CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Effective Control of Epstein-Barr Virus–Related Hemophagocytic Lymphohistiocytosis With Immunochemotherapy

By Shinsaku Imashuku, Shigeyoshi Hibi, Toshio Ohara, Asayuki Iwai, Masahiro Sako, Masahiko Kato, Hirokazu Arakawa, Manabu Sutomatsu, Satoshi Katoaka, Keiko Asami, Dajiro Hasegawa, Yoshiyuki Kosaka, Kimihiko Sano, Noboru Igarashi, Keiko Maruhashi, Ryouji Ichimi, Hajime Kawasaki, Naoko Maeda, Akihiko Taniyawa, Koji Arai, Takanori Abe, Hiroaki Hisakawa, Hitemasa Miyashita, and Jan-Inge Henter for the Histiocyte Society

The familial form of hemophagocytic lymphohistiocytosis (HLH) is a lethal disorder. Although the prognosis for Epstein-Barr virus-associated HLH (EBV-HLH) remains uncertain, numerous reports indicate that it can also be fatal in a substantial proportion of cases. We therefore assessed the potential of immunochemotherapy with a core combination of steroids and etoposide to control EBV-HLH in 17 infants and children who met stringent diagnostic criteria for this reactive disorder of the mononuclear phagocyte system. Treatment of life-threatening emergencies was left to the discretion of participating investigators and typically included either intravenous Ig or cyclosporin A (CSA). Five patients (29%) entered complete remission during the induction phase (1 to 2 months), whereas 10 others (57%) required additional treatment to achieve this status. In 2 cases, immunochemotherapy was ineffective, prompting allogeneic bone marrow transplantation. Severe but reversible myelosuppression was a common finding; adverse late sequelae were limited to epileptic activity in one child and chronic EBV infection in 2 others. Fourteen of the 17 patients treated with immunochemotherapy have maintained their complete responses for 4 to 39 months (median, 15 months), suggesting a low probability of disease recurrence. These results provide a new perspective on EBV-HLH, showing effective control (and perhaps cure) of the majority of EBV-HLH cases without bone marrow transplantation, using steroids and etoposide, with or without immunomodulatory agents.

From Division of Pediatrics, Children’s Research Hospital, and Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan; Division of Pediatrics, Iwate Prefectural Central Hospital, Iwate, Japan; Division of Pediatrics, Kagawa Children’s Hospital, Kagawa, Japan; Division of Pediatrics, Osaka City General Hospital, Osaka, Japan; Department of Pediatrics, Gunma University School of Medicine, Gunma, Japan; Division of Pediatrics, Niigata Cancer Center, Niigata, Japan; Department of Pediatrics, Kobe University, School of Medicine, Kobe, Japan; Division of Pediatrics, Toyama Prefectural Central Hospital, Toyama, Japan; Department of Pediatrics, Mie University School of Medicine, Mie, Japan; Division of Pediatrics, Seirei Hamamatsu General Hospital, Hamamatsu, Japan; Department of Pediatrics, Fukui Medical University, Fukui, Japan; Department of Pediatrics, Hiroaki University School of Medicine, Hiroaki, Japan; Division of Pediatrics, Kochi Red Cross Hospital, Kochi, Japan; Department of Pediatrics, Kochi Medical College, Kochi, Japan; and Department of Woman and Child Health, Childhood Cancer Research Unit, Karolinska Hospital, Stockholm, Sweden.

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Address reprint requests to Shinsaku Imashuku, MD, Division of Pediatrics, Children’s Research Hospital, Kyoto Prefectural University of Medicine, Hirokoji, Kawaramachi, Kamigyo-ku, Kyoto, Japan 602-0841.

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transplantation (BMT), which consistently induces disease resolution and seems to be curative in many cases, has been suggested as the treatment of choice for EBV-HLH, although it is associated with high morbidity.

The HLH Study Group (Chairman: Dr Jan-Ingge Henter, Stockholm, Sweden) of the Histiocyte Society as well as other study groups have long sought to improve clinical outcome in familial and virus-associated HLH by combining established cytotoxic induction regimens (epipodophyllotoxins, intrathecal methotrexate, and corticosteroids) with newer forms of immunomodulatory therapy (to maintain remissions until an acceptable marrow donor is found). Here, we report the results of the use of immunomodulation in 17 patients with EBV-HLH treated at various Japanese pediatric centers from 1992 to 1997 (see Table 1).

PATIENTS AND METHODS

Eligibility and Diagnostic Criteria

Infants and children younger than 16 years of age who met the diagnostic guidelines for HLH with a positive EBV genome in various biological specimens and had not received treatment for this disease were eligible for registration. Briefly, fever persisting beyond 7 days with peaks $>38.5^\circ$C, hepatosplenomegaly, cytopenia (affecting at least two lineages in the peripheral blood and not caused by a hypocellular or dysplastic bone marrow), and hypertriglyceridemia (fasting triglycerides $>2.0$ mmol/L) or hypofibrinogenemia (fibrinogen $<1.5$ g/L) raised strong suspicion of a hemophagocytic syndrome. Cytomorphologically, the bone marrow smears of all patients showed increased hemophagocytosis (median, 9.5%; range, 1.5 to 33.5% of nucleated cell counts; normal, $<1.0$%).

Serological tests for EBV infection were based on viral capsid antigen (VCA)-IgG, VCA-IgM, early antigen-DR-IgG, and Epstein-Barr virus nuclear antigen (EBNA) antibody titers. Peripheral blood mononuclear (PBM) cells, bone marrow cells, or other biological specimens, lymph nodes primarily, were routinely examined for EBV-DNA by the polymerase chain reaction (PCR). Approval was obtained from the Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

<table>
<thead>
<tr>
<th>Table 1. Clinical and Biological Status of 17 Patients With EBV-HLH</th>
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<tr>
<td><strong>Sex</strong></td>
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<td><strong>Median age (range) at diagnosis</strong></td>
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<tr>
<td><strong>Family history of HLH</strong></td>
</tr>
<tr>
<td><strong>Initial clinical presentation (no. of cases)</strong></td>
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<tr>
<td>Persistent fever ($&gt;38.5^\circ$C)</td>
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<tr>
<td>Hepatosplenomegaly</td>
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<td>Coagulopathy</td>
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<td>CNS symptoms</td>
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<td>Lymphadenopathy</td>
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<td>J aundice</td>
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<td>Pleural effusion</td>
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<tr>
<td><strong>Initial biological presentation (no. of cases)</strong></td>
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<tr>
<td>Cytopenia</td>
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<tr>
<td>Hypertriglyceridemia</td>
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<tr>
<td>Hypofibrinogenemia</td>
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<tr>
<td>Serum ferritin $&gt;1,000$ ng/mL</td>
</tr>
<tr>
<td>Serum LDH $&gt;1,000$ IU/L</td>
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<tr>
<td>Specimen positive for EBV-DNA</td>
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Clonality Testing

Clonality was assessed by Southern blot analysis of DNA samples probed for EBV and/or T-cell receptor (TCR) gene sequences using standard methods. Briefly, each DNA sample was digested with EcoRI, BamHI, and HindIII (TCR$\gamma$); EcoRI, BamHI, and Kpn I (TCR$\gamma$); and EcoRI and PstI (EBV). The samples were then electrophoresed and transferred to nylon membranes. The EBV probe was a $3^P$-labeled 1.9-kb Xho I fragment containing the tandem repeat sequence of EBV. The $3^P$-labeled TCR probes were the 0.4-kb Bgl II-EcoRV fragment of the cDNA that contained the constant region of the TCR$\gamma$ gene, and a 0.8-kb HindIII-EcoRI 3' end fragment that cross-hybridized with both J$\gamma$1 and J$\gamma$2 sequences (TCR$\gamma$).

Cytokine Assays

Serum concentrations of soluble interleukin-2 receptor (IL-2R) and interferon-gamma (IFN-\(\gamma\)) were determined with commercial immunoassay (T Cell Science, Cambridge, MA) and immunoradiometric kits, as previously described.

Treatment Regimens

**Induction chemotherapy.** The core elements of treatment were steroids and etoposide. In 9 of our 17 cases (53%), remission induction therapy followed the HLH-94 protocol, either closely or entirely. These 9 cases were among the more than 50 from Japan that have been registered in an international study to evaluate the HLH-94 protocol. Dexamethasone (starting dose: 10 mg/m$^2$ per day, orally [PO] or intravenously [IV]) and etoposide (150 mg/m$^2$ per dose, IV, initially twice weekly for 2 weeks, then weekly) were administered over 8 weeks. The remaining 8 patients were treated on independent protocols that uniformly included prednisolone (2 mg/kg per day, PO, for 2 to 4 weeks) and with one exception, etoposide (150 mg/m$^2$ per dose, IV, twice or three times a week). Intrathecal methotrexate was planned only for patients with clinical evidence of CNS progression or unimproved pleocytosis in cerebrospinal fluid. Short-term infusions of cyclosporin A (CSA; 6 mg/kg per day) were given to 3 patients, at the physician's discretion, to control the unrestricted release of cytokines and their receptors during periods of neutropenia.

Plasma exchange or exchange transfusion was used in 7 cases to stabilize patients in medical crises caused by advanced disease. Intravenous Ig was administered with therapeutic intent to 4 patients. Additional features of the regimens are given in Table 2.

**Continuation treatment.** Responses to induction therapy were considered complete if after 8 weeks there was unequivocal resolution of clinical signs and symptoms, as well as the normalization of laboratory findings, particularly serum levels of ferritin. Patients with persistent fever and other symptoms of HLH, or with abnormally high levels of serum ferritin in the absence of definitive symptoms, were judged to have partial responses and were placed on intensification or maintenance regimens until a complete response was induced. In 7 cases, these treatments followed the HLH-94 protocol: dexamethasone pulses every other week, daily CSA, and biweekly etoposide. Other continuation regimens for refractory cases were modeled on chemotherapy for Hodgkin's disease (HD) or non-Hodgkin's lymphoma (NHL). Specific agents and their sequences of administration are reported in Table 2.

RESULTS

Table 1 summarizes the initial clinical and biological findings in our 17 patients. Persistent fever, hepatosplenomegaly, and cytopenia were defining features, with coagulopathy, jaundice, and lymphadenopathy also noted in more than half the patients. Additionally, the serum ferritin concentration (normal range, 8 to 78 ng/mL) exceeded 1,000 ng/mL in 12 cases, whereas the
cases (4 and 10) are in complete remission for 4 to 7 months, with 2 still receiving treatment. At the time of the most recent follow-up evaluation (December 1997), all patients except one (case 4) were in complete remission for 4 to 39 months (median, 15 months), with 2 still receiving treatment.

There were no bacterial infections during the study. Adverse late sequelae were limited to epileptic activity associated with CNS lesions in one child (case 10) and EBV antibody titers indicative of chronic EBV infection in 2 other cases (cases 9 and 11). Complete remissions were induced in 5 patients during the first 8 weeks of therapy, 3 on HLH-94 protocol and 2 on independent treatment plans (Table 2). One of these responders (case 5) immediately relapsed and was placed on HLH-94 maintenance treatment; the remaining patients did not receive additional therapy for HLH. All other responses were considered partial, prompting a switch to continuation therapy that included dexamethasone pulses every other week, daily CSA, and biweekly etoposide (HLH-94 protocol) or diverse agents that have shown promising activity against HLH (etoposide, prednisolone, SA, and HD- or NHL-type regimens). Patients were provided therapy for a median of 7 months (range, 1 to 36 months). Two cases (cases 2 and 13) were considered refractory to chemotherapy, requiring allogeneic BMT at 2 months and 7 months postdiagnosis respectively. In addition, another case (case 4) awaits BMT after relapse. At the time of the most recent follow-up evaluation (December 1997), all patients except one (case 4) were in complete remission for 4 to 39 months (median, 15 months), with 2 still receiving treatment.
EBV-related hemophagocytic lymphohistiocytosis poses unusual challenges to pediatric hematologists. First of all, the reactive disorder may be difficult to distinguish from infectious mononucleosis, septicemia, certain hematologic malignancies, and systemic autoimmune disorders. All patients in our series met the HLH diagnostic criteria proposed by Henter et al,1 had EBV-DNA in one or more biological specimens by PCR or by Southern blot analysis, and lacked a family history of HLH. Thus, although the distinction between familial and virus-associated HLH is not always clear,39 we would contend that the constellation of presenting clinical and laboratory findings in our series, together with monoclonal or bclonal proliferation of EBV-infected cells (Table 3), adequately supports the diagnosis of EBV-HLH.

Second, because of the poor long-term results of chemotherapy only for familial HLH,40 it has been suggested that EBV-related disease might be similarly resistant, requiring allogeneic BMT; however, occasional reports indicate that some patients may resolve while they are receiving supportive care.17-22,24,25 Neither the medical literature nor our previous experience clearly distinguishes between patients in whom EBV-HLH has a fatal outcome and those who recover at the beginning of treatment. The dilemma, then, is whether to begin aggressive therapy immediately or to control the disease course until there is justification for BMT. Lacking clear risk factors or reliable epidemiological data on which to base therapeutic decisions, we chose instead to treat the disease with immunochemotherapy, switching to BMT only when recurrent disease developed. A total of 11 patients (65%) were treated on the HLH-94 protocol, either entirely or in conjunction with other agents (Table 2). Six others received treatment that was based on precedents in the medical literature,41,42 but that incorporated a core combination of a steroid and etoposide, as in the HLH-94 regimen. In one case (case 17), treatment was limited to steroids and CSA, with etoposide omitted altogether.

All but 2 of the patients achieved complete response on immunochemotherapy (2 cases attained complete response after BMT) and have maintained these responses for 4+ to 39+ months. Thus, it was possible to control EBV-HLH in a high proportion of cases without resorting to BMT. In earlier studies,23,40 patients with familial or virus-associated HLH have generally relapsed within 5 months of attaining complete response on different regimens of immunochemotherapy. In our EBV-HLH cases, 13 of the 17 cases needed continuation treatment with etoposide and steroids with or without CSA, or with other forms of chemotherapy. BMT was successfully performed in 2 cases that showed resistance to all available immunochemotherapy, and is being planned for one other patient. The median response duration in our series, 15+ months, suggests a low probability of disease recurrence in EBV-HLH; however, with the current poor understanding of HLH pathogenesis, we cannot rule out the possibility of delayed relapses.

Steroids and etoposide were common features of treatment, both in the induction phase and in the continuation phase for patients who showed resistance to other agents. Thus, we attribute the excellent outcome largely to judicious use of prednisolone or dexamethasone and etoposide. That dexamethasone crosses the blood-brain barrier more readily than prednisolone2 suggests that it would confer greater protection against HLH in the CNS, a frequent site of involvement in patients with this disease.43,44 CSA, which has not been widely used in therapy for EBV-HLH, seems to have controlled the dysregulated release of cytokines and their receptors in patients with neutropenia and therefore, should be considered a useful component of combination treatment of this disorder, as currently suggested.45-55 Plasma exchange or exchange transfusion56,57 was used at the investigators’ discretion when patients presented with advanced disease complicated by coagulopathy. Without appropriate controls, we are unable to assess the efficacy of these procedures, but would suggest that they be considered as possible effective means to stabilize the patient’s condition in preparation for induction therapy. The value of intravenous immunoglobulin (IVIG) administered early in the induction phase to 4 patients is also difficult to establish. However, reports of deleterious effects produced by IVIG in patients with hemophagocytic syndrome, caused by the presence of cytokines in the Ig preparations,58,59 together with its unimpressive activity against EBV-HLH in the present study (Table 2), indicate that this agent could be omitted from first-line protocols without significant loss of efficacy.

Ideally, one could select therapy for EBV-HLH on the basis of the relapse hazard, reserving BMT for cases with a very high risk of failure. In practice, however, the disease is extremely heterogeneous with few clinical and biological markers that accurately predict responsiveness to immunologic or cytotoxic agents.39 High serum levels of ferritin, lactate dehydrogenase, soluble IL-2R, and IFN-γ have all been implicated as factors that increase the risk of relapse in patients with HLH.14,15,23,60,61 but these associations need to be established in larger trials. One particularly ominous finding indicates that some cases of EBV-HLH involve clonal proliferations of EBV-infected T lymphocytes,9,13,62-64 so that treatment strategies must include methods to control transformed T cells as well as hemophagocytic histiocytes.

With these concerns in mind, we recommend the following therapeutic approach to newly diagnosed cases of EBV-HLH: (1) induction therapy with steroids and etoposide that follows the HLH-94 protocol (8 weeks) with or without exchange transfusion/plasma exchange to alleviate life-threatening complications at presentation; (2) for patients without CR after 8 weeks, continuation of the HLH-94 protocol including the addition of CSA (6 to 12 months); and (3) NHL- or HD-type or other chemotherapy for refractory cases as possible salvage. Although not used in the patients presented here, antithymocytoglobin may also be a reasonable therapeutic choice for refractory disease.45 Allogeneic BMT should be held in reserve and used only for patients with highly continuous

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refractory disease, as EBV-HLH seems to be much more responsible to immunochemotherapy than the familial form of HLH. As one of several types of hemophagocytic lymphohistiocytosis, it is important to conclude that EBV-HLH has a surprisingly good prognosis, using the immunochemotherapy included in the HLH-94 protocol. Because the ultimate utility and safety of the immunochemotherapy we describe will depend on the outcome of trials in larger numbers of patients, we urge greater cooperation among pediatric centers worldwide to ensure that all essential information on EBV-HLH is gathered and analyzed in a timely manner.

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