OVA peptide (data not shown), probably due to a very low number of Ag-specific T cells. We also tested whether another Notch ligand, Jagged2, has a similar ability to DL1. However, inoculation of DCs transduced with Jagged2 (Jagged2-DCs) did not suppress EG7 growth (Fig. 5*C*), and T cells from Jagged2-DCs did not exhibit cytolytic activity against OVA-pulsed EL4 cells (Fig. 5*D*). The *hes1* expression in T cells from Jagged2-DC–inoculated mice was higher than that from cont-DC–inoculated mice but lower than that from DL1-DC–inoculated mice (Supplemental Fig. 3). Taken together, these data indicate that manipulation of Notch signaling induced by DL1 could be beneficial as a new strategy to augment antitumor CTL activity.

Discussion

Notch signaling controls mature T cell differentiation and activation by directly regulating transcription of effector molecules (7, 8, 14, 15). In particular, our group has recently demonstrated that Notch2 signaling directly controls CTL effector molecules, including granzyme B, by integrating RBP-J and CREB1 (8). In this study, we revealed that Notch2, but not Notch1, signaling in $CD8^+$ T cells is required for efficient induction of antitumor CTLs. Furthermore, treating tumor-bearing mice with anti-Notch2 mAb or DL1-DCs strengthened antitumor CTL responses. These data indicate that Notch2 signaling is required for augmenting antitumor CTL activity and suggest that manipulation of Notch2 signaling might provide a new clinical approach for cancer immunotherapy.

We have recently demonstrated that Notch signaling controls cytotoxic responses in both CTL and NK cells (8, 9). These data suggest that Notch signaling is a crucial signaling pathway required for cytotoxic responses in immune cells. In the studies described in this report, we found that deficiency of Notch2 but not Notch1 decreased the antitumor responses in vivo, although Notch1 is highly expressed on activated $CD8^+$ T cells (8). These data suggest a lower affinity of Notch1 and Notch ligands present in our tumor model relative to those that activate the Notch2 pathway in $CD8^+$ T cells. Notch–Notch ligand interaction is tightly regulated by Notch glycosylation (16), which might be crucial for the distinct Notch receptor utilization controlling CTL responses that we observe. We also found that treatment with anti-Notch2 agonistic Ab in tumor-bearing mice increased antitumor responses. These data strongly suggest that the major target cells for anti-Notch2 mAb in terms of antitumor effects would be $CD8^+$ T cells, although we cannot completely deny the possibility that anti-Notch2 mAb interacts with Notch2 on non-$CD8^+$ T cells, which may indirectly affect Notch2 signaling in $CD8^+$ T cells. Notch2 is widely expressed on many tissues, and thus treatment with anti-Notch2 mAb might have some adverse effects for the host, although we have never seen any macroscopic changes in mice after Ab treatment. Nevertheless, to reduce the possibility of adverse effects of anti-Notch2 mAb, the appropriate route or dose must be considered.

Our previous study showed that DL1 is able to augment CTL responses in vivo (8). We demonstrated in this study that injection of DL1-DCs peritumorally suppresses tumor growth compared with cont-DCs. These data suggest a potential therapeutic strategy to augment antitumor CTLs by injecting DL1-DCs pulsed with tumor-specific Ags. However, we should be cautious with this approach in terms of clinical use because Notch signaling regulates angiogenesis, which nurtures tumor cells (17–19). Those studies revealed that delta-like 4 contributes to angiogenesis. Although the contribution of DL1 to angiogenesis around tumor cells has not been reported, DL1 might also be able to activate Notch receptors that control angiogenesis. Thus, injection of DL1-DCs around the tumor burden may promote angiogenesis for tumor growth, although DL1-DCs would also help to strengthen CTL-mediated killing of tumor cells. The i.v. or s.c. route as a method to transfer DCs has been used in human clinical trials for treating cancer patients. Therefore, it would be important to carefully evaluate whether i.v. or s.c. injection of DL1-DCs affects angiogenesis at the tumor site before applying those methods for clinical use.

In the present work, we have focused on investigating the role of Notch2 signaling in tumor immunity and the effect of anti-Notch2 mAb or DL1-DC treatment on tumor eradication. We show that these treatments enhance survival and decrease the size of the tumor by augmenting CTL activity. The data suggest that stimulation of Notch2 would be a new way to stimulate antitumor immune responses. Combining anti-Notch2 mAb with other therapeutic approaches, such as DC-mediated tumor vaccines, is likely to yield further clinical benefits.

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Disclosures

The authors have no financial conflicts of interest.

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Nationwide Survey of Single-System Single Site Langerhans Cell Histiocytosis in Japan

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Background. Since neither a standard treatment nor a protocol study for single-system single site (SS-s)-type Langerhans cell histiocytosis (LCH) exists, we conducted a nationwide survey in Japan to clarify the epidemiology and clinical outcome of this subtype. ***Procedure.*** Questionnaires regarding the clinical course of children with SS-s-type LCH diagnosed between 1995 and 2006 were sent to all members of the Japanese Society of Pediatric Hematology. ***Results.*** One hundred forty-six children with histologically proven SS-s LCH were evaluable. The most frequently affected organ was bone (82%), followed by skin (12%). Few patients (14%) had a CNS-RISK lesion defined by the Histiocyte Society. Patients with a skin lesion were diagnosed at a significantly younger age than patients with a bone lesion (median: 6 months vs. 5 years 11 months, $P < 0.001$). The treatment regimen varied, but one-third of the patients in total and 71% of patients with a CNS-RISK lesion received chemotherapy that did not include etoposide. All but one patient attained remission. Ten patients (7%) showed reactivation. Of these, all eight with an initial bone lesion only exhibited reactivation in the bone(s). One patient with an initial skin lesion exhibited reactivation in the thymus. None of the patients died from disease progression or treatment complications. ***Conclusions.*** Our retrospective study, in which a relatively large proportion of the patients received chemotherapy, reveals that patients with SS-s LCH have a good prognosis. A prospective study should be conducted to confirm this and to identify the most effective and least toxic therapy for SS-s LCH. Pediatr Blood Cancer 2010;54:98–102.

Key words: chemotherapy; epidemiology; Langerhans' cell histiocytosis; single system

INTRODUCTION

Langerhans cell histiocytosis (LCH) is the most common histiocytic disorder characterized by the uncontrolled clonal proliferation of Langerhans cells. Its clinical manifestations and course are highly variable, and range from a self-healing solitary lesion to fatal multiorgan involvement [1]. LCH is classified into three distinct forms: single-system single site (SS-s), single-system multisites (SS-m), and multisystem (MS) type. An epidemiological study in Japan [2] has reported that the SS-s, SS-m, and MS types of LCH are diagnosed at a ratio of almost 1:1:1.

Several clinical studies have been performed to improve the outcome of LCH. These include international clinical trials run by the Histiocyte Society [3,4] and a Japanese clinical study performed by the Japan LCH Study Group (JLSG) [5]. These studies have improved the outcome of SS-m and MS-type LCH. However, in terms of SS-s-type LCH, a standard treatment or a protocol study for it is lacking [6]. To date, only one study has examined a large number of patients with single-system LCH, namely, the prospective observational study denoted as DAL-HX 83/90 [7]. Because it appears that the prognosis of patients with SS-s-type LCH is generally good, it is less common that chemotherapy is applied to them [6]. However, the patients with the craniofacial bone(s) (orbital, temporal, mastoid, sphenoidal, zygomatical, ethomoidal bones, the maxilla, paranasal sinuses, or anterior or middle cranial fossa) with intracranial soft tissue extension (the so-called CNS-RISK lesion(s)) had higher risk for the development of diabetes insipidus (DI) [8], and the LCH-III protocol study conducted by the Histiocyte Society suggests that chemotherapy should be offered to these patients, even if there is only a single lesion [9].

To further clarify the epidemiology, clinical outcome of SS-s-type LCH, we conducted a nationwide survey of LCH in Japan. We found that the rates of reactivation and sequelae were remarkably low in our cohort of SS-s LCH, in which a relatively large proportion of the patients received chemotherapy.

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MATERIALS AND METHODS

Data Collection

To compile the clinical data of new pediatric patients (age younger than 18 years at the time of diagnosis) with SS-s-type LCH who were diagnosed and treated between 1995 and 2006, the HLH/LCH Committee of the Japanese Society of Pediatric Hematology (JSPH) sent questionnaires to all the hospitals in Japan in which pediatric hematologists (JSPH members) worked. The SS-s type of LCH was defined as the infiltration of LCH cells in one site of one affected organ, as confirmed by histology. The questionnaire asked about the diagnostic procedure, the age at diagnosis, the sex, the site of the lesion, the treatment, the occurrence of complications, and the outcome. We received replies from 294 of 320 hospitals (92%). Eventually, the details of 174 patients from 81 hospitals were

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complied. Of these, 28 patients were excluded from this study for following reasons: 5 because they had multisystem-type disease, 7 because they had multifocal bone type disease, and 16 because the diagnosis was not confirmed by biopsy and histology.

Statistical Analysis

The age of diagnosis of the patients was compared by using the Mann–Whitney *U*-test. In patients with bone lesion, the therapeutic modality and the factors affecting reactivation including gender, age at diagnosis, the region affected at onset, and type of initial treatment were analyzed by using the chi-square test. *P*-values less than 0.05 were considered significant.

RESULTS

One hundred forty-six patients with SS-s LCH from 71 hospitals were evaluable. The median observation time was 3.3 years. The diagnosis was based on the presence in the lesional cells of CD1a antigen and/or Birbeck granules (98 patients), langerin antigen (1 patient), and S100 protein (31 patients), or the hematoxylin–eosin staining findings (16 patients). There were 77 males and 69 females (Table I). The median age at diagnosis was 4.8 years, ranging from 0.0 to 16.8 years. The most frequently affected organ was bone (120 patients, 82%), followed by skin (18 patients, 12%). The site of the bone lesion was a CNS-RISK in 21 patients, the skull or facial bone other than a CNS-RISK lesion in 49, the vertebra in 8, the extremities in 26, the pelvis in 5, and the thorax in 11. The age of diagnosis of the patients with a CNS-RISK lesion was significantly lower than that with other bone lesions (median age: 3 years 7 months vs. 6 years 3 months, $P = 0.021$). Of the patients with a skin lesion, 61% were less than 1 year old and were significantly younger than those with a bone lesion (median age: 6 months vs. 5 years 11 months, $P < 0.001$). The patients with a bone lesion were more frequently male (male/female ratio: 1.22), especially in those with a lesion on an extremity (ratio: 2.25). In contrast, neither gender was more likely to have a skin lesion.

Of the patients with a bone lesion, 33% were treated with chemotherapy, 35% were treated with curettage, and 23% received a biopsy only. More than 70% in the patients with a CNS-RISK lesion and nearly two-third of patients with vertebral bone lesion received chemotherapy. The frequency of receiving chemotherapy in patients with a CNS-RISK lesion was significantly high compared to in patients with other bone lesions (15/21 vs. 24/99, $P < 0.001$).

Of the patients with a skin lesion, 28% were treated with chemotherapy, while 56% were treated with biopsy only and remaining patients received surgical treatment or corticosteroid therapy (Table II). Although the chemotherapy regimen used varied, none of the patients received etoposide. All but 1 patient (99%) attained remission, but 10 patients (7%) subsequently suffered a reactivation. None of the patients died of disease progression or treatment complications. At last follow-up, 144 of 146 (99%) did not have active disease (Table II).

All eight patients with reactivated disease and an initial bone lesion exhibited a skeletal reactivation only (two in the same site at onset, one in another site, and five in multiple sites). Of the two reactivated patients with an initial skin lesion, the reactivation occurred in the skin in one and in the thymus in the other. The median duration from diagnosis to reactivation was 4 months (range, 0.1–2.5 years) (Table III). Any factors including gender, age at diagnosis, the region affected at onset, and the type of initial treatment were not associated with reactivation of LCH involving a single bone in this analysis (Table IV).

Six patients (4%) had late sequelae. Four with an initial bone lesion had orthopedic sequelae. Two patients suffered developmental impairments: one patient with a thymus lesion had a developmental impairment due to hypoxia arising from airway obstruction, while the other patient, who had a lesion on the intracranial mass, had a developmental impairment because of damage during surgery. None of the patients had DI. There was no correlation between reactivation and the sequelae (Table III).

TABLE I. Characteristics of Patients With SS-s LCH

Site involved	n (%)	Gender (M/F)	Age at diagnosis (median)
Bone	120 (82)	66/54	5m to 16y 9m (5y 11m)
CNS-RISK lesion[a]	21 (14)	14/7	6m to 14y0m (3y7m)*
Non CNS-RISK lesion[b]	49 (34)	26/23	10m to 16y0m (7y4m)
Extremities	26 (18)	18/8	5m to 15y3m (4y5m)
Thorax/shoulder	11 (8)	1/10	1y7m to 9y8m (5y0m)
Vertebra	8 (5)	5/3	11m to 16y9m (11y2m)
Pelvis	5 (3)	2/3	2y6m to 13y2m (7y0m)
Skin	18 (12)	9/9	0m to 14y1m (6m)#
Soft tissue	2 (1)	1/1	3m and 4y3m
Oral mucosa	2 (1)	1/1	1m and 6y7m
Thymus	2 (1)	0/2	5m and 3y0m
Lymph node	1 (1)	0/1	1y6m
Intra cranial mass	1 (1)	0/1	1m
Total	146 (100)	77/69	0m to 16y9m (4y10m)

m, months; y, years. [a]Combined lesions in the orbital, temporal, mastoid, sphenoidal, zygomatical, ethomoidal bones, the maxilla, paranasal sinuses, or anterior or middle cranial fossa, with intracranial soft tissue extension; [b]Skull or facial bone lesion other than CNS-RISK lesion; *Significantly young compared to patients with other bone lesion ($P = 0.021$); #Significantly young compared to patients with the bone lesion ($P < 0.001$).

TABLE II. Initial Treatment and Outcome of SS-s LCH (n (%))

		Initial treatment					Outcome			
			Corticosteroid						Status at last follow-up	
Site involved	None	Curettage / resection	Local	Systemic	Radiation	Chemotherapy	Attained remission	Subsequent reactivation	NAD	AD	Sequelae
Bone	120	27 (23)	42 (35)	7 (6)	4 (3)	1 (1)	39 (33)	120 (100)	8 (7)	119 (99)	1 (1)	4 (3)
CSN-RISK lesion[a]	21	2 (10)	3 (14)	0	1 (5)	0	15 (71[#])	21 (100)	1 (5)	21 (100)	0	0
Non CNS-RISK lesion[b]	49	8 (16)	28 (57)	0	1 (2)	1 (2)	11 (22)	49 (100)	3 (6)	49 (100)	0	2 (4)
Extremities	26	11 (42)	5 (19)	3 (12)	1 (4)	0	6[c] (23)	26 (100)	2 (8)	26 (100)	0	1 (4)
Thorax/shoulder	11	3 (27)	4 (36)	2 (18)	1 (9)	0	1 (9)	11 (100)	2 (18)	10 (91)	1 (9)	0
Vertebra	8	1 (13)	0	2 (25)	0	0	5[c] (63)	8 (100)	0	8 (100)	0	1 (13)
Pelvis	5	2 (40)	2 (40)	0	0	0	1 (20)	5 (100)	0	5 (100)	0	0
Skin	18	10 (56)	1 (6)	1 (6)	1 (6)	0	5 (28)	17 (94)	2 (11)	17 (94)	1 (6)	0
Other	8	0	4 (50)	0	0	0	4 (50)	8 (100)	0	8 (100)	0	2 (25)
Total	146	37 (25)	47 (32)	8 (5)	5 (3)	1 (1)	48 (33)	145 (99)	10 (7)	144 (99)	2 (1)	6 (4)

NAD, no active disease; AD, active disease. [a]Combined lesions in the orbital, temporal, mastoid, sphenoidal, zygomatical, ethomoidal bones, the maxilla, paranasal sinuses, or anterior or middle cranial fossa, with intracranial soft tissue extension; [b]Skull or facial bone lesion other than CNS-RISK lesion; [c]Including one patient received treatment combined chemotherapy and radiation; [#]Significantly high incidence compared to patients with the other bone lesion ($P < 0.001$).

DISCUSSION

In this study, we retrospectively analyzed 146 patients with SS-s LCH. Although the pediatric hematologists in over 90% of the hospitals in Japan answered the questionnaire we sent, it remains possible that some patients were excluded because they were under the care of an orthopedist or dermatologist.

In our cohort, the organ that was most frequently affected was bone (over 80% of the patients had a lesion in bone), followed by skin. The patients with a skin lesion were younger than those with a bone lesion, while males developed SS-s LCH more frequently than women. These features were quite similar to those of the cohort studied by the DAL-HX study [7]. They were also consistent with the results of an epidemiological study that found, of unifocal LCH patients, 70% had a bone lesion, 77% of the patients with a skin lesion were less than 1 year old, and males were more often affected by the disease than females (male/female ratio: 1.3) [10].

The involvement of CNS-RISK lesion(s) carry an about threefold risk for the development of DI which is the hallmark of central nervous system involvement in LCH [8]. Of patients enrolled onto DAL-HX83/90, LCH-I, and LCH-II, majority of whom were MS or SS-m-type LCH, 43% had CNS-RISK lesion(s) [8]. In our SS-s cohort, only 14% of patients had a CNS-RISK lesion, who were significantly younger than patients with other bone lesion. The frequency of the CNS-RISK lesion might rise as SS-s, SS-m, MS, and the disease stage progress.

We found one-third of the patients with a bone lesion were treated with chemotherapy. In particular, more than 70% of patients with a CNS-RISK lesion and nearly two-thirds of patients with a vertebral bone lesion received chemotherapy. A considerable proportion of the patients with a skin lesion (28%) also received chemotherapy. In the DAL-HX study [7], only 8% of patients with a single bone lesion were given systemic treatment. In the LCH-III protocol study chemotherapy is offered to patients with vertebral lesion(s) as well as CNS-RISK lesion(s), even if only a single lesion is present [9]. However, in general, few patients with unifocal bone lesion are treated with chemotherapy. Indeed, in one report from a neurosurgeon, only 3 of 27 (11%) patients with unifocal LCH in a craniospinal site were treated [11].

Regardless of the type of treatment, almost all patients attained remission, and none of the patients died of disease progression or treatment complications. Some patients suffered from reactivation, mostly within a year after diagnosis. In patients exhibiting reactivation, all with only an initial bone lesion showed reactivation in bone(s), whereas some patients with a skin lesion suffered a reactivation in areas other than skin and progressed to multisystem-type LCH. These features were also similar to those of the cohort described by the DAL-HX study [7]. As previously reported [12], isolated cutaneous LCH in infants may be an aggressive disorder that can progress to multiorgan involvement.

The rates of both reactivation and sequelae of LCH involving a single bone in our study were low compared to the rates reported in the DAL-HX study (8/120 vs. 22/121; $P = 0.007$, and 3/120 vs. 25/121; $P < 0.001$, respectively) [7]. Four of the 120 patients (3%) with a bone lesion suffered from orthopedic consequences and two patients with lesions in special areas other than the skin or bone suffered from developmental impairment. In contrast, the DAL-HX study reported that sequelae were already present at diagnosis in 10% of patients with a bone lesion, and that more than half of the sequelae involved orthopedic disabilities, followed by neurologic

TABLE III. Characteristics of the Patients Who Suffered a Reactivation or Sequelae

Initial site	Gender	Age at diagnosis	Initial treatment	Reactivation		Sequelae
				Site	Interval[a]	
Bone						
CNS-RISK lesion[b]	M	1y3m	Chemotherapy	Multiple bone	1y0m	None
Non-CNS-RISK lesion[c]	M	6y1m	Curettage	Multiple bone	3m	None
Non CNS-RISK lesion[c]	F	12y10m	Curettage	Single same bone	1y0m	None
Non-CNS-RISK lesion[c]	F	12y7m	Systemic steroid	Single same bone	1m	None
Upper limb	M	4y7m	None	Multiple bone	3m	None
Lower limb	M	2y5m	None	Multiple bone	2m	None
Thorax	F	7y1m	Curettage	Multiple bone	3m	None
Shoulder	F	7y5m	Chemotherapy	Single other bone	2y6m	None
Skin	M	5m	None	Thymus	5m	None
	F	6m	Chemotherapy	Skin	9m	None
Bone						
Non-CNS-RISK lesion[c]	M	12y5m	Curettage	None		Bone defect
Non-CNS-RISK lesion[c]	F	8y5m	Curettage	None		Bone defect
Lower limb	M	2y0m	Chemotherapy	None		Bone fracture
Vertebra	M	16y9m	Chemotherapy	None		Flat bone
Thymus	F	5m	Chemotherapy	None		DD
Cranial mass	F	1m	Resection	None		DD

DD, developmental disorder; m, months; y, years. [a]Interval from diagnosis; [b]Combined lesions in the orbital, temporal, mastoid, sphenoidal, zygomatical, ethomoidal bones, the maxilla, paranasal sinuses, or anterior or middle cranial fossa, with intracranial soft tissue extension; [c]Skull or facial bone lesion other than CNS-RISK lesion.

consequences, and DI and/or anterior pituitary dysfunction. A retrospective study from Argentina had similar results as the DAL-HX study: of 161 patients with single-system unifocal LCH, reactivation occurred in 17.4%, and sequelae, mainly orthopedic problems, developed in 19.1% (the mean follow-up time was 4.8 years) [13]. However, this study did not include information on the type of treatment which these patients received [13].

No factor associated with reactivation of LCH involving a single bone was found in this analysis. We speculate that the low rate of patients with a CNS-RISK lesion, who have intrinsically high risk of DI, and the high rate of applying chemotherapy to these patients in our cohort could be responsible for this as well as the low rates of reactivation and sequelae in our cohort. Most reactivations occurred within 1 year from diagnosis in our study, which suggests that the observation time (median 3.3 years) is sufficient for determining the reactivation rate of our cohort. However, the observation time in our study is too short to draw conclusions with regard to the sequelae rate, because while DI usually developed within 3 years after diagnosis, the rates of neurological consequences increased rapidly 10 years after diagnosis, and the incidence of orthopedic abnormalities and growth retardation accrued with each passing year after diagnosis [14].

TABLE IV. Factors Affecting Reactivation in Patients With a Bone Lesion

Variables	Reactivation	*P*-value
Gender		
Male	4/66	
Female	4/54	0.769
Age at diagnosis[a]		
<6 years old	3/59	
>6 years old	5/59	0.464
Region		
CNS-RISK lesion[b]	1/21	
Other than CNS-RSK lesion	7/99	0.700
Treatment		
Chemotherapy	2/39	
Other than chemotherapy	6/81	0.639

[a]Data of age at diagnosis were missing in two patients; [b]Combined lesions in the orbital, temporal, mastoid, sphenoidal, zygomatical, ethomoidal bones, the maxilla, paranasal sinuses, or anterior, or middle cranial fossa, with intracranial soft tissue extension.

In conclusion, we conducted a retrospective study of patients with SS-s LCH in Japan and found that a relatively large proportion received chemotherapy and that the prognosis was generally good. A prospective study should be conducted to confirm these results and to identify the most effective and least toxic therapy for SS-s LCH.

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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.

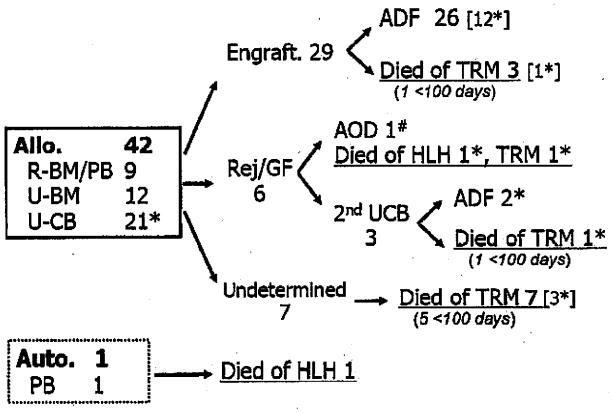

Fig. 1. Cohort diagram for the clinical outcome of 43 patients with familial hemophagocytic lymphohistiocytosis (FHL) who underwent stem cell transplantation (SCT). Of 42 patients after allogeneic SCT, 29 achieved engraftment (18 complete, 3 mixed) and 6 failed to engraft. One (#) with graft failure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

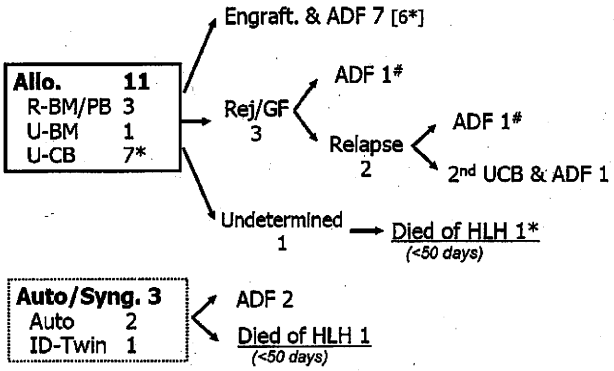

Fig. 2. Cohort diagram for the clinical outcome of 14 patients with Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) who underwent SCT. Among 11 patients after the first allogeneic SCT, 7 achieved successful engraftment and 3 failed to engraft. A total of 12 patients (86%) were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

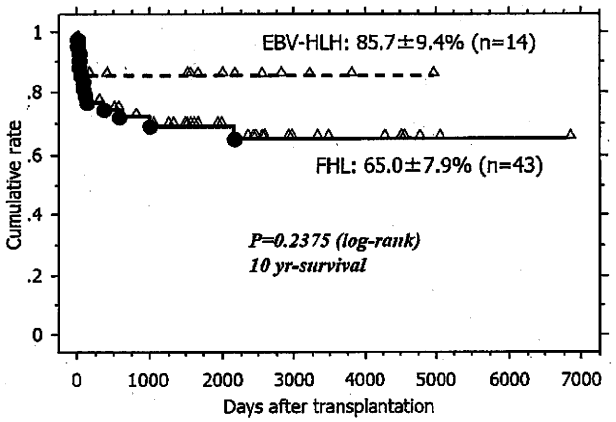

Fig. 3. Cumulative probability of post-transplant overall survival of FHL (*solid line*) and EBV-HLH patients (*dashed line*) who underwent SCT. Closed circle and open triangle represent deceased and alive patients, respectively. Each value indicates the 10-year overall survival rate plus or minus standard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

成人EBウイルス関連血球貪食症候群5例の検討

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Epstein-Barr ウイルス（EBV）関連血球貪食症候群（HLH）は，EBV 感染 T 細胞と活性化組織球の相互作用により血球減少や肝障害，凝固障害といった重症病態をひきおこし，小児での報告が多い。今回，成人 EBV-HLH5 症例を経験したので報告する。年齢は中央値 17 歳（16～40 歳）で，初感染後，2ヶ月以内に発症した例が 4 例であり，1 例は再活性化のパターンを示した。末梢血 EBV ゲノム量は $2\times10^2-3\times10^6$ コピー/ml と高値を示し，すべての症例で EBV 感染 T 細胞のクローン性増殖を認めた。治療法は，免疫抑制±エトポシドが主体であり，重症例の 2 例では血漿交換を施行した。3 例は完全寛解（観察期間 13, 19, 30 か月）を維持しているが，2 例は治療抵抗性となり，多剤併用化学療法も効果なく死亡した。

EBV-HLH は的確な初期診断，治療が重要であり，今後成人例に関しても認知度をあげていく必要がある。（臨床血液 **51**（1）：74～79，2010）

Key words : Adult, Epstein-Barr virus, Hemophagocytic lymphohistiocytosis

緒　言

伝染性単核症（infectious mononucleosis: IM）は Epstein-Barr virus（EBV）の初感染により，感染した B 細胞の増殖と，免疫応答として活性化された細胞傷害性 T 細胞の増殖をひきおこすが，一般的には self-limited な疾患である。EBV 関連血球貪食症候群（EBV-HLH: hemophagocytic lymphohistiocytosis）は，EBV の初感染あるいは再活性化に伴い，EBV が感染した T 細胞がクローン性増殖をきたし，活性化組織球との相互作用により血球減少や肝障害，凝固障害といった様々な重篤な病態をひきおこすが，主に小児例での報告が多く，成人領域での認知度は十分とはいえない。今回若年を含め成人 5 例の EBV-HLH 症例を経験したので報告する。

対象症例および方法

2002 年 8 月より 2008 年 2 月までに当科で経験した EBV-HLH5 症例に関して解析をおこなった。慢性活動性 EBV 感染症の経過中に発症した症例は除外した。血球貪食を示す検査値異常としては，Henter らの基準[1]を用いた。重症度の評価としては，血球貪食症候群のスコアリングシステム[2]を使用した。末梢血単核細胞を免疫磁気ビーズ法を用いて CD3 陽性細胞（T 細胞），CD16 陽性細胞（NK 細胞）に分離し，リアルタイム PCR 法で EBV ゲノム量を測定し，優位な細胞を EBV 感染細胞とした。EBV クローナリティの解析は，骨髄あるいは末梢血単核細胞を使用し，terminal repeat をプローブとしたサザンブロット法を施行した。一部の症例では T 細胞受容体 β 鎖遺伝子の再構成も検討した。

結　果

年齢は 16～40 歳（中央値 17 歳）で，4 例が 20 歳以下と若年者であった。性別は，男性 2 例，女性 3 例であり，免疫異常等の基礎疾患は認めなかった（表 1）。

症例 1-4 は，初感染（IM 発症）後，2 か月以内の短期間に EBV-HLH を発症した。症例 1，2 では発熱，咽頭痛，倦怠感等の初発症状が，一旦軽快した後に発症したが，症例 3，4 では初発症状に引き続き発症した。症例 5 は抗 EADR-IgG 抗体価が 640 倍と高値であり，EBV の再活性化にもとづく EBV-HLH と考えられたが，慢性活動性 EBV 感染症を疑う前駆症状は病歴上認めなかった。全例で血漿中の EBV ゲノム量の増加（cut off

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表1 EBV関連検査

症例	性	発症年齢	IM症状から発症までの期間	EBV-DNA 血漿 (コピー/ml)	EBV抗体価 VCA-IgM	VCA-IgG	EA-IgG	EBNA	clonality EBV/TCR	EBV感染細胞
1	男	16	約2か月	7×10^5	<×10	×160	<×10	×10	+/+	CD3
2	女	19	約1.5か月	9×10^4	×10	×160	<×10	<×10	+/+	CD3
3	女	16	約半月	2×10^3	<×10	×40	<×10	<×10	+/nd	CD3
4	女	17	約1か月	3×10^6	×10	×160	×80	<×10	+/nd	CD3
5	男	41	約1か月	1×10^6	<×10	×320	×640	×40	+/+	CD3

nd: 施行せず

表2 入院時検査所見

症例	WBC (Neutro)/μl	Hb g/dl	Plt ×10⁴/μl	AST/ALT IU/l	LDH IU/l	中性脂肪 mg/dl	Fib mg/dl	Ferritin ng/ml	sIL-2R IU/ml	Score
1	600 (520)	8.7	4.5	541/416	2,280	303	292	34,509	28,100	4
2	5,430 (1,411)	12.4	2.4	215/419	1,238	111	314	4,755	6,000以上	2
3	2,230 (740)	8.2	3.4	139/111	1,054	226	102	8,796	4,459.4	2
4	1,200 (540)	11.8	3.7	2,413/500	14,743	432	119	99,280	12,274	7
5	5,110 (4,011)	9.5	6.7	1,203/454	4,368	349	75	55,775	6,000以上	7

値：2×10^2コピー/ml未満）を認め，CD3陽性細胞（T細胞）に優位に感染していた。EBVのterminal repeatをプローブとしたサザンブロット解析では，全例でEBV感染細胞の単クローン性増殖を認め，T細胞受容体β鎖遺伝子の再構成は解析した3例で陽性であった（表1）。

検査所見では，全例で総ビリルビン（2.0～8.2 mg/dl，中央値4.3 mg/dl）およびFDPの高値（14.5～132.7 μg/ml，中央値41.9 μg/ml）を認めた。Henterらの HLH診断ガイドライン[1]に記載されている検査異常としては，貧血（Hb<9 g/dl）；2例，好中球減少（<1,000/μl）；3例，血小板減少（$<10\times10^4$/μl）；5例，中性脂肪高値（>265 mg/dl）；3例，フィブリノーゲン低値（<150 mg/dl）；3例，フェリチン高値（>500 ng/ml）；5例，可溶性IL-2レセプター高値（>2,400 IU/ml）を5例に認めた（表2）。血小板数に比較し好中球数，Hbは比較的保たれており，凝固異常を高率に認めた。全例でフェリチンおよび可溶性IL-2レセプターは著明高値を示した。血球貪食症候群の重症度[2]は，中等症から重症とされる4点以上を3例にみとめ，うち2例（症例4，5）は最高点の7点であった（表2）。この2例に関しては，血漿交換により初期の病勢のコントロールが可能であった。重症例に関しては，高サイトカイン血症をすみやかに是正するため血漿交換が有用と考えられた[3]。

当科ではメチルプレドニゾロン等のステロイドとシクロスポリンの併用による免疫抑制療法を第1選択としており，重症例に関しては，エトポシドの併用や血漿交換を随時追加している（表3）。症例2，3は免疫抑制のみで改善したが，症例1では，一旦改善傾向を認めるも，短期間で再燃し，同種造血幹細胞移植（臍帯血移植およびHLA3座不一致の母児間同種末梢血幹細胞移植）を含めた種々の治療に抵抗性であり，原疾患の悪化のため，発症後約7.5か月で死亡した。経過の詳細に関して，症例1，2に関してはすでに報告した[4]。再活性化が示唆された症例5では，血漿交換および免疫抑制＋エトポシドで一旦軽快するも，約1か月で再燃し，多剤併用化学療法を施行するも効果なく，原疾患の悪化にて発症後約6か月で死亡した。生存例は3例であるが，悪性リンパ腫との鑑別が困難であった症例4に関して詳述する。

症例4：17歳，女性で生来健康。2008年1月中旬より咽頭痛・発熱が出現し，解熱しないため2月初旬に前医を受診。EBVの抗VCA IgM抗体陽性，抗EBNA抗体陰性であり伝染性単核症と診断，血小板減少（5.0×10^4/μl）と肝機能障害を認め，経過観察を行っていたが，以後も39℃以上の発熱が5日間持続し，汎血球減少（白血球数1,200/μl，Hb 11.8 g/dl，血小板数3.7×10^4/μl）およびフェリチン（99,280 ng/ml），LDH（14,743 IU/l），FDP（132.7 μg/ml）の高値を認めたため，

表3 治療法および予後

症例	治　療	予後 (month)
1	m-PSL + CsA + ETP，多剤併用化学療法（CHOP 等），同種造血幹細胞移植（RIST2 回）	7.5 mo
2	m-PSL パルス，CsA＋m-PSL	30 mo＋
3	m-PSL パルス，CsA＋PSL	19 mo＋
4	血漿交換，m-PSL パルス，CPM＋ETP，PSL＋CsA＋ETP	13 mo＋
5	血漿交換，m-PSL パルス，CsA＋PSL＋ETP，多剤併用化学療法（CHASE）	6 mo

＋生存中

略語：(m)-PSL; (メチル) プレドニゾロン，CsA; シクロスポリン，CPM; シクロホスファミド，ETP; エトポシド，RIST: reduced intensity stem cell transplantation CHOP: シクロホスファミド＋ドキソルビシン＋ビンクリスチン＋プレドニゾロン CHASE: シクロホスファミド＋大量シタラビン＋デキサメタゾン＋エトポシド

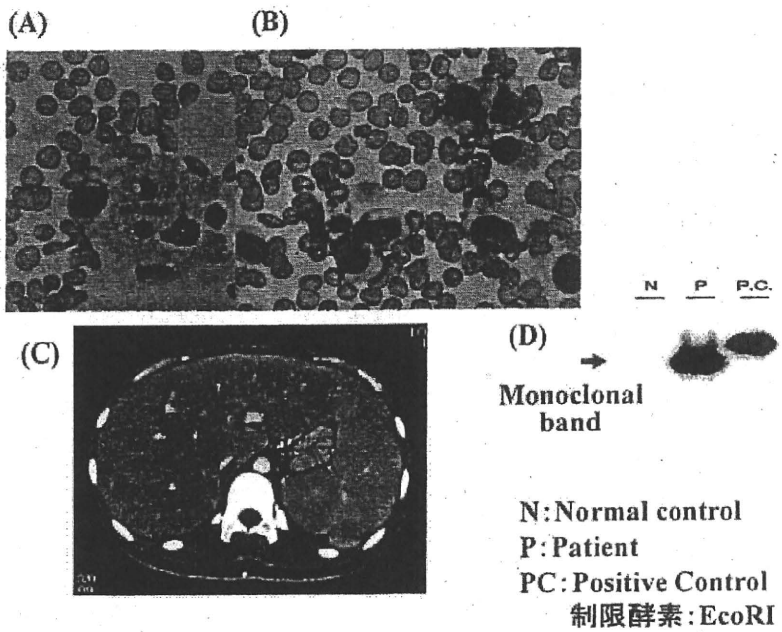

図1 症例4の入院時所見。骨髄塗抹標本（メイギムザ染色×1,000）：活性化組織球による血球貪食像（A）及び異型リンパ球を81.2%認めた（B）。腹部CT検査: 肝脾腫を認めた（C）。サザン法によるEBVクローナリティ解析（骨髄）：EBV感染細胞の単クローン性増殖を認めた（D）。

当院に救急搬送となった。当院来院時の骨髄塗抹標本では，血球を貪食した組織球が散見（図1A）される以外に，異型リンパ球を81.2%に認め，表面マーカーの検索では，リンパ球領域の解析で95%前後がCD3＋CD8＋HLA－DR＋であった（図1B）。また可溶性IL-2レセプターも12,274 IU/ml と著増し，肝脾腫も認めた（図1C）ため，当初は悪性リンパ腫との鑑別が困難であった。血漿中のEBVゲノム量は 3×10^6 コピー/ml と増加していた。高サイトカイン血症をすみやかに是正するため血漿交換やメチルプレドニゾロンパル

ス療法を施行し，末梢性T細胞リンパ腫が鑑別にあがるため，CHOP+エトポシド療法を予定したが，すでに血管漏出によると思われる肺水腫を併発しており，腫瘍崩壊にもとづく高サイトカイン血症を極力抑えるためシクロホスファミドとエトポシドの分割投与を施行した。治療中にも肺水腫の悪化あり，また全身状態も不良であり，ドキソルビシンとビンクリスチンの投与は断念したが，治療によく反応し血漿EBVゲノム量も1×10^3コピー/mlと減少した。本例では，入院時の骨髄単核細胞を使用したサザンブロット解析で，EBV感染細胞の単クローン性増殖が示された（図1D）が，免疫抑制+エトポシド治療の反応が良好であったことより，病歴などからEBV-HLHと診断し，HLH-2004プロトコール[1]を参考にプレドニゾロン+シクロスポリン+エトポシドの治療に変更した。その後の反応も良好であり，全身状態や検査所見の改善を認め，治療開始後1カ月で血漿EBVゲノムは検出されなくなった。エトポシドの投与は150 mg/m²を計8回施行し，プレドニゾロンおよびシクロスポリンは漸減中止した。現在，約1年経過するも，検査異常はすべて正常化しており再発は認めていない。本症例では，ステロイド，免疫抑制剤，エトポシド単独の抗がん剤治療に対する反応が良好で，通常の悪性リンパ腫とは異なる経過を示した。血球減少を認めてから，診断までの期間が約5日間と長引いたため，血球貪食に伴う血球減少と，異型リンパ球の増加が更に進行し，悪性リンパ腫との鑑別が困難であったと考えられた。

考　察

EBVの初感染によるIMは，self-limitedな経過をたどる良性の疾患であるが，EBV-HLHは，EBVの初感染あるいは再活性化後に，速やかにEBV感染細胞（活性化CD8陽性細胞）[5]の単クローン性増殖をきたし，血球減少，凝固障害，肝障害等をきたし急激な経過を辿る疾患であり，fulminant EBV+T−cell lymphoproliferative disorder等として報告されている[6]。感染細胞は単クローン性増殖を示し，リンパ増殖性疾患としての性格も有しているため，悪性リンパ腫との異同も問題となる。

EBV-HLHに関しては，標準治療は確立されていないが，HLH-2004プロトコール[1]等を参考に，Tリンパ球や組織球の活性化に関連する高サイトカイン血症を是正するため，まず免疫抑制剤が使用されることが多い。同時にEBV感染細胞および活性化組織球の制御のためエトポシドが併用される。2001年から2005年にかけての全国調査[7]では，EBV-HLH（163人）の約80%は14歳以下の小児であり，小児例では5年生存率が87.3%と良好であるのに対し，若年者も含めた成人例では68%前後と不良である。成人でのEBV-HLH症例の報告は少なく，多くは予後が不良とされているが[6~9]，EBVの感染状況（初感染あるいは再活性化等）が不明であったり，明らかなT/NKリンパ腫合併例が含まれていたり，HLH-2004プロトコールに準じた治療内容でないものも多いため，小児例との比較が困難である。成人例でのまとまった報告例であるImashukuらの報告[10]では，20例中9例は文献からの考察であり，情報が十分とはいえず，一定の限界はあるが，エトポシドを4週以内に使用した7人の2.5年生存率が85.7 ± 13.2%に対し，エトポシドを4週以内に使用しなかった13人の2.5年生存率は10.3 ± 9.4%と，エトポシドの早期必要性が示唆されている。すなわち，成人例であっても，早期からの機会を逸しない適切な治療が必要であり，初期治療に反応悪く，発熱等の臨床症状や検査所見の改善を認めない場合は，速やかにエトポシドを追加すべきとされている。

エトポシドは血球貪食症候群におけるkey drugであり，投与機会を逸してはならないが，2次発がんの問題もあり，過剰投与には注意が必要である。一部の症例では，EBV感染細胞のクローナルな増殖があっても，免疫抑制のみで治療可能である[11]ことから，エトポシドの至適投与時期や免疫抑制剤の至適投与期間等不明な点も多い。症例3ではシクロスポリンの内服を約50日で自己中断したため，その後，4~5日で発熱や，一旦正常化していたフェリチン値の上昇や，肝障害，血球減少の進行とともに，血漿中のEBVゲノム量の再増加を認めた。その後，シクロスポリン+プレドニゾロンの再投与で，再度寛解状態となったため，ステロイドは約3か月で中止し，シクロスポリンは，血中トラフ濃度が150~200 mg/lを維持するように，約6か月投与を続け，漸減中止したが，その後，再発を認めていない。Imashukuらは，EBV-HLHをHLHプロトコロールで治療するに際して，8週間のデキサメサゾン+エトポシドで完全寛解（臨床症状の消失および検査所見;特に血清フェリチンの正常化）に到達しない例には，シクロスポリンも含んだ6~12ケ月の維持療法を施行している[12,13]。

血球貪食症候群のスコアリングシステム[2]と予後との関連については，重症例では生存率が低下することが示されている[7]。今回，少数例の検討ではあるが，点数が低い群では免疫抑制療法のみ，高い群では血漿交換やエトポシドの併用等，より集約的治療が必要となる可能性が示唆された。重症度診断基準に基づく治療の層別化の可能性について今後より多数例での検討が必要である。

一方，免疫抑制剤にエトポシドを併用しても，一時的な効果しか示さない治療抵抗例も存在する。このような例に対しては，悪性リンパ腫に準じた多剤併用療法が選

択されるが，その効果は必ずしも十分とはいえず[7, 11]，早期の同種造血幹移植の介入等新たな治療戦略の確立が望まれる。

高サイトカイン血症に伴う病態は，時間～日単位で悪化していくため，早期の診断，治療が重要であり，EBV-HLH 症例の死亡例の半数は，診断後 2 か月以内の早期死亡（出血，感染）といった報告もある[12]。また，診断までの期間が長引くと，症例 4 のように悪性リンパ腫との鑑別が困難な場合も生じ得る。EBV-HLH は激烈な症状を呈する疾患であるが，今回の我々の経験や過去の報告[14]からは，初感染から移行するものは，若年成人例ではあっても，小児同様[7]，HLH プロトコールに準じた適切な治療で制御可能と考えられ，適切な早期診断治療により，良好な予後が望める疾患である。本邦でも近年，EBV 初感染年齢が欧米並みに青年期にシフトしている[15]ことより，IM のみならず EBV-HLH 等の EBV 関連重症疾患が，成人においても増加する可能性がある。IM の経過中，あるいは一旦改善した後も，持続する発熱や血球（特に血小板）減少，AST 優位で LDH 高値や肝脾腫を伴う肝障害，高フェリチン血症や凝固異常（FDP 高値，フィブリノーゲン低値等）を認める際は，速やかに専門施設へ紹介してもらうべく，血液内科のみならず，感染症内科や肝臓内科等，IM を診療する機会が多い診療科を含め，認知度をあげていく必要がある。

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