Case Report

Glycoprotein 350-targeted chimeric antigen receptor T-cell therapy for nonneoplastic chronic active Epstein-Barr virus infection: a case report

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Background: Chronic active Epstein-Barr virus (CAEBV) infection is a rare disease in which the Epstein-Barr virus (EBV) persists and replicates, causing chronic symptoms and fatal complications. The treatment of CAEBV is still evolving. Our case report showed a new therapy for CAEBV.

Case Description: A 14-year-old boy presented with a 10-month history of recurrent diarrhea, intermittent fever, abdominal pain, distension, dizziness, and fatigue. Physical examination findings included severe malnutrition and hepatosplenomegaly. The local hospital's test results showed that the load of EBV DNA in peripheral blood was 5.99×10^6 copies/mL. Despite treatment with acyclovir, chemotherapy, and supportive care, the symptoms persisted. We determined the lymphocyte subtypes of EBV infection by fluorescence quantitative polymerase chain reaction and the expression of EBV envelope glycoprotein 350 (gp350) in peripheral blood lymphocytes. EBV not only infects B cells but also T and NK cells. According to the clinical manifestations, elevated EBV DNA levels, and positive EBV-encoded small RNA (EBER) status, the patient was diagnosed with CAEBV infection. The patient received a conditioning regimen of fludarabine and cyclophosphamide and an intravenous infusion of gp350-targeted chimeric antigen receptor T (CAR T) cells. After infusion, the patient developed grade I cytokine release syndrome (CRS) and was discharged 10 days later. During the follow-up, the EBV-DNA count remained undetectable.

Conclusions: Our case report showed that CAR T-cell therapy is relatively safe and effective for treating CAEBV in children, with milder CRS compared to that in malignant tumors. However, a greater number of cases are needed to further evaluate the efficacy and safety.

Keywords: Chimeric antigen receptor T-cells therapy (CAR T-cells therapy); Epstein-Barr virus infection (EBV infection); chronic active Epstein-Barr virus infection (CAEBV infection); case report

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Introduction

Chronic active Epstein-Barr virus (CAEBV) infection is a rare disease characterized by systemic inflammation and clonal proliferation of Epstein-Barr virus (EBV)-infected T or natural killer (NK) cells, resulting in chronic symptoms and fatal complications. The treatment of CAEBV is still evolving and includes antiviral drugs, immune system regulators, and hematopoietic stem cell transplantation (HSCT). Here, we report the treatment with chimeric antigen receptor T (CAR T) cells in a child diagnosed with CAEBV infection, mainly involving the digestive system. Thus far, the effect of treatment is significant for the patient's symptoms or clinical indicators. Our case report shows that CAR T-cell therapy is relatively safe for treating CAEBV in children, and the degree of cytokine release syndrome (CRS) is relatively milder than that of malignant tumors. We present this article in accordance with the CARE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-24-292/rc).

Case presentation

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the parents of the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

Highlight box

Key findings

- We present a case of a child diagnosed with chronic active Epstein-Barr virus (CAEBV) infection, mainly involving the digestive system. So far, the effect of chimeric antigen receptor T (CAR T) cell treatment has been significant for the patient's symptoms and clinical indicators.

What is known and what is new?

- Research on CAR T-cell therapy in treating a variety of viral infections is ongoing.
- Our case highlights the potential of CAR T-cell therapy as a therapeutic option for patients with CAEBV infection.

What is the implication, and what should change now?

- The safety and efficacy of CAR T-cell therapy in CAEBV should be evaluated in future large-scale clinical trials.

The patient, a male aged 14 years old, had recurrent diarrhea more than ten times one day since October 2021. In addition, he has intermittent fever, the highest body temperature of 39.0 ℃, abdominal pain, abdominal distension, dizziness, fatigue. His weight has decreased by 15 kg since the onset of disease. Physical examination findings included severe malnutrition, hepatosplenomegaly, a weight of 31.15 kg, a height of 160 cm, and a body mass index (BMI) of 12.17 kg/m^2 [<3 standard deviation (SD)]. The local hospital's test results in September 2021 showed that the load of EBV DNA in peripheral blood was 5.99×10^6 copies/mL. Imaging studies showed a thickened intestinal wall in the duodenum, along with multiple enlarged lymph nodes in the mesentery and retroperitoneum. Histopathological analysis from a biopsy taken during a gastroenteroscopy confirmed a diagnosis of EBV-positive lymphoproliferative disease. Despite receiving treatment with antibiotics, chemotherapy, and supportive care, the patient's symptoms continued to persist. Notably, both his birth and family histories were unremarkable. *In situ* hybridization showed positivity for EBV-encoded small RNA (EBER), and the lymphocyte subtypes of EBV infection were determined by fluorescence quantitative polymerase chain reaction at Shanghai Children's Medical Center (SCMC) (*Figure 1*). EBV not only infects B cells, but also T and NK cells. Antibodies of autoimmune diseases were negative, and hematopoietic malignancy was ruled out by bone marrow biopsy. Whole-exon gene sequencing showed no gene variations. Imaging showed a thick duodenal wall and enlarged lymph nodes in the abdominal cavity. The ileum and colon biopsy revealed EBV-positive lymphoproliferative disease. Despite treatment with acyclovir, chemotherapy, and supportive care, the symptoms persisted. According to the clinical manifestations, elevated EBV DNA levels, and positive EBER status, the patient was diagnosed with CAEBV infection. Other laboratory results are shown in *Table 1*. The expression of EBV envelope glycoprotein 350 (gp350) in peripheral blood lymphocytes was detected via flow cytometry (*Figure 2*). After the ethical approval was obtained at SCMC, the patient and his parents signed the informed consent form for CAR T-cell therapy targeting gp350.

On August 30, 2022, 48 mL of whole blood was drawn from the patient's peripheral vein, the patient's peripheral blood mononuclear cells (PBMCs) were enriched from heparinized blood samples of patients by standard density centrifugation and CD3-positive T lymphocytes were collected by anti-CD3 dynabeads. Anti-CD3/CD28 dynabeads activated these T cells for two days, and then

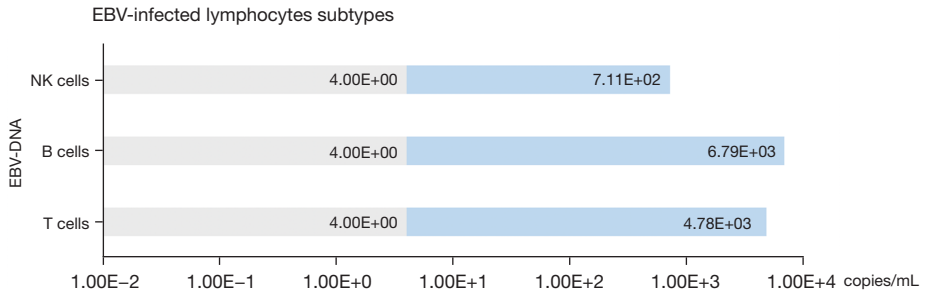

Figure 1 The lymphocyte subtypes of EBV infection determined by fluorescence quantitative polymerase chain reaction. The gray area indicates the normal range. EBV, Epstein-Barr virus; NK, natural killer.

Table 1 Laboratory results

Variables	Reference range	Admission evaluation	Discharge evaluation
Fibrinogen degradation products (μg/mL)	<5	83.7	3.2
Serum ion			
D-dimers (mg/L)	<0.3	11.9	0.55
Sodium (mmol/L)	137–145	130	135.5
Chloride (mmol/L)	98–107	94	100
Potassium (mmol/L)	3.5–5.1	3.47	4.07
Calcium (mmol/L)	2.23–2.80	1.86	2.1
Phosphorus (mmol/L)	1.45–2.10	1.11	1.31
Cellular immune			
NK cells (cells/μL)	90–600	217.25	–
$CD3^+$ (cells/μL)	700–2,100	1,200.17	–
$CD3^+/CD4^+$ (cells/μL)	300–1,400	363.09	–
$CD3^+/CD8^+$ (cells/μL)	200–900	793.02	–
$CD3^-/CD19^+$ (cells/μL)	100–500	332.71	–
CD4/CD8	0.98–1.94	0.46	–
EBV			
Anti-viral capsid antigen IgM	<40	<10	<10
Anti-viral capsid antigen IgG	<20	>750	>750
Anti-nuclear antigen IgG	<20	111	>150
Anti-early antigen IgG	<40	>150	238

Reference values are affected by many variables, including the patient population and the laboratory methods used. They may therefore not be appropriate for all patients. Because of long-term diarrhea, he had a disorder of water and electrolyte, and the blood coagulation index showed hyperfibrinolysis. EBV-related antibodies showed previous infection. The humoral and cellular immune functions were normal, and the $CD4^+/CD8^+$ decreased, consistent with the manifestation of virus infection. Positron emission tomography CT: (I) part of the colon and abdominopelvic part of the small intestine multiple patchy increased FDG metabolism, combined with pathology, is consistent with the manifestation of inflammatory disease. Abdominal and retroperitoneal multiple lymph nodes with increased FDG metabolism. (II) The spleen was large, multiple low-density nodules were in the splenic parenchyma, FDG metabolism was defective, and multiple patchy FDG metabolism increased foci in the spleen. (III) Multiple skeletal FDG metabolism is diffusely increased throughout the body, and bone marrow reactive hyperplasia may be considered. NK, natural killer; IgM, immunoglobulin M; IgG, immunoglobulin G; EBV, Epstein-Barr virus; CT, computed tomography; FDG, fluorodeoxyglucose.

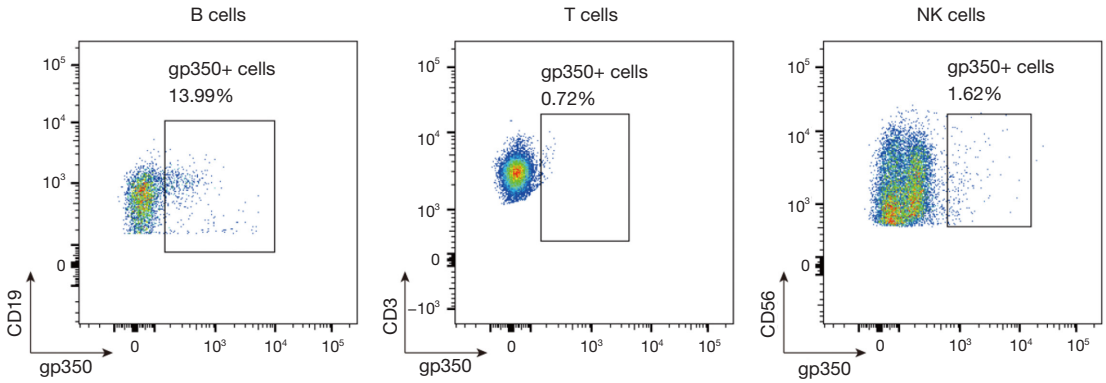

Figure 2 The expression of EBV envelope gp350 in peripheral blood lymphocytes according to flow cytometry. The expression of gp350 was 13.99%, 0.72%, and 1.62% in T, B, and NK cells, respectively. EBV, Epstein-Barr virus; gp350, glycoprotein 350; NK, natural killer.

transduced with second-generation anti-gp350 CAR lentivirus, containing the MYC label, single-chain fragment variable (scFv) sequences derived from mouse 72A1 mAb, with signals provided by the costimulatory molecules 4-1BB and CD3-zeta. Five days later, a sufficient number of cells were washed and resuspended in a saline solution with 2.5% human serum albumin. All cell processing steps are operated and double-checked by skilled technicians in good manufacturing practice (GMP). Transduction efficiency is determined on day 5 by fluorescence-activated cell sorting (FACS) using anti-MYC monoclonal antibodies. On September 2, 2022, the patient received a conditioning regimen of fludarabine (40 mg/m^2 for 3 days) and cyclophosphamide (0.4 g/m^2 for 3 days). On September 7, 2022, he received an intravenous infusion of CAR T cells (4×10^6/kg).

The vital signs, routine blood measures, and cytokine levels were consistently monitored. Three days after infusion, the patient developed grade I CRS according to the American Society for Transplantation and Cellular Therapy (ASTCT) grading system (1), as indicated by fever and diarrhea. He was given 160 mg of tocilizumab injection on the sixth day when the interleukin 6 (IL-6) level peaked (583.8 pg/mL) and was discharged 10 days after infusion.

One month later, he was readmitted for diarrhea and a reduced urine volume lasting 2 weeks. The milder symptoms suggested incomplete clearance of the infected cells by CAR T cells. After a week of acyclovir and supportive treatment, he was discharged again after his symptoms improved. At the time of discharge, an ultrasound of his abdomen revealed no enlargement of the liver and a slight enlargement of the spleen.

Subsequently, he received gamma globulin injections monthly for 6 months. During the 15-month follow-up, his weight increased to 60 kg, and hepatosplenomegaly and lymph node enlargement resolved. Regular virus level checks showed that the EBV-DNA count remained undetectable (*Figure 3*). He has now returned to school and daily activities.

Discussion

EBV is a widely prevalent herpes virus, with a global infection rate of around 95% (2). A small number of children with EBV infection may develop chronic or recurrent contagious mononucleosis-like symptoms, the infection may develop into CAEBV, and a variety of life-threatening complications may emerge, such as hemophagocytic lymphohistiocytosis (HLH), interstitial pneumonia, peptic ulcer, lymphoma, intestinal pneumoniae, vasculitis, uveitis, liver failure, coronary aneurysms, and central nervous system involvement (2,3). The prognosis for CAEBV varies. The primary purpose of treatment is to eliminate the infected T, B, and NK cells. The clinical treatment includes traditional antiviral therapy, immunosuppressive therapy, cytotoxic chemotherapy, CD20-targeted antibody therapy, and HSCT. Thus far, HSCT has shown promising results in some severe CAEBV cases (4). CAR T-cell therapy involves genetically engineering the autologous T cells to induce effector T lymphocytes to produce targeted cytotoxicity to specific cells, thus eliminating them. In adults, CAR T-cell therapy has been effective in treating posttransplant lymphoproliferative disorder (5). CAR T-cell therapy in children has shown success in treating recurrent, refractory tumors, demonstrating both efficacy and safety. The targeting of CAR T cells depends on how T cells express specific scFvs that recognizes target cell antigens. gp350,

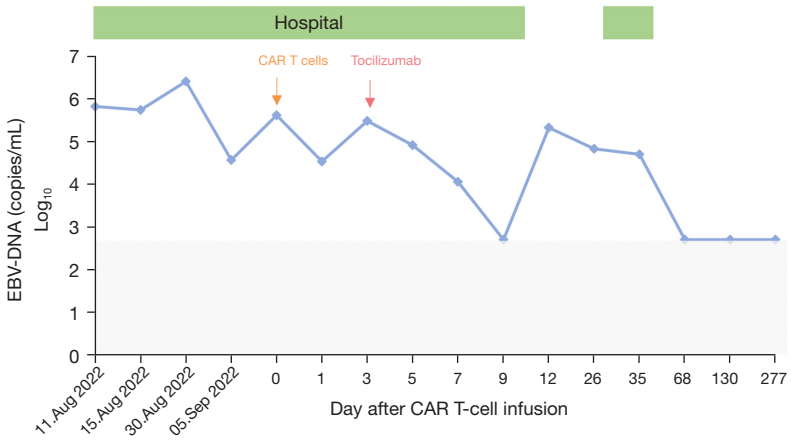

Figure 3 The changes of virus levels in plasma before and after CAR T-cell infusion. The gray line indicates the EBV-DNA-negative reference range of $<5\times10^2$ copies/mL. After the second discharge on October 12, 2022 (day 35 after infusion), regular checkups showed that the EBV-DNA count remained undetectable. CAR T, chimeric antigen receptor T; EBV, Epstein-Barr virus.

the most abundant glycoprotein on the cell membrane of EBV, plays a crucial role in initiating infection (6). During EBV activation, the expression of gp350 can be detected on the cell surface and occasionally in some EBV-related malignant tumors (7). Here, we report on one patient with CAEBV infection treated with CAR T cells that target gp350 epitopes, in which the gp350 reactive cytotoxic T cells could recognize and kill human leukocyte antigen-matched EBV-infected target cells.

Research on CAR T-cell therapy in treating a variety of viral infections is ongoing. The main achievements of CAR T cell therapy for viral infection include treatments targeting acquired immunodeficiency syndrome [human immunodeficiency virus (HIV)], hepatitis C virus (HCV), hepatitis B virus, human cytomegalovirus (CMV), and EBV. Anti-HIV CAR T-cell therapy has made significant progress and is currently in the clinical research stage. Related therapies for other viruses, including for HCV (8) and CMV (9), are still in the early stages of preclinical research. At least three clinical trials are underway on eradicating latent reservoirs, one using modified broadly neutralizing antibody-based (bNAb-based) CAR T-cell therapy (NCT03240328), one using CD4-based CAR T-cell therapy and the CC chemokine receptor 5 (CCR5) ablation (NCT03617198), and one using LVgp120duoCAR T cells. Anti-HIV CAR T-cell therapy has been successful *in vitro*, demonstrating safety, but its low efficacy *in vivo* remains a significant challenge. Other limitations include the susceptibility of CAR T cells to HIV infection, off-target effects, severe CRS, and neurotoxicity, all which require further in-depth study.

For EBV, preclinical trials have been conducted. In one study, prepared $CD8^+$gp350 CAR-T cells were verified in humanized mouse models of EBV infection and lymphoproliferative diseases; in 75% of the mice, the malignant lymphoid tissue proliferation of EBV-infected B cells was controlled or inhibited, tumor growth was suppressed, and inflammation was reduced (10). This is also consistent with the clinical data that we have observed.

Conclusions

Our case report supports CAR T-cell therapy as a relatively safe and effective treatment for CAEBV in children, with milder CRS compared to that observed in malignant tumors. Research on CAR T-cell therapy in treating infectious diseases is ongoing, but the results published thus far are consistent with our clinical observations. Nonetheless, a greater number of cases are needed to further evaluate the efficacy and safety of CAR T-cell therapy.

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Footnote

Reporting Checklist: The authors have completed the CARE

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Peer Review File: Available at https://tp.amegroups.com/article/view/10.21037/tp-24-292/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-24-292/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the parents of the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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