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Longitudinal follow-up of patients with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis

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A B S T R A C T

Background and Objectives. Although immunochemotherapy has been reported to be an effective initial treatment for patients with Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH), the long-term outcome of these patients remains unknown. The main purpose of this study was to determine the outcome of the EBV-HLH patients treated between 1992 and 2001.

Design and Methods. During this period, a total of 78 EBV-HLH patients were consecutively registered in 3 separate studies. The rates of initial response, reactivation, and survival as well as causes of death were analyzed. The outcome of the patients who received hematopoietic stem cell transplantation was also studied.

Results. With a median follow-up of 43 months, clinical reactivation was noted in 13 patients (19.4%) and a total of 12 patients needed hematopoietic stem cell transplantation, of whom 9 are alive and well. There had been 19 deaths: early deaths were due to hemorrhages and infections (n=11), while late deaths were related to late reactivation (n=4), transplant-associated causes (n=3) and secondary leukemia (n=1). Overall, after a median follow-up of 43 months, 59 (75.6%) of the 78 patients are alive and well.

Interpretation and Conclusions. The majority of successfully treated EBV-HLH patients have a good outcome and remain disease-free.

Key words: Epstein-Barr virus, hemophagocytic lymphohistiocytosis, chronic active anti-Epstein-Barr virus antibody titer, hematopoietic stem cell transplantation.

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It has recently been demonstrated that patients with Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (EBV-HLH) have lymphoproliferation predominantly in EBV-infected T cells and/or natural killer (NK) cells.[1-6] The disease thus falls into the category of EBV-associated T- or NK-cell lymphoproliferative disorders (T- or NK-LPD).[3,4] This observation has been made in Japan and Taiwan but also confirmed in other Western countries.[3-5] The clinical features of EBV-HLH are typical of those of other types of HLH defined by Henter *et al.*,[7] and include high fever, cytopenia, liver dysfunction and coagulopathy. Hemophagocytic lymphohistiocytosis comprises primary HLH (familial or hereditary, FHL) and secondary HLH.[7,8] Patients with FHL have a very poor prognosis and their only hope of cure is a hematopoietic stem cell transplantation (SCT).[9,10] By contrast, EBV-HLH is generally considered to be a secondary HLH, although the differential diagnosis from FHL or X-linked lymphoproliferative disease (XLP) must be kept in mind.[9-11] The prognosis of EBV-HLH has been assumed to be better than that of FHL; however, unless optimal treatment is rapidly instituted, the outcome of EBV-HLH is also dismal.[12-15] We have previously shown that immuno-chemotherapy as an induction treatment is very effective for patients with EBV-HLH,[14,15] To date, however, studies on the long-term outcome of EBV-HLH patients are not available. We report here the long-term therapeutic results of our prospective study of 78 Japanese patients with EBV-HLH, who we treated between 1992 and 2001.

Table 1. Longitudinal assessment of 78 patients with EBV-HLH.

Study number	*1*	*2*	*3*	*Total*
Study period	1992-1997	1995-1999	1996-2001	1992-2001
Patient details				
Number of patients	17	31*	30	78
Age (yrs.)				
<2	7	8	14	29 (37.2%)
2-15	10	20	14	44 (56.4%)
>15	0	3	2	5 (6.4%)
Sex (M/F)	4/13	14/17	11/19	29/49
Serological responses				
Patients with onset serology	17	24	21	62 (79.5%)
Previous exposure (a)	14	15	11	40
First exposure/ reactivation (b)	3	9	10	22
Repeat serology	15	16	13	44 (56.4%)
Transition from (a) to (b)	1	2	0	3 (3.8%)
Treatment results				
Response documented at 8 weeks	17	24	26	67
CR	5	19	23	47
PR	12	5	3	20
Clinical reactivation	3	4	6	13 (19.4%)
On therapy	2	3	3	8
Off therapy	1	1	3	5
SCT	3	5	4	12 (15.4%)
Subsequent (later than 9th week) CR	17	20	26	63 (80.8%)
Outcome***				
Alive	15	20	25	59 (75.6%)
Dead	2	12	5	19 (24.4%)
Early death (< 2 months)	0	7	4	11 (14.1%)°
Late death (> 2 months)	2	5	1	8 (10.3%)
Causes of death				
Early death due to:				
hemorrhages/infections	0	7	4	11 (14.1%)
Late death due to:				
active disease	1	2	1	4
transplant-related causes	0	3	0	3
secondary leukemia	1	0	0	1

*#see text for the description of Studies 1-3. *Study 2 enrolled a total of 47 patients, including the 31 new patients presented here and 16 patients previously enrolled in Study 1. °Eleven patients who died early after diagnosis could not be evaluated for their initial response to treatment at 8 weeks after diagnosis.*
****As of December 2002. The median (range) of follow-up periods for the patients were 78 (20-122) months in Study 1, 42 (1-96) months in Study 2, 43 (1-78) months in Study 3, and 43 (1 –122) months in all studies.*

Design and Methods

Patients

A total of 78 Japanese patients with EBV-HLH, treated between 1992 and 2001, were enrolled in this study (Table 1). The majority of patients (85%) were treated with etoposide-containing regimens, including 81% who were treated with the international protocol, HLH-94.[7] Some of these patients had participated in two earlier clinical studies. Study 1, which involved 17 patients, had tested the efficacy of immunochemotherapy for treating EBV-HLH.[14] Study 2 involved 31 new patients as well as the 16 patients in Study 1 and had assessed the efficacy of etoposide for treating EBV-HL.[15] In the current study we have grouped the remaining 30 patients, treated and registered after these two previous studies, into Study 3. Twenty-six of these patients received etoposide-containing-regimens, 23 of whom were treated with the HLH-94 protocol (Table 1). Of the 78 patients, 29 were boys and 49 were girls. Over half the patients were 2 to 15 years old (44/78, 56.4%) while most of the remaining patients were less than 2 years old (29/78, 37.2%). The median (range) of the follow-up period for the patients studied was 43 (1-122) months.

Diagnostic criteria

All patients fulfilled the diagnostic criteria for EBV-HLH,[13] namely, positivity for the EBV genome in the blood/bone marrow and other tissues (determined by polymerase chain reaction (PCR), Southern blot and/or *in situ* hybridization for EBER) and positive anti-viral capsid antigen (VCA)-specific-IgG. No cases of T or NK-lymphoma/leukemia not associated with EBV-related HLH were included.

Methods

EBV-DNA in the blood/bone marrow was detected with conventional PCR[16] or real-time PCR.[17] DNA specimens were also subjected to clonality studies with Southern blot analysis for the EBV termini as well as T-cell receptor rearrangements.[18,19] Serological tests assessed the presence of four different antibodies: anti-VCA-IgG, anti-VCA-IgM, anti-EADR-IgG, and anti-EBNA. The titers of these anti-EBV antibodies were determined by a fluorescence antibody method and allowed patients to be classified into having a pattern of (a) previous exposure or (b) first exposure/reactivation.[13] The serological features were assessed at disease onset for 62 of the 78 patients (79.5%). In addition, in 44 of the patients (56.4%), serological testing was performed more than once during the clinical course of the disease. Perforin expression was measured on $CD8^+$ T cells or on NK cells and perforin gene sequencing was performed in 8 patients as described previously.[20] SH2D1A expression or mutations, which would support a diagnosis of XLP, were tested in 12 male patients in two separate laboratories.[21,22]

Treatment and follow-up

Thirty-seven patients initially received treatment that did not involve etoposide, and consisted mostly of corticosteroids and intravenous immunoglobulin.[15] Of these, 25 patients were later switched to receive etoposide-containing regimens. The other 41 patients were treated with etoposide-containing regimens such as the HLH-94 protocol, from disease onset and onwards.[7,8,14,15] In the HLH-94 protocol, etoposide (150 mg/m^2, iv) is given twice weekly for the first 2 weeks, then once weekly for another 6 weeks. Dexamethasone is started at 10mg/m^2/day for the first 2 weeks, then tapered off stepwise and stopped over 8 weeks. The initial response to treatment was evaluated after eight weeks of treatment. In partially responding patients, maintenance therapy was implemented, this consists of etoposide (150 mg/m^2, iv) every second week, dexamethasone (10 mg/m^2/day for 3 days) every second week and oral cyclosporin A (6 mg/kg, daily) for an additional 6-12 months. Alternative intensification and continuation regimens, including chemotherapy for Hodgkin's disease or non-Hodgkin's lymphoma, were given to one patient with refractory disease because no donor was available for SCT.[14] SCT was employed for patients with refractory disease at the discretion of the physician in charge at each institute. The subsequent clinical course of each patient was monitored 2, 6, and 12 months, and 2, 3, 4, and 5 years after disease onset, using the HLH-94 follow-up sheets, to determine whether the patient was in clinical remission or had had any events such as late reactivation or death.[7] Another follow-up form was used to collect information on EBV status and serum immunoglobulin data were recorded. The follow-up sheets were filled in by the physician in charge at each institute. When the data were not complete, one of the authors (SI) solicited the missing information by telephone.

Treatment response

Complete response (CR) was defined as complete resolution of clinical signs and symptoms, as well as the normalization of laboratory findings, particularly serum levels of ferritin. When the patients continued to have fever and other symptoms of HLH, or had abnormally high levels of serum ferritin in the absence of definite symptoms, they were judged to have had a partial response (PR).[14] The PR patients were placed on intensification or maintenance regimens until a CR was induced. Clinical reactivation was defined as an exacerbation of clinical and laboratory findings compatible with HLH in a patient previously in CR or PR.

Statistical analysis

The χ^2 test was applied for the comparisons of the two groups. Differences were considered statistically significant if the two-tailed p value was <0.05.

Results

Disease characteristics

At the onset of EBV-HLH, 22 of the 62 patients whose serological parameters were tested at disease onset showed a pattern of first exposure/reactivation. Of the remaining patients, 40 showed a pattern of previous exposure. Serological testing was performed more than once in 44 patients. Of these, 4 patients with a pattern of previous exposure later demonstrated a reactivation pattern. Three of these 4 patients developed clinical reactivation and had a prolonged refractory clinical course, needing longer chemotherapy and/or SCT. Peripheral blood/ bone marrow cells or other tissues were positive for the EBV genome in all patients, as required for the diagnosis. The usefulness of cell-free EBV genome quantification at diagnosis and during follow-up was confirmed in 10 cases: these data have been published elsewhere.[17] Southern blot-

ting to assess the clonality of EBV-infected cells was performed for 33 patients. Of these, 25 patients had monoclonal, 6 had biclonal and 2 had polyclonal EBV disease (*data not shown*), while T-cell receptor genes were rearranged in 15 patients. Regarding underlying immunodeficiency, we were later informed that SH2D1A mutations had been found post-mortem in 2 male patients (one in Study 2 and the other in Study 3) who both died within 8 weeks of diagnosis, while perforin expression was reported to be normal in the 8 patients tested.

Therapeutic results

As summarized in Table 1, eleven patients (14.1%) died early, within two months of their diagnosis, of two major complications. Of these 11 patients, 7 died so quickly that they could not be evaluated at all for their response to treatment. Of the remaining 67 patients evaluated eight weeks after diagnosis, 47 showed a CR to induction therapy. The remaining 20 cases achieved a PR. Clinical reactivation occurred in 13 (19.4%) of these 67 patients. Twelve patients received SCT when they showed persistence of PR and/or reactivation and were judged to have refractory disease. Overall, 63 (80.8%) of the 78 patients showed a CR during the maintenance treatment period, including one refractory patient treated intensively with lymphoma-type treatment. Overall, 59 patients (75.6%) are alive as of December 2002. Although two XLP patients died early, no later development of hypogammaglobulinemia was noted among the male survivors. There were 19 deaths. As reported above, 11 patients died early, within two months of their diagnosis. The other 8 patients died late, after the first evaluation carried out 2 months post-diagnosis. These patients included one who developed secondary leukemia 31 months after diagnosis (Study 1)[23] and four patients who died from active disease due to reactivation, which occurred between 4 months and 20 months after diagnosis (Studies 1 - 3), and who did not undergo SCT. In addition, three patients died from transplant-related complications 2 weeks, 45 days, and 3 months after SCT (Study 2).

Follow-up of SCT recipients

As summarized in Table 2, twelve patients received allogeneic SCT, all while they were in PR. Eight of these patients received SCT within 12 months of diagnosis and the remaining four patients had their transplant between 14 and 33 months after diagnosis. Six patients received unrelated cord blood (U-CBT) while the other six received cells from related donors.

The most common conditioning regimen was a combination of busulfan/etoposide/cyclophosphamide (BU/VP16/CY). Successful engraftment was obtained in 10 patients, of whom three died of transplant-related complications and seven survived (58%). The two patients who rejected their transplant have been doing well following their autonomous recovery. As a result, nine of the 12 transplanted patients were doing well, as of December 2002. The details of the SCT of seven of these patients have been previously reported.[24-26]

Discussion

In 1999, we reported the results of a study of 17 patients showing that etoposide, dexamethasone and cyclosporin A could effectively control EBV-HLH.[14] This observation was then confirmed in a study with a larg-

Table 2. Details of SCT recipients transplanted for refractory EBV-HLH.

Cases	*Study*	*Age (yr.) Sex*	*SCT*	*Time of SCT from Dx (mo.)*	*Conditioning*	*GVHD prophylaxis*	*Engrafted*	*Outcome (survival from Dx, mo.)***
1	1	7/M	R-twin-BMT	2	BU/VP16/CY	CsA/sMTX	yes	Alive (69+)
2	1	4/F	R-BMT	7	TBI/BU/L-PAM	CsA/sMTX	yes	Alive (85+)
3	1	1.2/F	R-haplo	14	BU/VP16/CY	CsA	no	Alive (72+)
4	2	0.6/F	U-CBT	30	BU/VP16/CY	CsA/sMTX	yes	Alive (120+)
5	2	1.7/F	R-BMT	11	TBI/VP16/CY	CsA/sMTX	yes	Alive (66+)
6	2	31/F	R-BMT	6	TBI/VP16/CY	CsA	yes	Died (9)
7	2	3.5/F	U-CBT	2.5	BU/VP16/CY	mPSL	yes	Died (3)
8	2	4.3/F	U-CBT	28	BU/CY/VP16	CsA	yes	Died (29)
9	3	0.7/F	U-CBT	8	BU/VP16/CY	CsA	yes	Alive (36+)
10	3	4/F	U-CBT	33	TBI/VP16/CY	CsA/PSL	yes	Alive (9+)
11	3	1.6/M	R-BMT	12	BU/VP16/CY	CsA	no	Alive (29+)
12	3	21/F	R-PBSCT	6	TBI/CY/thiotepa	CsA	yes	Alive (28+)

*SCT: hematopoietic stem cell transplantation; Dx: diagnosis; R: related, U= unrelated; BMT: bone marrow transplantation; CBT: cord blood transplantation; PBSCT: peripheral blood stem cell transplantation; haplo: haploidentical; TBI: total body irradiation; BU: busulfan: VP16: etoposide; L-PAM: melphalan; CY: cyclophosphamide; CsA: cyclosporin A; sMTX: short course of methotrexate; mPSL: methylprednisolone; PSL: prednisolone. **As of December 2002.*

er cohort of 47 patients, which included 16 patients from the previous study,[15] followed by another more recent 30 patients. We have been following up these patients to determine their long-term outcome. With a combined total of 78 patients, this report describes one of the largest longitudinal follow-up studies on EBV-HLH patients. From the serology pattern at diagnosis, we assume that the majority of patients had been previously infected, thus it seems they did not develop HLH at initial infection with EBV. However, as an alternate explanation, these patients might have responded to EBV in an inappropriate immunologic manner due to an unknown immune deficiency. Two of the patients included in this series, who died early without having an opportunity to receive SCT, were later found to have had XLP. No definite cases of FHL were included in our study groups.

Regarding the therapeutic results, this study shows that a major obstacle to successful management of EBV-HLH is early death, occurring within 2 months of diagnosis. Of the 78 patients, 11 (14.1%) died early after diagnosis from hemorrhages and fatal infections. Of the 67 patients who had attained a CR or PR by eight weeks after diagnosis, 13 later showed clinical reactivation. Late deaths, which occurred in 10.3% of the overall series of patients, were mostly due to active disease (n = 3) or transplantation-related complications (n = 3). Secondary leukemia was noted in a single case. Twelve patients (15.4%) received a SCT. Half of these received the transplant within a year of diagnosis, because of refractoriness to immunochemotherapy. The remaining six patients received SCT 14, 28, 30 and 33 months after diagnosis due to late reactivation of the disease. Including the SCT recipients, 59 (75.6%) of the 78 EBV-HLH patients were alive and well after a median follow-up of 43 months. It can be concluded that the majority of successfully treated EBV-HLH patients have had a good outcome.

Henter *et al.* recently published a large international cohort study on 113 HLH patients treated with the HLH-94 protocol. In this study, 65 patients (57.5%) received SCT, 25 died prior to SCT, while 23 were alive without SCT, of whom 20, probably with secondary HLH, were alive and had been off therapy for more than 12 months.[8]

The data suggest that this international study included more patients with FHL and fewer with secondary HLH. Although our report did not include cases of FHL, being limited purely to EBV-HLH (except for 2 XLP patients), our data show that 15.4% of the patients needed SCT because of refractory EBV-HLH disease. It is currently reported that about 30% of FHL patients lack perforin expression; however, EBV-HLH patients so far screened expressed perforin normally.[20,27] The precise molecular mechanisms underlying the development of EBV-HLH remain to be determined.

Based on our experience, there are three major aspects of EBV-HLH therapy that crucially affect the long-term outcome. First, strategies to reduce the likelihood of early death due to fatal hemorrhages and severe opportunistic infections related to neutropenia must be immediately instituted after diagnosis. These strategies include careful monitoring of hemostatic parameters, antithrombin III administration and prompt introduction of cyclosporin A.[12,13] It should be remembered that patients with XLP might be among this early death group. Secondly, patients with PR at completion of the 8 weeks' induction therapy must receive intensification or maintenance therapy and should be watched for later reactivation of the disease. Thirdly, refractory cases of EBV-HLH must be quickly and precisely diagnosed so that patients can receive SCT at an appropriate time. Our data show that SCT effectively treated 58% of our patients with refractory EBV-HLH who were successfully engrafted. The diagnosis of refractory disease was based on unresolved clinical features as well as persistently high serum ferritin levels, but in future may be greatly facilitated by longitudinal quantification of EBV genome copies in biological materials during the course of treatment.[17,28] Prospective therapeutic strategies that include SCT therefore promise to be effective in treating patients with EBV-infected T-cell or NK-cell lymphoproliferative disease, resulting in a good long-term outcome.

Contributions. SI and AM contributed to the design of the study, collecting follow-up sheets, analyzing and interpreting data and drafting the article. They approved the final version. TT, YT and SH contributed to the study on EBV serology, clonality and analysis/interpretation of data. HT, YI, YO, MS, HT, and AW clinically contributed greatly in the study of refractory patients and transplantation. JIH contributed to the planning, conception and design of the study, and revising the manuscript critically. SI is responsible for the paper as well as for Tables 1 and 2.

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