TTV has a phylogenic similarity with chicken anemia virus. Taken together, it may raise the possibility that TTV leads to the maturation arrest and apoptosis of infected hematopoietic progenitor cells mimicking to human parvovirus B19. Of note, it has been reported that a putative TTV/1a-derived 105 aa protein induced apoptosis in cultured cells [4]. The copy number of TTV per MNCs was higher in PB than in BM. It might be explained by the fact that PB samples were taken from the patient 4 days earlier than BM samples during the convalescent phase of hepatitis. Alternatively, erythroid and myeloid progenitor cells with high TTV loads had been already depleted in the hypoplastic BM cells based on the different cellular tropism of TTV/1a. Significant TTV/1a load in hypoplastic BM cells implicated a direct effect of the virus on the hematopoietic progenitor cells but not always explain the continued aplasia following the disappearance of circulating TTV DNA. Until now, we have investigated three other patients with HAA in our institute; all showed negative TTV DNA in sera at the onset of illness (unpublished data).

Hence, TTV/1a may not be a major causative agent in HAA. However, the first identified case of TTV-HAA deserves further analyses on the pathological significance of TTV isolates/genotypes.

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Potential conflicts of interest All authors have no conflicts of interest.

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Hematopoietic Stem Cell Transplantation for Familial Hemophagocytic Lymphohistiocytosis and Epstein–Barr Virus-Associated Hemophagocytic Lymphohistiocytosis in Japan

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Background. Post-transplant outcomes of hemophagocytic lymphohistiocytosis (HLH) patients were analyzed in Japan where Epstein–Barr virus (EBV)-associated severe forms are problematic. ***Methods.*** Fifty-seven patients (43 familial HLH [12 FHL2, 11 FHL3, 20 undefined], 14 EBV-HLH) who underwent stem cell transplantation (SCT) between 1995 and 2005 were enrolled based on the nationwide registration. ***Results.*** Fifty-seven patients underwent 61 SCTs, including 4 consecutive SCTs. SCTs were employed using allogeneic donors in 93% of cases (allo 53, twin 1, auto 3). Unrelated donor cord blood transplantation (UCBT) was employed in half of cases (21 FHL, 7 EBV-HLH). Reduced intensity conditioning was used in 26% of cases. The 10-year overall survival rates (median ± SE%) were 65.0 ± 7.9% in FHL and 85.7 ± 9.4% in EBV-HLH patients, respectively. The survival of UCBT recipients was >65% in both FHL and EBV-HLH patients. Three out of four patients were alive with successful engraftment after second UCBT. FHL patients showed a poorer outcome due to early treatment-related deaths (<100 days, seven patients) and a higher incidence of sequelae than EBV-HLH patients ($P = 0.02$). The risk of death for FHL patients having received an unrelated donor bone marrow transplant was marginally higher than that for a related donor SCT ($P = 0.05$) and that for UCBT ($P = 0.07$). ***Conclusions.*** EBV-HLH patients had a better prognosis after SCT than FHL patients. FHL patients showed either an equal or better outcome even after UCBT compared with the recent reports. UCB might therefore be acceptable as an alternate SCT source for HLH patients, although the optimal conditioning remains to be determined. Pediatr Blood Cancer 2010;54:299–306.

Key words: central nervous system disease; Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis; familial hemophagocytic lymphohistiocytosis; hematopoietic stem cell transplantation; reduced intensity conditioning; umbilical cord blood transplantation

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is an immunohematologic emergency, characterized by fever, cytopenias, hepatosplenomegaly, hyperferritinemia, and disseminated intravascular coagulopathy (DIC) [1,2]. HLH comprises primary form of familial hemophagocytic lymphohistiocytosis (FHL) and secondary form occurring in association with infections, malignancies, and rheumatic diseases. FHL has currently been classified into FHL1 linked to chromosome 9, FHL2 with *PRF1* mutation, FHL3 with *UNC13D* mutation, and FHL4 with *STX11* mutation, although more than half of patients have no mutations of these genes [1]. HLH could also be a presenting symptom in patients with the other inherited disorders including X-linked lymphoproliferative disease (XLP), Griscelli syndrome, Hermansky–Pudlak syndrome, Chediak–Higashi syndrome and primary immunodeficiency diseases. HLH accounts for the common basis of hypercytokinemia arising from excessive immune activation, in which activated lymphocytes and hemophagocytosing-macrophages without malignant morphology infiltrate into systemic organs, including the bone

Additional Supporting Information may be found in the online version of this article.

Abbreviations: BM, bone marrow; BMT, bone marrow transplantation; CB, cord blood; CBT, cord blood transplantation; CNS, central nervous system; CT, computed tomography; EBV-HLH, Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis; EEG, electroencephalography; FHL, familial hemophagocytic lymphohistiocytosis; HLH, hemophagocytic lymphohistiocytosis; PB, peripheral blood; SCT, hematopoietic stem cell transplantation; MRI, magnetic resonance imaging; OS, overall survival; SCT, hematopoietic stem cell transplantation; TRM, treatment-related mortality; RIC, reduced intensity conditioning; VOD, venoocclusive disease; XLP, X-linked lymphoproliferative disease/syndrome.

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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.

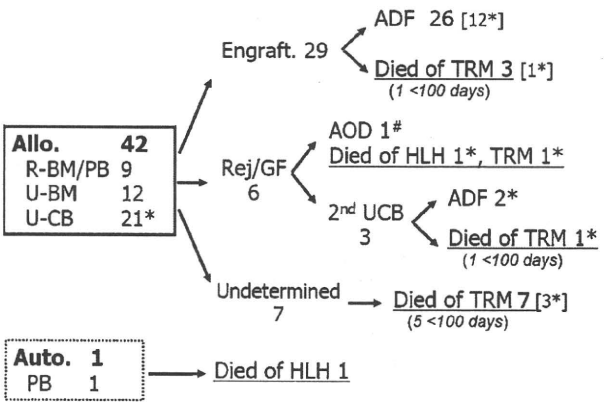

Fig. 1. Cohort diagram for the clinical outcome of 43 patients with familial hemophagocytic lymphohistiocytosis (FHL) who underwent stem cell transplantation (SCT). Of 42 patients after allogeneic SCT, 29 achieved engraftment (18 complete, 3 mixed) and 6 failed to engraft. One (#) with graft failure 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.

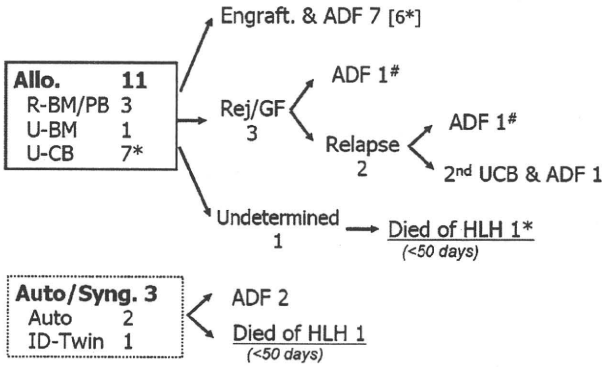

Fig. 2. Cohort diagram for the clinical outcome of 14 patients with Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) who underwent SCT. Among 11 patients after the first allogeneic SCT, 7 achieved successful engraftment and 3 failed to engraft. A total of 12 patients (86%) were 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.

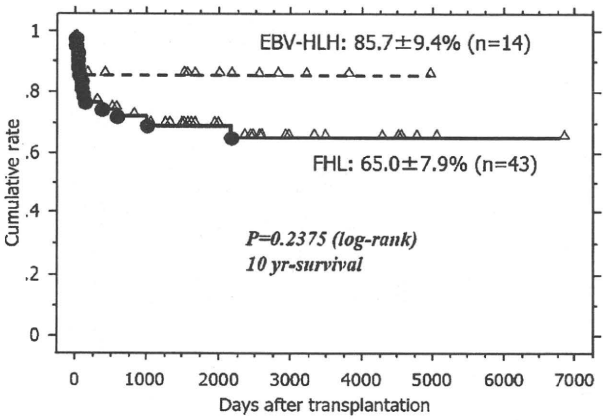

Fig. 3. Cumulative probability of post-transplant overall survival of FHL (*solid line*) and EBV-HLH patients (*dashed line*) who underwent SCT. Closed circle and open triangle represent deceased and alive patients, respectively. Each value indicates the 10-year overall survival rate plus or minus standard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

典型例とは異なる緩徐な臨床経過を示し，リボソームタンパク遺伝子 *RPL11* の変異の検出により診断した Diamond-Blackfan 貧血の 1 例

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A Diamond-Blackfan Anemia Patient with Mutation of *RPL11* Gene who Had Relatively Mild Clinical Course

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Abstract Diamond-Blackfan anemia (DBA) is a congenital pure red cell aplasia. Recently, mutations of ribosomal protein (RP) genes have been reported in about 50% of patients with DBA. We encountered an 8-year-boy who showed a relatively mild clinical course for DBA. It was difficult to make a diagnosis. DNA derived from peripheral blood of this patient was analyzed for 6 ribosomal protein genes (*RPS19, RPS24, RPS17, RPL5, RPL11, RPL35A*) known as the cause of DBA, and *RPS14*, recently identified as the responsible gene for 5q-syndrome. A novel mutation in exon 5 of *RPL11* (462delA) was identified in this patient, suggesting that he be diagnosed with DBA. In some cases showing relatively mild phenotype for DBA, identification of ribosomal protein gene mutation leads to a diagnosis of DBA.

要　旨 Diamond-Blackfan 貧血（DBA）は先天性の赤芽球癆であり，最近その原因遺伝子としてリボソームタンパク（RP）遺伝子の変異が約 50%の頻度で認められることが報告された．われわれは，典型的な DBA 症例に比べて緩徐な経過を示し，診断に難渋していた 8 歳男児の末梢血から DNA を抽出し，DBA で変異が報告されている 6 遺伝子（*RPS19, RPS24, RPS17, RPL5, RPL11, RPL35A*）および 5q-症候群の原因遺伝子として最近同定された *RPS14* について解析を行った．その結果，従来報告のない *RPL11* 遺伝子変異（exon 5 の 1 塩基欠失：462delA）を検出し DBA と診断した．本症例のように典型例とは異なり緩徐の経過を示す症例の中にも，RP 遺伝子変異から DBA の診断に至る症例が存在するものと思われた．

Key words: Diamond-Blackfan anemia, ribosomal protein gene, RPL11

I. はじめに

Diamond-Blackfan 貧血（DBA）は乳児期に発症する赤血球造血のみが障害される先天性の赤芽球癆である．近年，DBA の原因として，約 50%の頻度でリボソームタンパク（RP）遺伝子の変異が認められることが明らかになった[1]．その中で *RPS19* の変異が約半数を占め，そのほかにも *RPS24, RPS7, RPS17, RPL5, RPL11* および *RPL35A* の各遺伝子変異が報告され，DBA に関しては「リボソーム病」としての新たな疾患概念が確立しつつある[2]．今回，新規の *RPL11* 変異を伴った DBA の 1 例を経験したので報告する．

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II. 症　　例

症例：8 歳男児（日系ブラジル人）．

家族歴：母方祖父に白血病の既往あり．父には輸血後肝炎の既往あり（輸血が必要となった理由は不明）．

現病歴：1歳時に貧血のため近医に入院し，Epstein-Barr（EB）ウイルスによる transient erythroblastopenia of children（TEC）と診断された．4歳2カ月時には上気道炎を契機に貧血が進行し，ブラジルにて骨髄穿刺を含む検査が行われたが診断に至らず，無治療にて貧血は改善した．4歳10カ月時に再び上気道炎を契機に貧血が進行し，当院入院となった．

入院時現症（4歳10カ月時）：身長：103.3 cm（−0.5 SD），体重：13.5 kg（−1.7 SD），意識清明，眼瞼結膜は貧血様，眼球結膜黄染なし，病的リンパ節腫脹なし，咽頭発赤軽度，胸部聴診上異常所見なし，腹部は平坦かつ軟，肝脾腫なし，皮疹なし，外表奇形なし，発達は年齢相当であった．

1. 4歳10カ月時入院時検査所見

血液検査所見（Table 1）：正球性貧血を呈しており，網状赤血球は低値であった．鉄欠乏性貧血や溶血性貧血，巨核芽球性貧血は否定的であった．

骨髄像（Fig. 1）：有核細胞数は110,000/μl，巨核球数56.3/μl，M/E比3.9．赤芽球系のみ著しい低形成を認め，一部 myeloid 系に軽度の異形成を認めた．

コロニーアッセイでは CSF-GM, BFU-E ともに正常の5〜10倍のコロニー形成を認めた．

染色体検査（G-banding）：46, XY，染色体検査（FISH）：monosomy 7（−），trisomy 8（−），染色体断裂試験 2/100 break/gap.

2. 4歳10カ月時入院後経過

入院後赤血球輸血を行い，輸血後は Hb 8〜9 g/dl 台へ上昇したが，その後徐々に低下し輸血を行わない状態で，Hb 5.3〜5.8 g/dl（網状赤血球 2〜3‰）を推移したため，以後外来にて経過観察の方針とした．退院（5歳1カ月）後，当初は感冒罹患時に年1回程度の赤血球輸血を要していた（5歳10カ月時，6歳7カ月時）が，しだいに輸血頻度が増え（7歳8カ月時，7歳10カ月時，8歳0カ月時）たため，8歳1カ月時に骨髄穿刺を含めた再評価目的で入院となった．

3. 8歳1カ月時入院時検査所見

血液検査（Table 2）：前回同様，正球性貧血を認める．網状赤血球は9.6‰であった．

骨髄像（Fig. 2）：有核細胞数は321,000/μl，巨核球数156.3/μl，M/E比7.26．過形成で赤芽球系のみ著しい低形成を認め，芽球は7.3%（type I 7.0%, type II 0.3%）とやや多かったが急性白血病は考えにくく，pure red cell aplasia（PRCA）に近い像であった（日本小児血液学会 MDS 委員会/再生不良性貧血委員会中央診断による）．

コロニーアッセイでは，CSF-GM, BFU-E ともに正常のコロニー形成を認めた．

染色体検査（G-banding）：46, XY，染色体検査（FISH）：

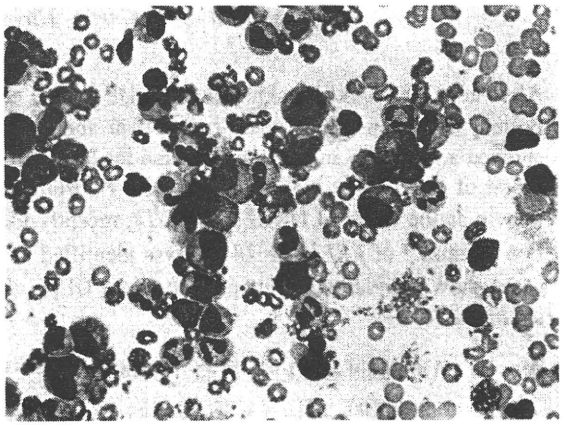

Fig. 1　Bone marrow findings (4y10mo)
Erythroid precursors are absent in normocellular bone marrow. Slight dysplastic changes in the myeloid cell are also seen.

Table 1　Laboratory findings (4y10mo)

Peripheral blood		Blood chemistry			
WBC	8,000 /μl	BUN	9.4 mg/dl	UIBC	39 μg/dl
RBC	188×10⁴ /μl	Cre	0.4 mg/dl	TIBC	226 μg/dl
Hb	6.4 g/dl	Na	137 mEq/l	Direct Coombs' test	(−)
Ht	17.3%	K	3.9 mEq/l	Indirect Coombs' test	(−)
MCV	91.8 fl	Cl	101 mEq/l	Haptoglobin	301 mg/dl
MCH	33.9 pg	AST	20 IU/l	Folic acid	12.5 ng/ml
MCHC	37.0%	ALT	9 IU/l	Vit. B12	440 pg/ml
PLT	31.2×10⁴ /μl	LDH	228 IU/l	Hemoglobin F	1%
Reticulo	0.3‰	CPK	94 IU/l	Erythropoietin	7,493.5 mU/ml
		Glu	95 mg/dl	HAM test	(−)
		CRP	1.7 mg/dl		
		Fe	187 μg/dl		
		Ferritin	120 ng/ml		

Table 2　Laboratory findings (8y1mo)

Peripheral blood		Blood chemistry			
WBC	4,900/μl	BUN	9.0 mg/dl	Ferritin	370 ng/ml
RBC	234×10⁴/μl	Cre	0.4 mg/dl	UIBC	80 μg/dl
Hb	8.0 g/dl	Na	142 mEq/l	TIBC	227 μg/dl
Ht	22.8%	K	4.2 mEq/l	Direct Coombs' test	(−)
MCV	97.2 fl	Cl	106 mEq/l	Indirect Coombs' test	(−)
MCH	34.2 pg	AST	17 IU/l	Haptoglobin	76 mg/dl
MCHC	35.2%	ALT	8 IU/l		
PLT	29.8×10⁴/μl	LDH	197 IU/l		
Reticulo	9.6‰	CPK	145 IU/l		
		Glu	91 mg/dl		
		CRP	0.1 mg/dl		
		Fe	147 μg/dl		

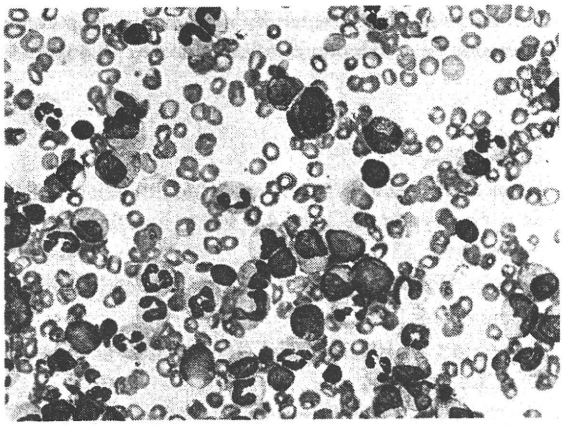

Fig. 2　Bone marrow findings (8y1mo)
Erythroid precursors are insufficient in hypercellular bone marrow.

monosomy 7 (−), trisomy 8 (−).

4. 8歳1カ月時入院後経過

PRCA がもっとも疑わしかったが，骨髄穿刺にて芽球を 7.3%認め，骨髄異形成症候群（MDS）との鑑別が必要であったことから，DBA の原因遺伝子である RP 遺伝子の変異についての解析を行った．解析に際しては，それまで DBA で変異の報告がある 6 遺伝子（*RPS19, RPS24, RPS17, RPL5, RPL11, RPL35A*）および 5q−症候群の原因遺伝子である *RPS14*[3] について変異の検索を行った．その結果，*RPL11* 遺伝子の exon 5 に 1 塩基欠失（462delA）を検出し，その結果 frameshift による premature stop codon が挿入されることがわかり，DBA と診断した（Fig. 3）．

退院後も輸血依存の状態が続いていた（8 歳 3 カ月，8 歳 4 カ月時に赤血球輸血を施行）が，RP 遺伝子変異の結果判明後 prednisolone 1 mg/kg/day の内服を開始したところ貧血の改善が得られ，ステロイド治療開始後 9 カ月現在，赤血球輸血が不要の状態で経過している．今後も同治療を継続しフォローしていく方針である．

III. 考　　察

1936 年 Josephs により 2 例[4]，2 年後には Diamond と Blackfan により 4 例[5] が報告されて以来，DBA の病因に関するさまざまな研究が行われてきたが，長らく病因は不明であった．1999 年に *RPS19* 遺伝子変異が DBA 患者の約 25%に認められることが報告されて以来[6]，DBA の原因遺伝子として複数の RP 遺伝子が同定されてきた．RP 遺伝子変異に伴うリボソームの機能障害のため生じる翻訳の異常が DBA の造血障害の中心的なメカニズムであることが明らかになりつつある[7]．これまでに同定された RP 遺伝子の異常により DBA の約半数について原因遺伝子の特定が可能であるが，まだ残り半数の DBA の原因遺伝子は不明である[1]．

本症例で認めた *RPL11* 遺伝子変異は従来報告のない新規のものであった．DBA の約 30%で奇形の合併を認め[1]，とくに *RPL5* では口唇，口蓋裂，先天性心疾患や母指の異常などの多発奇形がみられ，*RPL11* 遺伝子変異では母指単独の異常が高頻度で認められる[8-10] が，本症例では母指の異常を含め外表奇形は認めなかった．

RPL11 遺伝子変異例を含めて，DBA 患者のうち大部分は乳児期に発症し，赤血球輸血依存となることも多い[11] が，本症例は初発が 1 歳過ぎと遅く，赤血球輸血依存となったのも学童期に入ってからと，従来の報告よりも緩徐な経過を示していた．また経過中，網状赤血球数は必ずしも低値ではなく正常から低値にかけて変動を認めたこと，コロニーアッセイで BFU-E のコロニー形成が認められたことを含め，典型的な DBA とは異なる経過および検査結果のため診断に苦慮していたが，RP 遺伝子変異の検出から診断に至った．本症例での緩徐な症状の進行，典型例と異なる検査結果は新規の遺伝子変異と関連する可能性も考えられたが，この点についてはさらなる検討が必要である．

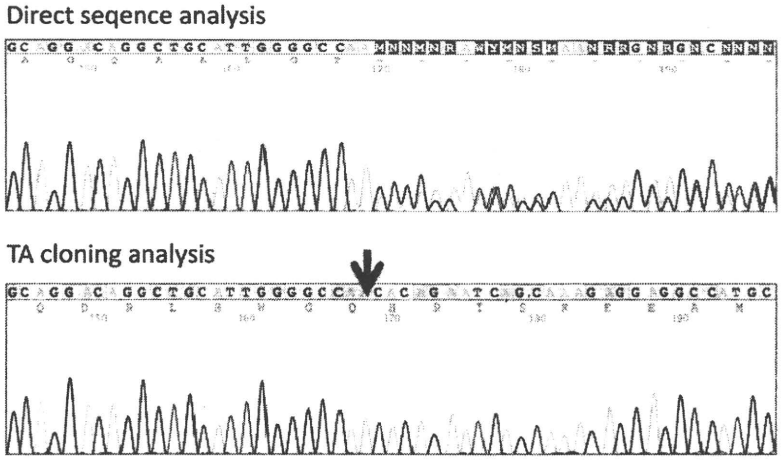

Fig. 3 Germline mutation analysis of the *RPL11* gene revealed the substitution of an alanine by a premature stop codon at amino acid position 153 (p.A153fs*40) resulting from a heterozygous one base deletion at nucleotide 462 (c.462delA) in exon 5.

一方，治療反応性に関して，*RPS19* 遺伝子変異のない症例ではステロイド治療の奏効率が70%を超えるのに対して，*RPS19* 遺伝子変異のある症例では奏効率46%と反応不良で，輸血依存性が高くなるとの報告がある[12]．*RPL11* 遺伝子変異の有無とステロイド治療反応性に関しては症例数も限られていることから報告はないが，本症例では治療開始前に輸血依存であったのがステロイド治療開始後は輸血が不要な状態が続いており，治療反応性は比較的良好であると考えている．

8歳1カ月時の骨髄穿刺で芽球の増多（7.3%）を認めMDSとの鑑別に苦慮したが，DBAではAMLやMDSなどを発症するリスクも高いとされる[13]ため，今後注意深い経過観察が必要である．

IV. 結　　語

典型的なDBAとは異なり，緩徐な経過を示す症例の中にもRP遺伝子を検索することによりDBAの診断に至る症例が存在する．今後，臨床的にDBAが疑われる症例について，RP遺伝子変異の検索を行うことは診断に必須の検査になってくると思われる．

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