Outcome of L-DEP Regimen for Treatment of Pediatric Chronic Active Epstein-Barr Virus Infection

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Research Article
Abstract

**Purpose** We intended to investigate the clinical features of pediatric patients with chronic active Epstein-Barr virus infection (CAEBV) and effectiveness of the L-DEP regimen before HSCT (hematopoietic stem cell transplantation).

**Methods** A retrospective analysis was performed on 35 patients with CAEBV at Beijing Children's Hospital from January 2016 to January 2020. The efficacy and adverse events of the L-DEP regimen were evaluated.

**Results** The median age of 35 patients was 7.0 years (range 2.5-17.5 years). 28 patients achieved clinical response (80.0%, 22 in clinical CR, 6 in clinical PR) after L-DEP. In terms of virological response, 7 patients (20%) were assessed as virological CR and 23 patients (65.7%) were virological PR. Finally, 29 patients underwent allo-HSCT. Median survival time was 18 months (2-50 months). The 3-year overall survival rates in patients treated with chemotherapy only (n=6), chemotherapy followed by HSCT (n=25) were 33.3% and 75.4%, respectively. After L-DEP 1st treatment, the amount of EBV-DNA loads in blood and plasma were significantly reduced than that before chemotherapy (median: $4.29 \times 10^5$ vs. $1.84 \times 10^6$, Mann-Whitney U, $P=0.0004$; $5.00 \times 10^2$ vs. $3.17 \times 10^3$, Mann-Whitney U, $P=0.003$). And, compared with liver and spleen size before chemotherapy, the size of liver and spleen shrank significantly after L-DEP 2nd (median 3.8cm vs. 1.9cm, $P=0.003$; 3.8cm vs. 0cm, $P=0.008$). In addition, after L-DEP treatment, there was no difference in clinical or virological response rate whether HLH was accompanied or not (clinical response: 77.3% vs. 84.6%, $P=0.689$; virological response: 90.9% vs. 76.9%, $P=0.337$).

**Conclusion** L-DEP regimen is an effective therapy in treating CAEBV for bridging to allo-HSCT.

Introduction

Chronic active Epstein-Barr virus infection (CAEBV) is a rare lymphoproliferative disorder (LPD), which typically presents as persistent infectious mononucleosis-like disease and/or hemophagocytic lymphohistiocytosis (HLH) [1-3]. It is mainly due to inflammation accompanied by EBV infection of T or NK cells. EBV-infected T or NK cells could proliferate and infiltrate clonally into multiple organs, leading to different clinical behaviors from indolent disease to rapidly life-threatening disease. The clinical manifestations include fever, skin rashes, hepatomegaly, splenomegaly, lymphadenopathy, liver dysfunction, and higher EBV-DNA load in blood or plasma [3]. In terms of treatment, allogeneic-HSCT (allo-HSCT) is the only curative treatment, for eliminating EBV-infected T or NK cells [4]. Unfortunately, active disease conditions, which is at the beginning of the conditioning treatment of allo-HSCT, is significantly associated with poor allo-HSCT outcomes [1,5]. Therefore, to improve the prognosis, patients should receive chemotherapy before allo-HSCT to resolve disease activity [1].

However, the response rate of different chemotherapy regimens on CAEBV differ greatly. Many studies have proposed different chemotherapy regimens, including cooling therapy (combination of cyclosporine
A, steroids and etoposide), CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone), the combination of the two (cooling therapy, CHOP, Capizzi and ESCAP), but the total response rate of patients is less than 40%, even as low as 10% \[^{1,6-7}\]. To improve outcomes of CAEBV, it is indispensable to establish a more effective chemotherapy.

In 2016, Wang et al used PEG-asparaginase, liposomal doxorubicin, etoposide, and high-dose methylprednisolone (L-DEP regimen) as salvage therapy for controlling refractory EBV-related hemophagocytic lymphohistiocytosis (EBV-HLH) and gained significant EBV-DNA decrease and good outcome (OR: 85.7\%) \[^{8}\]. In 2020, Zhao et al showed that L-DEP regimen could also benefit pediatric patients with refractory EBV-HLH, with a OR rate of 61.5\% \[^{9}\]. As both EBV-HLH and CAEBV are EBV-related LPD, and CAEBV is often associated with HLH, we speculate L-DEP regimen may be benefit to children with CAEBV.

In this study, we treated children with CAEBV with L-DEP regimens, and analyzed the clinical characteristics, prognosis factors, and effectiveness of L-DEP regimens. The results showed that L-DEP regimen is effective therapy in treating CAEBV for bridging to allo-HSCT.

**Patients And Methods**

Retrospectively, single-center data were collected from 35 patients with CAEBV treated at the hematology oncology center of Beijing Children's Hospital between January 2016 and January 2020. According to World Health Organization (WHO) classification 2016, children who fulfilled all the followed criteria were diagnosed as CAEBV: (1) Sustained or recurrent IM-like symptoms persist for more than 3 months; (2) Elevated EBV genome load in the peripheral blood (PB) or the tissue lesion; (3) EBV infection of T or NK cells in the affected tissues or the PB; (4) Exclusion of other possible diagnoses: primary infection of EBV (infectious mononucleosis), autoimmune diseases, congenital immunodeficiencies, HIV, and other immunodeficiencies requiring immunosuppressive therapies or underlying diseases with potential immunosuppression \[^{7}\]. The clinical diagnosis of HLH is based on the HLH-2004 diagnostic criteria \[^{10}\].

Clinical data, including demographic characteristics, laboratory findings, treatment outcomes and mortality, were collected. Written informed consent was obtained from the parents, and the study was approved by the Institutional Review Board of Beijing Children's Hospital, Capital Medical University. This clinical trial assessing the L-DEP regimen for CAEBV patients was retrospectively registered (Identifier: ChiCTR1900020574).

**Treatments**

All patients were treated with L-DEP regimens after diagnosis. The L-DEP regimen included PEG-asparaginase (2000 U/m\(^2\), day 5), liposomal doxorubicin (25 mg/m\(^2\), day 1), etoposide (100 mg/m\(^2\) once a week, days 1, 8 and 15), methylprednisolone (2 mg/kg, days 1-7; 1 mg/kg, days 8-14; 1 mg/kg, and tapering, days 15-21). The L-DEP regimen used for at least 2 courses, and up to 3 courses. The treatment
response was evaluated after 3 weeks (L-DEP 1\textsuperscript{st}) and/or 6 weeks (L-DEP 2\textsuperscript{nd}) \cite{8}. Once a patient achieved remission, allo-HSCT was recommended.

After chemotherapy, a total of 29 patients underwent allo-HSCT, the other 6 patients did not undergo HSCT because of financial problem or disease deterioration.

**Evaluation of response to L-DEP regimen**

The outcomes of the L-DEP regimen were evaluated and classified as follows: clinical complete resolution (CR) was defined as no symptoms of inflammation including fever, liver dysfunction, progressive skin lesions, vasculitis, accompanied by a significant decrease in EBV-DNA or not. Resolution of a part of the above symptoms of disease was defined as clinical partial resolution (PR) \cite{1}. Progressive disease (PD) was defined as exacerbation of active disease, or development of new findings of disease activity; and stable disease (SD) represented non-improvement of disease activity or no findings of novel active disease \cite{1}. In our article, we defined virological CR as a significant decrease EBV-DNA load in both blood and plasma (<10\textsuperscript{2.5} copies/μg DNA). A 50% drop in EBV-DNA load in either blood or plasma can be defined as virological PR.

**Monitoring the size of liver and spleen**

We use Doppler ultrasound to monitor and evaluate the size of the liver and spleen. The value was marked according to the measured size under the costal margin. We have conducted multiple evaluations, including at diagnosis, after L-DEP 1\textsuperscript{st} and L-DEP 2\textsuperscript{nd}, and before allo-HSCT.

**Evaluation of cytokines**

The common cytokines including IFN-γ (<2.1 pg/ml), TNF-α (1.30-8.5 pg/ml), IL-10 (1.2-4.55 pg/ml), IL-6 (<2.05 pg/ml) were determined by flow cytometry. sCD25 (<6400 pg/ml) which is often used as a signal of inflammation response was determined by ELISA.

**Survival and follow-up**

Overall survival (OS) was estimated from the date of diagnosis until the date of death due to any reasons or the last contact with the patients. The last follow-up date was February 2021.

**Statistics**

The SPSS 20.0 package (IBM, Armonk, USA) was used for all statistical analyses. The normality of numerical variables was evaluated using the Shapiro-Wilks test. The t-test or Mann-Whitney test were used to determine differences between numerical variables with a normal or a skewed distribution between two groups respectively. The chi-square test was used to determine whether there were differences in qualitative variables between groups. Survival analyses were carried out by Kaplan-Meier,
and differences in OS were compared with a two-tailed log-rank test. A P value <0.05 was considered statistically significant. GraphPad Prism 6.0 (Inc., San Diego, CA, USA) was used to draw graphs.

Results

Clinical characteristics of the CAEBV patients

Thirty-five patients with CAEBV were enrolled in this study. Of these patients, there were 20 girls and 15 boys, with a female-male ratio of 1.33:1. The median age was 7.0 years (range 2.5-17.5 years). All patients had lymphadenopathy. Fever, splenomegaly, and hepatomegaly were also common in patients with CAEBV. We also found elevated level of EBV-DNA copies in blood, and positive EBV-encoded RNA (EBER) in situ hybridization in bone marrow. T or NK cells were infected by EBV in all patients as indicated by histopathological biopsy in lymph nodes, liver, skin or spleen. Patients’ clinical characteristics at diagnosis were shown in Table 1.

Genetic characteristics

There was no patient with an affected sibling (family history). Whole exome sequencing showed that only two patients carried mutations possibly related to CAEBV, spontaneous heterozygous mutation in PIK3CD [11] (c.3061G>A in exon 24) and compound heterozygous mutations in IFNGR1 [12][13] (c.961G>A, c.85+5C>T), respectively.

In this study, the patient with PIK3CD c.3061G>A mutation was a 8 years old boy and had a history of multiple respiratory infections after birth. Clinical tests showed cellular immune deficiency. All subtypes of lymphocytes were lower than normal, including total T cell (50.7%), CD4/CD8 (0.49), total B cell (8.5%), T-helper cell (14.8%), T-regular cell (30.4%). The number of EBV-infected T cell was increased (5.41×10^5 per 10^6 cell). He was treated with allo-HSCT, with a survival period of 37 months, and eventually died of severe respiratory infection after transplantation.

The patient with IFNGR1 c.3061G>A mutation was a 4 years old girl and had abnormal humoral immunity and cellular immunity, decreased NK activity. Thus, this mutation was possibly related to the pathogenesis of CAEBV. She was treated with allo-HSCT, with a survival period of 14 months so far.

Response to L-DEP regimen

We evaluated response rate of chemotherapy on L-DEP treatment. The results showed that 28 patients achieved clinical response (80.0%, 22 in clinical CR, 6 in clinical PR) after chemotherapy. One patient (2.9%) was evaluated with PD and 6 patient (17.1%) were SD. In terms of virological response, 7 patients (20%) were assessed as virological CR and 23 patients (65.7%) gained virological PR. Thus, the rate of clinical remission and virological remission were up to 80% and 85.7% in our patients with CAEBV, respectively.
Ultimately, a total of 29 patients underwent allo-HSCT and 6 patients were not treated with HSCT because of financial problem or disease deterioration. Among them, 11 patients eventually died. They were died of progressive deterioration of disease before allo-HSCT (n=4) and complications of death after transplantation (severe infection (n=2), multiple organs failure (n=2) and graft versus host disease (n=3). Median survival time was 24 months (4-57 months). Among all the transplanted patients, 19 patients were transplanted in our transplant center, with all using reduced-intensity conditioning regimen. Ten patients with CAEBV chose the outside transplant center. The conditioning regimens were treated with either myeloablative conditioning or reduced-intensity conditioning. Follow up of the patients with CAEBV was revealed as the flowchart in Fig. 1.

In addition, L-DEP 1st and L-DEP 2nd therapy significantly reduced hepatosplenomegaly: the sizes of liver and spleen shrank from 3.8cm (1-6.5cm) and 2.8cm (1.0-13.0cm) under the costal margin at diagnosis to 2.9cm (0-5.5cm) and 1.3cm (0-10.0 cm), 1.9cm (0-4.8cm) and 0cm (0-8.0cm) after L-DEP 1st and L-DEP 2nd respectively (P=0.003 and 0.007; both P<0.0001, Fig.2).

It was noteworthy that L-DEP treatment made no effect on response rate between the groups of patients with (n=22) or without HLH (n=13), clinical response: 77.3% vs. 84.6%, P=0.689; virological response, 90.9% vs. 76.9%, P=0.337.

**Impact of L-DEP regimen on EBV-DNA load in blood and plasma**

All patients had elevated level of EBV-DNA copies in blood. After L-DEP 1st course, the amount of EBV-DNA loads in blood and plasma were significantly reduced than that before chemotherapy (blood: 4.29×10^5 copies vs. 1.84×10^6 copies, Mann-Whitney U: P=0.0004; plasma: 5.00×10^2 copies vs. 3.17×10^3 copies, Mann-Whitney U: P=0.003). After L-DEP 2nd course, the load of EBV-DNA was also lower than that before chemotherapy (blood: 2.27×10^5 copies vs. 1.84×10^6 copies, Mann-Whitney U: P=0.0001; plasma: 5.00×10^2 copies vs. 3.17×10^3 copies, Mann-Whitney U: P=0.003), although it was similar to that after L-DEP 1st. In addition, the EBV-DNA load in plasma turned negative in 74.2% and 91.4% of patients after L-DEP 1st and 2nd respectively. Therefore, L-DEP therapy resulted in decreases in EBV-DNA load in blood or plasma.

**Impact of L-DEP regimen on cytokine levels**

CAEBV is often complicated with abnormal elevation of inflammatory cytokines. As shown, L-DEP significantly decreased the levels of IFN-γ, from 61.04 pg/ml at diagnosis to 15.19pg/ml and 7.76pg/ml after L-DEP 1st and L-DEP 2nd, respectively (P=0.015 and 0.006 respectively, Fig.3A). The same effect was also observed on the levels of IL-10, decreased from 116.63 pg/ml at diagnosis to 6.7 pg/ml and 7.7pg/ml after L-DEP 1st and L-DEP 2nd respectively (both P<0.0001, Fig. 3B). However, there is no effect of L-DEP therapy on levels of TNF-α (at diagnosis, L-DEP 1st and L-DEP 2nd: 26.74 pg/ml, 6.08 pg/ml and 4.00 pg/ml; P=0.388) and IL-6 (15.43 pg/ml, 20.74 pg/ml and 16.35 pg/ml; P=0.352).
**Adverse effects of L-DEP therapy**

In our patients, diarrhea was the most common adverse effect, observed in 22 patients (62.8%). Other common adverse effects included abnormal coagulation in 17 patients (48.5%), myelosuppression in 15 patients (42.8%), high liver enzymes in 15 patients (42.8%), pancreatic injury in 14 patients (40.0%), infection in 6 patients (28.5%), myocardial damage in 8 patients (21.0%). Acute pancreatitis and gastrointestinal bleeding were also observed in 4 patients (11.4%) and 3 patients (8.5%) respectively.

We treated these patients with symptomatic treatment, including plasma transfusion, antibiotics, glutathione, octreotide, and so on. Most of the adverse events could be alleviated and disappeared. However, 3 patients had serious treatment-related complications and died after treatment failure. Two patients with elevated liver enzymes and abnormal coagulation received plasma transfusion and glutathione therapy, but neither recovered. Both patients died of gastrointestinal bleeding and liver failure. The last one with myelosuppression gained virological PR, nevertheless died of uncontrolled HLH.

**Treatment outcome**

By the end of February 2021, 24 patients were alive and 11 patients died. The short-term cause of death was progressive deterioration of disease before allo-HSCT (n=4, 36.4%). The long-term cause of death (more than 2 years) were mostly due to complications post-transplantation including severe infection (n=2, 18.2%), multiple organs failure (n=2, 18.2%) and graft versus host disease (n=3, 27.3%). Median survival time was 24 months (4-57 months). The probability rate of OS at 1-year, 3-year, and 5-year were 82.9%, 79.7%%, 57.8% (Fig.4). In addition, we found a trend of increase in 3-year OS in patients treated with chemotherapy only (n=6) or chemotherapy followed by transplantation (n=29; 33.3% vs. 75.4%, P=0.098).

**Prognostic factors associated with effectiveness of L-DEP regimen**

In order to analyze the prognostic factors in CAEBV, we divided the patients into two groups according to each of the common clinical, and laboratory features. We used the 75th percentile or reference value of each of the numerical features as cutoff value for grouping. However, correlation of treatment outcome was not observed with age (≥ 4.5 years), time of EBV infection to diagnosis (≥ 12 months), the size of liver and spleen, or EBV-DNA load in blood and plasma, chemotherapy (≥ 3 course), clinical CR/PR or virological CR/PR, complicated with HLH, cytokine level (see in Table 2).

**Discussion**

CAEBV is an EBV-associated lymphoproliferative disorder, which affects T and NK cells. It is related with some manifestations including severe mosquito bite allergy, hydroa vacciniforme, or HLH [14]. CAEBV is a rare disorder and occurs frequently in east Asia for unknown reasons, easily progressing into extranodal NK/T lymphoma or aggressive NK cell leukemia [15]. CAEBV is characterized by clonal proliferation of EBV positive T and/or NK cells, followed by a dismal prognosis and resulting in prolonged or recurrent IM-
like symptoms (eg, fever, node swelling). Recently, the underlying mechanisms of CAEBV have gradually become clear \[7\]. EBV infection of T cells or NK cells can occur with a high EBV load in blood. In addition, it was informed that cytotoxic T cells decreased in numbers or showed dysfunction in CAEBV \[7\]. These findings suggest that undetermined immunosuppressive disorders may underlie persistent infection of T or NK cells, which is similar to the pathogenesis of HLH. In 2020, some new genes somatic mutations in host's cell are confirmed to be related to the pathogenesis of CAEBV, such as \textit{DDX3X, KMT2D, BCOR, KDM6A} and \textit{TP53} \[16\]. This supports that CAEBV patients have genetic abnormalities and needs to be treated with HSCT.

The CAEBV “3-step therapy” which is proposed by Japanese scholars has improved the overall survival rate. However, the remission rate of CAEBV disease activity before HSCT in “the 3-step strategy” was only 20-30\% \[6\]. In addition, there are other chemotherapies to bridge HSCT for treating CAEBV, however, the effectiveness of these options was unsatisfactory, especially without virological CR, and did not agree with each other \[1\]. The development of an effective treatment is urgently needed. Recently, Wang has used L-DEP regimen to treat refractory EBV-HLH and 85.7\% of the patients achieved overall response \[8\]. It is well known that PEG-asparaginase could attack EBV-infected T- and NK-cells, which may not be able to synthesize L-asparagine themselves \[8,17\]. In EBV-infected cells, PEG-asparaginase induces hydrolysis of L-asparagin (essential amino acids for protein syntheses), thus preventing these cells from synthesizing the corresponding proteins, ultimately inhibiting cellular proliferation and resulting in decline in EBV-DNA \[17\]. An in vitro study by Jinta et al \[18\] demonstrated that L-asparaginase dose-dependently reduces the number of EBV-positive T and NK cells, while not affecting the peripheral blood mononuclear cells of normal donors, suggesting the inhibition of L-asparaginase on the proliferation of EBV-positive T cells and NK cells \[8,18\]. Thus, it was of great interest to explore the role of L-DEP regimen in treating CAEBV.

In this study, we found that the amount of EBV-DNA loads in blood and plasma were significantly reduced by 8.1 times and 6.34 times than that at diagnosis, respectively, similarly to the decrease of 10 times in serum 4 weeks after L-DEP therapy in EBV-HLH patients \[9\]. In addition, clinical and virological responses were up to 80\% and 85.7\% respectively. Therefore, L-DEP regimen was effective in reducing EBV-infected cells and EBV-DNA load in patients with CAEBV.

It has been reported that good clinical response is associated with good outcome after allo-HSCT \[1,5\]. In our study, we found that the clinical remission was 80.0\% after L-DEP chemotherapy in CAEBV before bridging transplantation. This may have something to do with the introduction of PEG-asparaginase at the beginning of treatment. This result may be associated with good allo-HSCT outcomes \[1,5\]. However, in this study, 3-year OS after HSCT was 75.4\%, lower than that of “the 3-step strategy” \[4\]. It may be attributed to the delay of HSCT due to unavailability of suitable donors or economic reasons. So, our results shown that L-DEP chemotherapy was sufficient to resolve disease activity before HSCT. But, to improve survival in patients with CAEBV, the patients should undergo allo-HSCT as soon as possible after L-DEP chemotherapy to achieve better survival, and we should reduce transplant-related mortality as well.
Recently, somatic mutations in *DDX3X, KMT2D, BCOR, KDM6A* and *TP53* were confirmed to be related to the pathogenesis of CAEBV\[^{16}\]. In this study, there were two patients with mutations in *PIK3CD* and *IFNGR1*, respectively. Whether these mutations were pathogenic factors of CAEBV was unknown. Although *PIK3CD* somatic mutation c.3061G>A detected in one of our patients has not been reported to be the pathogenic site of CAEBV, gain-of-function mutation of *PIK3CD* was confirmed to be related to the pathogenesis of Activated PI3 kinase delta syndrome (APDS) which is a primary immunodeficiency and can lead to EBV infection\[^{19,20}\]. The apparent PIK3CD deficiency-related clinical phenotype of this patient (history of repeated infections, cellular immune deficiency, increased EBV-infected T cell) indicated the likely pathogenic role of this mutation. In addition, this site had been verified by SIFT and Polyphen2 software as the pathogenic site. Up to now, the binding of IFN-γ to INFGR1 has been confirmed to activates the JAK-STAT pathway, and IFNGR1 was an upregulated gene in patients with CAEBV. So far, more than 40 different mutations have been found in all 7 exons of IFNgR1 gene, which mainly lead to IFN-γ R1 deficiency\[^{21}\]. However, the pathogenicity of compound heterozygous mutation of *IFNGR1* (c.961G>A, c.85+5C>T) still needs confirmative study in CAEBV.

Until now, the factors related to poor prognosis in CAEBV were not clear. Kimura et al had reported that patients with T cell-type CAEBV had poorer prognosis than those with NK cell-type CAEBV\[^{22}\]. The prognosis of the childhood-onset group was better than that of the adolescent/adult- and elderly-onset groups\[^{1}\]. However, in this study, we did not observe any factors associated with poor prognosis. It may be due to fewer patients or insufficient follow-up time, as slow progression is common in CAEBV. In the future study, more CAEBV cases and longer follow-up time are needed.

**Conclusion**

In summary, our findings suggest that patients with CAEBV achieved higher clinical remission rate and virological remission rate after L-DEP regimen treatment and the amount of EBV-DNA load in blood or plasma could be significantly reduced to 6 times, enlarged liver and spleen also shrunk through this regimen. So, L-DEP regimen is an effective therapy in treating CAEBV for bridging allo-HSCT. Although this was a small study, we have provided some clinical evidence for the efficacy of the L-DEP regimen in CAEBV patients. Our current prospective and large-scale clinical trial will further assess the L-DEP regimen for patients with CAEBV.

**List Of Abbreviations**

EBV Epstein-Barr virus; IM infectious mononucleosis; HLH hemophagocytic lymphohistiocytosis; CAEBV chronic active Epstein–Barr virus infection; EBER Epstein-Barr virus -encoded small RNA; L-DEP PEG-Aspegaspargase, doxorubicin, etoposide and methylprednisolone; CTL cytotoxic T cell; LMP latent membrane protein; VEGF vascular endothelial growth factor; JAK-STAT Janus kinase-signal transducer and activator of transcriptions; IL interleukin; NF-κB nuclear factor κB; HSCT hematopoietic stem cell transplantation
Declarations

Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Beijing Children's Hospital, Capital Medical University.

Consent for publication: All authors have read and approved the final manuscript. All parents signed informed consent forms and approved the final manuscript.

Availability of data and materials: The data that support the findings of this study are available on request from the corresponding author.

Competing interests: No

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Authors' contributions:

Dr Honghao Ma, Dr Liping Zhang and Dr Ang Wei conceptualized and designed the study, and drafted the initial manuscript.

Dr Jun Yang, Dr Dong Wang and Dr Yunze Zhao Dr Li Zhang, designed the data collection instruments, collected data.

Dr Qing Zhang, Dr Sitong Chen and Dr Hongyun Lian carried out the initial analyses

Dr Chunju Zhou and Dr Maoquan Qin reviewed and revised the manuscript.

Dr Tianyou Wang, Dr Rui Zhang and Dr Zhigang Li conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

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Tables

Table 1. Clinical characteristics of 35 patients with CAEBV
| Clinical Feature                                      | No. of Patients (%) or Median of Clinical Features (range) |
|-------------------------------------------------------|----------------------------------------------------------|
| **Gender**                                            |                                                          |
| Male                                                  | 15 (42.8%)                                               |
| Female                                                | 20 (57.2%)                                               |
| Median age at diagnosis (year)                        | 7.0 (2.5-17.5)                                           |
| EBV infection time before diagnosis (month)           | 3 (1-60)                                                 |
| **Fever**                                             | 31 (88.6%)                                               |
| Splenomegaly                                          | 31 (88.6%)                                               |
| Hepatomegaly                                          | 29 (82.9%)                                               |
| Lymphadenopathy                                       | 35 (100%)                                                |
| Skin lesions                                          | 9 (25.7%)                                                |
| Coronary artery dilatation                            | 4 (11.4%)                                                |
| With HLH                                              | 22 (62.9%)                                               |
| WBC ($\times 10^9$/L)                                 | 4.67 (0.95-12.2)                                         |
| Neutrophil ($\times 10^9$/L)                          | 1.89 (0.27-6.72)                                         |
| Hb (g/L)                                              | 104 (83-133)                                             |
| PLT ($\times 10^9$/L)                                 | 189 (39-515)                                             |
| Albumin (g/L)                                         | 36.7 (21.6-44.8)                                         |
| AST (U/L)                                             | 80.4 (13.8-1314.0)                                       |
| ALT (U/L)                                             | 60.3 (9.8-1275.3)                                        |
| Bilirubin ($\mu$mol/L)                                | 10.43 (3.81-99.83)                                       |
| Triglyceride (mmol/L)                                 | 1.90 (0.58-4.09)                                         |
| Fibrinogen (g/L)                                      | 2.12 (0.84-34.1)                                         |
| Ferritin (ng/ml)                                      | 146.9 (24.7-4353.00)                                     |
| NK activity (%)                                       | 15.43 (7.80-17.48)                                       |
| Level of cytokines                                    |                                                          |
| IFN-γ (pg/ml)                                         | 12.72 (1.62-769.91)                                      |
| TNF-α (pg/ml)                                         | 2.82 (0.00-244.33)                                       |
|                      |                  |                  |
|----------------------|------------------|------------------|
| IL-10 (pg/ml)        | 5.31 (1.35-103.42) |                  |
| IL-6 (pg/ml)         | 32.9 (1.75-2500.00) |                  |
| SCD25                | 10831 (437.0-44000.0) |                  |
| EBV-DNA copies       |                  |                  |
| In blood             | 1.84×10^6 (5.00×10^3-3.96×10^7) |                  |
| In plasma            | 3.17×10^3 (5.00×10^2-5.30×10^6) |                  |

No., number; EBV, Epstein-Barr Virus; WBC, white blood cell; Hb, Hemoglobin; PLT, Platelets; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; IL, interleukin; NK, natural killer.

**Table 2. Analysis of putative prognostic factors in CAEBV patients.**
| Variables                                      | Univariate analysis |                |
|-----------------------------------------------|---------------------|----------------|
|                                               | Hazard Ratio (95% CI) | P-value       |
| Age (≥ 4.5 years)                             | 4.72 (0.82-10.68)   | 0.097         |
| Time of EBV infection to diagnosis (≥ 12 months) | 1.56 (0.39-7.80)    | 0.485         |
| Liver (≥3.9 cm)                               | 0.76 (0.18-3.19)    | 0.726         |
| Spleen (≥4.1 cm)                              | 1.38 (0.38-5.19)    | 0.602         |
| AST (≥150U/L)                                 | 0.64 (0.17-2.59)    | 0.570         |
| ALT (≥100 U/L)                                | 0.64 (0.17-2.60)    | 0.567         |
| Fib (<1.5 g/L)                                | 0.51 (0.11-2.88)    | 0.511         |
| Complicated with HLH                          | 1.10 (0.32-3.70)    | 0.878         |
| EBV-DNA in blood (≥5.0×10^6 copies)           | 0.93 (0.25-3.44)    | 0.918         |
| EBV-DNA in plasma (≥1.0×10^5 copies)          | 1.15 (0.23-5.83)    | 0.850         |
| Chemotherapy (≥ 3 course)                     | 0.31 (0.04-2.07)    | 0.235         |
| Clinical CR                                   | 0.68 (0.19-2.27)    | 0.520         |
| Clinical PR                                   | 1.19 (0.24-6.16)    | 0.818         |
| Virological CR                                | 1.48 (0.28-9.26)    | 0.607         |
| Virological PR                                | 1.96 (0.57-6.28)    | 0.301         |
| EBV-T cell                                    | 0.53 (0.10-1.68)    | 0.249         |
| EBV-NK cell                                   | 0.56 (0.09-3.52)    | 0.543         |
| EBV-T/NK cell                                 | 0.38 (0.05-2.95)    | 0.355         |

CI, confidence interval of ratio; HLH, hemophagocytic lymphohistiocytosis.

**Figures**
Figure 1

Patients' treatment and follow-up. Pt, patients; L-DEP 1st, the L-DEP first course; L-DEP 2nd, the L-DEP second course; L-DEP 3rd, the L-DEP third course; CR, complete response; PR, partial response; HSCT, hematopoietic stem cell transplantation.

Figure 2

L-DEP therapy reduced the enlargements of liver (A) and spleen (B).
Figure 3

The levels of IFN-γ and IL-10 decreased obviously after L-DEP therapy. (A) IFN-γ, (B) IL-10.

Figure 4
Overall survival of 35 patients with CAEBV. The probability rate of overall survival at 1-year, 3-year, and 5-year after CAEBV diagnosis were 82.9%, 65.0%, 57.8%. 