Bone Marrow Transplantation (2000) 26, 235–237

www.nature.com/bmt

Case report

Unrelated partially matched peripheral blood stem cell transplantation with highly purified CD34$^+$ cells in a child with Wiskott–Aldrich syndrome

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Summary:

Stem cell transplantation is the only curative approach to the treatment of Wiskott–Aldrich syndrome. However, using grafts from partially matched unrelated donors is associated with increased risk of graft rejection and graft-versus-host disease. In an attempt to prevent these problems, a 6-year-old boy with Wiskott–Aldrich syndrome lacking a suitable family donor, was transplanted with large numbers of unrelated highly purified CD34$^+$ peripheral blood stem cells mismatched at one C locus. Conditioning consisted of busulfan 16 mg/kg body weight, cyclophosphamide 200 mg/kg body weight and antithymocyte globulin 20 mg/kg body weight × 3 days. The boy had a rapid hematopoietic engraftment and showed immunologic reconstitution by day +92. Although he did not receive prophylactic immunosuppression he did not develop any graft-versus-host disease and is well and alive up to now, 25 months after transplantation. *Bone Marrow Transplantation* (2000) **26,** 235–237.
Keywords: Wiskott–Aldrich syndrome; peripheral blood stem cell transplantation; MACS; CD34-selection

Wiskott–Aldrich syndrome (WAS) is a rare X-linked disease, characterized by the clinical triad of thrombocytopenia, eczema, primary progressive T cell immunodeficiency and impaired antipolysaccharide antibody production.[1–3] Patients may present these abnormalities with different degrees of severity. Boys are at high risk of thrombocytopenic/thrombocytopathic bleedings, infections and malignancies. Recently, the gene mutation responsible for WAS was identified. It encodes the WAS-P protein, which is able to interact with a large number of other proteins assumed to be involved in the regulation of signal transduction and cytoskeletal organisation.[4] Despite intensified supportive therapy, such as prophylactic administration of antibiotics and immunoglobulins, use of platelet transfusions and splenectomy, the survival of these patients remains poor.[5,6] The only curative approach is allogeneic stem cell transplantation.[7,8] Most successful transplants utilized HLA-identical sibling donors.[9–13] The results of unrelated matched or mismatched donor transplantations are associated with an increased risk of graft rejection and graft-versus-host disease (GVHD).[14–16] In an attempt to overcome these limitations a 'megadose' of highly purified CD34$^+$ peripheral blood stem cells (PBSC) from an unrelated C-locus mismatched donor was used in this patient. This report details the clinical outcome, as well as the hematopoietic and immunologic reconstitution and follow-up after this kind of unrelated peripheral blood stem cell transplant in a patient with WAS.

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Received 17 January 2000; accepted 3 April 2000

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The diagnosis of WAS was established at the age of 10 months by the presence of thrombocytopenia, abnormal small 'microplatelets', eczema and immune deficiency. At the age of 13 months, the patient was splenectomized due to a massive intestinal bleeding refractory to platelet transfusions. This intervention resulted in normal platelet counts for 4 years. The boy was treated with 7-s immunoglobulin infusions (1 g/kg body weight) every 3 to 6 months, and received oral decontamination consisting of trimetoprim-sulfamethoxazole (3 mg/kg/day) for prophylaxis of *Pneumocystis carinii* for 4 days each week, penicillin-G and fluconazole. Despite this treatment the patient experienced several severe bacterial infections prior to and after splenectomy. At the age of 5 years platelet counts decreased again ($\leqslant$20 000/μl) and a banal head injury resulted in a massive bilateral orbital hematoma (Figure 1). At this time diagnosis of WAS was confirmed by the deletion of the C-nucleotide of exon 10 (position 1065) which was found after amplification of exon 1–12 of the WASP-gene by polymerase chain reaction (PCR) followed by direct sequencing of the PCR products.

In the absence of an HLA-identical family donor, an international search for potential unrelated donors was per-

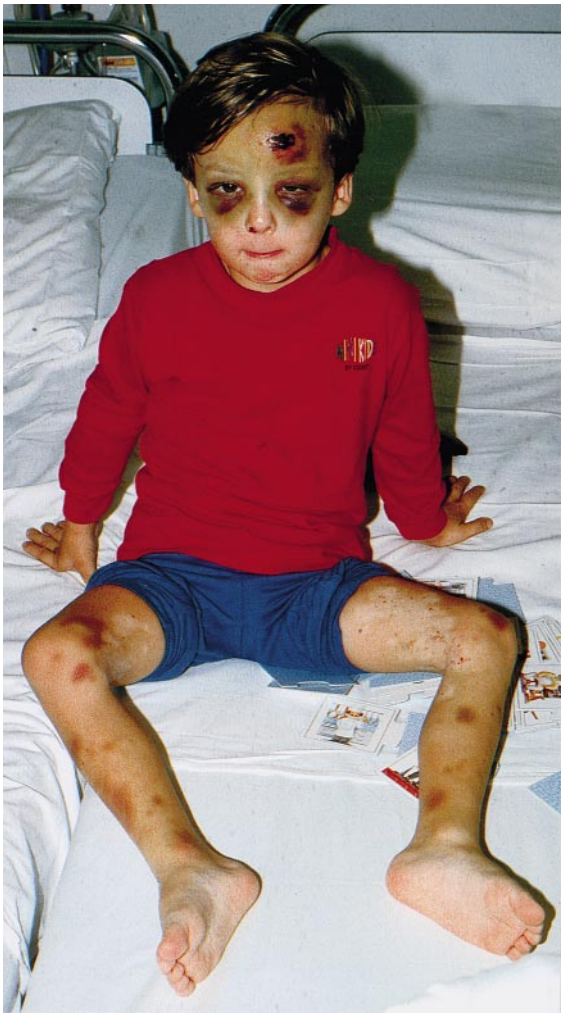

Figure 1 Bilateral orbital hematoma after a banal head injury in the thrombocytopenic child with WAS.

formed by the Austrian Bone Marrow Donor Registry and a suitable donor was found by the Deutsche Knochenmarsspenderdatei (DKMS, Tübingen, Germany). The donor was mismatched at one HLA-C locus (patient: A 1,3; B 38,60; C w2; DRB1 0801, 1301; DRB3 0101; DQB1 0402, 0603; donor: A 1,3; B 38,60; C w3; DRB1 0801,1301; DRB3 0101; DQB1 0402, 0603). After obtaining informed consent the donor cells were mobilized with G-CSF (5 μg/kg body weight/day) for 5 days.

A single leukapheresis was performed and yielded 9.04×10^{10} nucleated cells (NC) with 7.23×10^8 $CD34^+$ cells. After positive selection of $CD34^+$ cells by magnetic cell sorting (MACS) the number of NC was 3.7×10^8 with 3.67×10^8 $CD34^+$ cells (purity 99.34%, recovery 50.7%). Contamination with $CD3^+$ cells was 1.5×10^5. The patient received 24.5×10^6 $CD34^+$ cells/kg body weight together with 1×10^4 $CD3^+$ cells/kg/body weight after conditioning with busulfan (4 mg/kg body weight $\times$ 4), cyclophosphamide (50 mg/kg body weight $\times$ 4) and antithymocyte globulin (ATG, horse; Sero Merieux, Vienna, Austria) (20 mg/kg body weight $\times$ 3). He was given G-CSF 5 μg/kg/day subcutaneously starting at day +4 and erythropoietin (EPO) at a dosage of 150 IU/day subcutaneously until white blood cell counts stabilized at $\geqslant 5 \times 10^9$/l and hemoglobin levels $\geqslant$10 g/dl without erythrocyte transfusions for 10 consecutive days.

The boy showed complete hematological reconstitution of donor origin with ANC $\geqslant 0.5 \times 10^9$/l on day +8, platelets $\geqslant 20 \times 10^9$/l on day +11 and 0.9% reticulocytes on day +11. A measurable number of circulating lymphocytes appeared 1 month after transplantation. The majority of these early lymphocytes were analyzed as $CD56^+/CD16^+$ natural killer (NK) cells. The patient showed a constant increase in T lymphocytes ($CD3^+$) and also an impressive rise in B lymphocytes ($CD19^+$) within 9 months after transplantation. T lymphocytes showed a normal proliferative response to polyclonal activators (phytohemagglutinin, concanavalin A, pokeweed mitogen) within 12 months after transplantation (Table 1).[17] Repeated chimerism analysis of peripheral blood revealed permanent 100% donor hematopoiesis and the underlying gene defect (deletion of the C-nucleotide of exon 10, position 1065) could not be detected in the peripheral blood or the bone marrow cells. Eczema disappeared 2 weeks after starting conditioning chemotherapy, but reappeared within 5 weeks after transplantation due to an allergic reaction against yeast and milk protein. This was proven by elevation of serum levels of IgE up to 1386 U/ml, by radio-immunosorbent test (RIST), radio-allergosorbent test (RAST) and histological examination of repeated skin biopsies and disappeared after a milk-free diet. As of 1 May 2000, the patient is alive and well without any further treatment for 25 months.

Discussion

Despite intensive supportive care such as splenectomy, infusion of immunoglobulins, platelet transfusions and prophylactic administration of broad-spectrum antibiotics, the only curative approach in patients with WAS is allogeneic stem cell transplantation.[7–13] An HLA-identical sibling graft is the preferred stem cell source, whereas the outcome with HLA-non-identical bone marrow or peripheral blood stem cells with or without T cell depletion is rather poor because of graft rejection and acute/chronic GvHD.[14–16] Therefore Ozsahin *et al*[11] concluded that transplants from partially incompatible donors should be restricted to patients with severe complications of WAS. On the other hand, patients should be transplanted in optimum condition, before severe complications from the underlying disease develop. In our patient the transfusion of large numbers ('megadose') of highly purified T cell-depleted $CD34^+$ stem cells was used, assuming that megadoses of $CD34^+$ cells might overcome the HLA-mismatch in the unrelated donor setting and abrogate resistance to engraftment.[18–21] Magnetic cell sorting (MACS) of peripheral blood $CD34^+$ cells proved to be an excellent procedure recovering sufficient numbers of stem cells with high purity and low T cell contamination.[22,23]

The patient showed rapid and sustained hematological engraftment with complete immunological reconstitution within 9 months and did not receive prophylactic immunosuppressive treatment.

To our knowledge this is the first report of the clinical

Table 1 Immunological reconstitution

Time (months)	*Lymphocytes ($\times 10^7$/l)*						*Stimulation (cpm/lymphocyte)*		
	CD3	*CD4*	*CD8*	*CD19*	*CD56/CD16*	*Ratio CD4/8*	*Con A*	*PHA*	*PWM*
1	157	144	27	41	500	5.33		ND	
3	449	204	224	92	469	0.91		ND	
7	3538	2798	864	82	329	3.23		ND	
11	1800	1504	312	704	299	4.80	41.6	24.7	7.78
15	1300	890	400	640	110	2.2		ND	

outcome, hematopoietic and immunologic reconstitution and follow-up of a child with WAS who underwent transplantation with unrelated highly purified $CD34^+$ cells mismatched at one C-locus. The favorable outcome in this patient might encourage further investigation of the role of using 'megadoses' of unrelated matched or mismatched $CD34^+$-selected peripheral blood stem cells in patients with WAS at an earlier stage of disease before severe and life-threatening complications might interfere negatively with the transplantation outcome.

References

1 Krivit W, Good RA. Aldrich's syndrome (thrombocytopenia, eczema, and infection in infants): studies of the defence mechanisms. *Am J Dis Child* 1959; **97**: 137–153.
2 Remold-O'Donnel E, Rosen FS. Sialophorin (CD43) and the Wiskott–Aldrich syndrome. *Immunodev Rev* 1990; **2**: 151–174.
3 Hagemann TL, Kwan S. The identification of two promotors and the complete genomic sequence for the Wiskott–Aldrich syndrome gene. *Biochem Biophys Res Commun* 1999; **5**: 104–109.
4 Kirchhausen T. Wiskott–Aldrich syndrome: a gene, a multifunctional protein and the beginnings of an explanation. *Mol Med Today* 1998; **4**: 300–304.
5 Litzman J, Jones A, Hann I *et al*. Intravenous immunoglobulin, splenectomy and antibiotic prophylaxis in Wiskott–Aldrich syndrome. *Arch Dis Child* 1996; **75**: 436–439.
6 Mullen CA, Anderson KD, Blaese RM. Splenectomy and/or bone marrow transplantation in the management of the Wiskott–Aldrich syndrome: long-term follow-up of 62 cases. *Blood* 1993; **82**: 2961–2966.
7 Bach FH, Albertini RJ, Joo P *et al*. Bone marrow transplantation in a patient with Wiskott–Aldrich syndrome. *Lancet* 1969; **2**: 1364–1366.
8 Parkmann R, Rappeport J, Geha R *et al*. Complete correction of the Wiskott–Aldrich syndrome by allogeneic bone marrow transplantation. *New Engl J Med* 1978; **298**: 921–927.
9 Rosales F, Peylan-Ramu N, Cividalli G *et al*. The role of thiotepa in allogeneic bone marrow transplantation for genetic diseases. *Bone Marrow Transplant* 1999; **23**: 861–865.
10 Huang MT, Chou CC, Jou ST *et al*. Successful bone marrow transplantation in a Chinese boy with Wiskott–Aldrich syndrome. *Asian Pac J Allergy Immunol* 1996; **14**: 57–63.
11 Ozsahin H, Le-Deist F, Benkerrou M *et al*. Bone marrow transplantation in 26 patients with Wiskott–Aldrich syndrome from a single centre. *J Pediatr* 1996; **129**: 238–244.
12 Gaspoz JM, Waldvogel F, Cornu P *et al*. Significant and persistent improvement of thrombocytopenia after splenectomy in an adult with the Wiskott–Aldrich syndrome and intra cerebral bleeding. *Am J Hematol* 1995; **48**: 182–185.
13 Kapoor N, Kirkpatrick D, Blaese RM *et al*. Reconstitution of normal megakaryocytopoiesis and immunologic function in Wiskott–Aldrich syndrome by marrow transplantation following myeloablation and immunosuppression with busulphan and cyclophosphamide. *Blood* 1981; **57**: 692–696.
14 Lenarsky C, Weinberg K, Kohn DB, Parkman R. Unrelated donor BMT for Wiskott–Aldrich syndrome. *Bone Marrow Transplant* 1993; **12**: 145–147.
15 Brochstein JA, Gillio AP, Ruggiero M *et al*. Marrow transplantation from human leukocyte antigen-identical or haploidentical donors for correction of Wiskott–Aldrich syndrome. *J Pediatr* 1991; **119**: 907–912.
16 Fischer A, Landais P, Friedrich W *et al*. Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. *Blood* 1994; **8**: 1149–1154.
17 Mangge H, Beaufort F, Samitz M, Schauenstein K. Peripheral blood of non-leukemic lymphoma patients exhibit aberrant expression of T cell activation markers after polyclonal stimulation *in vitro*. *Cancer* 1990; **66**: 677–683.
18 Aversa F, Tabilio A, Velardi A *et al*. Treatment of high-risk acute leukemia with T cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *New Engl J Med* 1998; **339**: 1186–1193.
19 Handgretinger R, Schumm M, Lang P *et al*. Transplantation of megadoses of purified haploidentical stem cells. *Ann N Acad Sci* 1999; **872**: 351–362.
20 Schwinger W, Urban Ch, Lackner H *et al*. Unrelated peripheral blood stem cell transplantation with 'megadoses' of purified $CD34^+$ cells in three children with refractory severe aplastic anemia. *Bone Marrow Transplant* 2000; **25**: 513–517.
21 Bachar-Lustig E, Rachamim N, Li HW *et al*. Megadose of T cell-depleted bone marrow overcomes MHC barriers in sublethally irradiated mice. *Nature Med* 1995; **1**: 1268–1273.
22 McNiece I, Briddell R, Stoney G *et al*. Large-scale isolation of $CD34^+$ cells using the Amgen cell selection device results in high levels of purity and recovery. *J Hematother* 1997; **6**: 5–11.
23 Miltenyi S, Müller W, Weichel W, Radbruch A. High gradient magnetic cell separation with MACS. *Cytometry* 1990; **11**: 231–238.