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Phenotypic and Genotypic Correction of WASP Gene Mutation in Wiskott-Aldrich Syndrome by Unrelated Cord Blood Stem Cell Transplantation

We present two cases of Wiskott-Aldrich syndrome (WAS), in which nonsense mutations in the *WASP* gene were corrected phenotypically as well as genotypically by unrelated cord blood stem cell transplantation (CBSCT). Two male patients were diagnosed with WAS at the age of 5-month and 3-month and each received unrelated CBSCT at 16-month and 20-month of age, respectively. The infused cord blood (CB) units had 4/6 and 5/6 HLA matches and the infusion doses of total nucleated cells (TNC) and CD34+ cells were 6.24×10^7/kg and 5.08×10^7/kg for TNC and 1.33×10^5/kg and 4.8×10^5/kg for CD34+ cells, for UPN1 and UPN2, respectively. Complete donor cell chimerism was documented by variable number tandem repeat (VNTR) with neutrophil engraftment on days 31 and 13 and platelets on days 58 and 50, respectively. Immunologic reconstitution demonstrated that CBSCT resulted in consistent and stable T-, B-, and NK-cell development. Flow cytometric analysis for immunologic markers and sequence analysis of the *WASP* gene mutation revealed a normal pattern after CBSCT. These cases demonstrate that CBs can be an important source of stem cells for the phenotypical and genotypical correction of genetic diseases such as WAS.

Key Words : *Wiskott-Aldrich Syndrome; WASP; Unrelated Cord Blood Stem Cell Transplantation*

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INTRODUCTION

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency characterized by a clinical triad of immunodeficiency, thrombocytopenia, and eczema (1, 2). WAS is caused by mutations in the gene encoding Wiskott-Aldrich syndrome protein (WASP) that affect all hematopoietic stem cells including lymphocytes, monocytes, neutrophils and platelets (3). Various WASP gene mutations have been reported in patients with WAS and X-linked thrombocytopenia, a clinically mild allelic variant (4-9).

The treatment of WAS depends on the severity of the immunodeficiency. In severe cases of WAS, the immunodeficiency limits the life expectancy of the patient, and immune reconstitution is the treatment of choice. Imai et al. (10) demonstrated correlations between the clinical phenotype, the extent of the mutation, and the presence or absence of WASP and also recommended hematopoietic stem cell transplantation (HSCT) especially for the patients with *WASP*-negative WAS. Recently, there have been reports of successful treatment of WAS by unrelated HSCT with cord blood (CB) as well as bone marrow transplant when a matched sibling donor was unavailable (11-16). Although phenotypic corrections for immunologic and hematologic parameters have been reported, genotypic corrections for WAS with cord blood stem cell transplantation (CBSCT) have not been demonstrated.

In this report, we demonstrate 2 cases of WAS which were phenotypically and genotypically corrected with unrelated CBSCT.

CASE REPORT

Patient 1 (UPN 1) is a male who was diagnosed with WAS at the age of 5 months. He presented with incidentally detected thrombocytopenia (23,000/μL) with skin eczema and severe, recurrent otitis media and diarrhea on admission. The second male patient (UPN 2) presented with neonatal thrombocytopenia at birth and received intermittent intravenous immunoglobulin (IVIG). Thereafter he experienced skin ec-

zema and recurrent infections such as cellulitis and pneumonia, until he visited our hospital at 3 months old. Flow cytometric analysis of peripheral blood mononuclear cells (PBMC) for these 2 patients revealed a defect in *WASP*, leading to the diagnosis of WAS. Subsequently, the nonsense mutations, Arg211stop and Arg13stop, were confirmed by genomic analysis (17). Before transplantation, these patients were treated with monthly infusions of IVIG as well as supportive treatment but there was no clinical improvement.

CBSCT was performed in a laminar air flow room with conventional supportive therapy. The pre-transplantation conditioning regimen for the 2 patients was 1 mg/kg of busulfan intravenously every 6 hr on days -9 through -6. This was followed by 50 mg/kg of intravenous cyclophosphamide on days -5 through -3 and 30 mg/kg of intravenous antithymocyte globulin (ATG) on days -3 through -1. Prophylaxis for acute graft versus host disease (GVHD) included continuous infusion of cyclosporine A beginning on day -1, targeting whole blood levels to be 200 to 400 ng/mL, and 1 mg/kg/dose of methylprednisone every 12 hr on days 5 through 19, and then a taper.

The degree of human leukocyte antigen (HLA) match confirmed by high resolution DNA typing between the infused CB and the patients was 4/6 for UPN 1 and 5/6 for UPN 2. Infused cell doses of TNC and CD34+ cells for UPN1 and UPN2 were 6.24×10^7/kg and 5.08×10^7/kg for TNC, respectively, and 1.33×10^5/kg and 4.8×10^5/kg for CD34+ cells, respectively.

T-, NK-, and B-cell enumeration and quantitative immunoglobulin studies (for immunoglobulin [Ig] G, A, M, D, and E) were performed. Cytofluorographic analyses of lymphocyte subpopulations were performed with murine monoclonal antibodies conjugated to either fluorescein (FITC) or phycoerythrin (PE) and then analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, CA, U.S.A.).

Heparinized venous blood samples from patients and family members were fractionated on a Ficoll-Hypaque gradient to isolate PBMCs. For mutational analysis, genomic DNA was extracted from the peripheral lymphocytes, and 12 *WASP* gene exons were amplified by polymerase chain reaction (PCR) followed by direct sequencing according to the protocol of Sasahara et al. (18).

Hematopoietic reconstitution following CBSCT was uneventful, with an absolute neutrophil count (ANC) of more than 500/μL on days 31 and 13 and a platelet count of more than 20,000/μL on days 58 and 50, for UPN 1 and UPN 2, respectively. Molecular chimerism studies using the VNTR method showed a complete donor cell type for these patients (data not shown). Acute GVHD did not occur, even after the infusion of HLA 1 or 2 antigen mismatched CB. UPN 1 experienced 1 episode of sepsis with *B. cepacia* during the pre-engraftment period without any complications. UPN 2 expe-

Table 1. Cord blood stem cell transplantation data in children with WAS

	UPN 1	UPN 2
Age at transplantation (month)	16	20
Degree of HLA mismatch	2	1
Preparative regimen	Bu, Cy, ATG	Bu, Cy, ATG
Infused cell dose		
TNC (10^7/kg)	6.24	5.08
CD34+ cell (10^5/kg)	1.33	4.8
Time to engraftment (day)		
ANC (>500/μL)	31	13
Platelet (>20,000/μL)	58	50
Follow-up duration (month)	60	55

WAS, Wiskott-Aldrich Syndrome; HLA, human leukocyte antigen; TNC, total nucleated cells; ANC, absolute neutrophil count.

Table 2. Immunological laboratory data of pre-/post-transplantation in children with WAS

	UPN 1					UPN 2				
	Pre-transplant	Post-transplant				Pre-transplant	Post-transplant			
		7 mo	16 mo	48 mo	60 mo		7 mo	12 mo	24 mo	55 mo
Total eosinophil (μ/L)	800	200	288	360	160	1,600	300	90	110	100
IgE (IU/mL)	786	44.4	25.7	134.9	66.1	1,713	216	84.77	84.77	41.9
IgG (mg/dL)	1,912	NA	589	1,010	1,030	NA	NA	NA	NA	1,087
IgA (mg/dL)	72.3	NA	29.2	104	105	NA	NA	NA	NA	172
IgM (mg/dL)	30.4	NA	270	254	246	NA	NA	NA	NA	198
IgD (mg/dL)	0.397	NA		70	81	NA	NA	NA	NA	58.3
Lymphocyte subset										
T3 (CD3) (%)	47	NA	60.6	58	64	36	NA	43.3	62.6	70.1
T4 (CD4) (%)	30	NA	35.8	29	29	31	NA	29.3	33	43.9
T8 (CD8) (%)	7	NA	26	28	32	5	NA	13.1	22.3	23.9
T4/T8 ratio	5.7:1	NA	1.38:1	1.04:1	0.91:1	6.2:1	NA	2.2:1	1.5:1	1.8:1
B (CD19) (%)	32	NA	33.9	19	26	32	NA	49.7	25	23.2
NK (CD16/56) (%)	18	NA	6.19	19	9	31	NA	13.8	12.1	5.9

WAS, Wiskott-Aldrich Syndrome; NA, not available; mo, months.

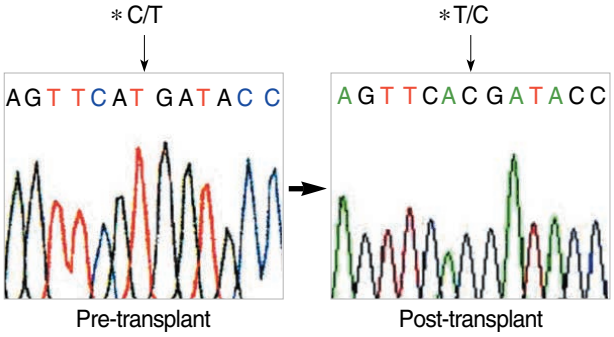

Fig. 1. Sequence analysis showing genetic correction of the *WASP* gene in UPN 1. DNA sequences are shown that encompass a single point mutation (C to T) or a correction in exon 7 of the *WASP* gene. The asterisk denotes a C to T transversion or the T to C correction.

rienced fever and respiratory distress with hypoxemia due to pulmonary edema as well as skin rashes on the trunk in the pre-engraftment period (from days 8 to days 10), which mimics engraftment syndrome. He recovered with oxygen supply via endotracheal tube and fluid restriction as well as methylprednisone therapy. Both patients were clinically well without eczematous skin and recurrent infections at 60 months (UPN 1) and 55 months (UPN 2) post CBSCT. Clinical data regarding CBSCT are shown in Table 1. The elevated total eosinophil counts and serum IgE levels have been normalized since 7 months post-transplantation in both children (Table 2). Other immunologic parameters including IgG, IgA, IgM, IgD and lymphocyte subsets are summarized at Table 2.

To determine whether the genotype was corrected by CBSCT, we analyzed the *WASP* gene sequence before and after CBSCT in UPN 1. UPN 1 had a single base substitution (C665T) in exon 7 that results in an amino acid change in codon 211 (Arg211stop) before CBSCT. Following CBSCT, UPN1 had a normal sequence at the mutation site in exon 7 of the *WASP* gene (Fig. 1).

DISCUSSION

The gene responsible for WAS (*WASP*) consists of 12 exons with 1,823 bp. *WASP* encodes a 502 amino acid protein that is expressed selectively in hematopoietic stem cell-derived lineages (4). To date, approximately 100 mutations in the *WASP* gene have been described. These mutations consist of frameshift mutations, missense mutations, or splice-site mutations, which all give rise to aberrant transcription (4-9). A correlation between clinical phenotype and genotype was reported independently by several investigators (7, 9). Imai et al. (10) observed that patients with missense mutations were *WASP*-positive, but patients with nonsense mutations, large deletions, small deletions, and small insertions were *WASP*-negative. Patients with splice anomalies were either *WASP*-positive or *WASP*-negative. Lack of *WASP* expression was associated with susceptibility to bacterial, viral, fungal, and *Pneumocystis jiroveci* infections and with severe eczema, intestinal hemorrhage, death from intracranial bleeding, and malignancies. They also revealed that the rates for overall survival and event-free survival were significantly lower in *WASP*-negative patients. Conclusively, they recommended HSCT to improve prognosis, especially for WASP-negative patients.

Recently, HSCT has been the principal modality for correction of immune deficiencies such as WAS. Since CB has been successfully transplanted to reconstitute patients with WAS, as first reported in 1994 (11-16), CB appears to be an alternative donor source compared with matched unrelated bone marrow with successful engraftment associated with no to mild acute GVHD and without development of chronic GVHD. In this study, we identified 2 WAS patients who had nonsense mutations in WASP and we successfully treated them by unrelated CBSCT with engraftment with no associated GVHD.

In CBSCT, the immunologic reconstitution resulted in consistent and stable T-cell, B-cell, and natural killer-cell development. The kinetics of recovery of phenotypic expression and function of the T cells occurred between 60 and 100 days and that of natural killer cells at approximately 180 days (19). The immunologic parameters, especially CD8 of our patients also demonstrated normal values after CBSCT.

Furthermore, following CBSCT, we found that UPN1 had a normal sequence at the mutation site in the exon 7 of *WASP* gene. While CBSCT has been successfully performed for phenotypic correction including clinical features and immunological parameters in WAS, our study emphasizes the evidence of genetic correction as well as phenotypic correction by CBSCT in WAS. To our knowledge, this is the first report documenting a genetic correction by CBSCT in WAS.

Collectively, our data demonstrate that CBs could be an important source of stem cells for the phenotypic as well as genotypic correction of genetic diseases such as WAS.

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