Successful unrelated cord blood transplantation for a patient with CD40 ligand deficiency

We report a CD40 ligand deficiency (CD40LD) patient who was successfully treated with unrelated cord blood transplantation (URCBT). Conditioning regimen was busulfan and cyclophosphamide. The clinical course was uneventful and durable engraftment was achieved. This successful case encourages the use of URCB as an alternative donor source for CD40LD patients.

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CD40 ligand deficiency (CD40LD) is an X-linked primary immunodeficiency disease due to a mutation of *CD40LG* coding CD40L.[1] The clinical spectrum of this disorder includes recurrent bacterial sinopulmonary infection and opportunistic infections such as pneumocystis jiroveci pneumonia (PCP).[2-4] Serum levels of IgG and IgA are low or absent, whereas IgM is normal or elevated. European studies showed that the long-term survival rate of CD40LD patients is low[4] and that hematopoietic stem cell transplantation (HSCT) is the only curative therapy.[5] Although ours is not the only study to report successful cases of bone marrow transplantation (BMT) for CD40LD,[5-7] unrelated cord blood transplantation (URCBT) has been reported in only one patient. In this case, conditioning consisted of busulfan (BU), cyclophosphamide (CY), etoposide (VP-16) and anti-thymocyte globulin (ATG).[8]

We report a successful URCBT for a CD40LD patient with the conditioning of BU+CY and investigated the immunologic reconstitution during the period of URCBT.

The patient was the first born child of non-consanguineous Japanese parents with no family history of immunodeficiency. At the age of one he developed PCP and laboratory examination revealed neutropenia (130/μL) and hypogammaglobulinemia (IgG 34, IgA 20 mg/dL) with a high level of serum IgM (505 mg/dL). CD40L was not detected on his activated T lymphocytes (Figure 1) and the genetic analysis of *CD40LG* revealed a large deletion including whole exons (*data not shown*). He was, therefore, diagnosed as CD40LD. He made a full recovery, and cotrimoxazole and intravenous immunoglobulin (IVIG) were continued.

Since a HLA genotype-matched URBM donor was not available, URCBT was undertaken when he was 3 years old with the written informed consent of his parents. At this time, he was in good clinical condition and had no active infection. The infused cord blood unit matched 6/6 serologically and 5/6 genotypically in HLA-A, B, DRB1 loci (recipient: A 2402, B 5101/4002, DRB1 1201/1501, donor: A 2402, B 5101/4002, DRB1 1202/1501), nucleated cell count was 8.1×10^7 cells/kg body weight and CD34 positive cell count was 2.0×10^5 cells/kg. Both donor and recipient were CMV seronegative and their blood types were B (+). Before transplant, the patient was given 1 mg/kg BU for the pharmacokinetic (PK) study, and BU average steady-state plasma concentration (BU-Css) at this dose was 390 ng/mL. Accordingly, BU was orally administered 8 mg/kg/day (2 mg/kg/dose × 4/day) for 4 days (day –9 to –6) and estimated Bu-Css by this dose was approximately 780 ng/mL. CY was administered intravenously 50 mg/kg/day for 4 days (day –5 to –2). Prophylaxis against GVHD consisted of cyclosporine A (CsA) from day –1 and 1 mg/kg/day of prednisolone (PSL) from day +1. CsA was administered intravenously in two divided doses and dosage was adjusted to maintain a trough level of 200 ng/mL. CsA was gradually tapered from day 80 and was stopped on day 350. Supportive therapies were cotrimoxazole, oral amphotericin B, IVIG, intravenous acyclovir and G-CSF.

Neutrophil engraftment (absolute neutrophil count $>0.5\times10^9$/L) was achieved on day 19 and complete donor chimerism in peripheral blood was confirmed by short tandem repeat method on day 16 and on day 65. The last platelet transfusion was on day 38 post-transplant. Grade 1 acute GVHD limited to the skin occurred on day 20 and resolved without further therapy. No life-threatening infection or other transplant-related organ damage, including mucositis and hepatotoxicity, occurred. CD40L expression on activated T lymphocytes was normalized after CBT (Figure 1). T cell receptor excision circles (TRECs), which are known to be a surrogate marker for T cell neogenesis, gradually increased after transplantation and reached to the normal range after 350 days post-transplant (Figure 2A). Class switched memory B cell ($IgD^-IgM^-CD27^+CD19^+$) counts were almost non-existent before CBT, but increased after CBT (Figure 2B). The last

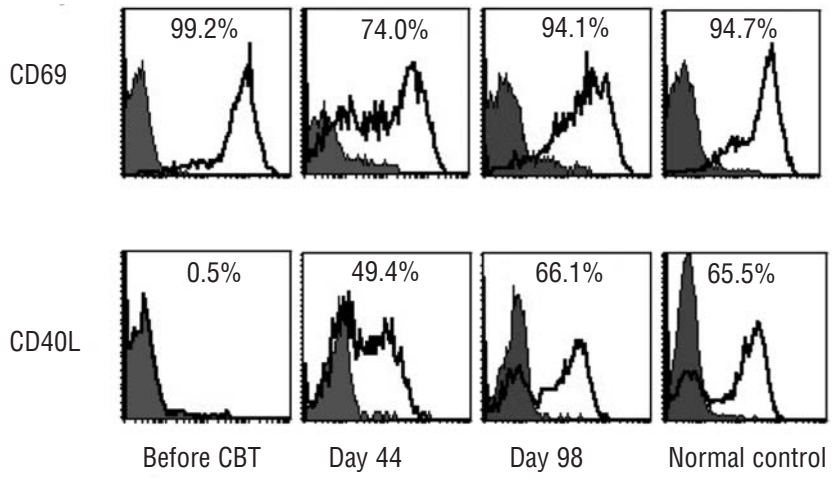

Figure 1. CD40L on activated T cells was expressed after URCBT. Patient fluorescence histograms are shown before transplantation, 44 days and 98 days after URCBT and for normal control. All studies were gated on $CD3^+$ cells either unstimulated (filled) or stimulated (not filled) with PMA and ionomycin. Percentages shown in each histogram represent the percentage of CD69 (upper panel) or CD40L (lower panel) positive cells.

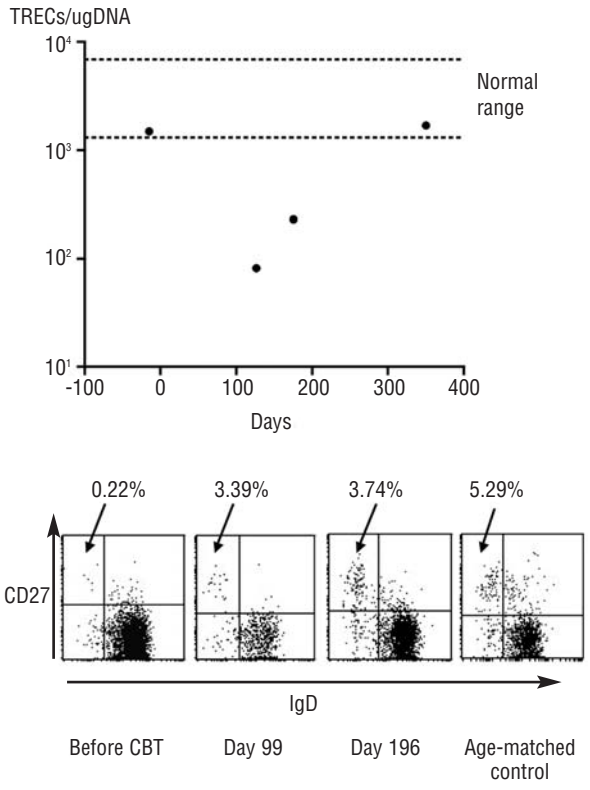

Figure 2. TRECs were recovered (A) and class-switched memory B cells were detected (B) after URCBT. A. TRECs/μg DNA in whole blood is shown. Quantitative real-time PCR for δRec-ψJα signal joint TRECs was performed as described by others (9) using the ABI PRISM 7300 Sequence Detection Systems (Applied Biosystems). Normal range was determined by age-matched control subjects (*data not shown*). B. B cells were analyzed using anti-CD19, anti-CD27, and anti-IgD mAb (Beckton Dickinson) and analyzed by flowcytometer (FACS Calibur, Beckton Dickinson). Percentages shown in each histogram represent the percentage of class-switched memory B cells (IgD-CD27⁺CD19⁺) in CD19⁺ B cells.

IVIG was administered on day 93 post-transplant. Now, 24 months after URCBT, the patient has a good clinical condition and requires no medication. Although the conditioning of the previously reported URCBT was BU, CY, VP-16 and ATG,[8] our patient underwent URCBT with BU+CY without VP-16 to avoid secondary malignancies and without ATG to avoid overimmunosuppression. The clinical course of our patient was uneventful and full donor chimerism was achieved. In T cell lineage, CD40L expression and TRECs became normal. B cell reconstitution was confirmed by the appearance of class switched memory B cells and independence from IVIG. The infused cell dose was higher than the cell dose usually required, which may have been an advantage for full engraftment. This successful case encourages the use of URCB as an alternative donor source for CD40LD patients.

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