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Successful Allogeneic Peripheral Blood Stem Cell Transplantation in Wiskott-Aldrich Syndrome Patients: First Report in Thailand

Suradej Hongeng^{1,3}, Suwat Benjaponpitak¹, Pimpan Tardtong², Wandee Varavithya¹, Ampaiwan Chuansumrit¹, Amornsri Chunharas¹, Watana Chaisiripoomkere³ and Phongjan Hathirat^{1,3}

Wiskott-Aldrich syndrome (WAS), an X-linked recessive disorder, is characterized by primary progressive T cell immunodeficiency, impaired antipolysaccharide antibody production, eczema, and thrombocytopenia.^{1,2} Laboratory studies demonstrated variable but generally reduced response to T cell mitogens and allogeneic cells, markedly decreased serum concentration of IgM and isoagglutinins, normal concentration of IgG₂, and a small platelet volume associated with impaired platelet aggregation. The primary defect in the X-linked WAS gene maps to the proximal short arm of the X-chromosome (Xp 11.23).³ This gene was found to encode the WAS protein, a component of the cytoskeletal surface membrane complex of hematopoietic cells.⁴ Allogeneic bone marrow transplantation (BMT) can correct both lymphoid and platelet abnormalities associated with WAS.⁵⁻⁷ Here we additionally report the use of allogeneic peripheral blood stem cell transplantation (PBSCT) in this disease which we described previously.⁸

SUMMARY Wiskott-Aldrich syndrome (WAS), an X-linked recessive disorder, is characterized by primary progressive T cell immunodeficiency, impaired antipolysaccharide antibody production, eczema, and thrombocytopenia. Stem cell transplantation is the only curative therapy. To evaluate the use of allogeneic peripheral stem cell transplantation (PBSCT) in this group of patients, we performed allogeneic PBSCT in two WAS patients (3 and 12 years old). The conditioning regimen consisted of busulfan 4 mg/kg/day for 4 days, and cyclophosphamide 50 mg/kg/day for 4 days. Graft-versus-host disease prophylaxis was consistent with cyclosporin A and methotrexate. Peripheral blood stem cells were collected from their brother donors (6 and 16 years old) by continuous flow leukapheresis after mobilization with granulocyte-colony-stimulating factor at a dose of 7.5 µg/kg/day for 5 days. Both recipients achieved neutrophils engraftment on days 11 and 12. The first patient achieved platelets engraftment on day 30. The second patient did not have platelet count below 20.0 x 10⁹/l during PBSCT procedure. Both did not develop acute or chronic graft-versus-host disease. At present, they are healthy after PBSCT. The follow up time after transplantation is 1,170 days and 269 days, respectively. Allogeneic PBSCT is economically feasible for WAS. The cost of PBSCT in Thailand is 20 to 30% less than bone marrow and cord blood stem cell transplantation. The cost of the transplant procedure for each patient in Thailand is US \$ 12,000. This study is the first report of a successful stem cell transplantation in WAS patients in Thailand.

PATIENTS REPORT

Patient 1

The diagnosis of WAS was made in January 1998 in a 3-year-old boy presenting with several bouts of bloody diarrhea, pneumonia, otitis media, persistent eczematous rash on the face, trunk, and

extremities, and persistent thrombocytopenia since a newborn. Physical examination revealed generalized lymphadenopathy. His labora-

From the ¹Departments of Pediatrics, ²Pathology, and ³Bone Marrow Transplant Program, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
Correspondence: Suradej Hongeng

tory profiles are shown in Table 1. We tested the T cell function in this patient with a panel of antigens (Merieux; Pasteur Merieux, Lyon, France) consisting of proteus 8, trichophyton, candida, tetanus toxoid, diphtheria, streptococcus, tuberculin, and glycerin control. The results showed non-reactivity to all antigens. We evaluated CD4⁺ and CD8⁺ lymphocyte numbers and ratio in this patient, showing a CD4⁺ count of 3,900 cells/mm³ (47%), a CD8⁺ count of 680 cells/mm³ (8%), and a CD4⁺/CD8⁺ ratio of 5.9. This finding is consistent with WAS which usually has a low CD8⁺ count.

Patient 2

A 12-year-old boy was allogeneic HLA-identical sibling

diagnosed with WAS in June 2000. He had several bouts of otitis media, pneumonia, autoimmune hemolytic anemia, persistent thrombocytopenia, and persistent purpuric skin lesions since 6 years of age. These purpuric skin lesions were consistent with leukocytoclastic vasculitis. His laboratory profiles are shown in Table 2. We also tested the T cell function in this patient with a panel of antigens as mentioned above. The result showed non-reactivity to all antigens as well. T cell enumeration revealed a CD4⁺ count of 731 cells/mm³ (42%), a CD8⁺ count of 157 cells/mm³ (9%), and a CD4⁺/CD8⁺ ratio of 4.6.

PBSCT. Patient 1 received peripheral blood stem cells (PBSCs) from his 6-year-old brother who weighed 25 kg and patient 2 received PBSCs from his 16-year-old brother who weighed 60 kg. PBSCs from the donors were obtained by continuous flow leukapheresis with COBE Spectra (Cobe, Lakewood, CO, USA) after mobilization with granulocyte colony-stimulating factor (G-CSF) at a dose of 7.5 µg/kg/day subcutaneously for 5 days. The conditioning regimen consisted of busulfan 4mg/kg p.o. in divided doses daily for 4 days (day -9 to day -6) and cyclophosphamide 50 mg/kg once daily i.v. for 4 days (day -5 to day -2). Graft-versus-host disease prophylaxis was consistent with cyclosporin A at a dose

Table 1 Laboratory findings pre- and post-PBSCT in patient 1

| Laboratory data | Pre-PBSCT | | Day +44 post-PBSCT | Day +545 post-PBSCT | Day +1,000 post-PBSCT |
|----------------------------------|-----------|-------|-----------------------|------------------------|--------------------------|
| | Recipient | Donor | | | |
| Platelets (x 10 ⁹ /l) | 12.0 | 462 | 60.0 | 290.0 | 270.0 |
| Mean platelet volume (fl) | 4.5 | 6.3 | 7.0 | 7.1 | 6.0 |
| IgG (mg/ml) | 13.2 | 12.7 | 14.3 | 12.9 | 13.1 |
| IgA (mg/ml) | 1.9 | 1.47 | 1.29 | 1.12 | 1.22 |
| IgM (mg/ml) | 0.32 | 2.67 | 0.51 | 2.02 | 2.37 |

Table 2 Laboratory findings pre- and post-PBSCT in patient 2

| Laboratory data | Pre-PBSCT | | Day +40 post-PBSCT | Day +150 post-PBSCT | Day +217 post-PBSCT |
|----------------------------------|-----------|-------|-----------------------|------------------------|------------------------|
| | Recipient | Donor | | | |
| Platelets (x 10 ⁹ /l) | 30.0 | 357 | 73.0 | 374.0 | 282.0 |
| Mean platelet volume (fl) | 4.1 | 6.9 | - | 6.77 | 7.0 |
| IgG (mg/ml) | 13.5 | 14.0 | 18.0 | 15.4 | 18.7 |
| IgA (mg/ml) | 2.62 | 1.78 | 2.29 | 1.49 | 2.38 |
| IgM (mg/ml) | 0.72 | 2.69 | 0.92 | 1.5 | 1.58 |

of 3 mg/kg i.v. q 12 h until day +30 and then orally for 100 days, and methotrexate (15 mg/m² i.v. on day +1, 10 mg/m² on day +3, +6, and +11).

The number of infused mononuclear cells and CD34⁺ cells was 5.1 x 10⁸ cells/kg and 6.27 x 10⁶ cells/kg in patient 1 and 9.9 x 10⁸ cells/kg and 7.16 x 10⁶ cells/kg in patient 2, respectively. Both recipients received GM-CSF 250 µg/m² after PBSCs infusion on day +1 until neutrophil engraftment. Engrafted neutrophils were visible on days 11 and 12 in patients 1 and 2, respectively, and platelets on day 30 in patient 1. The platelet count of patient 2 did not drop below 20 x 10⁹/l during the PBSCT procedure. Serial hematologic findings and immunoglobulin levels were followed after the PBSCT procedure as shown in Tables 1 and 2. None

of the patients developed acute or chronic GVHD. At present, they are healthy after PBSCT. The follow up time is 1,170 days and 269 days in patients 1 and 2, respectively. We also repeated T cell function in both patients. The result showed reactivity to all antigens at 2 years for the first patient and at 8 months for the second patient after the transplant procedure. Chimerism studies show full donor engraftment in both patients as Figs. 1 and 2.

DISCUSSION

This current report is aimed to describe successful allogeneic PBSCT in WAS patients. It is important to emphasize that initially following PBSCT, we have demonstrated post transplantation immune reconstitution by the evidence of normal immunoglobulin levels and

normal T-cell function to specific antigens with delayed type skin testing. Because WAS is characterized by T cell function deficiencies, a more comprehensive immunologic evaluation should be performed in future work including serum antibody responses to specific antigens, lymphocyte surface marker analysis on various immunocompetent cells (T, B, and NK cells) involving their function, and a polarized cytokines (Th1 and Th2) study. This information would be more useful with regard to GVHD and other complications in PBSC transplanted WAS patients.

Results from large centers in the United States and Europe indicate that busulfan and cyclophosphamide, when used to prepare patients for HLA-matched marrow grafts, have led to full correction, long-term survival, and disease free

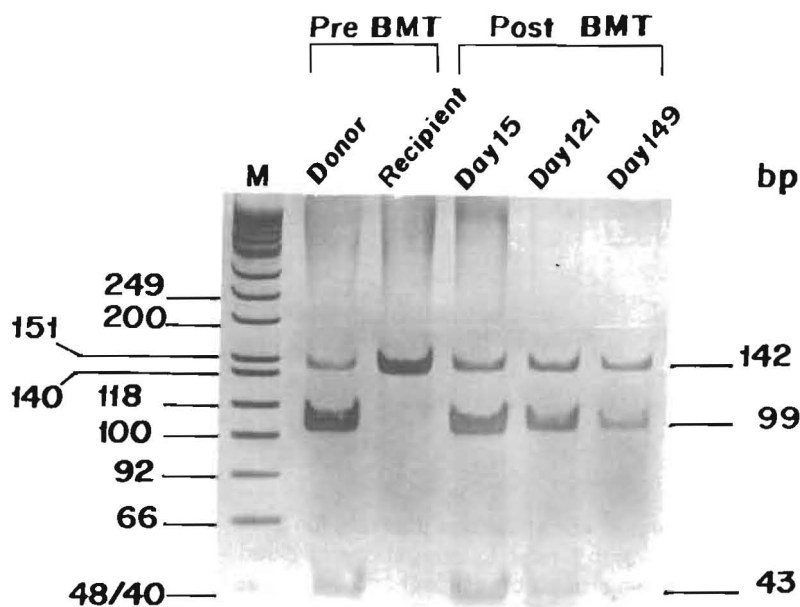


Fig. 1 *Bcl* I RFLP of intron 18 of factor VIII gene to detect chimerism pre- and post-peripheral blood stem cell transplantation. The amplified product of intron 18 of factor VIII gene is 142 base pairs. If polymorphism is present, the amplified product will be digested by *Bcl* I restriction enzyme into 99 and 43 base pairs.

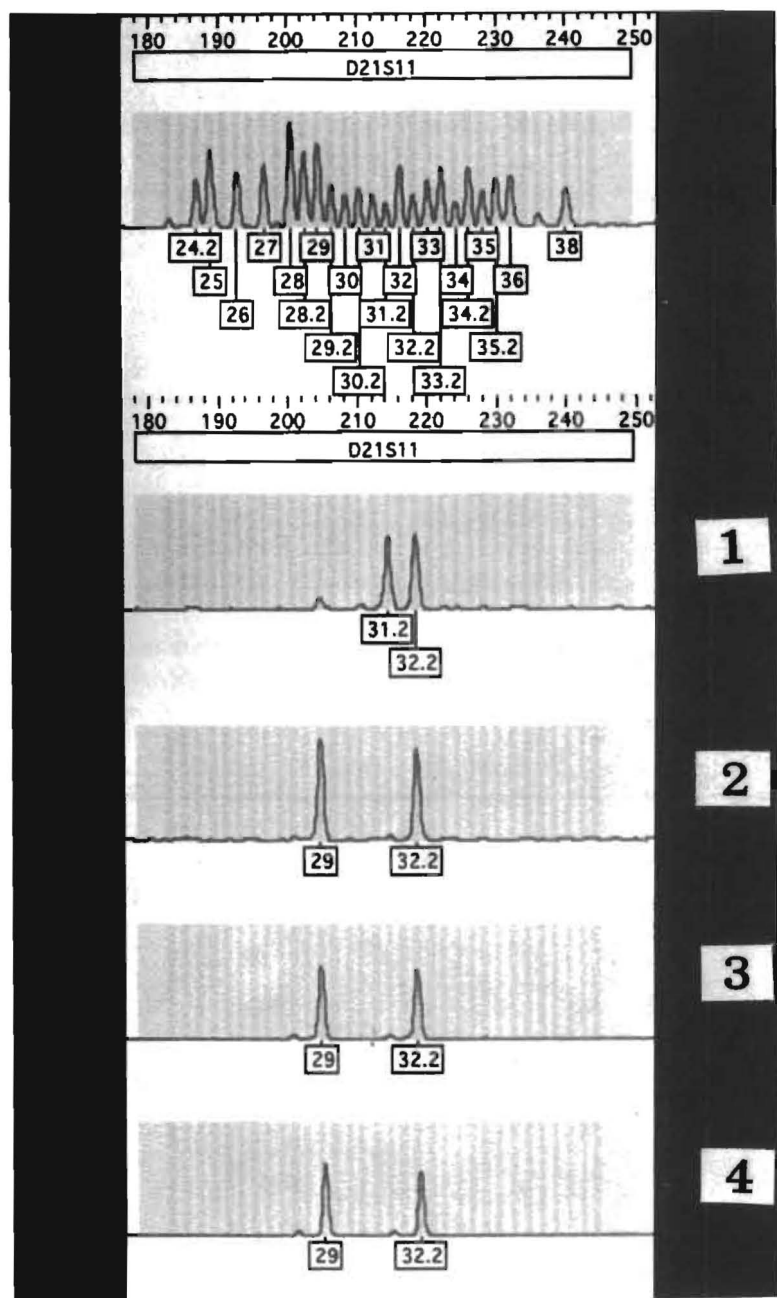


Fig. 2 DNA profiles of recipient and donor at various intervals for STR allele of locus DS1S11. 1 and 2 refer to pre-peripheral blood stem cell transplantation allelic profiles of recipient and donor, respectively. 3 and 4 refer to allelic profiles of recipient on day +40 and +170 post-peripheral blood stem cell transplantation, respectively.

survival for 36 of 41 (88%) patients transplanted.⁹⁻¹¹ Single-center studies of HLA-identical related BMT have long-term survival rates of 80% at 1.5 to 16.5 years' follow-up ($n = 10$)⁶ and 91% at 7.5 to 19.5 ($n = 11$)⁷ years post BMT, respectively. A worldwide review from 1968 through 1995 reported that 57 of 65 children with WAS survived long term after HLA-matched BMT.¹² A successful HLA-matched related cord blood transplant for WAS has been reported.¹³ Because of inevitable poor outcome in untreated WAS patients and the lack of any other curative treatment modality, stem cell transplantation should be performed whenever an HLA-identical donor is available. For patients without an HLA-matched sibling, transplants from closely matched unrelated donors are a very viable therapeutic option.¹⁴ In the future, cord blood transplants, with their lowered risk of acute and chronic GVHD even when HLA-mismatched, will likely add to the therapeutic options for children who lack a suitable donor. Regarding to HLA haplotype-disparate BMT in WAS, the results are still yet controversy.^{6,15} The cost of PBSCT in Thailand is 20 to 30% less than BMT and cord blood stem cell transplantation costs because of the shorter time of the transplantation procedure and lack of need for general anesthesia for the stem cell collection. The cost of the transplant procedure for each patient in Thailand is US\$ 12,000. We would like to emphasize that allogeneic PBSCT is economically feasible for curation of WAS. This study represents the first report of a successful stem cell transplantation in WAS patients in Thailand.

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