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Case Report

CD45RA+ Depleted Lymphocyte Infusion for Treatment of Refractory Cytomegalovirus Disease in Complete DiGeorge Syndrome: A Case Report

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ABSTRACT

Complete DiGeorge syndrome (cDGS) refers to DGS with profound T cell deficiency. Herein, we present the case of an infant with cDGS suffering from refractory cytomegalovirus (CMV) infection and who was treated with CD45RA+ depleted lymphocyte infusion. The patient was diagnosed with cDGS by fluorescence in situ hybridization which verified 22q11.2 deletion and as well as by the observed profound T cell deficiency (CD3+ T cells 69/μL, CD4+ T cells 7/μL). On the 45th day of age, CMV viremia was first detected with a plasma viral load (VL) of 120,000 IU/mL. Ganciclovir treatment effectively reduced VL post 56 days of treatment; however, VL subsequently rebounded. A CMV UL97 phosphotransferase M460V mutation conferring ganciclovir resistance emerged and foscarnet was incorporated. Despite this, high titers of CMV viremia (VL 2,820,000 IU/mL) and CMV retinitis were complicated. To restore T cell immunity and treat refractory CMV infection, CD45RA+ depleted CMV-specific lymphocytes from the patient's father were infused twice on the 196th and 207th days after birth. After receiving the second infusion, a decline in CMV VL was observed, with a decrease to 87,100 IU/mL by the tenth day following infusion, despite the failure in maintaining T cell increase. The patient died of *Pneumocystis jirovecii* pneumonia and *Elizabethkingia meningoseptica* sepsis on the 222nd day after birth. CD45RA+ depleted lymphocyte infusion may be a therapeutic option for refractory CMV disease in cDGS patients.

Keywords: DiGeorge syndrome; Cytomegalovirus; Severe combined immunodeficiency

INTRODUCTION

DiGeorge syndrome (DGS) occurs in approximately 1:4,000 births. DGS is mostly associated with a 22q11.2 deletion and its typical features include cardiac anomalies, hypoparathyroidism, and a hypoplastic thymus.[1] Abnormal thymic development in DGS leads to impaired T cell immunity, and DGS with athymia and profound T cell deficiency (CD3+ T cells < 50/μL) is referred to as complete DGS (cDGS) and resembles the immunocompromised states of severe combined immunodeficiency (SCID).[2] Despite antimicrobial prophylaxis and immunoglobulin (Ig) replacement therapy, severe infections

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Presentation

This study was partially presented in the Fall Meeting of the Korean Society of Pediatrics in 2021.

Conflict of Interest

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

Author Contributions

Conceptualization: Choi EH, Kang HJ; Data curation: Chin HJ, Park HJ; Formal analysis: Chin HJ; Investigation: Chin HJ, Ryu YH, Kang DY, Hong KT, Choi JY, Yun KW, Lee B; Supervision: Choi EH; Visualization: Chin HJ, Ryu YH; Writing - original draft: Chin HJ; Writing - review & editing: Choi EH.

occurring in the cDGS necessitate T cell immunity reconstitution. Thymus transplantation, hematopoietic stem cell transplantation (HSCT), and unmobilized peripheral blood lymphocyte (PBL) infusion are the available therapeutic options for cDGS.[3-9] Viral infections before these immune reconstitution therapies complicate the clinical course, limit the time for searching for matched donors, and increase the risk of graft failure.[6] Infusion of mononuclear cells from a donor with known immunity to the virus has been used as salvage therapy in such circumstances in cDGS.[7,10] However, this treatment modality is associated with a risk of graft-versus-host disease (GvHD), and often requires additional immunosuppression even in cases involving matched sibling donors.[6] Infusion of CD45RA+ depleted lymphocytes minimizes the risk of GvHD and has been utilized to treat viral infections in conditions deficient in cellular immunity, such as cytomegalovirus (CMV) infection in a recent HSCT recipient.[11]

Herein, we present the case of an infant with cDGS who suffered from severe refractory CMV infection and was treated with a CD45RA+ depleted lymphocyte infusion.

CASE

The girl was born at a gestational age of 38 weeks with a birth weight of 2.32 kg. Her nose was bulbous, and both auricles were low-set and hypoplastic. No thymic shadows were observed on plain radiography at the day of birth (**Fig. 1**). An echocardiogram revealed pulmonary atresia with ventricular septal defect. In suspicion of DGS, fluorescence in situ hybridization (FISH) was conducted on the 2nd day after birth. No fluorescence of the TUPLE1 probe was detected in any of the analyzed cells, which was compatible with DGS. Hypocalcemia was detected on the 3rd day after birth. Further evaluation of immunologic status found profound T cell deficiency (CD3+ T cells 69/μL, CD4+ T cells 7/μL, and CD8+ T cells 14/μL,) and confirmed the diagnosis of complete DGS. CD19+ B cell (481/μL) and CD16+/CD56+ NK cell (564/μL) counts were not decreased. Although IgG level was within the normal range (985mg/dL) at birth, intravenous Ig was replaced four times through regular monitoring of the levels. Serological evaluations for congenital infections, including CMV, Herpes simplex virus, and Toxoplasma, conducted on the day of birth were all negative. Prophylaxis for *Pneumocystis jirovecii* pneumonia (PJP) with trimethoprim-sulfamethoxazole (TMP-SMX) and antifungal prophylaxis with fluconazole were initiated.

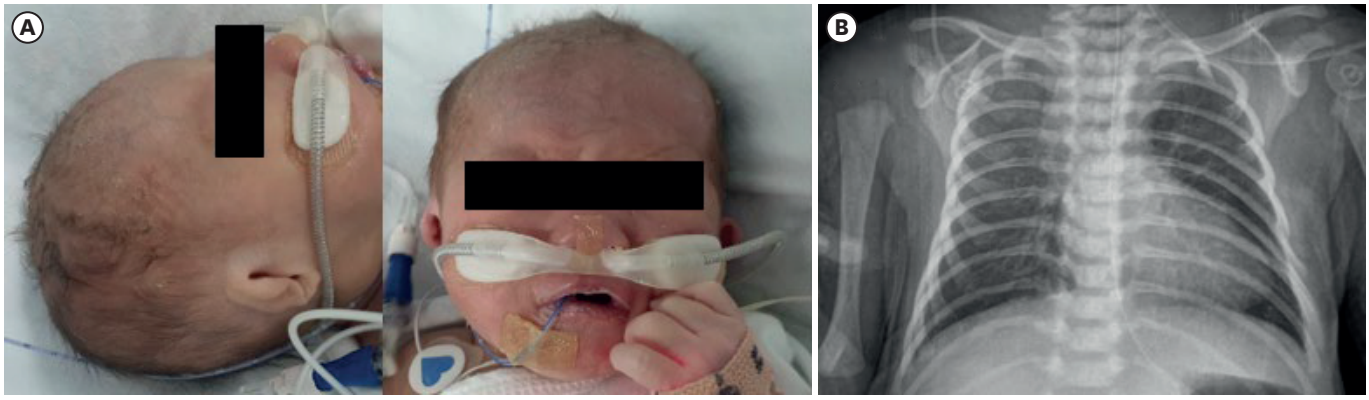

Fig. 1. Syndromic features of the patient. (A) Facial anomalies of low-set, hypoplastic auricles, and bulbous nose. (B) Absent thymic shadow on chest radiograph taken at the day of birth.

The increase in transaminase levels at 42nd day after birth prompted testing for CMV as part of the etiological evaluation of hepatitis, and at 45th day, CMV viremia was first detected, with a plasma viral load (VL) of 120,000 IU/mL. Viral detection tests for Epstein-Barr virus yielded negative result. The patient never had received transfusion before the detection of CMV viremia, and although she was fed breastmilk, CMV culture and polymerase chain reaction (PCR) of the breastmilk were negative. To treat CMV infection, ganciclovir treatment was initiated intravenously at an induction dose of 6 milligrams per kilograms per dose twice a day. This effectively reduced the VL (910 IU/mL) post 56 days of treatment, and the ganciclovir dose was reduced to once a day as maintenance. However, subsequently, VL rebounded, and the ganciclovir dose was re-escalated as before. The patient's hemodynamics became unstable with a rhinovirus infection, prompting corrective surgery for cardiac anomalies. Total correction of the cardiac anomaly was done at the age of 4 months. High titers of CMV viremia ensued (VL 2,820,000 IU/mL) and CMV retinitis was complicated. No intravitreal antiviral treatments were administered. The CMV UL97 phosphotransferase M460V mutation conferring ganciclovir resistance emerged, and foscarnet was incorporated as 180 mg/kg per day on the 153rd day of age (**Fig. 2**). Episodes of fever without identifiable infection other than CMV persisted, leading to unstable hemodynamics. At six months of age, a high CMV VL of 3,700,000 IU/mL was detected in the cerebrospinal fluid, with a concurrent plasma VL of 704,000 IU/mL (**Fig. 2**). Despite antiviral therapy, severe CMV disease was refractory, highlighting the limitations of this treatment approach. Therefore, CMV targeted T cell therapy was deemed necessary. Thymus transplantation was unavailable, and waiting for immune reconstitution post transplantation was not feasible within the given timeframe and non-availability for donor. Peripheral blood lymphocyte (PBL) infusion was considered; however, the patient had no siblings, and no suitable matched unrelated donors were identified. Considering these circumstances, CD45RA+ depleted lymphocytes were obtained from the patient's father, who had a 6/10 human leukocyte antigen (HLA) match and tested positive for CMV IgG. No stimulation with granulocyte-colony stimulating factor was conducted on the donor. Lymphocytes were separated from the whole blood of the donor using the CliniMACS system (Miltenyi Biotec GmbH, Bergish Gladbach, Germany). CD45RA+ cells were depleted using CliniMACS CD45RA reagent according to the manufacturer's protocol. The obtained CD45RA+ depleted lymphocytes were infused on the 196th day of age (0.77×10^6 CD3+ T cells/kg and 0.88×10^6 CD45RO+ cells/kg). The graft was administered without conditioning regimen or GvHD prophylaxis. The plasma CMV titer increased to >10,000,000 IU/mL. The CD19+ B cell (541/μL) and CD16+/CD56+ NK cell counts (344/μL) were comparable before the infusion. Increase in peripheral T cells was not observed on day 10 post infusion (CD3+ T cells 6/μL, CD4+ T cells 5/μL, CD8+ T cells 1/μL), and a second infusion with twice the dose of CD3+ cells was conducted at day 11 from the first infusion (1.49×10^6 CD3+ cells/kg, 1.7×10^6 CD45RO+ cells/kg). Nonetheless, T cell increase and immune recovery were not achieved (CD3+ T cells 36/μL, CD4+ 1/μL, CD8+ 4/μL on day 25 from the first infusion). However, a decline in the VL of CMV was observed, with a decrease to 87,100 IU/mL by day 10 post second infusion (**Fig. 2**). No signs of GvHD were observed.

On the 198th day of age, the patient suffered from respiratory failure which was later confirmed to be PJP by PCR method, while concurrent tests for *Mycobacterium tuberculosis* and nontuberculous mycobacteria were negative. In addition, central line-related bloodstream infection caused by *Elizabethkingia meningoseptica* was detected on the 200th day of age when the serum Ig level was low (172 mg/dL). With the worsening of multiorgan dysfunction, gentamicin and minocycline were added to the preexisting treatment regimen, which included teicoplanin, meropenem, colistin, TMP-SMX, and voriconazole. Despite ventilator

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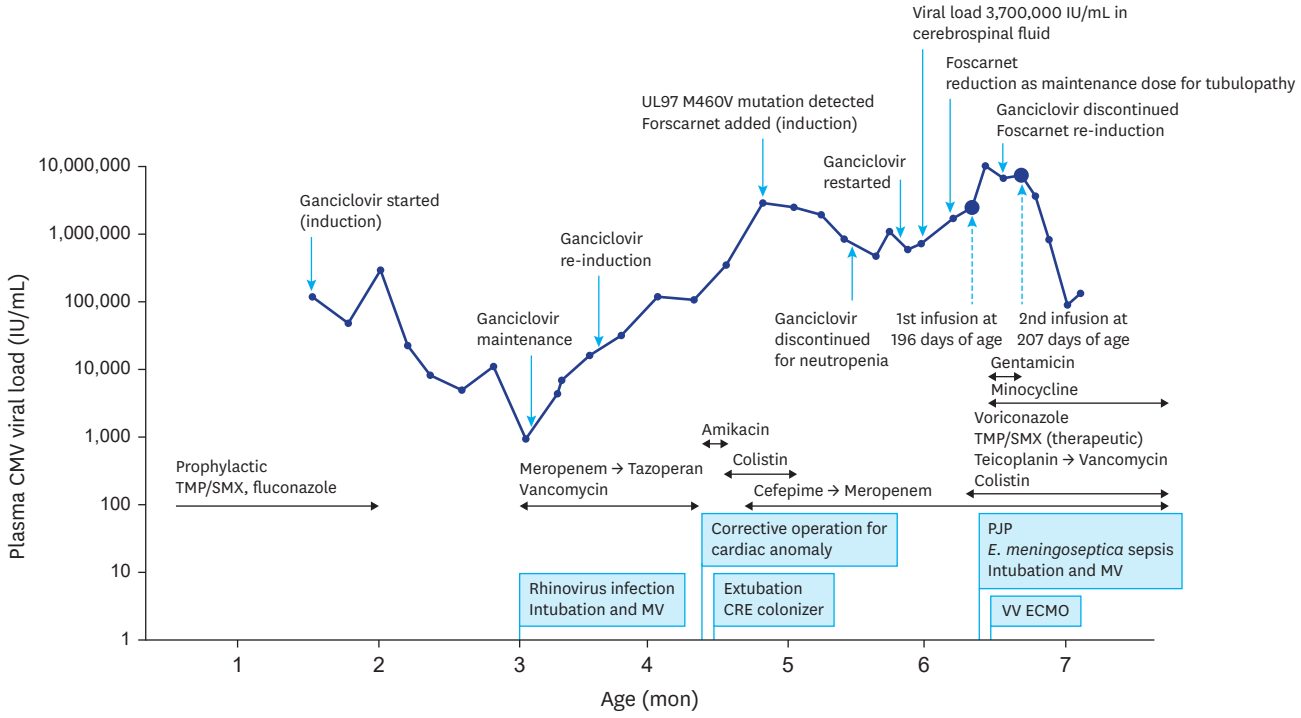

Fig. 2. Plasma CMV viral load of the patient according to age with major clinical events. Note that the viral load is expressed in logarithmic scale. 1st and 2nd infusion refer to CD45RA+ depleted lymphocyte infusions.
Abbreviation: CMV, cytomegalovirus; TMP/SMX, trimethoprim/sulfamethoxazole; CRE, carbapenem-resistant Enterobacteriaceae; PJP, *Pneumocystis jirovecii* pneumonia; VV ECMO, veno-venous extracorporeal membrane oxygenation; MV, mechanical ventilation.

support, extracorporeal membrane oxygenation, and continuous renal replacement therapy, the patient died due to multiorgan failure from PJP and possible *Elizabethkingia meningoseptica* sepsis on the 222nd day of her life.

This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No.: H-2307-035-1447), which waived the need for informed consent.

DISCUSSION

The patient presented in this case report was an infant with a rare disease, cDGS, who experienced severe CMV disease and exhibited a UL97 phosphotransferase M460V mutation conferring ganciclovir resistance. The possibility of congenital CMV infection was low, considering the negative CMV serology, normal brain sonography and normal liver enzyme and platelet counts in the neonatal period. Managing progressive refractory CMV disease in babies with profound T cell deficiency poses significant challenges, further compounded by the limited treatment options. Despite the administration of antimicrobial prophylaxis and Ig replacement therapy, severe viral infections in cDGS necessitate therapies that can restore T cell immunity. Postnatal allogeneic thymus transplantation for cDGS has demonstrated promising outcomes, with an overall survival rate of 75%; however, immune reconstitution following transplantation typically requires approximately 5 months.[4] Given the limited availability of this procedure in Korea, alternative treatment options must be explored to effectively manage this rare case.

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Historically, HSCT or PBL infusion have been attempted; however, they exhibit a lower overall survival rate of 41%.[2] Conversely, matched sibling donor transplantation yields higher overall survival rates ranging from 62–75%, with reports of persistent and functioning donor T lymphocytes observed over several years of follow-up. GvHD remains a major concern following HSCT/PBL.[5,6,12-14] To mitigate GvHD, CD45RA+ depleted lymphocyte infusion has been introduced, which selectively removes naïve T cells (CD45RA+) while preserving virus-specific memory T cells, particularly in haploidentical settings.[8,11] In contrast to thymus transplantation, rapid immune reconstitution within a few days to weeks can be expected in HSCT/PBL, which makes possible its use for salvage treatment in severe CMV disease that does not respond to adequate antiviral therapy for cDGS and other T cell immunodeficiencies.[7,10]

Thus, in the circumstance where the patient had no siblings and no suitable matched unrelated donor, CD45RA+ depleted CMV-specific lymphocytes infusion was tried. While this method decreased the CMV VL, T cell increase was not demonstrated post 10 days. While direct comparison with HSCT may be challenging, a previous study on post-HSCT outcome in DGS demonstrated no increase in CD4+ T cells at 1 or 2 months post HSCT.[5] Mortality triggered by bacterial sepsis after the infusion may be attributed to low CD19+ B cells and Ig levels during sepsis. Moreover, impaired T cell function may have led to further humoral immunity impairment.

Optimal treatment options for refractory CMV disease in cDGS patients remain obscure. Reports highlighting the outcomes of thymus transplantation emphasize that severe viral infection (especially preexisting CMV infection) represents one of the highest risk factors associated with failure of thymus transplantation.[2,15] In a report on cord blood transplantation (CBT) for CMV infection in DGS, there was improvement after CBT but later CMV infection relapsed with mortality.[16] Successful resolution of refractory adenovirus infection and immune reconstitution in DGS has been reported by bone marrow transplantation from a completely HLA-matched sibling donor.[10]

Additionally, our case supports the necessity of introducing neonatal T-cell receptor excision circles (TREC) screening in Korea for early diagnosis of cDGS as well as SCID.[17,18]

In this case report, we present our experience with CD45RA+ depleted lymphocyte infusion in an infant with cDGS and refractory CMV disease. Although we were able to demonstrate a decline in the CMV VL, this treatment approach was not successful in restoring T cell immunity. Further investigations are required to explore optimal management strategies to address exhaustion of infused lymphocytes and to achieve successful engraftment in the cDGS.

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요약

완전 디죠지 증후군(complete DiGeroge syndrome)은 디죠지 증후군 중 심한 T세포면역결핍의 양상을 보이는 경우이다. 본 증례보고에서는 치료 불응성 거대세포바이러스 감염 상태에 있는 완전 디죠지 증후군 영아에게 T 세포기능 회복을 위해 CD45RA+ 선별제거 림프구 주입을 시행한 경험을 공유하고자 한다. 증례는 형광제자리부합법으로 22q11.2 결실이 확인된 만삭출생 여아로, T 세포면역결핍(T 세포 수 CD3+ 69/μL, CD4+ 7/μL)이 동반되어 완전 디죠지 증후군으로 진단되었다. 생후 45일에 거대세포바이러스 감염증(혈중 바이러스역가 120,000 IU/mL)이 처음 진단되었고, 간시클로버 치료 56일째까지는 혈중 바이러스역가가 감소하였으나 이후 다시 증가하였다. 간시클로버 저항성을 부여하는 것으로 알려진 거대세포바이러스 UL97 단백 인산화효소의 M460V 변이가 확인되어 포스카넷과의 병합요법으로 치료하였으나, 혈중 바이러스역가 2,820,000 IU/mL 로 상승하며, 거대세포바이러스 망막염이 동반되었다. T 세포기능 회복과 불응성 거대세포바이러스 감염의 치료를 위해, 생후 196일과 207일에 환아의 아버지에게서 채집한 CD45RA+ 선별제거 거대세포바이러스 특이 림프구를 2회 주입한 결과, T세포 증가를 이루지 못하였으나, 거대세포바이러스 역가의 감소는 관찰되었다(2차 주입 후 10일째 바이러스역가, 87,000 IU/mL). 환아는 폐포자충 폐렴과 *Elizabethkingia meningoseptica* 패혈증으로 생후 222일에 사망하였다.