Epstein-Barr virus (EBV) is a ubiquitous herpes virus, and over 95% of the human population is infected during their lifetime. Primary EBV infection is usually asymptomatic, and those infected recover completely. However, some people develop infectious mononucleosis, which is characterized by a triad of symptoms including fever, pharyngitis, and lymphadenopathy which last for one to four weeks. Infectious mononucleosis is usually resolved within one to two months without treatment.

Uncommonly, primary EBV infection is complicated by hemophagocytic syndrome, which has been called EBV-associated hemophagocytic lymphohistiocytosis (HLH). EBV-associated HLH is defined by the criteria for HLH-94 and HLH-2004, and patients must fulfill five of eight criteria, including fever, splenomegaly, bicytopenia, hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis, low/absent natural killer (NK)-cell activity, hyperferritinemia, and high soluble interleukin-2 receptor levels, unless the family history or molecular diagnosis is consistent with HLH. By definition, the patient has no evidence of malignancy, but some patients may transform to T- or NK-cell malignancy during the course of the disease.

EBV-positive systemic T-cell lymphoproliferative disease (LPD) of childhood may develop after primary EBV infection and has been reported previously under the names of fulminant EBV-positive T-cell LPD of childhood, sporadic fatal infectious EBV-Positive T/NK-Cell Lymphoproliferative Disease of Childhood

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Background: Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (HLH), EBV-positive systemic T-cell lymphoproliferative disease (STLPD) of childhood, and chronic active EBV (CAEBV) infection may develop after primary EBV infection. This study reviewed the clinicopathological spectrum of EBV-associated T- and natural killer (NK)-cell LPD, including STLPD and CAEBV infection, with an analysis of T-cell clonality. Methods: Clinicopathological features of seven patients with EBV-associated HLH or STLPD and 12 patients with CAEBV infection were reviewed. Immunohistochemical staining and a T-cell receptor (TCR) gene rearrangement study were performed. Results: STLPD and EBV-positive HLH showed significantly overlapping clinicopathological findings. One patient with STLPD and one patient with EBV-positive HLH demonstrated moderate to severe atypia of the infiltrating lymphocytes, whereas the remaining patients lacked significant atypia. Twelve patients had CAEBV infection, four of whom suffered mosquito-bite hypersensitivity, five showed NK lymphocytosis, and one suffered hydroa vacciniforme. Infiltrating lymphocytes were predominantly small and devoid of atypia. Hemophagocytic histiocytosis was found in seven of 11 patients. Monoclonality was detected in three (50%) of the six patients with successful TCR gene analysis. Conclusions: EBV-positive HLH and STLPD share similar clinicopathological findings and may constitute a continuous spectrum of acute EBV-associated T- or NK-cell proliferative disorders. The distinction of EBV-positive T-cell LPD from EBV-positive HLH may be difficult during routine diagnoses because of the technical limitations of clonality assessment.

Key Words: Epstein-Barr virus infections; Lymphoma, T-cell; Killer cells, natural; Lymphoproliferative disorders; Clonality
mononucleosis, fulminant hemophagocytic syndrome, fatal
EBV-associated hemophagocytic syndrome, and severe chronic
active EBV (CAEBV) infection.4 The disease shares significant
clinicopathological similarities with EBV-associated HLH, but
is defined as a clonal disease of EBV-infected T-cells with an ac-
tivated cytotoxic phenotype in the 2008 World Health Organiza-
tion (WHO) classification.4 The disease occurs most often in
children and young adults after a primary EBV infection,
but can also occur in adult patients.5 Histologically, the proliferat-
ing cells often lack cytologic atypia. Hemophagocytic histiocy-
tosis in the hepatic sinusoids or bone marrow is the main histol-
ogical change. Even the infiltrating cells appear benign, and
the patients follow a fulminant clinical course arising from he-
emophagocytic syndrome and multiorgan failure.4

A minority of patients with primary EBV infection develop
CAEBV infection, which is initially defined by chronic or re-
current infectious mononucleosis-like symptoms that persist for
at least six months and by an unusual pattern of anti-EBV anti-
bodies.1 CAEBV infection is not a simple EBV infection, but a
LPD induced by EBV infection. Patients with CAEBV infec-
tion may present with a variety of clinical signs and symptoms,
including fever, hepatosplenomegaly, lymphadenopathy, and
skin lesions, as well as hypersensitivity to mosquito bites and
hydroa vacciniforme.4 Laboratory findings include non-specific
abnormalities, such as liver dysfunction, thrombocytopenia,
anemia, and EBV-related abnormalities, including elevated an-
tibody titers against viral capsid antigen and/or early antigen
and an elevated EBV DNA load. The pathological changes are
variable, ranging from reactive hyperplasia to monomorphc T-
or NK-cell proliferation, which can be polyclonal or oligoclo-
nal. Monoclonal T-cell LPD arising in CAEBV infection was
classified as systemic T-cell lymphoproliferative disease (STL-
PD) of childhood in the 2008 WHO classification. The prognosis is
variable, and a substantial proportion of patients die from the
disease. The main causes of death are hemophagocytic syndrome
and T- or NK-cell lymphoma. Adverse prognostic factors in
CAEBV infection include the T-cell lineage, an age of onset old-
er than eight years, and liver dysfunction.4

Because EBV-positive T- or NK-cell LPD of childhood shows
variable histological changes and the infiltrating cells look de-
ceptively benign, the distinction between reactive lymphoprol-
iferation and aggressive neoplastic lymphoid proliferation is
very difficult when based on histological changes. Clonality is
regarded as a criterion distinguishing EBV-positive STLDP of
childhood from other EBV-positive hemophagocytic syndromes
and CAEBV infection,3 but the prognostic impact of T-cell or

EBV clonality on EBV-associated LPD is unclear. The purpose
of this study was to review the clinicopathological spectrum of
EBV-associated T- and NK-cell LPD of childhood, including
STLDP of childhood and CAEBV infection, with an analysis of
T-cell clonality.

**MATERIALS AND METHODS**

**Patients**

CAEBV infection was defined as 1) persistent or recurrent
symptoms related to EBV infection for more than three months;
2) high EBV genome levels in affected tissues or peripheral
blood; 3) chronic illness that could not be explained by other
known disease processes at diagnosis, and 4) no specific under-
lying immunological abnormality.7

To identify EBV-positive systemic T-cell LPD of childhood
based on the 2008 WHO classification, all patients with EBV-
positive hemophagocytic syndrome presenting at our institu-
tion were enrolled in the present study. Patient inclusion was
based on the fulfillment of the diagnostic criteria for HLH-2004
in addition to the presence of a high viral load in the tissues or
peripheral blood.3

The surgical pathology database of Samsung Medical Center
from 1994 to 2012 was screened with keywords including “CA-
EBV,” “EBV,” and “hemophagocytosis.” Of the 96 cases re-
trieved using “EBV” and “hemophagocytosis” as keywords, 84
patients were excluded because LPD had arisen in immuno-
compromised patients, such as those receiving organ transplan-
tation or patients with congenital immune deficiency syndrome.
Fifteen patients were diagnosed with CAEBV infection, and 12
patients had paraffin blocks available for examination. Among
the remaining 12 patients, seven had paraffin blocks available
for examination and were enrolled in the present study. Formal-
lin-fixed paraffin-embedded blocks of liver, bone marrow, and
lymph node tissues were used for further investigation.

**Immunohistochemistry**

Immunohistochemical analysis was performed on the paraffin
sections using monoclonal and polyclonal antibodies to detect
lineage-specific or lineage-characteristic antigens, including
CD3 (Dakopatts, Copenhagen, Denmark), CD20 (Novocastra,
Newcastle upon Tyne, UK), CD56 (Novocastra), CD4 (Novo-
castra), and CD8 (Novocastra).

**T-cell receptor (TCR) gene rearrangement**

For the polymerase chain reaction (PCR) amplification of the
TCR γ locus, DNA was extracted from the paraffin-embedded formalin-fixed tissues with the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany). Conventional PCR followed by single-stranded conformational polymorphism analysis was performed, as previously described. When conventional PCR for TCR γ genes produced negative results, further analysis was performed using the TCR β and γ primers, according to the recommendations of the BIOMED-2 protocols (Invivoscribe, San Diego, CA, USA).

RESULTS

All patients (12 males and eight females) were Korean, ranging from 4 to 61 years of age.

EBV-positive hemophagocytic syndrome

TCR gene rearrangement

TCR gene rearrangement analysis revealed monoclonality in two patients (28.6%) and polyclonality in four patients (57.1%) (Fig. 1). The analysis of one patient failed because the DNA quality was poor. Two patients with T-cell monoclonality were classified as suffering systemic T-cell LPD and the remaining patients as suffering EBV-positive HLH.

Clinicopathological findings of systemic T-cell LPD and EBV-positive HLH

Of the two patients with systemic T-cell LPD, one was a young female and the other an adult male. The EBV-positive HLH patients included two children, two young adults, and one elderly adult, with ages ranging from 4 to 61 years (median, 20 years). The clinicopathological findings for the two conditions were similar, and almost all patients had acute-onset fever. Their symptoms included lymph node enlargement and hepatosplenomegaly. The duration of the symptoms was two months in patients with systemic T-cell LPD and ranged from three days to one month (median, 1 month) in EBV-positive HLH patients. Two patients with systemic T-cell LPD received chemotherapy but died after two and three months, respectively. Among the five patients with EBV-positive HLH, four received chemotherapy or steroid and died after a median period of one month, whereas the one patient treated with the HLH-2004 regimen recovered completely and was alive at the final follow-up. The EBV load in the peripheral blood was analyzed using whole blood, and the loads during the clinical course are

Fig. 1. T-cell receptor (TCR) gene rearrangement. (A) TCR gene rearrangement demonstrates monoclonal peak on TCR-β tube A (Table 1, patient 2). (B) TCR gene rearrangement demonstrates polyclonality on TCR-β tube C (Table 1, patient 3).
Table 1. Clinicopathological findings of systemic T-cell lymphoproliferative disease and EBV-positive hemophagocytic lymphohistiocytosis patients

| No. | Sex | Age (yr) | Symptom | Onset | Biopsy site | Hemophagocytic histiocytes | Cell size/Atypia | EBV-PCR (copy/µL whole blood) | EBV-ISH | EBV serology | IHC | TCR β gene | Treatment | Follow-up | Course |
|-----|-----|----------|---------|-------|-------------|-----------------------------|-----------------|-------------------------------|---------|--------------|-----|-----------|-----------|----------|--------|
| 1   | F   | 7        | Fever, cervical lymph node enlargement | 2MA   | LN, BM      | Present                     | Medium/moderate atypia | 42 (initial), 2.5-42 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(+), EBNA(+) | CD8+ | Monoclonal | VHR, L-Asp | 3 mo      | Dead   |
| 2   | M   | 43       | Fever, splenomegaly | 2MA   | BM          | Present                     | Small/mild atypia   | 142 (initial), 0-142 | Positive | GC             | CD8+ | Monoclonal | IMVP-16/ PD | 2 mo      | Dead   |

EBV-positive hemophagocytic lymphohistiocytosis

| 3   | F   | 4        | Fever, jaundice, hepatomegaly, leukocytopenia | 2WA   | LN, BM, liver | Present                     | Medium/severe atypia | 506 (initial), 4.77-6,422 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(+), EBNA(+) | CD8+ | Polyclonal | VHR | 3 mo      | Dead   |
| 4   | M   | 10       | Cervical lymph node enlargement, persistent fever, hepatosplenomegaly, LFT abnormality, pancytopenia, facial petechiae, gingival swelling | 1MA   | BM          | Present                     | Medium/moderate atypia | 5,810 (initial), 8.4-5,810 | Failed | NA             | CD8+ | No band | HLH-2004 | 69 mo | Alive |
| 5   | F   | 20       | Headache, fever, night sweat, abdominal pain, hepatosplenomegaly | 3DA   | BM          | Present                     | Small/mild atypia   | 8.5 | Positive | NA             | CD8+ | Polyclonal | CHOP | 1 mo      | Dead   |
| 6   | M   | 33       | Fever | 1MA | Liver       | Present                     | Small/mild atypia   | 105.7 | Positive | NA             | CD8+ | Polyclonal | Steroid | 3 days | Dead   |
| 7   | F   | 61       | Fever, chill | 4WA | Spleen, BM  | Present                     | Small/mild atypia   | NA | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(+), EBNA(+) | CD8+ | Polyclonal | Steroid | 1 mo | Dead   |

Treatment regimen: VHR: prednisolone, cyclophosphamide, daunorubicin, vincristine, L-asparaginase, intrathecal methotrexate, L-Asp: L-asparaginase, IMVP-16/ PD: ifosfamide, methotrexate, etoposide, prednisolone, 106B: prednisolone, cyclophosphamide, daunorubicin, vincristine, L-asparaginase, CHOP: cyclophosphamide, doxorubicin, vincristine, prednisolone. LPD, lymphoproliferative disease; EBV, Epstein-Barr virus; PCR, polymerase chain reaction; ISH, in situ hybridization; IHC, immunohistochemistry; TCR, T-cell receptor; F, female; MA, months ago; LN, lymph node; BM, bone marrow; EB-VCA, EB viral capsid antigen; EBV-EA, EBV early antigen complex; EBNA, EBV nuclear antigen; M, male; WA, weeks ago; LFT, liver function test; NA, not available; DA, days ago.

*HLH-94/2004: dexamethasone, cyclosporin, intravenous Ig.
shown in Table 1. The levels were variable, and two patients with EBV-positive HLH showed high viral loads in their peripheral blood.

Biopsies were obtained from the bone marrow of six patients, lymph nodes of two patients, liver of one patient, and spleen of one patient. In the bone marrow, liver, and spleen samples, the number of lymphocytes was slightly increased and showed mild to equivocal atypia. Hemophagocytic histiocytosis was observed in all patients. The lymph node biopsies from patient 1 (systemic T-cell LPD) and patient 3 (EBV-positive HLH) demonstrated diffuse effacement of the nodal architecture by the infiltration of monotonous medium-sized atypical lymphocytes (Fig. 2). The lymphocytes had hyperchromatic nuclei and irregular nuclear contours. At the first liver biopsy of patient 3, EBV-infected cells were small to medium size and showed mild to equivocal atypia (Fig. 3A, B). After three months, the lymph nodes of patient 1 were effaced and infiltrated by medium-sized atypical EBV-positive lymphocytes (Fig. 3C-F). On initial examination, the histological findings suggested neoplastic proliferation, but patient 3 displayed polyclonality according to the TCR gene rearrangement (Table 1). The bone marrow of the two patients showed similar findings to the observations in the lymph nodes.

The phenotype of the infiltrated cells was CD3-positive, CD20-negative, and CD56-negative in all cases. Systemic T-cell LPD showed mixed CD4- or CD8-positive cells. EBV-positive HLH was CD8-positive T-cell dominant in three patients and CD4-positive T-cell dominant in two patients. In situ hybridization used to detect EBV in biopsy tissues using the EBV-encoded early RNA 1 probe showed the presence of EBV-infected cells in six of seven patients (Fig. 2). In one patient, in situ hybridization failed to detect EBV because the tissue was improperly fixed. The numbers of EBV DNA copies in the peripheral blood varied, ranging up to 5,810 copies/μL.

### CAEBV infection

The CAEBV group consisted of 12 patients (eight males and four females), with ages ranging from 10 to 59 years (median, 21 years) (Table 2). Nine patients were children or young adults, one patient was elderly, and their clinical manifestations varied. Eight patients presented with hepatosplenomegaly and lymphadenopathy, and six patients complained of fever. Mosquito-bite hypersensitivity was observed in four children, and three patients had NK lymphocytosis. One child experienced hydroa vacciniforme-like eruption. Some patients manifested unusual clinical findings, such as bowel perforation, immunoglobulin A nephropathy, choreic movement, and brain infarction. Biopsies of the bone marrow, skin, gastrointestinal tract, or lung were performed. The histology varied according to the site of biopsy. EBV-positive lymphocytes were scattered, and infiltrated lymphocytes were predominantly small and devoid of atypia (Fig. 4). Hemophagocytic histiocytosis was found in seven of 11 bone marrow tissues. On immunohistochemistry, CD4-positive T-cells were dominant in three patients and CD8-positive cells were dominant in three patients.

Monoclonality was detected in three of the six patients in whom PCR analysis of the TCR gene was successful. Among the three polyclonal patients, one was CD56-positive, with a skewed killer cell immunoglobulin-like receptor (KIR) phenotype, suggesting monoclonal NK-cell proliferation. Among the three patients with polyclonal T-cell proliferation, two were alive and one was alive 30 months after diagnosis. Of the three
patients with monoclonal T-cell proliferation, two patients with monomorphic histology died of their disease after ten months and seven months, respectively, and one patient with polymorphic histology had persistent disease six months after diagnosis. The median survival was 18 months. Comparison of patient survival according to clonality was not statistically meaningful because of the limited number of patients included in the study, but monoclonal patients tended to have poorer prognosis.

**DISCUSSION**

EBV-positive HLH is a clinical entity described in the literature that includes a wide spectrum of illnesses, ranging from EBV-associated reactive polyclonal lymphoproliferation to monoclonal disease. EBV-associated HLH can be divided into three evolutionary stages. The innate immune response phase in the first week of EBV infection is followed by the T-cell activation stage in the second week, which is followed later by the macrophage activation stage. During the last evolutionary phase, a substantial percentage of patients may progress to monoclonal T-cell LPD with or without an abnormal karyotype, which is equivalent to EBV-positive systemic T-cell LPD of childhood. In vitro studies have suggested that the tumor necrosis factor (TNF) receptor secreted by EBV-infected T-cells and the TNF-receptor-associated factors/nuclear factor-κB/extracellular signal-related kinase pathway activated by the EB viral protein, LPM-1, play a role in the progression to T-cell lymphoma.

The “EBV-positive systemic T-cell LPD of childhood” in the 2008 WHO classification corresponds to the neoplastic stage of EBV-positive HLH. However, the separation of systemic T-cell LPD from EBV-positive HLH based on clonality raises two issues: difficult diagnosis due to the technical limitations of clonality assessment, and the clinical impact of clonality on EBV-positive NK- or T-cell LPD.

The clonality of EBV-infected LPD can be determined by various methods, including TCR gene rearrangement, cytogenetic studies, and investigation of the terminal repeats of the EBV genome. TCR gene rearrangement, detected by conventional PCR analysis or Southern blot hybridization, is inadequate to examine the clonality of T-cell EBV-infected LPD because of its low sensitivity. EBV-infected cells defined as polyclonal by TCR analysis can be monoclonal based on EBV terminal repeat analysis or cytogenetic analysis. The sensitivity of the recently developed BIOMED-2 assay is superior to the conventional PCR assay. In addition to low sensitivity, a TCR gene rearrangement study is disadvantageous because it cannot
Table 2. Clinicopathological findings of CAEBV infection

| No. | Sex | Age (yr) | Symptom | Onset | Biopsy site | Hemophagocytic histiocytosis | Associated lymphoma | EBV-PCR (copy/µL whole blood) | EBV-ISH | EBV serology | IHC | TCR γ gene | Treatment | Follow-up | Course |
|-----|-----|----------|---------|-------|------------|-----------------------------|---------------------|-----------------------------|---------|-------------|-----|-----------|------------|-----------|--------|
| 1   | M   | 10       | LFT abnormality, hepatosplenomegaly, multiple enlarged lymph node (posterior neck, axilla and inguinal area) mosquito-bite hypersensitivity | 2YA LN, BM | Present | Peripheral T-cell lymphoma | 77.6 (initial), 71.24-2366 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(+) | CD3+ | CD3+ | Monoclonal/monomorphic | 106B | 10 mo | Dead |
| 2   | F   | 14       | Fever, sore throat, IgA nephropathy, hydroa vacciniforme | Infancy | Skin, BM | Present | T/NK cell lymphoma | NA | NA | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(+) | CD3+ | CD3+ | CD3+>CD8 CD56- | Monoclonal/monomorphic | HLH-2004a | 7 mo | Dead |
| 3   | M   | 15       | Nausea, weight loss, LFT abnormality, Herpes zoster infection | 2MA Liver, BM | Absent | None | 258.5 (initial), 22-258.5 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Monoclonal/monomorphic | CD3+ | 15 mo | Alive |
| 4   | M   | 15       | Mosquito-bite hypersensitivity, palpable neck mass, NK lymphocytosis | 2WA LN, BM | Absent | None | 529.8 (initial), 40-529.8 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(±) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Polyclonal/polymorphic | CD3+ | 15 mo | Alive |
| 5   | M   | 16       | Fever and skin lesion (4YA), bowel perforation (7YA) mosquito-bite hypersensitivity, NK lymphocytosis | Infancy | Skin, BM | Present | None | 2,290 (initial), 7-2,290 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(±) | EBNA(±) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Monoclonal/polymorphic | CD3+ | 15 mo | Alive |
| 6   | M   | 21       | Mosquito-bite hypersensitivity, fever, epigastric pain, nausea, NK lymphocytosis | 7MA Liver, BM | Present | None | NA | NA | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | NC | NC | IMVP-16PD | IMVP-16PD | 3 mo | Dead |
| 7   | M   | 21       | Chorea movement, hepatosplenomegaly, severe oral ulcer, history of pneumonia, thrombocytopenia, NK lymphocytosis | 2YA Liver, BM | Present | None | 29 (initial), 29-73.5 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Polyclonal/polymorphic | CD3+ | 15 mo | Alive (improved) |
| 8   | F   | 20       | Fever, dizziness, nausea, hepatosplenomegaly, pancytopenia, LFT abnormality, diffuse lung infiltration | 2WA LN, lung, BM | Absent | None | 189.2 (initial), 189.2-1,897 | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(±) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Monoclonal/polymorphic | CD3+ | 15 mo | Alive (persistent) |
| 9   | M   | 33       | Fatigue, hepatosplenomegaly, NK lymphocytosis | 7MA Liver, BM | Present | None | 3,446 (initial), 1,918.6-12,112 | Positive | NA | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+ | CD3+ | No band | CVP, CHOP, IMVP-16/PD, VP | 8 mo | Dead |
| 10  | F   | 41       | Fever, hepatomegaly, abdominal pain, cerebral infarct | 2YA BM | Present | Medium/ mild atypia | 1,231.8 (initial), 1,231.8-16,188 | Positive | NA | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+>CD8 CD56- | CD3+ | CD3+ | Polyclonal/polymorphic | CD3+ | 15 mo | Alive |
| 11  | M   | 44       | LFT abnormality, hepatosplenomegaly, palpable neck mass | 8MA LN, Iver | Absent | None | NA | Positive | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+ | CD3+ | No band | CVP, CHOP, IMVP-16/PD, VP | 8 mo | Dead |
| 12  | F   | 59       | Fever, myalgia, NK lymphocytosis | 7MA BM | Present | None | 65.28 (initial), 36.6-2,934 | Negative | NA | EB-VCA, IgG(+) | EB-VCA, IgM(-) | EBV-EA(-) | EBNA(-) | CD3+ | CD3+ | CD3+ | CD3+ | No band | CVP, CHOP, IMVP-16/PD, VP | 8 mo | Dead |

Treatment regimen: 106B: prednisolone, cyclophosphamide, daunorubicin, vincristine, L-asparaginase, CHOP: cyclophosphamide, daunorubicin, vincristine, prednisolone, ESHAP: etoposide, methylprednisolone, high-dose cytarabine, cisplatin, ABVD: adriamycin, bleomycin, vinblastine, dacarbazine, IMVP-16/PD: ifosfamide, methotrexate, etoposide, prednisolone, ICE: ifosfamide, carboplatin, etoposide, CVP: cyclophosphamide, vincristine, prednisolone.

CAEBV: chronic active Epstein-Barr virus; EBV: Epstein-Barr virus; PCR: polymerase chain reaction; ISH: in situ hybridization; IHC: immunohistochemistry; TCR: T-cell receptor; M: male; LFT: liver function test; YA: years ago; LN: lymph node; BM: bone marrow; EB-VCA: EB viral capsid antigen; EBV-EA: EB early antigen complex; EBNA: EBV nuclear antigen; F: female; NK: natural killer; NA: not available; MA: months ago; WA: weeks ago; KIR: killer cell immunoglobulin-like receptor.

HLH-94/2004: dexamethasone, cyclosporinA, intravenous lg.
be used for EBV-positive HLH of NK-cell types. EBV usually infects B-cells and uncommonly T-cells, but NK-cells can also be infected. A recent study by Kimura et al.17 demonstrated that a minor proportion of EBV-positive HLH is actually an NK-cell disorder. Human NK-cells use a sophisticated system of inhibitory and stimulatory receptors of the KIR gene family, which are expressed in a clonally distributed manner. NK-cell KIR gene phenotyping can help determine the clonality of NK-cells,18 but fresh samples are required. Thus, this technique has limited utility in the analysis of paraffin-embedded tissues. Analysis of the EBV terminal repeat may be the most sensitive method because it can be applied in T-, B- and NK-cell disorders. However, EBV clonality is difficult to analyze with routine diagnostic biopsies because large amounts of freshly sampled DNA for Southern blot hybridization are required; chromosomal studies share the same issue.

The clonality of EBV-positive HLH has been reported in a few studies from Asia.12,19,20 A study using conventional PCR reported monoclonal TCR gene rearrangements in ten of 30 adult patients and immunoglobulin gene rearrangements in eight of the same 30 patients.19 The study by Imashuku et al.,12 who analyzed the EBV terminal repeat with Southern blotting, revealed a monoclonal pattern in 20 of 24 patients, a biclonal pattern in two patients, and a polyclonal pattern in two patients. In their study, the TCR gene rearrangement was monoclonal in 15 of 25 patients and polyclonal in ten of the same 25 patients. Clonal cytogenetic abnormalities were observed in seven of 25 patients.12 Recently, Matsuda et al.20 analyzed the TCR gene with the BIOMED-2 protocol, the most recent and advanced technique for gene rearrangement studies and found at all six children with HLH showed a clonal band. According to the above-mentioned studies, a significant proportion of EBV-posi-
tive HLH is a clonal disease that would be classified as STLPD of childhood by the current WHO classification.

In previous studies that analyzed the clonality of EBV and proliferating cells in EBV-positive HLH, clonality itself apparently had no clinical impact on patient outcome. Imashuku et al. reported that the clonality of T- and B-cells has no prognostic significance, whereas all patients with cyrogenetic abnormalities experience an aggressive clinical course. The clinical significance of a clonal karyotypic abnormality was also reported in other studies. During the transformation from benign reactive lymphoid proliferation to overt neoplastic disease, a small clone of EBV-infected T- or NK-cells may emerge and transform into more malignant cells as genetic changes accumulate. Early clonal LPD in this neoplastic transformation may show a similar treatment response to polyclonal EBV-positive HLH, but overt malignant lymphoma with chromosomal abnormality will behave differently.

In terms of patient management, the clinician may use chemotherapy as the initial treatment when the patient is diagnosed with systemic T-cell LPD; however, in patients with EBV-positive HLH, the clinical selection of a therapeutic modality depends on the risk factors for EBV-positive HLH: persistently high EBV genome copy number in the serum, acute fulminant EBV infection mainly involves T- or NK-cells. The clonality of EBV and EBV-infected T- or NK-cells varies and may be polyclonal, oligoclonal, or monoclonal. As the disease progresses from polyclonal lymphoproliferation to monoclonal disease, histological atypia increases. Oshshima et al. proposed the categorization of CAEBV infection into three groups, polymorphic and polyclonal, polymorphous and monoclonal, or monomorphic and monoclonal, based on the clonality and histological changes. In the series reported by Oshshima et al., eight of 48 patients with CAEBV infection were polyclonal for TCR gene rearrangement, and the infiltrated cells displayed polymorphic histomorphology; 15 patients showed polymorphic morphology and biclonal or monoclonal TCR gene rearrangement; and 25 patients showed monomorphic histomorphology and monoclonal TCR gene rearrangement. Patients with the monomorphic and monoclonal type of CAEBV infection had poorer prognosis than patients with polymorphic polyclonal or polymorphic monoclonal disease. The survival of the polymorphic polyclonal and polymorphic monoclonal groups did not differ significantly. According to the 2008 WHO classification, systemic T-cell LPD corresponds to the polymorphic monoclonal and monomorphic monoclonal groups of CAEBV infection. Despite the limitations of a small sample size in our study, the patients with monoclonal CAEBV infection tended to have a poorer prognosis.

In conclusion, EBV-positive HLH and systemic T-cell LPD share similar clinicopathological findings and may constitute a continuous spectrum of acute EBV-associated T- or NK-cell proliferative disorders. The distinction of EBV-positive T-cell LPD from EBV-positive HLH may be difficult during routine diagnoses due to technical limitations of clonality assessment. The clinical impact of clonality in acute EBV-associated T- or NK-cell proliferative disorders is unclear, whereas monoclonal CAEBV infection disease tends to have a worse prognosis.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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