—Short Communication—

Changes in Cytokine Profile during Initial Treatment of Pediatric Hemophagocytic Lymphohistiocytosis Associated with Epstein-Barr Virus

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Hemophagocytic lymphohistiocytosis (HLH) associated with Epstein-Barr virus (EBV) infection can be self-limiting, severe/aggressive, or fatal. We report a case of EBV-HLH with persistent fever, severe pancytopenia, hypertriglyceridemia, and hypofibrinogenemia in a 4-year-old boy. Levels of plasma cytokines and chemokines were measured with a Bio-Plex system at 1, 2, 3, 4, 5, and 8 days after hospital admission. Administration of steroid and high-dose intravenous immunoglobulin (1 g/kg) did not alleviate fever or reduce cytokine production; however, after administration of etoposide (an antineoplastic agent), fever decreased immediately, the patient's general condition improved, and levels of IL-6, IL-10, IL-8, MCP-1, IFN-γ, and TNF-α declined after etoposide administration. In particular, IFN-γ production sharply declined, from 1,104.1 pg/mL to 101.5 pg/mL, and IL-6 level decreased from 229.8 pg/mL to 11.0 pg/mL, on the day after initial etoposide administration. There was no later recurrence of symptoms during treatment with dexamethasone, etoposide, and cyclosporine A. This case suggests that early etoposide administration is critical for treatment success and indicates that etoposide promptly inhibits cytokine production. (J Nippon Med Sch 2020; 87: 166–170)

Key words: Epstein-Barr virus, hemophagocytic lymphohistiocytosis, immunochemotherapy, cytokine, etoposide

Introduction

Primary Epstein-Barr virus (EBV) infection is usually asymptomatic but may cause infectious mononucleosis. Hemophagocytic lymphohistiocytosis (HLH) in children is characterized by persistent fever, splenomegaly with cytopenia, hypertriglyceridemia, and hypofibrinogenemia. Infiltration of histiocytes with hemophagocytic activity is usually observed in bone marrow. HLH is generally classified as primary and secondary. Primary HLH is caused by genetic defects, such as those in *PRF1* (which encodes perforin)[1]. EBV-associated HLH (EBV-HLH) is considered a major subtype of secondary HLH. Recently, the pathogenesis of HLH was found to involve impaired activation of T lymphocytes after stimulation by immune responses. This resulted in substantial production of inflammatory cytokines, which promote macrophage infiltration and cytokine network formation[1]. Studies of cytokine profiles of HLH patients reported elevated concentrations of many proinflammatory cytokines, such as interferon gamma (INF-γ), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6)[2].

Here, we report a case of EBV-HLH in a 4-year-old boy. We investigated changes in cytokine profile during the first 8 days of EBV-HLH treatment. To our knowledge, no previous study has examined changes in cytokine profile during initial treatment of pediatric EBV-HLH.

Case Report

A 4-year-old boy was admitted with a 10-day history of persistent high fever, cervical lymph node swelling, and general malaise. His past medical history and family history were unremarkable. On admission, blood examination revealed a white blood cell count of 0.9×10^9/L, in-

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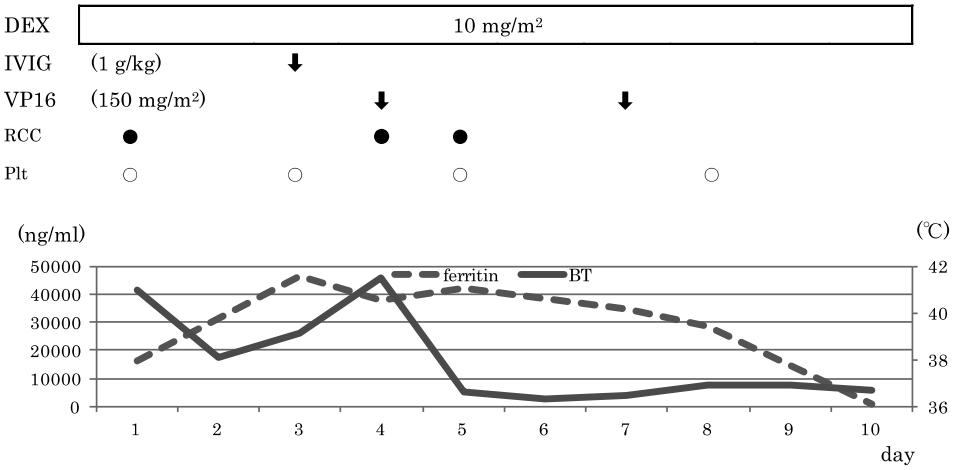
https://doi.org/10.1272/jnms.JNMS.2020_87-307
Journal Website (https://www.nms.ac.jp/sh/jnms/)



WBC (/μL)	900	300	200	700	600	900
Hb (g/dL)	6.6	8.1	7.4	10.5	10.4	10.4
Plt (10^4/μL)	2.4	3.0	4.4	5.2	2.4	7.3
LDH (U/L)	4021	6912	5166	4164	2633	1611

Fig. 1 Clinical course showing sequential changes in ferritin levels and fever status during treatment. Day 1 denotes hospital admission.
BT, body temperature; DEX, dexamethasone; IVIG: intravenous immunoglobulin; Hb, hemoglobin; LDH, lactate dehydrogenase; Plt, platelet transfusion; RCC, red blood cell transfusion; VP-16, etoposide; WBC, white blood cells.

cluding 1% atypical lymphocytes, a hemoglobin level of 6.6 g/dL, and a platelet count of 2.4×10^9/L. Coagulation examination showed a fibrinogen level of 83.6 mg/dL (normal range, 170-400 mg/dL) and a fibrin degradation product level of 28.7 μg/mL (<10 μg/mL). Other laboratory findings were total bilirubin 0.3 mg/dL (<0.85 mg/dL), aspartate aminotransferase 217 IU/L (<40 IU/L), alanine aminotransferase 43 IU/L (<28 IU/L), lactate dehydrogenase (LDH) 4,021 IU/L (<350 IU/L), blood urea nitrogen 10.6 mg/dL (<19.5 mg/dL), creatinine 0.37 mg/dL (<0.42 mg/dL), triglyceride 369 mg/dL (<149 mg/dL), ferritin 15,954 ng/mL (<140 ng/mL), soluble interleukin 2 receptor (sIL2-R) 13,237 U/mL (<496 U/mL), EBV viral capsid antigen (EBV-VCA) IgM titer 10, EBV-VCA IgG titer 160, and negative for EBV-associated nuclear antigen. Bone marrow aspiration at the time of initial diagnosis showed hypoplastic marrow with marked hemophagocytosis. Cytogenetic analysis of bone marrow showed the 46,XY (20/20) karyotype. EBV DNA was positive in peripheral blood (5×10^6 copies/mL), and we diagnosed EBV-HLH after excluding other malignancies.

Dexamethasone (10 mg/m^2) administration was started on admission, and high-dose intravenous immunoglobulin (1 g/kg) was administered on Day 3. Despite this, his fever persisted, and laboratory findings revealed thrombocytopenia and severe neutropenia (180/μL). Ferritin level increased to 46,580 ng/mL, about three times the level on Day 1 (on hospitalization), and LDH increased to 6,912 IU/L (**Fig. 1**). We administered etoposide on Day 4. Before etoposide therapy, levels of IL-6, IFN-γ, and monocyte chemoattractant protein (MCP-1) were increasing, and IL-10 and TNF-α levels were stable; expression levels were high from Day 3 to Day 4. However, after etoposide therapy, fever decreased immediately, and expression levels of several critical cytokines sharply decreased from Day 4 to Day 5 (**Fig. 1** and **Table 1**).

Thereafter, the patient received dexamethasone, etoposide, and cyclosporine A. The final dose of etoposide was given on Day 25 (6 administrations; total dose, 600 mg/m^2), and DEX administration was maintained at 5 mg/m^2. On Day 43, DEX dosage was reduced to 2.5 mg/m^2. After confirming the absence of symptom recurrence and that the EBV DNA copy number on Day 49 was negative, the patient was discharged. No serious adverse events were noted during treatment. He was subsequently discharged on Day 56. His general health since discharge has been good, and symptoms have not recurred during the approximately 1-year period since completion of treatment.

Methods

We analyzed plasma cytokine and chemokine levels on Days 1, 2, 3, 4, 5, and 8 after hospital admission by using a Bio-Plex system. Serum aliquots (50 μL) were collected

Table 1 Serum cytokine levels, by day of hospitalization

Cytokine (pg/mL)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
IFN-γ	790.6	1,259.8	825.9	1,104.1	101.5	14.2
IL-6	117.6	36.2	25.1	229.8	11.0	4.5
IL-10	225.4	20.1	12.9	12.2	7.5	5.9
TNF-α	5.7	74.4	34.4	57.4	52.5	14.3
MCP-1	434.0	480.3	322.8	1,152.2	284.0	93.4
IL-8	34.0	169.4	92.4	228.2	128.1	19.5
IL-13	2.1	4.0	1.3	1.3	1.6	5.9
Il-17	5.2	55.8	20.3	36.1	36.8	16.5
RANTES	482.3	3,788.5	546.1	405.8	260.4	1,004.2
IL-1β	1.3	3.6	0.86	2.59	2.0	0.8
IL-1Ra	1,077.2	1,426.2	817.1	3,345.9	2,007.3	4,957.8
G-CSF	17.9	102.4	21.0	68.4	39.5	19.8
GM-CSF	146.3	172.6	177.8	172.9	162.7	135.5
IL-12	OOR<	9.5	OOR<	81.0	41.8	OOR<
PDGF-β	81.4	115.2	67.3	82.6	45.1	301.8
MIP-1β	88.9	253.2	71.4	103.0	49.1	32.1
IP-10	11,321.3	80,694.3	74,015.6	78,186.0	76,922.3	3,138.6
FGF basic	30.1	91.1	52.2	63.2	70.0	40.4
Eotaxin	11.7	47.6	30.5	37.8	46.3	35.0
MIP-1α	5.0	8.7	2.5	4.3	2.1	0.5
VEGF	24.3	29.2	17.3	22.1	28.7	20.1
IL-9	22.17	80.16	18.7	26.23	16.0	21.2
IL-15	OOR<	50.79	OOR<	80.99	41.85	OOR<
IL-7	1.39	3.02	1.60	3.21	1.80	2.62
IL-2	OOR<	3.60	OOR<	2.96	2.23	OOR<
IL-4	0.55	1.48	0.58	1.04	0.99	1.81
IL-5	OOR<	OOR<	OOR<	OOR<	OOR<	OOR<

* Hospital days 1, 2, 3, 4, 5, and 8

IFN-γ, interferon gamma; IL, interleukin; MCP, monocyte chemoattractant protein; TNF-α, tumor necrosis factor alpha; RANTES, Regulated upon Activation, Normal T-cell Expressed and Secreted; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; PDGF, platelet-derived growth factor; MIP, macrophage inflammatory protein; VEGF, vascular endothelial growth factor; OOR, out of range.

from peripheral blood and analyzed with the Bio-Plex Pro Human Cytokine Grp 1 Panel 27-plex Assay, in accordance with the manufacturer's protocol (Bio-Rad, Hercules, CA, USA). Cytokine and chemokine concentrations were calculated with Bio-Plex Manager 3.0 software (Bio-Rad, Tokyo, Japan). A five-parameter curve-fitting algorithm was applied for calculations of the standard curve.

Discussion

We evaluated the effects of therapeutic drugs on cytokine production and clinical symptoms by measuring cytokine production in the very early stages of EBV-HLH treatment. Etoposide is critical in the treatment of severe EBV-HLH. EBV-HLH manifests as uncontrolled activation of the immune system and hypercytokinemia[3,4]. Imashuku et al reported that etoposide treatment within 4 weeks of HLH diagnosis improved outcomes of patients with EBV-HLH[5]. Although hypercytokinemia ceases with treatment, there are no reports of cytokine measurements obtained at short, regular intervals (i.e. daily) during therapeutic drug administration in the early stages of treatment.

In our patient, both symptoms and serum cytokine levels worsened even when steroid and immunoglobulin therapies were started in the initial treatment stage; however, fever resolved and cytokine production was markedly suppressed on the day after etoposide administration was started. Early use of immunoglobulin and/or corticosteroid is sometimes useful for controlling HLH activity, but the effect is transient[1]. In a recent retrospective nationwide survey, more than half of patients with EBV-HLH were treated with immunochemotherapy, based on the HLH-94/HLH-2004 protocol in Japan,

which includes dexamethasone, etoposide, and cyclosporine A. An additional 30% were treated with a corticosteroid-based therapy, and 10% received supportive therapy only. Complete remission was seen in 90% of patients after initial therapy[3]. Among several prognostic factors, patients with both hyperbilirubinemia (>1.8 mg/dL) and hyperferritinemia (>20,300 ng/mL) at the time of diagnosis had significantly poorer outcomes than those with low serum bilirubin and ferritin levels[3].

The disease state in which CD8-positive cytotoxic T lymphocytes (CTL) are the main EBV-infected cells is often classified as EBV-HLH. The presence of clonal CTL proliferation suggests that CTL overactivation by direct infection with EBV should be considered a basic pathological condition[1]. We examined several cytokines during early treatment (on Days 1, 2, 4, 5, and 8). Han reported that IL-6, IL-10, and INF-γ levels were significantly elevated in EBV-HLH patients[6]. In our patient, steroid and intravenous immunoglobulin administration did not reduce fever; cytokine production and pancytopenia progressed, and ferritin level increased to 46,580 ng/mL. However, IL-6, IL-10, IL-8, MCP-1, IFN-γ, and TNF-α levels were suppressed by etoposide administration. IL-6, IFN-γ, and TNF-α are proinflammatory cytokines regarded as "gatekeepers of inflammation". IL-8, a CXC chemokine, activates neutrophils, and MCP-1 is involved in monocyte/macrophage infiltration. Both are significantly elevated in patients with active HLH[7]. In our patient, IFN-γ level decreased from 1,104.1 pg/mL on Day 4 to 101.5 pg/mL on Day 5. On Day 8, the day after a second dose of etoposide was administered, IFN-γ level was 14.8 pg/mL. IL6 level also decreased from 229.8 pg/mL on Day 4 to 11.0 pg/mL on Day 5 to 4.5 pg/mL on Day 8; MCP-1 level decreased similarly.

These results indicate that etoposide promptly inhibits cytokine production. Furthermore, suppression of the inflammatory cytokines IL-17 and RANTES from Day 2 to Day 3 was likely attributable to steroid treatment. However, on Day 8, RANTES and PDGF levels were again elevated, suggesting that continued treatment Is necessary. Among anti-inflammatory cytokines, IL-10, IL-4, and IL-13 showed little change. However, the level of IL-1Ra, a receptor antagonist of the inflammatory cytokine IL-1, increased from the start of treatment and peaked on Day 8. Interestingly, IL-1β did not increase. IL-1Ra suppresses IL-1 (α and β) activity by competitively inhibiting binding of IL-1 to its receptor[8]. Thus, if IL-1Ra production greatly exceeds that of IL-1, IL-1 activity could be suppressed, thus diminishing inflammatory response, as well.

The role of IFN-γ in EBV-HLH is not well understood. Studies of primary HLH in perforin-deficient mice reported that anti-IFN-γ antibodies were effective for treatment and that IFN-γ is a driver cytokine in HLH onset[9]. Interestingly, recent evidence from mouse experiments indicates that etoposide selectively and rapidly removes activated T cells[10]. Our analysis suggests that this phenomenon is also involved in actual clinical treatment, as indicated by the rapid decrease in inflammatory cytokines after etoposide administration. Ishii recommends initial treatment with high-dose immunoglobulin and/or corticosteroid therapy for all patients with EBV-HLH, followed by immunochemotherapy for those resistant to initial therapy or with several risk factors[1]. In our case, when no initial treatment response was obtained, early administration of etoposide was effective in immediately suppressing levels of several cytokines. The mechanism of action of etoposide (a known antineoplastic agent) in the treatment of EBV-HLH is not fully understood. Although the present evidence advances our understanding, collection of data from multiple cases will be necessary in order to confirm the effects of etoposide on cytokine kinetics during early EBV-HLH treatment.

Acknowledgments: The authors thank Dr. Hikaru Takeshita for technical assistance. This work was supported in part by a Japan Leukemia Research Fund Award from the Friends of Leukemia Research Fund.

Conflict of Interest: The authors declare no conflicts of interest.

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(Received, December 11, 2019)
(Accepted, February 6, 2020)
(J-STAGE Advance Publication, March 31, 2020)