Letter to the Editor

Successful T-cell reconstitution after unrelated cord blood transplantation in a patient with complete DiGeorge syndrome

To the Editor:

DiGeorge syndrome (DGS) is a congenital disorder characterized by thymic aplasia, conotruncal cardiac defect, craniofacial abnormalities, and hypoparathyroidism. More than 55% of patients with clinical features compatible with DGS have a heterozygous 22q11.2 deletion[1]; among these, less than 1% develop severe T lymphopenia ($CD3^+$ T cell < 50/mm^3). This so-called complete DGS resembles a severe combined immunodeficiency. To achieve immunological reconstitution, these patients require allogeneic transplantation of the thymus tissue or hematopoietic stem cells. Although thymus transplantation could be considered the best choice of treatment, it is not available in most countries including Japan. Here, we report a female with complete DGS who received unrelated cord blood transplantation (CBT) and achieved T-cell reconstitution.

The patient was born at 37 weeks of gestational age with 2622 g birth weight. Her family history was unremarkable and there was no evidence of consanguinity. The patient was admitted to the neonatal intensive care unit because of poor breast-feeding and heart murmur at age 2 days. She presented with hypocalcemia (6.8 mg/dL), a low level of parathyroid hormone (8.6 pg/mL), a small ventricular septal defect, and the absence of thymus. Fluorescent *in situ* hybridization analysis confirmed the presence of a hemizygous deletion in the 22q11.2 region. Lymphocyte subset analysis revealed a marked decrease in $CD3^+$ T lymphocytes (1%, 26/mm^3) and normal $CD19^+$ B lymphocytes (78%, 2028/mm^3). T-cell proliferative assays for PHA and concanavalin A markedly decreased and the stimulation index was 0.9 and 0.5, respectively. Targeted next-generation sequencing analysis covering 349 primary immunodeficiency-related genes did not identify diagnostic mutations.[2] She was diagnosed with complete DGS.

CBT from a sex- and ABO-matched unrelated donor was performed. HLA disparity was examined (patient: A 24:02/33:03, B 15:11/59:01, Cw 01:02/03:03, DRB1 08:02/09:01, DRB3/4/5 4*01:03/−, DQA1 03:02/04:01, DQB1 03:03/04:02, DPA1 02:02/−, and DPB1 02:01/05;01; donor: A 24:02/−, B 46:01/59:01, Cw 01:02/−, DRB1 08:03/09:01, DRB3/4/5 4*01:03/−, DQA1 01:03/03:02, DQB1 03:03/06:01, DPA1 01:03/02:02, and DPB1 02:01/05;01). The conditioning regimen consisted of 30 mg/m^2/d of fludarabine on days −7 to −4 and 70 mg/m^2/d of melphalan on days −3 to −2. Graft-versus-host disease prophylaxis consisted of the continuous infusion of tacrolimus (0.02 mg/kg/d) from day −1 and short-term methotrexate, 15 mg/m^2/d on day +1 and 10 mg/m^2/d on day +3, +6, and +11. Granulocyte colony-stimulating factor was administered from day +5. A total of 1.9×10^7/kg of mononuclear cells (4.0×10^5/kg of $CD34^+$ cells) were infused. Neutrophil and $CD3^+$ T-cell engraftment was achieved on day +14. Neither acute nor chronic graft-versus-host disease was observed, and no serious complications occurred.

To assess the generation of T lymphocytes posttransplantation, we consecutively measured T-cell receptor excision circles (TRECs)[3] (see Table E1 in this article's Online Repository at www.jacionline.org). TRECs were not detectable in the peripheral blood before CBT; however, they were detected (1.6×10^3/μg DNA) on day +24 after CBT along with the elevation in $CD3^+$ T cells (1628/mm^3; Fig 1). At day +123, TRECs were detectable (4.6×10^3/μg DNA) with a sustained number of $CD3^+$ T cells (1296/mm^3). TRECs were detected from αβ T cells (9.9×10^2/μg DNA) but not from γδ T cells (<detection limit). However, TRECs became undetectable at days +239, +553, and +750. Indeed, short tandem repeat-based chimerism analysis confirmed the donor origin of detected $CD3^+$ T cells (93% at day +37, 92% at day +126, 94% at day +234, and 100% at day +886). Donor myeloid chimerism was also confirmed (80% in $CD11b^+$ cells at day +886).

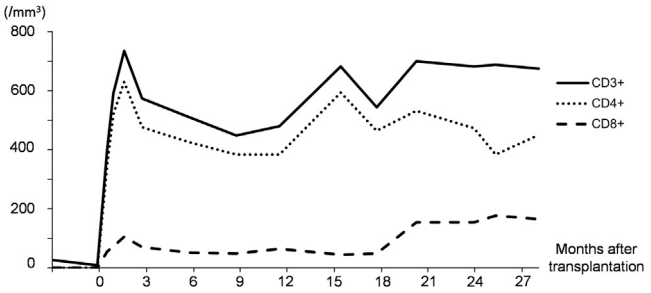

FIG 1. T-cell counts. Chronological change in the number of T cells after CBT.

We detected the naive/memory phenotype and T-cell receptor (TCR) diversity of patient's T cells at day +123. Both $CD4^+$ and $CD8^+$ fractions of the patient contained 18% and 21% of T cells with a naive phenotype ($CD45RA^+CCR7^+$), respectively (Fig 2, *A*). Repertoire analysis of TCR β chain showed a diverse pattern in each cell subsets. The naive phenotype T cells and TCR diversity were retained at day +750 (Fig 2, *B*). Both $CD31^+$ and $CD31^-$ naive T cells were present (Fig 2, *C*). Presence of class-switched memory B cells was confirmed at day +921 (Fig 2, *D*). T-cell proliferative stimulation index with PHA and concanavalin A stimulation at day +886 was 204 (normal range, 101-2643) and 108 (normal range, 74-1793), respectively. Furthermore, we observed several specific immune responses in response to various vaccinations (see Table E2 in this article's Online Repository at www.jacionline.org). These observations support that naive phenotype T cells with diverse TCR repertoire were sustained after allogeneic CBT. At age 31 months, she remains alive without any serious infections and does not require immunoglobulin administration.

Theoretically, thymic transplantation is a choice of treatment for patients with complete DGS. In fact, the survival after thymic transplantation is excellent, and patients develop host-derived naive T cells with normal TCRs and mitogen responses.[4] However, this approach is possible at only 2 institutions worldwide. In contrast, hematopoietic stem cell transplantation is performed worldwide. Bone marrow transplantation (BMT) has also been successful in restoring immune competence; however, the main limitation is the availability of HLA-matched donors. Therefore, CBT was performed on our patient as a salvage therapy. Three cases of CBT in DGS and CHARGE

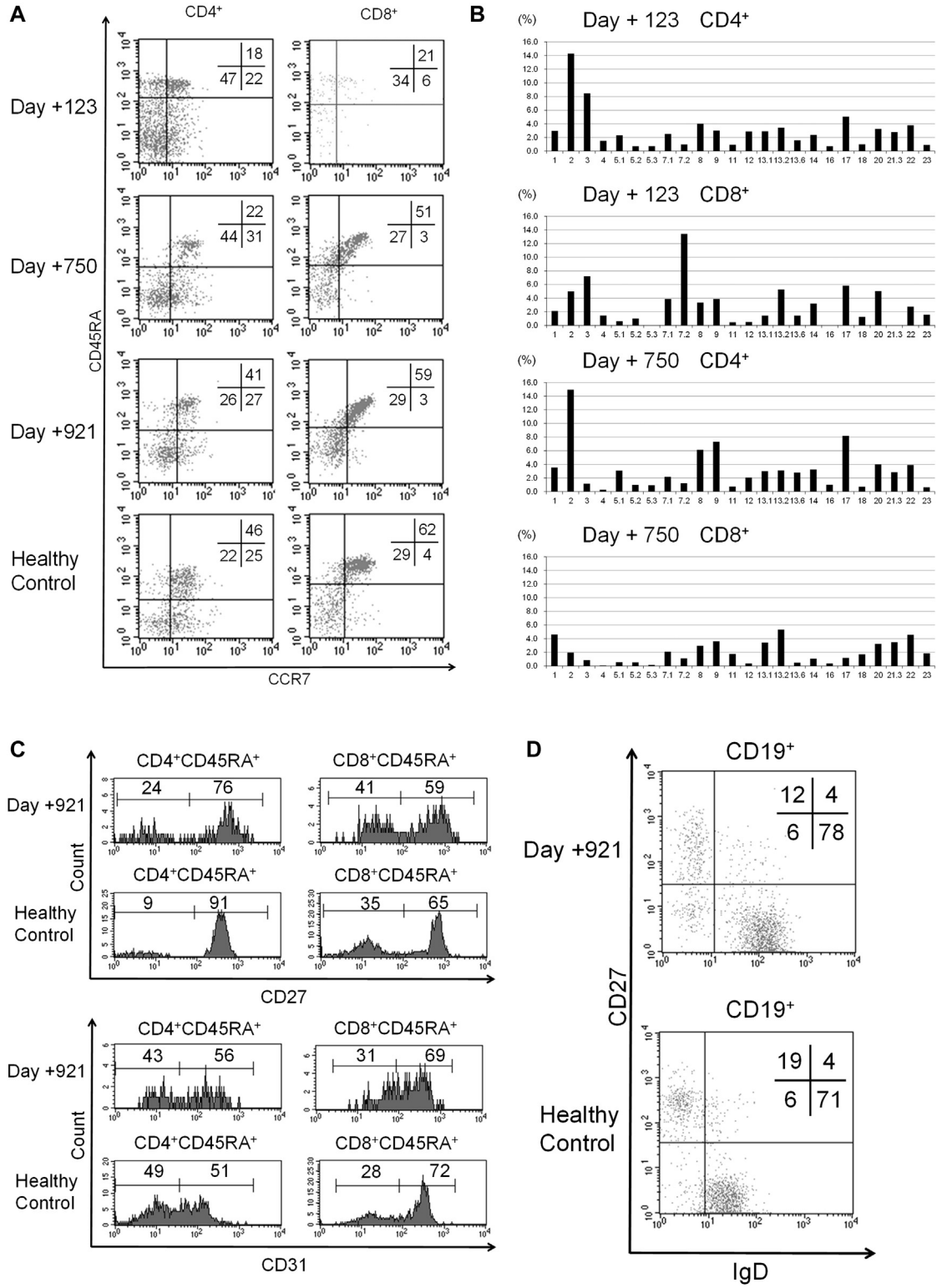

FIG 2. Flow cytometric analysis. **A,** Naive/memory phenotype of $CD4^+$ and $CD8^+$ T-cell subsets at day +123, day +750, and day +921 posttransplantation. Numbers indicate the percentage of cells in each quadrant. **B,** TCR Vβ repertoire of $CD4^+$ and $CD8^+$ T-cell subsets. **C,** CD27 and CD31 expression in T cells. **D,** Status of CD27 and IgD expression in $CD19^+$ B cells.

syndrome showing DiGeorge sequence have been reported so far (see Table E3 in this article's Online Repository at www.jacionline.org). All cases showed T-cell engraftment, but all cases died because of infection or heart failure.[5-7] The difference in clinical outcome might be explained by complications related to the molecular pathogenesis that causes DGS; at least

1 case died of heart failure related to a *CHD7* mutation. Complications other than immunodeficiency were mild in our case, which assisted successful CBT. In addition, none of the previous reported cases received conditioning regimen, whereas our case received reduced-intensity conditioning to reinforce engraftment of donor cells.

A previous report demonstrated that in patients with DGS who underwent BMT, reconstitution had predominantly occurred through the expansion of the donors' mature T-cell pool. Circulating T cells exhibited a memory phenotype with a restricted repertoire and were devoid of TRECs.[8] In contrast, we observed naive phenotype T cells and a fairly diverse T-cell receptor repertoire even 2 years after CBT. Because cord blood contains more naive T cells than the bone marrow and the T-cell repertoire in cord blood is diverse, CBT might be superior than BMT for the reconstitution of naive T cells compared.[9]

Recent studies showed that newborn screening for severe combined immunodeficiency can efficiently detect infants with complete DGS before they suffer from opportunistic infections.[10,11]

We suggest that CBT with reduced-intensity conditioning is a therapeutic option for complete DGS, which can particularly be useful for uninfected patients who are identified through newborn screening. Further reports should be accumulated to confirm the effectiveness of our procedure as a curative treatment for patients with complete DGS.

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REFERENCES

1. Rope AF, Cragun DL, Saal HM, Hopkin RJ. DiGeorge anomaly in the absence of chromosome 22q11.2 deletion. J Pediatr 2009;155:560-5.
2. Kojima D, Wang X, Muramatsu H, Okuno Y, Nishio N, Hama A, et al. Application of extensively targeted next-generation sequencing for the diagnosis of primary immunodeficiencies. J Allergy Clin Immunol 2016 [Epub ahead of print].
3. Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal guthrie cards. J Pediatr 2009;155:823-33.
4. Markert ML, Devlin BH, Alexieff MJ, Li J, McCarthy EA, Gupton SE, et al. Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. Blood 2007;109:4539-47.
5. Ohtsuka Y, Shimizu T, Nishizawa K, Ohtaki R, Someya T, Noguchi A, et al. Successful engraftment and decrease of cytomegalovirus load after cord blood stem cell transplantation in a patient with DiGeorge syndrome. Eur J Pediatr 2004;163:747-8.
6. Gennery AR, Slatter MA, Rice J, Hoefsloot LH, Barge D, McLean-Tooke A, et al. Mutations in CHD7 in patients with CHARGE syndrome cause T-B + natural killer cell + severe combined immune deficiency and may cause Omenn-like syndrome. Clin Exp Immunol 2008;153:75-80.
7. Inoue H, Takada H, Kusuda T, Goto T, Ochiai M, Kinjo T, et al. Successful cord blood transplantation for a CHARGE syndrome with CHD7 mutation showing DiGeorge sequence including hypoparathyroidism. Eur J Pediatr 2010;169:839-44.
8. Land MH, Garcia-Lloret MI, Borzy MS, Rao PN, Aziz N, McGhee SA, et al. Long-term results of bone marrow transplantation in complete DiGeorge syndrome. J Allergy Clin Immunol 2007;120:908-15.
9. Neller MA, Ladell K, McLaren JE, Matthews KK, Gostick E, Pentier JM, et al. Naive CD8(+) T-cell precursors display structured TCR repertoires and composite antigen-driven selection dynamics. Immunol Cell Biol 2015;93:625-33.
10. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol 2012;129:622-7.
11. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA 2014;312:729-38.

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REFERENCES

E1. Ohtsuka Y, Shimizu T, Nishizawa K, Ohtaki R, Someya T, Noguchi A, et al. Successful engraftment and decrease of cytomegalovirus load after cord blood stem cell transplantation in a patient with DiGeorge syndrome. Eur J Pediatr 2004;163:747-8.

E2. Gennery AR, Slatter MA, Rice J, Hoefsloot LH, Barge D, McLean-Tooke A, et al. Mutations in CHD7 in patients with CHARGE syndrome cause T-B + natural killer cell + severe combined immune deficiency and may cause Omenn-like syndrome. Clin Exp Immunol 2008;153:75-80.

E3. Inoue H, Takada H, Kusuda T, Goto T, Ochiai M, Kinjo T, et al. Successful cord blood transplantation for a CHARGE syndrome with CHD7 mutation showing DiGeorge sequence including hypoparathyroidism. Eur J Pediatr 2010;169:839-44.

TABLE E1. Quantitative analysis of TRECs/sjKRECs /cjKRECs

	Pre-CBT PBMCs	Day +24 PBMCs	Day +123			Day +239 PBMCs	Day +553 PBMCs	Day +750 PBMCs	Age-matched healthy control
			PBMCs	αβ T cells	γδ T cells				
TRECs	ND	1.6×10^3	4.6×10^3	9.9×10^2	ND	ND	ND	ND	9.9×10^2 - 4.4×10^4
sjKRECs	2.0×10^4	ND	3.7×10^4	—	—	—	—	—	—
cjKRECs	7.0×10^4	ND	9.2×10^4	—	—	—	—	—	—

Values represent copies/μg DNA.

cjKREC, Coding joint kappa-deleting recombination excision circle; *sjKREC*, signal joint kappa-deleting recombination excision circle; *ND*, not detected.

TABLE E2. Specific antibody responses to vaccinations

Vaccine	Patient	Protective level
Diphtheria	0.31 IU/mL	≥0.01
Tetanus	0.34 IU/mL	≥0.5
Polio (NT)	1:8 dilution	≤1:4
Pertussis (EIA)		
PT-IgG	23 IU/mL	≥10
FHA-IgG	45 IU/mL	≥10

EIA, Enzyme immunoassay; *FHA*, filamentous hemagglutinin; *NT*, neutralization test; *PT*, pertussis toxin.

TABLE E3. List of patients with complete DGS who received allogeneic CBT

Case	Sex	Molecular defect	Age at transplantation (mo)	Conditioning	GvHD prophylaxis	Acute GvHD	Chronic GvHD	Pretransplant CD3+ cell (/mm^3)	Autologous expansion of T cells	Posttransplant CD3+ cell (/mm^3)	Length of follow-up from the CBT (mo)	Outcome	Cause of death	Reference
1	M	Unclear	3	None	CyA	None	None	5	−	1690	1	Dead	CMV infection	E1
2	M	*CHD7*	4	None	CyA + steroid	Skin, stage 2	None	142	+	504	6	Dead	Parainfluenza viral pneumonitis	E2
3	M	*CHD7*	4	None	TAC + MTX	Skin	None	8	−	973		Dead*	Heart failure*	E3
4	F	del22q11.2	5	FLU + MEL	TAC + MTX	None	None	26	−	1628	27	Alive	−	Our case

CMV, Cytomegalovirus; *CyA*, cyclosporin A; *F*, female; *FLU*, fludarabine; *GvHD*, graft-versus-host disease; *M*, male; *MEL*, melphalan; *MTX*, methotrexate; *TAC*, tacrolimus.
*Personal communication, Inoue H, et al, 2010.

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