Original Article

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Dynamics of Epstein-Barr virus after cord blood transplantation: A nationwide survey in Japan

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Abstract

Epstein-Barr virus (EBV) is a common virus that latently infects most adults and has a tropism to B lymphocytes. In 1988, two cases of EBV infection were reported to be eradicated by hematopoietic stem cell transplantation from an EBV-negative donor. However, the dynamics of EBV after cord blood transplantation (CBT), namely, the kinetics of anti-EBV antibodies, the incidence of negative/adverse seroconversion (from positive to negative), and the clinical course of re-infection (second primary infection) by EBV, have not yet been characterized in detail. Therefore, we performed a nationwide survey that focused on the dynamics of EBV after CBT 1 year or later after CBT. Negative seroconversion occurred in 23% of previously EBV-infected patients. The incidence of late-onset EBV-associated events was 1.9% (13/674): 5 infectious mononucleosis, 2 hemophagocytic lymphohistiocytosis (HLH), and 6 remaining typical lymphoproliferative disease. HLH occurred in newly infected patients (primary or second primary) and also in those with reactivation and was fatal. The annual monitoring of anti-EBV antibody titers may facilitate the early detection of these late-onset EBV-associated events and treatment initiation before disease progression.

Key words: Epstein-Barr virus, hemophagocytic lymphohistiocytosis, lymphoproliferative disease, post-transplant lymphoproliferative disease, cord blood transplantation

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Introduction

The first successful bone marrow transplantation (BMT) from a sibling to treat a patient with acute leukemia was performed in 1969[1]. The application of allogeneic hematopoietic stem cell transplantation (HSCT) subsequently expanded worldwide. In 1988, the eradication of EBV by BMT from an EBV-negative donor was demonstrated in two recipients previously infected with EBV[2]. These findings provided proof of the concept of EBV infecting humans and persisting for life as a latent infection concealed in a certain subset of lymphocytes (B cells) after the primary infection (lytic infection).

The Japanese Cord Blood Bank Network was established in 1999. Cord blood transplantation (CBT) has since become popular in Japan. CBT is unique in that an EBV-positive patient (more than 95% of adults are positive for EBV) receives HSCT from an EBV-negative donor. However, the dynamics of EBV after CBT, namely, the kinetics of the anti-EBV antibody, the incidence of negative/adverse seroconversion (from positive to negative), and the clinical course of re-infection (second primary infection) by EBV, have not yet been elucidated in detail, there have been except for a few case reports[3-5].

Therefore, we performed a nationwide questionnaire survey with a focus on changes in the EBV status(anti-EBV antibody titers before and after CBT)and the clinical manifestations of late-onset EBV-associated events. The present study was approved by the Research Ethics Committee of Osaka Women's and Children's Hospital(#610).

Patients and Methods

In 2014, letters requesting participation in our survey were sent to 262 institutes in Japan, followed by questionnaires to the 146 institutes that responded.

Data collection and eligibility criteria

Eligibility criteria were as follows: recipients of CBT before December 31st, 2012, CBT as the first allogeneic HCT, complete donor chimerism(≥95%), and event-free survivors for more than 1 year after CBT. Events included relapse/progression of the primary disease, second malignancy, any death, and re-transplantation. Patients with congenital immunodeficiency affecting T cells, NK cells, and/or B cells were excluded.

EBV-specific questionnaire items included the following: previous EBV infection before treatment, anti-EBV antibody titers 1 year or later after CBT, late-onset EBV-associated events occurring 1 year or later after CBT, and EBV-related death. As a minimum requirement of anti-EBV antibody titers, information was obtained on the serum level of immunoglobulin G against the viral capsid antigen (VCA IgG)[6,7]. A fluorescent antibody test (FA) was commonly used to measure anti-EBV antibody titers, and negative serology was defined as lower than 10.

Definition and statistical analysis

Previous EBV infection before CBT was defined serologically by the detection of the anti-EBV antibody prior to the first blood transfusion. EBV-associated events were as follows: infectious mononucleosis (IM), hemophagocytic lymphohistiocytosis (HLH), and lymphoproliferative disease (LPD). In overlapping cases, patients were diagnosed according to the cardinal symptom. LPD indicates the uncontrolled neoplastic proliferation of lymphoid cells with end-organ manifestations[8]. However, LPD may also be used as a comprehensive term over a wide variety of EBV-associated diseases. Therefore, LPD without HLH is hereafter described as "typical LPD". The chi-squared test was used in statistical analyses.

Results

We received case reports from 83 institutes. Incomplete reports (n=4) were excluded, and the remaining 674 patients were analyzed. Patient characteristics are shown in **Table 1**. As the EBV status before CBT, the number of EBV-positive, EBV-negative, and EBV-indeterminate patients were 499(74.0%), 58(8.6%), and 117 (17.4%), respectively.

Table 1. Patient characteristics

Category	Patient number (n=674)
Sex	
Female/Male	322/352
Age, y	
Median (range)	34y (0-76y)
Age group	
Children (0-19y)	230
Adults (≥20y)	444
EBV status before CBT	
Previously infected	499
Uninfected	58
Not known	117
Diagnosis	
Myeloid neoplasms (n=321)	
Acute myeloid leukemia	245
Myelodysplastic syndromes	49
Others	27
Lymphoid neoplasms (n=305)	
Acute lymphoblastic leukemia	194
Mature B-cell lymphoma	26
Mature T/NK-cell lymphoma	25
Adult T-cell leukemia/lymphoma	27
EBV+ T/NK-cell LPDs	16
Others	17
Benign hematological disorders (n=28)	
Acquired aplastic anemia	12
Familial/primary HLH	6
Others	10
Non-hematological diseases (n=20)	
Congenital metabolic disorders	11
Malignant solid tumors	9

HLH, hemophagocytic lymphohistiocytosis; EBV+ LPDs, EBV-associated lymphoproliferative diseases; y, years old at CBT.

Kinetics of the anti-EBV antibody after CBT

Among EBV-positive patients before CBT, the anti-EBV antibody was measured 1 year or later after CBT in 81 patients(**Table 2**), with negative seroconversion of the anti-EBV antibody titer being observed in 19 (23%). In the age-oriented analysis, the ratio of negative seroconversion was as high as 40% (14/35) in children (0-19 years old at CBT) and 11% (5/46) in adults (≥20 years old at CBT) ($P<0.01$). However, anti-EBV antibody titers were not measured in 82.8% (558/674) of all patients and in 83.8%(418/499)of EBV-positive patients before CBT.

The anti-EBV antibody was monitored in 13 out of 19 patients with negative seroconversion, with 8 subse-

Table 2. Anti-EBV antibody titers before and after CBT

Anti-EBV Ab (before CBT)	Total (n)	Anti-EBV Ab (after CBT)			Negative seroconversion ratio in tested patients
		Pos	Neg	nk	
Pos	499	62	19	418	23% (19/81)
0-19y	134	21	14	99	40% (14/25)
≥20y	365	41	5	319	11% (5/46)
Neg	58	4	14	40	—
0-19y	53	4	13	36	—
≥20y	5	0	1	4	—
nk	117	8	9	100	—
Total	674	74	42	558	—

The anti-EBV antibody (Ab) was represented by serum VCA IgG. The Ab titer before CBT was measured prior to the first blood transfusion. The Ab titer after CBT was measured 1 year or later after CBT.
neg, negative; nk, not known; pos, positive; y, years old at CBT.

quently showing re-infection (positive seroconversion) by EBV: the clinical manifestation was IM in one and asymptomatic in seven.

Incidence of late-onset EBV-associated events

Late-onset EBV-associated events were documented in 13 out of 674 patients (1.9%) more than 1 year after CBT (**Figure 1**).

(i) ***Patients persistently positive for EBV before and after CBT***

Among 62 patients who were persistently positive for the anti-EBV antibody before and after CBT, 2 (3.3%) developed late-onset EBV-associated events, with both showing typical LPD as the reactivation of EBV.

(ii) ***Patients negative for EBV after CBT***

Three (7.1%) out of 42 patients who were negative for the anti-EBV antibody after CBT (regardless of the EBV status before CBT) developed late-onset EBV-associated events. The EBV status before CBT was positive in one patient, negative in one, and unknown in one. All three patients showed IM as the new infection (primary infection or re-infection/second primary infection) by EBV.

(iii) ***Patients with an indeterminate EBV status after CBT***

Eight (1.4%) out of 558 patients, whose anti-EBV antibody titers were not measured after CBT, developed late-onset EBV-associated events. All patients were positive for EBV before CBT. EBV-associated events were typical LPD in 4 patients, IM in 2, and HLH in 2. Among the 4 patients with typical LPD, the EBV status in one patient was presumed to be reactivation based on the anti-EBV antibody titer measured after its occurrence, and remained unknown in the remaining 3 patients.

Mortality of late-onset EBV-associated events

Late-onset EBV-associated events were fatal in 3 out of 13 patients (23%) (**Table 3** and **4**).

(i) ***Patients persistently positive for EBV before and after CBT***

Both patients with typical LPD were successfully treated and are alive.

(ii) ***Patients negative for EBV after CBT***

All three patients with IM recovered and are alive.

(iii) ***Patients with an indeterminate EBV status after CBT***

Among the 4 patients with typical LPD, one died and the remaining 3 were successfully treated. Two patients with IM recovered without any disease-specific treatment, and are currently disease-free. On the other hand, two patients with HLH died despite treatments.

Discussion

In the present study, HLH was presumedly due to EBV reactivation and was fatal in both patients. There were 2 additional cases of HLH among 5 Japanese patients with late-onset EBV-associated events in the literature, as

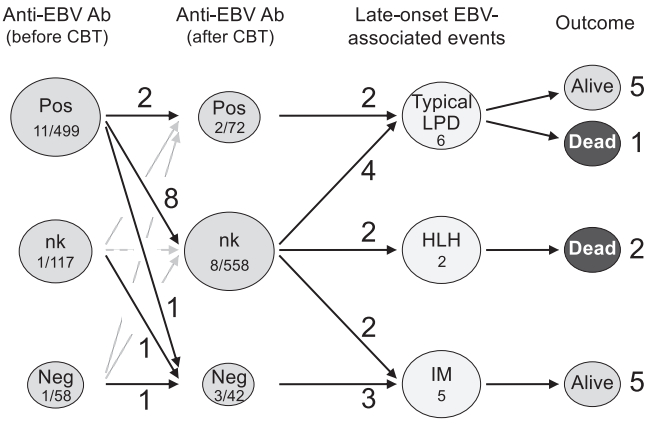

Figure 1. Late-onset EBV-associated events
Late-onset EBV-associated events in 13 patients were examined before CBT and more than 1 year after CBV. The types of events and their outcomes were shown.
Ab, antibody; neg, negative; nk, not known; pos, positive.

Table 3. Characteristics of patients with late-onset EBV-associated events

Pt #	Age at CBT	Sex	Diagnosis	MAC or RIC	GVHD prophylaxis	Administration of ATG/ALG	Acute GVHD		Chronic GVHD		References
							Grade	Treatment	Severity	Treatment	
1.	39y	F	AML (M2)	MAC	nk	no	Ⅰ	PSL	0	—	Present study
2.	28y	F	CAEBV	RIC	Tac/MTX/MMF	no	0	—	Limited	CsA	
3.	28y	F	ALL (BCP)	nk	CsA/MTX	nk	Ⅱ	PSL	Limited	no	
4.	1y	M	AML (M7)	RIC	CsA/MTX	no	0	—	Limited	no	
5.	41y	F	MDS (RA)	MAC	CsA/MTX/MMF	no	Ⅱ	MMF	Extensive	Steroid/CsA/MMF	
6.	57y	M	AML (M2)	RIC	Tac	no	Ⅱ	PSL	0	—	
7.	23y	F	CAEBV	MAC	Tac/MTX	no	Ⅱ	no	Limited	no	
8.	48y	F	AML (M4)	MAC	Tac/MTX	no	0	—	Limited	Topical only	
9.	65y	M	ALL (BCP)	MAC	CsA/MTX	no	Ⅱ	CsA/MTX	0	—	
10.	41y	F	NHL (T/NK)	MAC	CsA/MTX	no	0	—	Extensive	CsA	
11.	58y	F	AML (NOS)	RIC	Tac/MMF	no	0	—	0	—	
12.	61y	F	AML (M2)	RIC	CsA/MMF	no	0	—	0	—	
13.	23y	F	CAEBV	RIC	Tac/MTX	no	Ⅱ	PSL	ne	—	
Additional cases from the literature											
14.	50y	F	AML	RIC	Tac	no	Ⅱ	PSL/Tac	Limited	nk	[9]
15.	59y	M	SAA	RIC	Tac/MMF	no	Ⅰ	Tac/MMF	Limited	nk	
16.	55y	M	ALL (Ph+)	nk	nk	nk	nk	nk	nk	Steroid/CsA	[10]
17.	43y	F	ALL (MLL+)	MAC	Tac	no	0	—	nk	Tac	[3, 4]
18.	34y	M	MDS (RAEB)	nk	nk	nk	nk	nk	Extensive	PSL	[11]

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia (FAB classification in parentheses); ATG/ALG, anti-thymocyte globulin/anti-T-lymphocyte globulin; BCP, B-cell precursor; CAEBV, chronic active EBV infection; CsA, cyclosporine A; F, female; GVHD, graft-versus-host disease; M, male; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MTX, methotrexate; ne, not evaluable; NHL, non-Hodgkin lymphoma; nk, not known; PSL, prednisolone; RA, refractory anemia; RAEB, RA with excess blasts; RIC, reduced-intensity conditioning; SAA, severe aplastic anemia; Tac, tacrolimus; y, year (s)/year (s) old.

Table 4. Clinical course of patients with late-onset EBV-associated events

Pt #	EBV before CBT	EBV after CBT	Late-onset EBV-associated events after CBT					Final obs day	Alive/Dead (cause of death)	References
			Day	Disease type	EBV status (presumed)	IS drugs	Treatment			
1.	Infected	pos	4.5y	Typical LPD	reactivation	yes	IS reduction	5.4y+	Alive	Present study
2.	Infected	pos	1.8y	Typical LPD	reactivation	nk	Chem	11.5y+	Alive	
3.	Infected	neg	4.1y	IM	re-infection	nk	None	10.8y+	Alive	
4.	Uninfected	neg	5.0y	IM	primary infection	nk	None	8.5y+	Alive	
5.	nk	neg	6.8y	IM	nk	nk	nk	7.9y+	Alive	
6.	Infected	nk	6.5y	Typical LPD	(reactivation)	nk	nk	8.1y	Dead (EBV)	
7.	Infected	nk	1.2y	Typical LPD	nk	nk	Rtx	4.4y+	Alive	
8.	Infected	nk	1.0y	Typical LPD	nk	nk	Rtx	5.9y+	Alive	
9.	Infected	nk	6.8y	Typical LPD	nk	Yes	Chem	7.8y+	Alive	
10.	Infected	nk	3.8y	IM	nk	nk	None	5.0y+	Alive	
11.	Infected	nk	2.5y	IM	nk	Yes	None	5.6y+	Alive	
12.	Infected	nk	7.2y	HLH	(reactivation)	nk	Steroid/Etp	7.3y	Dead (EBV)	
13.	Infected	nk	5.8y	HLH	(reactivation)	no	Steroid/Rtx	5.9y	Dead (EBV)	
Additional cases from the literature										
14.	nk	nk	2.2y	Typical LPD	nk	no	Rtx	nk	Dead (AML)	[9]
15.	nk	nk	1.1y	Typical LPD	nk	Yes	IS reduction, Rtx	nk	Alive	
16.	Infected	nk	2.7y	Typical LPD	nk	Yes	IS stop, Rtx, Chem	3.0y	Dead (EBV)	[10]
17.	Infected	nk	1.1y	HLH	(re-infection)	Yes	IS stop, steroid/CsA	1.1y	Dead (EBV)	[3, 4]
18.	Infected	nk	4.6y	HLH	(reactivation)	Yes	steroid/CsA/Etp	0.4y+	Alive	[11]

#18: co-infection with varicella-zoster virus.
Chem, chemotherapy; Etp, etoposide; HLH, hemophagocytic lymphohistiocytosis; IM, infectious mononucleosis; IS, immunosuppressive/immunosuppressant; LPD, lymphoproliferative disease; neg, negative; nk, not known; pos, postive; Rtx, rituximab; y, year (s)/year (s) old.

shown in **Table 3** and **4**[3,4, 9-11]. One case of HLH was attributed to the re-infection by EBV after negative seroconversion, and the patient died[3,4]. The other case was presumedly due to the reactivation of EBV (with co-infection with varicella-zoster virus) and the patient is currently alive[11]. Therefore, HLH may occur in patients with new EBV infection and EBV reactivation, and its mortality was as high as 75% (3/4).

The negative seroconversion of the anti-EBV antibody might indicate the eradication of EBV after CBT. We used anti-EBV titer at least 1 year after CBT, based on our previous study[5], to avoid a false positive by residual antibodies early after CBT and the adoptive antibodies from blood transfusions. Negative seroconversion, in the present study, was estimated to occur in 23% of previously EBV-infected patients, which was lower than that in the previous report[5]. In that report, the eradication rate was as high as 43%, however, the patient number was small, and the patient age was mostly under 10 years old. Indeed, in the present study, the rate of EBV eradication was as high as 40% in children (0-19 years old at CBT), comparing 11% in adults. One explanation of the different rate by age may be attributed to the difference between adults and children in personal activities such as deep kissing. Also, acute lymphoblastic leukemia is common in children, and the drugs for ALL have much suppressive effect on lymphocytes (including EBV-infected B cells). Another explanation may be the spread of EBV to organs other than B cells with aging, such as EBV infection to gastric epithelial cells, which accounts for 7.2% of gastric cancer[12]. In addition, the intensity of conditioning regimen can affect the rate of negative seroconversion, because the incidence of PTLD was reported to be higher for patients receiving reduced-intensity conditioning than that for patients receiving myeloablative conditioning[13]. Intensive conditioning might help eradicating EBV-infected B cells and EBV itself.

Among 42 patients negative for EBV after CBT regardless of the EBV status before CBT, the anti-EBV antibody titer was monitored in 28 patients. Sixteen of these patients were newly infected by EBV more than 1 year after CBT: 3 (11%) showed IM and 13 (89%) were asymptomatic, and generally recovered with no treatment or only supportive care. However, it is important to note that although it is rare, life-threatening (primary and second primary) EBV infection may occur[3,4].

Late-onset EBV-associated events were observed in 5 out of 116 patients (4.3%) whose anti-EBV antibody titers were measured after CBT, and in 8 out of 558 patients (1.4%) whose titers were not. The incidence of these events was significantly lower in the latter group ($P=0.04$). In contrast, none of the 5 patients whose titers were measured died of EBV-associated events, while 3 out of 8 patients whose titers were not evaluated died. Although the mortality rate was high in the latter group, it was not significant ($P>0.1$). Therefore, monitoring of the EBV status may have led to early detection and treatment initiation, whereas a delayed diagnosis resulted in severe illness.

In conclusion, among EBV-positive patients before CBT, negative seroconversion of the anti-EBV antibody was observed in 23% (19/81) more than 1 year after CBT. The incidence of late-onset EBV-associated events was 1.9% (13/674), and the mortality rate was 23% (3/13). Due to the life-threatening late events of EBV, it is important to monitor anti-EBV antibody titers annually after CBT. When EBV-associated events are suspected, the EBV-DNA load needs to be measured. The identity of the EBV-infected subset of lymphocytes and whether its origin is recipient- or donor-derived also need to be clarified. Early diagnosis and treatment initiation may prevent late-onset EBV-associated mortality.

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Author Contributions

A. S. designed and performed the research, analyzed data, and wrote the manuscript. K. K. designed the research, analyzed data, and supervised the manuscript. S. T. and S. T. analyzed data and provided helpful comments. M. I., Y. O., M. T., H. H., M. K., and A. N. reported data and provided helpful comments.

Conflicts of Interest

The authors declare no conflict of interest associated with this article. Disclosure forms provided by the authors are available here.

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