Requirement for Etoposide in the Treatment of Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis

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Purpose: **We sought to identify the clinical variables most critical to successful treatment of Epstein-Barr virus (EBV)–associated hemophagocytic lymphohistiocytosis (HLH).**

Patients and Methods: **Among the factors tested were age at diagnosis (< 2 years or ≥ 2 years), time from diagnosis to initiation of treatment with or without etoposide-containing regimens, timing of cyclosporin A (CSA) administration during induction therapy, and the presence or absence of etoposide.**

Results: **By Kaplan-Meier analysis, the overall survival rate for the entire cohort of 47 patients, most of whom had moderately severe to severe disease, was 78.3% ± 6.7% (SE) at 4 years. The probability of long-term survival was significantly higher when etoposide treatment was begun less than 4 weeks from diagnosis (90.2% ± 6.9% *v* 56.5% ± 12.6% for patients receiving this agent later or not at all; *P* < .01, log-rank test). Multivariate analysis with the Cox proportional hazards model demonstrated the independent prognostic significance of a short interval from EBV-HLH diagnosis to etoposide administration (relative risk of death for patients lacking this feature, 14.1; 95% confidence interval, 1.16 to 166.7; *P* = .04). None of the competing variables analyzed had significant predictive strength in the Cox model. However, concomitant use of CSA with etoposide in a subset of patients appears to have prevented serious complications from neutropenia during the first year of treatment.**

Conclusion: **We conclude that early administration of etoposide, preferably with CSA, is the treatment of choice for patients with EBV-HLH.**

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THE MAJORITY of patients with hematologic disorders such as leukemia, lymphoma, and aplastic anemia are treated on well-controlled protocols that allow one to evaluate the effectiveness and adverse effects of different therapeutic regimens.[1-3] A conspicuous exception can be found in virus-associated hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH),[4-9] for which treatment tends to vary from patient to patient and among small cohorts. Consequently, there is no consensus on the best therapy for HLH associated with Epstein-Barr virus (EBV) infection, a reactive, proliferative disorder that is often fatal.[8] In the past, intravenous immunoglobulin (IVIG), corticosteroids, or a combination of these agents were considered first-line treatment for EBV-HLH, because this disease was thought to represent a reactive and polyclonal lymphohistiocytic proliferation not requiring intensive cytotoxic chemotherapy.[4-10] More recently, it was found that many cases of EBV-HLH have a monoclonal origin[11-13] and respond well to etoposide-containing regimens.[14] This observation was confirmed by us in 17 patients treated according to the international HLH-94 protocol or with related regimens.[15,16] Additional patients recruited onto similar programs have not fared as well, primarily because of severe hypercytokinemia-associated and probably etoposide-induced neutropenia.[17] This complication, together with reports of etoposide-related acute myeloid leukemia (AML),[18] has made physicians reluctant to include an epipodophyllotoxin in first-line treatments for EBV-HLH.

To better assess the role of etoposide in the management of EBV-HLH, we reviewed the clinical records of 47 patients with this disorder, all of whom were treated by the HLH study group in Japan. Particular attention was paid to survival experience as related to the timing and type of primary treatment. The overriding question was whether or not etoposide is essential for successful management of this hemophagocytic disease. Our findings indicate that IVIG-based primary regimens may not be adequate for most patients with EBV-HLH and that etoposide-containing reg-

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imens, supported by cyclosporin A (CSA) to prevent neutropenia-associated opportunistic infections, are more likely to decrease the high mortality rates often associated with this disease.

PATIENTS AND METHODS

Patients

Forty-seven children and young adults registered between 1992 and 1999 at the Japanese HLH Registration Center in Kyoto with a diagnosis of EBV-HLH were studied. This total included 17 previously described cases.[16] Nineteen of the 47 patients were initially treated according to the international HLH-94 protocol,[15] whereas either individualized protocols or HLH-94 preceded by different agents were used for the remaining cases. The criteria for diagnosis of EBV-HLH are described in a previous publication.[19] When registration was made, the disease appeared to have developed in all apparently immunocompetent patients. However, later it was revealed that one case of X-linked lymphoproliferative disease (XLP) and another case of familial hemophagocytic lymphohistiocytosis (FHL) were included. The remaining cases showed no features showing any possible hereditary disease, although no molecular screening was performed to rule out the diagnosis of XLP or FHL. To examine the impact of types of primary treatment on the prognosis was one of the main issue of this study, so that the patients' clinical features are compared in Table 1 between two groups initially treated conventionally (group 1) and with etoposide (group 2). Regarding the therapeutic measures, informed consent was obtained from the patients or families in all cases by the participating physicians.

Treatment

Of the 47 patients, 21 were treated first with corticosteroids alone, IVIG alone, CSA alone, or a combination of these drugs without etoposide (group 1) (Tables 1 and 2). Seventeen of the patients in this group (six on individualized protocols and 11 on the HLH-94 protocol) were later switched to etoposide-containing regimens. Four patients did not receive etoposide (one recovered with conventional treatment alone and three died before protocol switch). The remaining 26 patients (seven on individualized protocols and 19 on the HLH-94 protocol) received etoposide-based regimens first (group 2) (Table 1). Induction therapy in the HLH-94 protocol consisted of etoposide and dexamethasone (two doses of etoposide during weeks 1 and 2, and weekly doses from weeks 3 to 8); most of the individualized protocols specified two to five doses of etoposide during week 1 and variable doses thereafter. Etoposide (150 mg/m^2/dose) was given two doses (three cases), three doses (three cases), four doses (six cases), and more than five doses (31 cases), with a median (range) of six (two to 10) doses during the first 4 weeks. Total doses of etoposide during the 8 weeks were a median (range) of 10 (two to 15) doses.

In 19 cases, treatment was begun within a week of diagnosis, in 21 cases between weeks 1 and 4, and in the remaining seven cases at 4 weeks or later. Thirty of the 43 patients treated with etoposide received the drug within 4 weeks of diagnosis, whereas 13 received it at 4 weeks or later. CSA was administered during induction therapy to six patients in group 1 (four because of severe neutropenia and two because of persistent fever and suspicion of collagen disease–associated EBV-HLH), and to eight in group 2 (seven because of severe neutropenia and one because of relapse at 6 weeks). Altogether, 10 patients received etoposide and CSA early in the same induction regimen. CSA was used to maintain complete remissions in 12 of the cases in group 1 and 13 in group 2. Exchange transfusion, plasma exchange, plasmapheresis, or hemodialysis was used during induction therapy in five cases in group 1 and 10 cases in group 2. Hematopoietic stem cell transplantation was attempted in six patients (two in group 1 and four in group 2) when it became apparent that immunochemotherapy alone would probably not be effective in controlling EBV-HLH. Details of the procedure may be obtained by writing the senior author (S.I).

Laboratory Studies

Absolute neutrophil counts (ANCs) and concentrations of serum lactate dehydrogenase, serum ferritin, total bilirubin, and C-reactive protein were routinely determined, as previously reported.[20] Chromosome analysis was performed by a standard trypsin-Giemsa banding procedure.[21]

Definitions, Clinical Endpoints, and Statistical Analysis

At the latest statistical update (end of 1999), the surviving patients had been followed for a median of 29 months (range, 1 to 86 months). A complete clinical response was defined as the disappearance of all clinical signs and symptoms of the disease, with normalization of laboratory findings. Worsening of the presenting clinical manifestations or laboratory profile was considered evidence of disease progression. Neutropenia was indicated by an ANC of less than 1,000/μL.

Four-year overall survival was the major endpoint of the univariate and multivariate analyses, with certain exceptions (see footnotes to Table 3). In this report, the phrase "long-term survival" is synonymous with "4-year survival." Survival times were measured from the date of diagnosis (onset of disease) to death or to the last contact with surviving patients. Patients undergoing stem cell transplantation were censored on the date of that procedure. Six clinical variables were analyzed for their relationship to overall survival: age ($<$ 2 years *v* $\geq$ 2 years), time from diagnosis to initial therapy, time from diagnosis to etoposide administration ($<$ 4 weeks *v* $\geq$ 4 weeks or no etoposide), time from diagnosis to CSA administration (early *v* no or delayed CSA in group 2 or in neutropenic patients), and overall treatment strategy (group 1 *v* group 2). Survival curves were constructed by Kaplan-Meier life-table methods and analyzed with the log-rank test. The Cox proportional hazards model was used in the multivariate analyses of prognostic factors, and the χ^2 test was applied to categorical data in comparisons between two groups. Differences were considered statistically significant if the two-tailed *P* value was .05 or less.

RESULTS

The clinical characteristics, laboratory data, and treatment variables for groups 1 and 2 are reported in Table 1. With the exception of time to introduction of etoposide and serum concentration of C-reactive protein, these features were distributed similarly across groups. Comparison of the laboratory findings indicated that approximately 75% of the patients in each cohort had moderately severe to severe disease.[19]

Initial Responses to Treatment

Only one of the conventional regimens induced a complete response, leading to substitution of etoposide-based

Table 1. Characteristics of the Patient Population and Treatment Results*

Feature	No. of Patients		P†
	Group 1 (N = 21)	Group 2 (N = 26)	
Age, years			.325
Median	5.2	4.7	
Range	0.8-31	0.6-17	
Sex			.738
Male	10	10	
Female	11	16	
Underlying disease			.99
None	20	21	
XLP/FHL	1	1	
Neutropenia			.166
< 500 ANC/μL	12	8	
500-1,000 ANC/μL	5	12	
> 1,000 ANC/μL	4	6	
LDH			.387
< 2,000 IU/L	6	6	
2,000-5,000 IU/L	8	7	
> 5,000 IU/L	6	13	
Ferritin			.333
< 3,000 ng/mL	3	4	
3,000-10,000 ng/mL	9	6	
> 10,000 ng/mL	9	16	
Total bilirubin			.303
< 2.0 mg/dL	12	9	
2.0-10.0 mg/dL	8	15	
> 10.0 mg/dL	1	2	
C-reactive protein			.002
< 1.0 mg/dL	10	0	
1.0-10.0 mg/dL	7	12	
> 10.0 mg/dL	4	7	
Karyotype of bone marrow cells			.859
Abnormal	3	2	
Normal	10	13	
Weeks from diagnosis to initial therapy			.113
< 1	5	14	
1-4	12	9	
> 4	4	3	
Treatment with an etoposide-containing regimen	17	26	.072
Weeks from diagnosis to etoposide therapy			.002
< 4	7	23	
≥ 4	10	3	
Weeks from diagnosis to CSA therapy‡			.364
< 4	6(2)	8(8)	
≥ 4	4	1	
Treatment with ET, PE, PP, or HD	5	10	.449
Off treatment within 2 months	4	9	.330
Maintenance with CSA required	12	13	.846
Hemopoietic stem cell transplantation required	2	4	.99
Kaplan-Meier survival estimates (mean ± SD) at 4 years after diagnosis, %	68.5 ± 10.9	85.7 ± 8.0	.112
Deaths from acute complications	4	3	.459

Abbreviations: LDH, lactate dehydrogenase; ET, exchange transfusion; PE, plasma exchange; PP, plasmapheresis; HD, hemodialysis; ANC, absolute neutrophil count; CSA, cyclosporin A.

*The number of patients with laboratory data does not always equal the total number in the group, indicating that samples were either not available or not assessable.

†All *P* values refer to comparisons between group 1 and group 2.

‡Numbers in parentheses represent patients in the early-etoposide subgroup.

Table 2. Initial Treatment of Patients (group 1) Subsequently Switched to an Etoposide-Based Regimen

Case No.*	Treatment	Start Time After Diagnosis (weeks)	Duration (weeks)	Reason for Switch to Etoposide	Time to Etoposide After Diagnosis (weeks)
1	PE/PSL/IVIG	1	3	PD	4
4	mPSL	2	2	PD	4
5	PSL/IVIG	1	2	PD	3
7	PSL, mPSL pulse	3	4	PF	7
8	PSL/IVIG	1	2	RF	3
9	CSA	1	2	PF	3
10	Dex/CSA	2	1	PF	3
11	CSA	1.4	0.7	NR	1.9
12	ET/PE/IVIG/Dex	4	0.4	PD	4.4
13	PP/IVIG/PSL	4	1	PD	5
14	PSL/IVIG	3	1	NR	4
15	IVIG/PSL	2	2	RF	4
16	Dex	3	1	NR	4
17	PSL/IVIG	4	2	PF	6
18	PSL/IVIG, Dex/CSA	2	3	PD	5
19	IVIG/PSL, mPSL pulse	2	1.5	NR	3.5
20	PE/PSL	3	1	PD	4

Abbreviations: PE, plasma exchange; PSL, prednisolone; IVIG, intravenous immunoglobulin; Dex, dexamethasone; mPSL, methylprednisolone; CSA, cyclosporin A; ET, exchange transfusion; PP, plasmapheresis; PD, progression of disease; PF, persistent fever; RF, reemergence of fever; NR, no response.

*Four cases in group 1 were excluded because of early death (three cases) or complete response (one case).

treatments in 17 patients (group 1) (Table 2). Specific reasons for this switch were no response/disease progression with respiratory failure or disseminated intravascular coagulation (11 cases), persistent fever (four cases), and reemergence of high fever after a transient response (two cases). The median time to introduction of etoposide was 4 weeks (range, 1.9 to 7 weeks). Thirteen of the 15 patients who underwent exchange transfusion, plasma exchange, plasmapheresis, or hemodialysis in groups 1 and 2 survived the acute phase of the disease. There was no notable morbidity attributed to these therapeutic measures. Overall, etoposide-containing regimens induced 34 complete responses (13 in group 1 and 21 in group 2) and nine partial responses (four in group 1 and five in group 2).

Table 3. Univariate and Multivariate Analyses of Overall Survival by Risk Factor

Variable*	Univariate Analysis† *P*	Multivariate Analysis‡		
		RR	95% CI	*P*
Age, years				
< 2 (13 of 14) *v* ≥ 2 (24 of 33)†	.15	3.93	0.41-37.0	.23
Time from diagnosis to initial therapy				
Early (33 of 39) *v* late (4 of 8)	.01	0.98	0.21-4.70	.98
Time from diagnosis to etoposide				
Early (28 of 30) *v* late/no (9 of 17)	$< .01$	14.1	1.16-166.7	.04
Timing of CSA during induction therapy				
Early (12 of 14) *v* late/no (28 of 33)§	.32	2.74	0.62-12.1	.18
Timing of CSA in early etoposide group				
Early (10 of 10) *v* late/no (16 of 20)‖	.91	—	—	—
Timing of CSA for neutropenic patients				
Early (7 of 11) *v* late/no (17 of 22)	.49	—	—	—
Group 1 (15 of 21) *v* group 2 (22 of 26)	.11	2.00	0.25-15.9	.51

Abbreviations: CSA, cyclosporin A; RR, relative risk; CI, confidence interval.

*Numbers in parenthesis represent surviving/total patients.

†Log-rank analysis of Kaplan-Meier plots.

‡Cox proportional hazards model. RR refers to the risk of death associated with the first versus the second variable.

§Survival at 3 months.

‖Survival at 12 months.

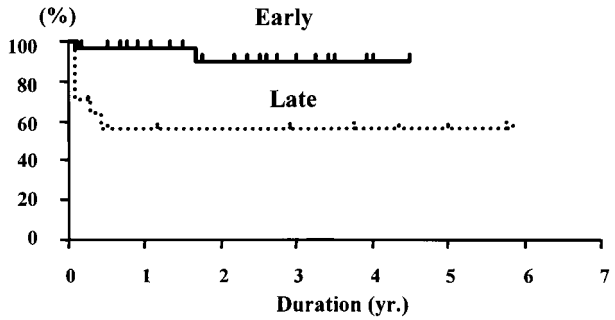

Fig 1. Kaplan-Meier analysis of survival times for patients receiving etoposide early (n = 30) versus late or not at all (n = 19). The difference between the curves is significant at the $P < .01$ level.

Rapidly Fatal Cases

Seven deaths (four in group 1 and three in group 2) occurred in the earliest phase of treatment (0 to 2 months). Four cases were attributed to pulmonary infection and hemorrhage, one to sepsis and pneumonia, one to fungal infection, and one to progressive hemorrhage. Comparison of the initial laboratory findings in this subgroup versus surviving patients revealed a significantly higher proportion with C-reactive protein concentrations more than 10 mg/dL (five of seven *v* six of 33, $P = .016$), although the distributions of ANC less than 500/μL were comparable (five of seven *v* 15 of 40, $P = .21$). These seven deaths were noted significantly higher in patients who received zero to three doses (seven of 10) versus four to 10 doses (zero of 33) of etoposide during the first 4 weeks ($P = .0001$). Karyotype analysis demonstrated a higher frequency of abnormal chromosomes in the bone marrow cells of rapidly fatal cases (four of five *v* one of 23, $P = .0008$). Details of karyotypes in these cases have been described previously.[21]

Univariate and Multivariate Analyses of Survival Data

By Kaplan-Meier analysis, the 4-year probability of survival for all 47 patients was 75.0% ± 6.7%. Univariate relationships of potentially important prognostic factors to overall survival are shown in Table 3. Patients whose initial treatment was begun within 4 weeks of diagnosis had a significantly higher probability of long-term survival than did patients with longer intervals to initial therapy ($P = .01$). The time from diagnosis to introduction of an etoposide-based regimen exerted the strongest influence on survival. Of 30 patients who received etoposide within 4 weeks of diagnosis, 28 were long-term survivors, compared with only nine of 17 who were given the agent at later intervals. Kaplan-Meier life-table estimates for these two groups were significantly different at the $P < .01$ level (90.2% ± 6.9% *v* 56.5% ± 12.6% at 4 years) (Fig 1). Early introduction of an etoposide-based regimen retained its favorable prognostic status after adjustment for all other variables (relative risk of death, 1.00 *v* 14.1 for patients not receiving this agent or receiving it at > 4 weeks after diagnosis; 95% confidence interval, 1.16 to 166.7; $P = .04$).

None of the other variables examined were significantly related to overall survival. Clinical data between the two groups treated early or late/not at all with etoposide were compared as shown in Table 4. No significant difference was identified besides survival and acute death rate. Although use of CSA to ameliorate neutropenia in the early-etoposide group appeared related to a higher survival rate during the first 12 months of treatment (10 of 10 *v* 16 of 20 among patients receiving CSA later or not at all), this difference was not significant by either Kaplan-Meier or Cox regression (Table 3).

Postremission Toxicity

There were three deaths among the 34 complete responders (two in group 1 and one in group 2), at 3, 5, and 20 months after remission. One of the patients died of a fungal infection and one of transplant-related acquired respiratory distress syndrome/multiple organ failure. The death at 20 months was because of reactivation of disease followed by ileocecal perforation/multiple organ failure. Secondary AML (*FAB M2*, no *MLL* gene rearrangement) was diagnosed in one patient (group 1) at 2 years after therapy with etoposide. This 8-year-old girl was once successfully treated with allogeneic bone marrow transplantation, but relapsed and was in active disease at the last statistical update.

DISCUSSION

Although etoposide-containing immunochemotherapy has become the recommended treatment for EBV-HLH,[16] immunomodulatory therapy with corticosteroids, IVIG, or a combination of these agents continues to be widely used. To identify the treatment components and other clinical variables most closely associated with a better outcome in patients with this disease, we studied 47 cases of EBV-HLH that had been treated with or without etoposide. Twenty-one of the patients were initially given conventional immunomodulatory therapy consisting of corticosteroids, IVIG, and CSA, with 17 subsequently switched to etoposide-containing regimens. Twenty-six cases were treated promptly with etoposide.

The prognostic implications of familial inheritance, serum cytokine levels, and cytogenetic abnormalities in HLH have been reported in previous publications,[21-24] but little is known regarding the treatment combinations most likely to secure long-term survival. Most of the problems that arise in conventional treatment of EBV-HLH can be appreciated

Table 4. Comparison of Clinical Data Between the Two Groups Treated Early or Late/No With Etoposide (< 4 weeks or > 4 weeks/no from diagnosis)

Feature	No. of Patients		P†
	Early-Etoposide Group (N = 30)	Late/No-Etoposide Group* (N = 17)	
Age, years			.129
Median	4.7	4.5	
Range	0.8-16	0.6-31	
Sex			.448
Male	14	6	
Female	16	11	
Neutropenia			.364
< 500 ANC/μL	13	7	
500-1,000 ANC/μL	9	8	
> 1,000 ANC/μL	8	2	
LDH			.671
< 2,000 IU/L	8	6	
2,000-5,000 IU/L	9	5	
> 5,000 IU/L	13	5	
Ferritin			.194
< 3,000 ng/mL	7	6	
3,000-10,000 ng/mL	6	6	
> 10,000 ng/mL	17	5	
C-reactive protein			.425
< 1.0 mg/dL	4	6	
1.0-10.0 mg/dL	12	7	
> 10.0 mg/dL	5	6	
Weeks from diagnosis to CSA therapy			.056
< 4	10	4	
≥ 4	1	4	
Treatment with ET, PE, PP, or HD	7	8	.176
Off treatment within 2 months	8	5	.99
Maintenance with CSA required	19	6	.121
Hemopoietic stem cell transplantation required	4	2	.99
Kaplan-Meier survival estimates (mean ± SD) at 4-years after diagnosis, %	90.2 ± 6.9	56.5 ± 12.6	< .01
Deaths from acute complications	1	6	.011

Abbreviations: LDH, lactate dehydrogenase; ET, exchange transfusion; PE, plasma exchange; PP, plasmapheresis; HD, hemodialysis; ANC, absolute neutrophil count; CSA, cyclosporin A.

*Consists of patients with late etoposide (n = 13) and no etoposide (n = 4).

†All *P* values refer to comparisons between the two groups.

from a survey of published case reports. Poor responses to IVIG or corticosteroids are often reported, and many patients who respond to subsequent therapy with etoposide may have prolonged neutropenic episodes accompanied by opportunistic infections. In one prototypical case described by Hatta et al,[25] a 20-year-old woman did not respond to methylprednisolone (1 g/d for three doses) and had persistently high fever with unimproved laboratory values. Although responding to subsequent treatment with etoposide, she failed to recover from severe leukopenia, and eventually developed meningitis. Intensive supportive therapy abolished her symptoms and promoted recovery of her leukocyte counts and laboratory values. Approximately half of our cases were similar to the above example.

The treatment of EBV-HLH with IVIG was once thought to be beneficial,[10,26,27] yet definitive evaluations of either IVIG or an IVIG plus corticosteroid combination in this disease are lacking. Of our 21 cases initially treated with immunomodulatory agents alone (IVIG in 12 cases), 17 ultimately required etoposide, underscoring the limited efficacy of immune modulation against this lymphohistiocytic disorder. We assume that physicians chose this type of treatment because they considered the disease to be mild, as reflected in the significantly low levels of C-reactive protein in this group, which may be related to milder disease activity or no severe opportunistic infection at the onset of disease (Table 1). Similarly, IVIG induced only a transient remission in a case of lupus erythematosus complicated by

macrophage activation syndrome.[28] By contrast, Su et al[14] reported that immunochemotherapy incorporating etoposide and IVIG was effective in controlling the progression of the hemophagocytic process in a substantial number of patients with virus-associated hemophagocytic syndrome. Chen et al[8,9] in the same group reported that the etoposide plus IVIG combination had other beneficial effects, such as the alleviation of fever and the correction of hematologic and hepatic abnormalities. Additionally, in their series three of the five patients initially treated with etoposide attained complete remissions, compared with none of 14 who first received two doses of IVIG (in the latter group, seven patients subsequently had complete responses to etoposide-containing regimens).[9]

These observations suggest the importance of early introduction of an etoposide-containing regimen in patients with EBV-HLH. Whether such therapy is universally needed or could be restricted to cases of moderately severe to severe disease remains to be determined. For patients with mild disease, it may be possible to begin treatment with immunomodulatory agents, switching to an HLH-94–like protocol at the first sign of failure. Support for this strategy comes from the observation that overall survival rates in groups 1 and 2 were not significantly different. Any firm conclusions will likely require additional data on the efficacy of IVIG. Although this immunomodulator can produce positive effects against HLH, its role in first-line regimens is unclear and will require careful evaluation in future prospective trials. The exact mechanism by which IVIG exerts its immunomodulatory effects is uncertain,[29] but may include alteration of T-cell activation and cytokine production.[30]

The results of our comparative analysis indicate that prompt use of etoposide ($<$ 4 weeks from diagnosis) is a critical factor in successful therapy for EBV-HLH. Additionally, higher doses of etoposide during the first 4 weeks of treatment was linked with better prognosis, because rapid death was found in patients treated with smaller doses of etoposide during this period. These findings may simply indicate that it was not possible to administer sufficient amounts of etoposide because of poor conditions in these patients. Since our findings indicated that etoposide administration within 4 weeks of diagnosis had the most importance prognostically, we compared clinical data between the group treated early with etoposide and the other group (Table 4). In the former group, significantly less incidence of acute death was noted, but no other variables were different between these two groups. Regardless of these beneficial effects, the potential for neutropenia-associated opportunistic infections[17] in patients treated with etoposide raises serious questions about intensive use of this agent. We therefore studied the relationship between the introduction of CSA among early etoposide recipients and survival. None of the 10 patients who received these agents within 4 weeks after being diagnosed with EBV-HLH had fatal opportunistic infections, compared with 16 of 20 who were given etoposide with delayed or no CSA. Although not attaining statistical significance by log-rank analysis, this result is provocative and suggests the need for further evaluation of CSA infusions as a means to ameliorate the complications associated with etoposide-induced or cytokine-induced neutropenia. Alternative strategies, such as plasmapheresis, exchange transfusion, plasma exchange, or hemodialysis,[31-33] might also be considered. Even with these supportive measures, the likelihood of encountering a "very-high-risk" subgroup appears high. Such patients may already harbor opportunistic infections at diagnosis, considering the presence of high C-reactive protein values ($>$ 10 mg/dL) in over half of our rapidly fatal cases. The case of secondary AML identified in this study underscores the increased risk of leukemogenesis associated with clinical use of the epipodophyllotoxins,[18] as well as the necessity of careful long-term follow-up of all etoposide recipients. Ideally, other less hazardous agents with therapeutic effects equivalent to or better than those of etoposide will be found in future prospective studies.

Comparison of treatment results for large cohorts of patients with EBV-HLH is difficult. Only recently have investigators begun to study the EBV variant apart from other virus-associated cases, and even in those instances the therapeutic strategies were not prospectively assigned in a controlled manner. Nonetheless, the 4-year 78.3% $\pm$ 6.7% survival rate in the present study represents considerable improvement over the clinical outcomes reported by Chen et al[9] and by us[20] for children with nonfamilial HLH (40.9% $\pm$ 10.5% and 57.2% $\pm$ 6.2%, respectively).

We conclude that early introduction of an etoposide-based regimen is a critical factor in securing long-term survival in patients with EBV-HLH. The addition of CSA to such regimens may reduce the frequency of fatal infections associated with neutropenia. Whether or not corticosteroids, IVIG, and other immunomodulatory agents should be incorporated with etoposide and CSA into first-line regimens for EBV-HLH remains to be evaluated.

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APPENDIX
Participating Institutions and Physicians

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Shinsyu University School of Medicine	S. Yamada
Nagano Red Cross Hospital	S. Kobayashi
University of Chiba School of Medicine	S. Suwabe
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Gunma University School of Medicine	M. Kato
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Fukui Medical University	A. Tanizawa
Iwate Prefectural Central Hospital	T. Ohara
Hamanomachi Hospital	E. Ishii
Yamaguchi University School of Medicine	H. Ayukawa
Kobe University School of Medicine	Y. Kosaka
Kyoto Prefectural University of Medicine	S. Hibi
Tokuyama Central Hospital	M. Uchida
Tomakomai City Hospital	T. Ohara
National Kumamoto Hospital	K. Takagi
Osaka City General Hospital	M. Sako
Ehime Prefectural Central Hospital	Y. Ishida
Nakamichi General Hospital	A. Watanabe
Sapporo Kohsei General Hospital	M. Konno
Kagawa Children's Hospital	A. Iwai
Akashi Municipal Hospital	Y. Kinoshita
Mie University School of Medicine	H. Kawasaki
Kochi Medical College	H. Hisakawa
Aichi Medical College	K. Konno
Nagoya 2nd Red Cross Hospital	M. Ishii
Ichinomiya City Hospital	S. Takeda
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