—Case Reports—

Umbilical-cord Blood Cell Transplantation Conditioned with a Reduced Intensity-regimen is a Practical Salvage Therapy for Severe Aplastic Anemia Refractory to Immunosuppressive Therapy with Antithymocyte Globulin/Ciclosporin

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Abstract

Immunosuppressive therapy and stem cell transplantation from an HLA-identical donor are the major effective treatments for severe aplastic anemia. However, treatments still need to be developed for patients who do not have a HLA-identical donor and have not shown a clinical response to immunosuppressive therapy. We herein report on 2 patients in whom this problem could be overcome by transplantation of HLA-mismatched umbilical cord blood from unrelated donors. Two Japanese patients with severe aplastic anemia underwent conditioning with fludarabine, cyclophosphamide, and low-dose total-body irradiation and then received transplants of umbilical cord blood. Engraftment of the three lineages occurred without problems. We conclude that umbilical cord blood transplantation with a reduced-intensity conditioning regimen of fludarabine, cyclophosphamide, and total-body irradiation for patients with aplastic anemia is a practical treatment and may be an attractive alternative for patients who does not have an HLA-identical donor and have shown no clinical response to immunosuppressive therapy.

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Key words: severe aplastic anemia, cord blood transplantation, reduced intensity conditioning

Introduction

Stem cell transplantation and immunosuppressive therapy with antithymocyte globulin (ATG) are the two most effective therapies for severe aplastic anemia[1,2]. However, only about 30% of patients can receive stem cell transplantation from HLA-identical sibling donors. Even though unrelated donors are also available, many patients still cannot find a suitable donor. Other patients who cannot receive transplantation are treated with immunosuppressive therapy. However, the response rate with immunosuppressive therapy is only 50% to 80%, and relapse after immunosuppressive therapy is a major problem[1–3]. Therefore, another salvage treatment is necessary for patients who do not have a suitable stem cell donor and have not shown a good response

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Table 1 Patient and cord blood characteristic

	Patient-1	Patient-2
Age/gender/weight (kg)	25/female/42.5	36/male/69.0
Time from diagnosis to CBT * (M)	41	23
Transfusion from diagnosis to CBT * (times)		
RBC	20	6
Platelet	7	14
Treatment before CBT *	ATG (horse)/CSA/G-CSF/PSL	ATG (horse)/CSA/G-CSF/PSL ATG (rabbit)/CSA/G-CSF/PSL
HLA type (patient/cord blood)		
HLA-A, B	A2,B48,55/A2,B48,51	A24,26,B35,56/A24,26,B35,7
HLA-DR	DR4,6/DR4,14	DR4,8/DR4,9
ABO blood type (patient/cord blood)	B/O	A/A
Gender (patient/cord blood)	female/male	male/female
Transplanted cell dosage		
Mononuclear cell dosage ($\times 10^7$/kg)	2.12	2.5
CD34 positive cell dosage ($\times 10^5$/kg)	1.25	0.6
Follow-up periods after CBT * (month)	25	17
Acute/chronic GVHD	none/limited (skin)	none/none

CBT *, cord blood transplantation; ATG, antithymocyte globulin

to immunosuppressive therapy. Recently, we have started to perform umbilical cord blood stem cell transplantation with a reduced-intensity conditioning regimen of fludarabine, cyclophosphamide, and low-dose total-body irradiation (TBI) for such patients. We present two patients with severe aplastic anemia treated with transplants of umbilical cord blood cells.

Case Reports

The patient and umbilical cord blood characteristics are shown in **Table 1**. The patients were a 25-year-old Japanese woman and a 36-year-old Japanese man. Severe aplastic anemia had been diagnosed, but suitable bone marrow donors could not be identified. Therefore, the patients received one or two courses of immunosuppressive therapy with ATG, ciclosporin, granulocyte-colony stimulating factor, and prednisolone. The effects of immunotherapy, however, were transient, and pancytopenia recurred in both patients. In particular, the platelet counts decreased to less than 1×10^{10}/L. Therefore, written informed consent was obtained for unrelated cord blood transplantation. The intervals from diagnosis to cord blood transplantation were 41 months and 23 months. The patients each received multiple transfusions of red blood cells and platelets before transplantation (**Table 1**). The reduced-intensity conditioning regimen consisted of fludarabine (30 mg/m^2/day from day -5 to -3), cyclophosphamide (750 mg/m^2/day from day -5 to -3), and TBI (2 Gy/day from -2 to -1). The unrelated HLA-mismatched umbilical cord blood cells were then transplanted. Prophylaxis for acute graft-versus-host disease (GVHD) was performed only with tacrolimus (0.03 mg/kg/day, continuous infusion) from day -3.

Both of the patients showed smooth engraftment after the umbilical cord blood cell transplantation. The engraftments of the white blood cells, red blood cells, and platelets were performed on days 19, 35, and 70, respectively, in patient 1 and on days 17, 32, and 33 in patient 2. The hematological recovery after transplantation is shown in **Figure 1**. In patient 1, engraftment syndrome, such as fever and skin rash (about 60% of body surface), was found on day 8 and was successfully treated with prednisolone. Chimerism analysis of the bone marrow cells using fluorescence in-situ hybridization showed the patient to be a complete donor type on day 35. Seventy-three days after transplantation, the patient began to have lower abdominal pain, dysuria, urgency, and macroscopic hematuria. The discovery of adenovirus

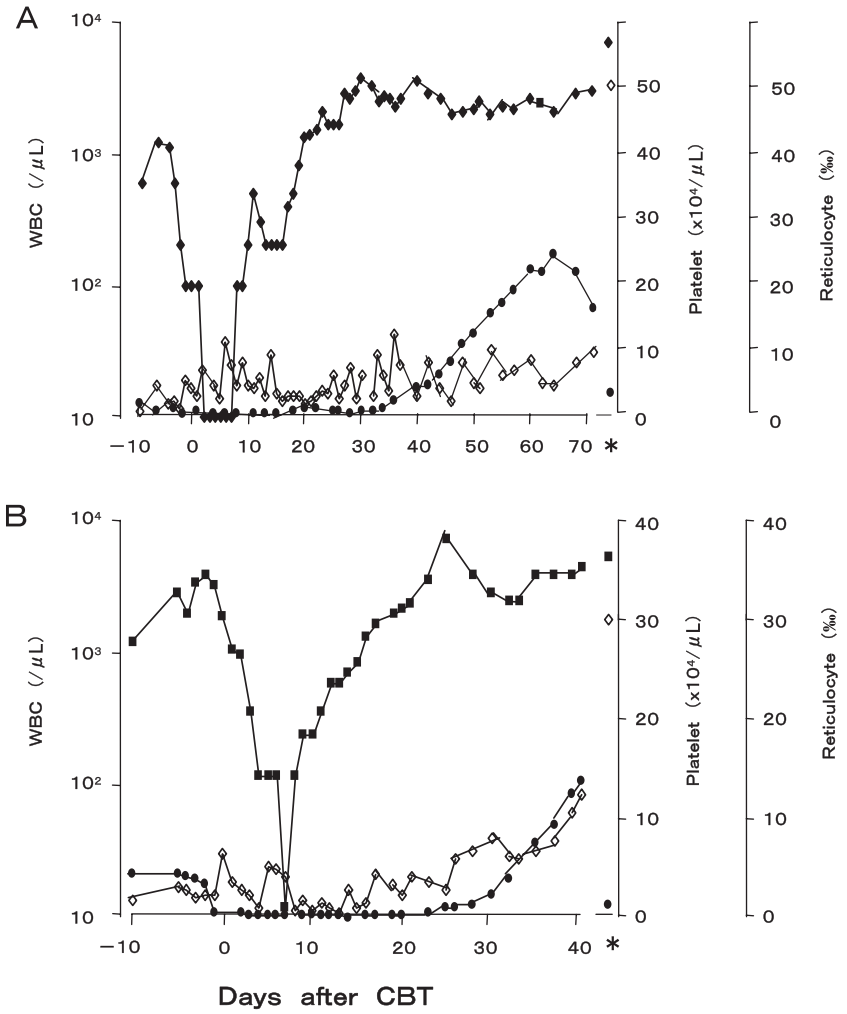

Fig. 1 Hematological recovery after umbilical cord blood transplantation in two patients.
A: patient 1; **B**: patient 2. ■: white blood cells; ◇: platelets; ●: reticulocytes; * : data from July 2007

antigen in the urine led to a diagnosis of adenoviral cystitis. These symptoms persisted for 1 month and then resolved spontaneously. A localized varicella-zoster virus infection of the right side of the abdomen developed in November 2005 and was successfully treated with acyclovir for 2 weeks. Eighteen weeks after transplantation, the patient complained of nausea and vomiting, which were caused by intestinal GVHD. Prednisolone and tacrolimus were effective in treating this episode.

In patient 2, the clinical course after transplantation was uneventful. Only engraftment syndrome, consisting of fever, skin rash, and diarrhea, developed on day 8 and was effectively treated with hydrocortisone at a dose of 200 mg/day. Fluorescence in-situ hybridization analysis of bone marrow cells showed complete chimerism on day 28. Neither an active viral infection nor GVHD developed after transplantation.

Both patients are being followed up as outpatients and are doing well 1 or 2 years after transplantation.

Discussion

Bone marrow transplantation is a good strategy for the treatment of severe aplastic anemia, with 66% to 94% of patients achieving long-term survival[1,2,4–8]. Some patients, however, do not have suitable stem cell donors. Alternative stem cell sources are a major concern for patients who do not have suitable HLA-identical donors or have not shown a good response to immunosuppressive

Table 2 Recent reports

case	stem cell source	conditioning regimen	GVHD prophylaxis	ANC engraftment	chimerism	aGVHD	cGVHD	outcome (Months)	reference
1	sibling BM	Flu/CY/ATG	CSA	12	Donor	No	No	Alive, 91	13
2	sibling BM	Flu/CY/ATG	CSA	16	Donor	No	No	Alive, 96	13
3	sibling BM	Flu/CY/ATG	CSA	18	mixed chimera	No	No	Alive, 90	13
4	sibling BM	Flu/CY/ATG	CSA	14	Donor	grade I	No	Alive, 88	13
5	sibling BM	Flu/CY/ATG	CSA	10	mixed chimera	No	No	Alive, 72	13
6	sibling BM	Flu/CY/ATG	CSA	10	Donor	grade I	No	Alive, 64	13
7	father BM	Flu/CY/ATG	CSA	NE	NE	NE	NE	Dead, day 10	13
8	father BM	Flu/CY/ATG	CSA	11	Donor	grade II	limited	Alive, 29	13
9	cousin BM	Flu/CY/ATG	CSA	13	Donor	No	No	Alive, 17	13
10	sibling BM	Flu/CY/ATG	CSA	26	Donor	No	No	Dead, day 107	13
11	sibling BM	Flu/CY/ATG	CSA	15	Donor	No	No	Alive, 6	13
12	sibling BM	Flu/CY/ATG	CSA	24	Donor	grade I	No	Alive, 5	13
13	sibling BM	Flu/CY/ATG	CSA	15	Donor	No	limited	Alive, 3	13
14	CB	CY/ATG	CSA/MTX	10	mixed chimera	No	No	Alive, 69	16
15	CB	CY/ATG	CSA/MTX	21	mixed chimera	No	NE	Alive, 53	16
16	CB	CY/ATG	CSA/MTX	25	mixed chimera	No	No	Dead, 3	16
17	CB	CY/ATG	CSA/MTX	11	mixed chimera	No	limited	Alive, 41	16
18	CB	CY/ATG	CSA/MTX	27	mixed chimera	No	NE	Alive, 41	15
19	CB	CY/ATG	CSA/MTX	15	NE	No	No	Dead, 2	16
20	CB	CY/ATG	CSA/MTX	14	mixed chimera	No	No	Alive, 11	16
21	CB	CY/ATG	CSA/MTX	19	mixed chimera	No	No	Alive, 6	16
22	CB	CY/ATG	CSA/MTX	9	Donor	No	No	Alive, 4	16
23	CB	Bu/CY/ATG	CSA/PSL	18	Donor	grade II	No	Alive, 12	18
24	CB	Flu/Mel/TBI	CSA	12	Donor	grade II	No	Alive, 40	19
25	CB	Flu/CY/TBI	Tacrorimus	19	Donor	No	limited	Alive, 25	Ours
26	CB	Flu/CY/TBI	Tacrorimus	17	Donor	No	No	Alive, 17	Ours

BM, bone marrow; CB, cord blood; Flu, fludarabine; CY, cyclophosphamide; ATG, antithymocyte globurin; TBI, total body irradiation CSA, ciclosporin; MTX, methotrexate; Mel, melpharan; NE, not evaluated

therapy[9]. HLA-mismatched family donors, including one haplo-identical related donor, and mismatched unrelated donors have recently been used as alternative donors[9]. However, complications, such as rejection, GVHD, and viral infection, have made these procedures difficult to perform in usual clinical practice. Therefore, another approach may need to be explored.

Unrelated umbilical cord blood transplantation and conditioning with cyclophosphamide and TBI were first performed in children with severe aplastic anemia. The rejection rate, however, was high, indicating that conditioning with cyclophosphamide and TBI was insufficient to eradicate host immune cells before transplantation. A new conditioning regimen that can eradicate host immune cells effectively but is less toxic should therefore be considered. Recently, fludarabine, which is a potent immunosuppressive agent, became available, and several reduced-intensity regimens containing fludarabine, such as fludarabine + cyclophosphamide[10], fludarabine + cyclophosphamide + ATG[11–13] and fludarabine + cyclophosphamide + alemtuzumab[14], have been tried in stem cell transplantation from HLA-identical related or unrelated donors. **Table 2** shows the results of transplantation with reduced-intensity conditioning regimens in patients with aplastic anemia, including our two patients. Resnick et al. have reported promising results in allogeneic bone marrow transplantation with a reduced-intensity conditioning regimen of fludarabine, cyclophosphamide, and ATG[13]. Using bone marrow cells as a stem cell source they obtained firm engraftment with stable complete donor chimerism in many cases, and both acute and chronic GVHD were reported to be mild.

Mao et al. first showed sustained hematopoiesis by umbilical cord blood transplantation and conditioning with cyclophosphamide and ATG in patients with severe aplastic anemia[15,16]. Although microsatellite DNA fingerprinting showed a sustained and stable mixed chimerism after transplantation, these cases show the possibility of umbilical cord blood cell transplantation with a reduced-intensity conditioning regimen for patients with severe aplastic anemia.

Cyclophosphamide, fludarabine, and TBI are each potent immunosuppressive agents, and the combination of these agents may have a strong immunosuppressive effect. Barker et al. have shown that the conditioning regimen of fludarabine, cyclophosphamide, and TBI achieves rapid and complete donor chimerism after transplantation of unrelated umbilical cord blood in adults with hematologic malignancy[17]. For this reason, we used this nonmyeloablative conditioning regimen for two patients with aplastic anemia. In our patients the hematological recovery after transplantation was uneventful, no severe complications were found, and complete donor chimerism was achieved. The degree of toxicity related to the conditioning in our cases was trivial. Engraftment syndrome consisting of fever and skin rash developed 8 to 9 days after transplantation in both patients but was easily treated with prednisolone. Acute GVHD did not develop in either patient, and limited chronic GVHD was found in only one patient. Viral infections by adenovirus and varicella-zoster virus developed in one patient, which indicates that viral infection remains a major concern after umbilical cord blood transplantation and that close observation for viral infections is necessary in such cases.

On the basis of these experiences we conclude that a conditioning regimen with fludarabine, cyclophosphamide, and TBI is a practical means for obtaining long-term donor hematopoiesis after umbilical cord blood transplantation in patients with severe aplastic anemia.

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