, 52% in the brother, and 98% in the farther (normal 75~131%). All three family members were in good health and experienced no thromboembolic episodes. Both the mother and brother carried the same heterozygous mutation as the patient.

Discussion

Acute infectious purpura fulminans mostly occurs in young children. The major causative agents include meningococcus, pneumococcus, streptococcus, and varicella-zoster virus [2, 3, 15]. Only four patients with Hib-associated

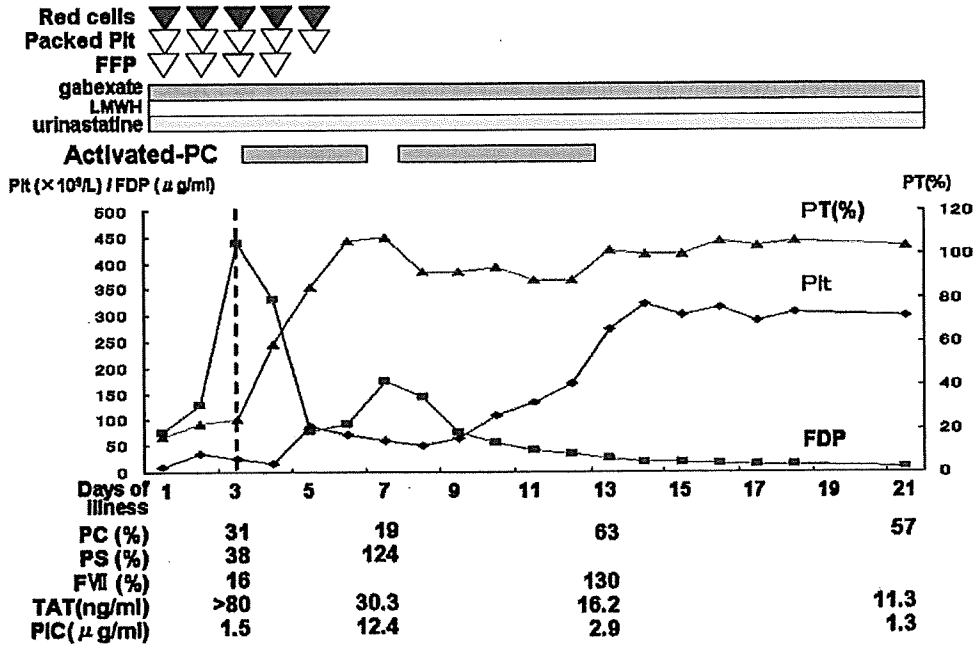

Fig. 3 Treatment course of the infant with *Haemophilus influenzae* meningitis complicated with purpura fulminans. FDP: fibrinogen and fibrin degradation products; FFP: fresh frozen plasma; FVII: factor VII; LMWH: low molecular weight heparin; PC: protein C; PIC: plasmin α2–antiplasmin complex; Plt: platelet; PS: protein S; PT: prothrombin time; TAT: thrombin–antithrombin complex

purpura fulminans have been reported [3, 4, 6, 17]. No purpura fulminans has been reported in heterozygous PC deficiency. According to a recent study of 16 purpura fulminans children in Turkey [8], six patients carried FVL (Arg506Gln) and one patient had heterozygous PS deficiency, but none had congenital PC deficiency. All six patients with transient low PC activities were <2 years of age, and four of them had severe infection [3]. FVL, the major responsible gene for Caucasian thrombophilia, is extremely rare in Asia. On the other hand, the frequency of heterozygous *PROC* mutation in Japanese deep vein thrombosis was three times higher than in Caucasians [13]. The observed prevalence of PC deficiency in Japan is estimated to be 1/620 [16]. Hib is still the leading cause of bacterial meningitis in Japanese children because no available vaccine has been introduced. These foundations might involve the first documentation of Japanese infants.

Heterozygous PC-deficient mice have been used in experiments to determine the exacerbated septic and endotoxemic responses and poor outcome [5, 12]. Lay et al. [11] reported that the excessive inflammatory reactions depend on the dose of lipopolysaccharide and the endogenous PC levels using various lines of the PC-deficient mice model. On the other hand, heterozygous FVL mice had a potential survival benefit in sepsis due to elevated thrombin and increased aPC generation [13]. The cytoprotective effects of aPC are facilitated directly on cells via the distinct receptors of endothelial PC receptor and protease-activated receptor-1 expressed on leukocytes, endothelial cells, and smooth muscle cells. Both mice models [9, 11] suggested a critical role of the anti-inflammatory and cytoprotective function of the aPC pathway in severe infection. In the clinical trial PROWESS, the 28-day mortality was lower in FVL heterozygotes (13.9%) than in non-FVL patients (27.9%) [13]. However, it was unknown whether congenital human PC-deficient heterozygotes have a higher risk of DIC and adverse outcome during sepsis. In the present patient, the rapid deterioration at the onset of meningitis with the marginal rise of circulating endotoxin might recapitulate the results of PC-deficient mice models [11, 12]. Effective life-support of the shock infant allowed us to observe the unique early-onset DIC-presenting thrombotic thrombocytopenic purpura-like hemolysis, multiple cerebral infarction, and acral gangrene, along with persistent high ratio of TAT to PIC (Fig. 3). Prolonged aPC therapy successfully overcame the progressive septic response in our patient with congenital type I PC deficiency. This patient is the first to exemplify the aggravated immune/hemostatic reactions and the effective aPC therapy in human congenital PC-deficiency.

In the PROWESS trial [1], aPC therapy has had clinical success in reducing the mortality of sepsis/SIRS adult patients. On the other hand, the RESOLVE study failed to find any benefit for the use of aPC in children with severe sepsis [14]. This discrepancy might depend on the different sample size and/or distinct mortality in the placebo group (PROWESS 31%, RESOLVE 17%). The clinical utility of aPC replacement might be limited in severe cases, as shown in our patient or mice models [11]. There are several reports indicating that lower levels of PC, as would be expected in PC-deficient heterozygotes, correlates with worse outcomes in sepsis [19]. The beneficial effect of aPC infusion was not dependent on the actual PC level in the PROWESS trial [1]. However, the ENHANCE trial of drotrecogin alfa (recombinant aPC) showed a significant association between increased 28-day mortality and decreased end-of-infusion PC levels [7]. More recently, Shorr et al. [18] recommended the sufficient infusion of drotrecogin alfa according to the PC level, based on the result that the PC level was the only variable correlated with both the effect of aPC therapy and survival. To reduce the adverse bleeding and to maximize the cytoprotective effect of aPC, monitoring the plasma PC activity and appropriate aPC replacement will improve the outcome of severe sepsis in children. Further study is needed in order to establish the optimal aPC therapy for sepsis patients.

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