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Cord blood stem cells

Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim

H Nagayama[1,2], K Misawa[1], H Tanaka[4], J Ooi[1], T Iseki[1], A Tojo[1], K Tani[1], Y Yamada[1], H Kodo[3], TA Takahashi[1], N Yamashita[1], S Shimazaki[4] and S Asano[1]

[1]*Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [2]*Keio University School of Medicine, Tokyo, Japan;* [3]*Fuchu Metropolitan Hospital, Tokyo, Japan; and* [4]*Kyorin University, Tokyo, Japan*

Summary:

We performed stem cell rescue and allogeneic skin transplantation on a lethally neutron-irradiated nuclear accident victim. HLA-DRB1 mismatched unrelated umbilical cord blood cells (2.08×10^7/kg recipient body weight) were transplanted to an 8–10 Gy equivalent neutron-irradiated patient because of a lack of a suitable bone marrow or peripheral blood donor. Pre-transplant conditioning consisted of anti-thymocyte γ-globulin alone, and GVHD prophylaxis was a combination of cyclosporine (CYA) and methylprednisolone (mPSL). Granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), and thrombopoietin (TPO) were concurrently administered after transplantation. The absolute neutrophil count reached 0.5×10^9/l on day 15, the reticulocyte count rose above 1% on day 23, and the platelet count was over 50×10^9/l on day 27, respectively. Cytogenetic studies of blood and marrow showed donor/recipient mixed chimerism. Rapid autologous hematopoietic recovery was recognized after withdrawal of CYA and mPSL. Repeated pathological examinations of the skin revealed no evidence of acute GVHD. Eighty-two days after the irradiation, skin transplantation was performed to treat radiation burns. Almost 90% of the transplanted skin engrafted. Immunological examination after autologous hematopoietic recovery revealed an almost normal T cell count. However, immune functions were severely impaired. The patient died from infectious complication 210 days after the accident.

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Keywords: umbilical cord blood; hematopoietic stem cell; skin transplantation; neutron irradiation; acute radiation syndrome

Correspondence: Dr H Nagayama, Department of Hematology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan
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The feasibility and efficacy of allogeneic hematopoietic stem cell transplantation for accidentally irradiated individuals remain unclear because of the high incidence of transplant-related mortality including acute graft-versus-host disease (GVHD) and ensuing complications, that are exacerbated by coincident radiation injury.[1] Target organs of acute GVHD are mainly the skin, gastro-intestinal tract, and liver, which are also susceptible to acute radiation syndrome (ARS).[2] Main causes of death after allogeneic bone marrow transplantation for irradiated individuals are burns, acute GVHD and interstitial pneumonitis with or without adult respiratory distress syndrome (ARDS).[1,2]

Umbilical cord blood is a useful stem cell source because the incidence and extent of acute GVHD associated with its use are low as compared to conventional bone marrow,[3–10] allowing for three HLA loci donor/recipient mismatching.[4,7,8] In addition, umbilical cord blood can be used for urgent triage for numerous victims without donor acquisition delay, because it has already been screened and cryopreserved. Thus, umbilical cord blood is an ideal stem cell source for accidentally irradiated individuals.

Neutrons cause severe cutaneous blistering and desquamative changes on irradiated skin fields several weeks after irradiation.[2] Systemic neutron irradiation induces fatal systemic destruction of the skin barrier that plays an important role in host defense against exogenous pathogens.

In the present study, we examined the feasibility and efficacy of unrelated HLA-disparate umbilical cord blood and cadaver skin transplantation for the rescue of a lethally irradiated recipient. We also examined donor/recipient chimerism and immunological reconstitution in the early phase of umbilical cord blood transplantation (CBT) for ARS.

Case report

A 39-year-old male, who had been lethally irradiated with neutrons at 8–10 Gy equivalent (Eq) in a nuclear accident,[11] was referred to our hospital on 4 October 1999 for hematopoietic stem cell transplantation. He had been irradiated by a critical fission reaction at the uranium-processing plant by JCO, Co., Ltd, in the village of Tokai in the Ibaraki Prefecture, Japan, on 30 September 1999. Symptoms

Table 1 Dosimetry of the patient based on (A) lymphocyte count nomogram, (B) time of ^{24}Na decay from peripheral blood samples, and (C) ringed chromosome analysis. The relative biological effect of neutrons was calculated as 1.7-fold that of γ-rays. (R.B.E., 1.7)

(A) Estimated irradiated dose using decreased curve of lymphocyte count	6–10 GyEq
(B) Estimated irradiated dose calculated by the half-life of ^{24}Na contained in the peripheral blood	10.4 GyEq
(C) Estimated irradiated dose using ringed chromosome analysis	7.8 GyEq

immediately after the accident were vomiting within 30 min, redness and edema of the skin, and marked lymphocytopenia with granulocytosis on examination of the blood. His consciousness level was normal. Bone marrow examination suggested that there was little hope of autologous hematopoietic reconstitution, and the same results were suggested by dosimetry (Table 1).

We intended to treat him with stem cell transplantation to avoid infection during the granulocytopenic period of ARS.[2] However, there was no suitable related bone marrow or peripheral blood stem cell donor, and several months would have been required to co-ordinate an unrelated donor from the Japan Marrow Donor Registry. We searched for an HLA-matched stem cell source at the Japan Cord Blood Bank Network, with permission from the Ministry of Health and Welfare, Japan. We finally found an HLA-DRB1 one locus-mismatched unrelated umbilical cord blood in the Tokai Cord Blood Bank, a member of the Japan Cord Blood Bank Network. Characteristics of this umbilical cord blood are shown in Table 2. It was serologically negative for HBV, HCV, HIV1/2, ATLL, and syphilis. Both the maternal and cord blood were negative for CMV-IgG and CMV-IgM (examined by ELISA; enzyme-linked immuno-solvent assay). Physical examination and screening tests for inherited disorders of the donor infant were normal 6 months after delivery. The institutional review board consented to this transplant. After signed informed consent had been obtained, the umbilical cord blood was transplanted. Before the transplant, both sternal and iliac bone marrow became markedly hypocellular. The bone marrow nucleated cell counts decreased to 0.5×10^9/l and 0.7×10^9/l (normal 7–20 $\times 10^9$/l in our institute), respectively.

Conditioning consisted of anti-thymocyte equine γ-globulin (ATG) (2.5 mg/kg/day) for two successive days (total 5 mg/kg). No cytotoxic drugs were used to avoid regimen-related toxicities. The dose of ATG was modified to a half of the conventional dose to avoid delay in lymphocyte recovery during the post-transplant period. After conditioning, a total of 2.08×10^7 umbilical cord blood-derived mononuclear cells was infused. GVHD prophylaxis consisted of cyclosporine (CYA; 3 mg/kg once daily i.v. infusion over 10 h from the day before CBT; day −1) and methylprednisolone sodium succinate (mPSL; 4 mg/kg i.v. on day −3, 2 mg/kg from day −2 to day 0). These drug doses were gradually tapered (see Figure 1a). Granulocyte colony-stimulating factor (G-CSF) (5 μg/kg/day from day −4, and 10 μg/kg/day from day −1 to day 16), erythropoietin (EPO) (100 IU/kg/day from day −1 to day 20), and thrombopoietin (TPO) (5 μg/kg/day from day 3 to day 16) were concurrently administered intravenously to assist in a rapid recovery of tri-lineage hematopoiesis. On day 15, the neutrophil count reached 0.5×10^9/l. We therefore considered that the umbilical cord blood had successfully engrafted. The reticulocyte count rose above 1% of total red blood cells on day 23, and the platelet count reached 50×10^9/l on day 27, respectively (Figure 1a–c).

On day 9, the fluorescence *in situ* hybridization technique using X- and Y-chromosome-specific probes (Y-probe FISH) showed that almost half of the cells analyzed had donor-derived XX signals and the others recipient-derived XY signals, indicating that mixed chimerism was present (Figure 2a–c). Throughout the post-transplant course, no evidence of acute GVHD was seen, but blistering of the right hand developed, rapidly extending to both the forearms and plantars. Repeated examination of cutaneous (right hand and leg on day 24, right forearm, right shank, right buttock, and abdominal skin on day 40, and both hips on day 94), and gastro-duodenal mucosae (day 40) revealed no evidence of acute GVHD. As we had obtained mixed chimerism that could induce immunological tolerance between donor and recipient, we reduced the doses of CYA

Table 2 HLA typing of recipient, umbilical cord blood donor, and skin-graft donor. Condensed type shows mismatched HLA antigen, and italic type shows high-resolution DNA typing using the PCR-SBT method

	Recipient		*Umbilical cord blood donor*		*Skin-graft donor*	
HLA-A	A11	A24	A11	A24		
	1101	*2402*			*2402101*	
HLA-B	B52	B62	B52	B62		
	5201	*15011*			*15011*	
HLA-C	Cw9		Cw3	Cw12		
	w0303	*w1202*				
HLA-DRB1	**DR14**	DR15	**DR13**	DR15		
	1406	*1502*	***1302***	*1502*	**04051**	**15011**

Characteristics of infused cord blood.
Collected volume 114 ml at collection (recipient BW 66 kg).
Total nucleated cells 2.08×10^7/kg.
CFU-GM 4.61×10^4/kg.
$CD34^+$ cells (at thawing) 7.11×10^4/kg.

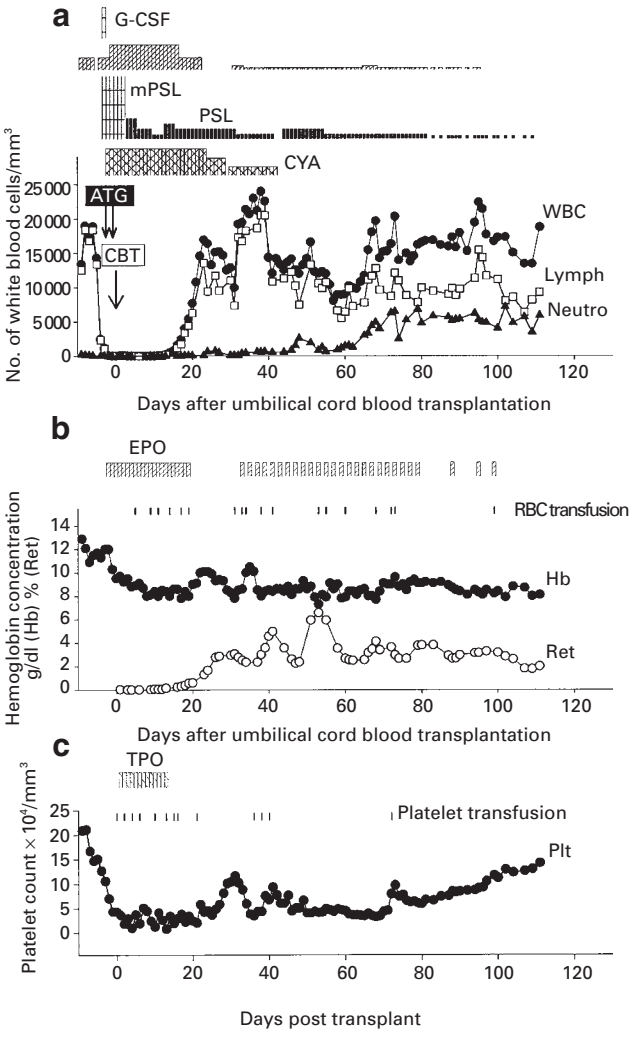

Figure 1 Treatment course and hematopoietic recovery from acute radiation syndrome. (**a**) Medication and recovery of white blood cells: ●, white blood cell count; ▲, neutrophil count; □, the lymphocyte count; G-CSF, granulocyte colony-stimulating factor; mPSL, methylprednisolone; PSL, prednisolone; CYA, cyclosporine; ATG, anti-thymocyte equine gammaglobulin; CBT, umbilical cord blood transplantation. (**b**) Hemoglobin concentration and reticulocyte count: ●, hemoglobin concentration; ○, reticulocyte count; EPO, erythropoietin; |, transfusion of red blood cells. (**c**) Platelet count: ●, platelet count; TPO, thrombopoietin; |, transfusion of platelets.

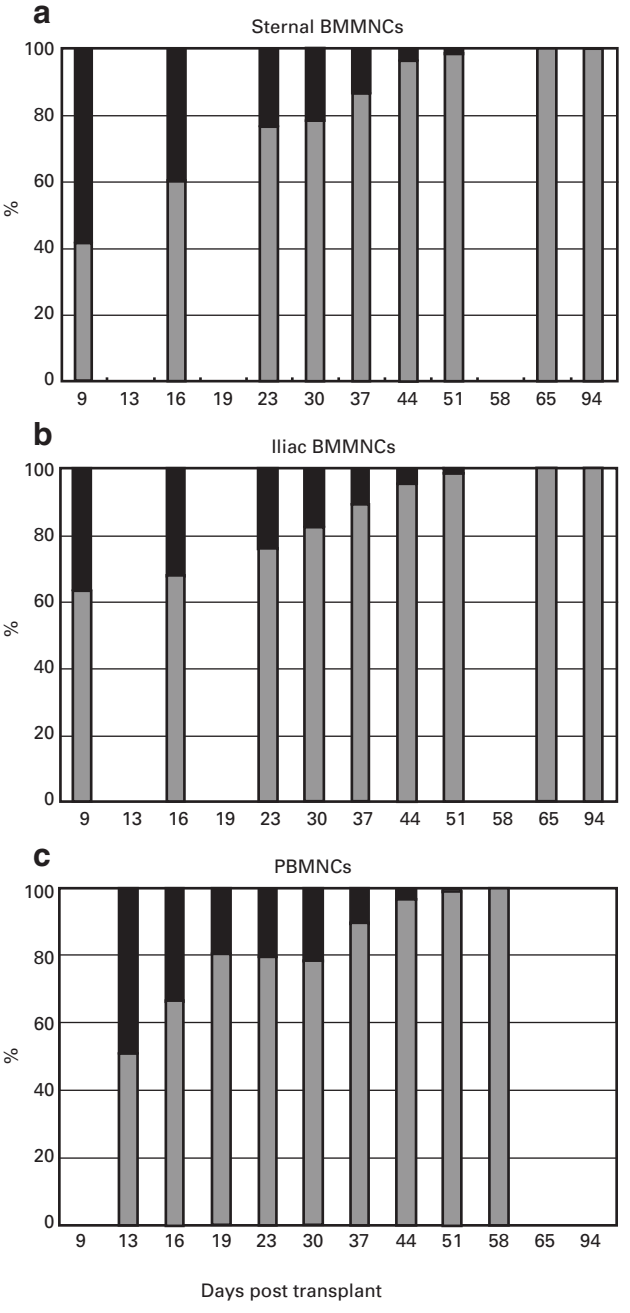

Figure 2 The chimeric state analysis of the patient-derived BM and PBMNCs using fluorescence-labeled *in situ* hybridization of the X and Y chromosome-specific probes: solid columns, percentages of donor-derived XX signals; shaded columns, percentages of recipient-derived XY signals. (**a**) Analysis of sternal bone marrow (sternal), (**b**) iliac bone marrow (iliac), and (**c**) peripheral blood mononuclear cells (PBMNCs).

and mPSL to ameliorate the post-transplant immunosuppressive state. Thereafter, unexpected rapid autologous hematopoietic recovery occurred, and almost all the PBMNCs analyzed developed recipient-derived XY signals by Y-probe FISH up to 50 days after CBT.

In spite of there being no evidence of acute GVHD, neutron-burn induced blistering and desquamation occurred. Deep dermal neutron burns progressed to cover 67% of the total body surface area (BSA) including the face, and both upper and lower extremities from day 60 to day 80 after CBT. Palliative care including skin-dressing and hygiene treatment was not effective and methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated from skin exudates. In order to relieve the pain, the exudates, and the infections of the desquamated skin lesions, we transplanted skin from a cadaver donor to both the victim's hands and arms (15% of BSA) on day 72 after CBT. Allogeneic cultured skin grafts were also transplanted to both legs (20% of BSA) on day 80 after CBT, and to the face (10% of BSA) on day 114. Almost 90% of this skin engrafted, relieving the pain, massive exudates, and infection.

On day 131 after CBT, his stool became positive for occult blood, and thereafter he developed progressive anemia. Finally, he became red blood cell transfusion depen-

dent. Gastric fiberoptic examination revealed acute gastric mucosal lesions in the antrum of the stomach causing diffuse bleeding. He suffered from obstructive sleep apnea due to ARS-related stomatitis, and clot formation in his buccal cavity. He aspirated the purulent exudates from the nasal cavity and developed MRSA pneumonia on day 153 after the accident. Acute renal failure developed as a result of the long-term administration of nephrotoxic antibiotics, including vancomycin and arbekacin. He was diagnosed as having ARDS based on the criteria outlined at a consensus conference of ARDS. We failed to resuscitate him with a tracheotomy, the open lung approach based on the protective ventilation strategy, and the use of mPSL 2 mg/kg/day. In spite of all our efforts, he died from respiratory and multi-organ failure on day 201 after CBT (day 210 after the accident).

Patient and methods

Patient materials

All materials examined were collected from this patient after signed informed consent, and used with his permission.

Dosimetry

Dosimetry was based on the lymphocyte count, blood ^{24}Na concentration, and chromosome analysis of peripheral blood mononuclear cells, as described previously.[2,11]

Analysis of chimerism

A fluorescence-labeled *in situ* hybridization (FISH) technique using X and Y sex chromosome-specific probes was employed to examine bone marrow (BM) and peripheral blood (PB) mononuclear cells (MNCs). Chromosomal analyses of BM and PBMNCs were also performed. On day 20 after transplantation, we separated the peripheral blood mononuclear cells by density gradation using Lymphoprep LY (Nycomed, Oslo, Norway), Percoll LY (Amersham Pharmacia Biotech, Uppsala, Sweden), and Percoll GR (Amersham Pharmacia Biotech). After the separation, we analyzed the karyotypes of the separated mononuclear cells and granulocytes.

Monitoring of immunological reconstitution

An absolute lymphocyte count, including $CD3^+CD4^+$ and $CD3^+CD8^+$, was carried out using a Biometric IMAGN 4T8 assay kit (Becton Dickinson, Mountain View, CA, USA). Cytokine production by patient-derived PBMNCs was assessed using a semi-quantitative RT-PCR method. Amplification of 1 μg cDNA from PMA- and IoM-stimulated patient- and normal healthy volunteer-derived PBMNCs was performed with a SuperTaq Premix kit (Sawady Technology, Tokyo, Japan) using specific primers for β-actin, GM-CSF, IL-4, IL-6, IL-12p40, IL-12p35, IFN-γ, and TNF-α (Continental Laboratory Products, San Diego, CA, USA).

For the detection of mitogenic responses of patient-derived lymphocytes, the patient's PBMNCs were isolated by Ficoll density gradation, and 5×10^5 cells were incubated in each well of a 96-well plate, stimulated with 20 μg/ml phytohemagglutinin (PHA), or 7 μg/ml concanavallin-A (ConA) for 64 h, and then pulsed with 12.5 μCi ^{3}H-labeled thymidine for 8 h. Pulsed cells were harvested and the β-irradiation was measured with a scintillation counter. Normal values for mitogenic responses among volunteer donor PBMNCs were 26000–53000 counts per minute for PHA-L stimulation, and 20000–48000 counts per minute for Con-A stimulation.

Allogeneic mixed leukocyte reaction (allo-MLR) was performed as below. Patient- or HLA-DRB1 disparate three donor-derived PBMNCs (5×10^4 cells) were irradiated to 150 Gy as stimulator cells and co-cultured with 5×10^4 donor- or patient-derived PBMNCs as responder cells, for 5 days in 96-well plates, and then pulsed with 1 μCi ^{3}H-labeled thymidine for 8 h. Pulsed cells were harvested and the β-irradiation was measured.

Immunoglobulin concentrations from the patient were also monitored.

Results

Dosimetry

Dosimetry of the patient is described in Table 1. As is usual with neutron irradiation, distribution of the estimated dose was heterogeneous. It is very difficult to estimate accurately the dose of irradiation received by victims during the ideal transplantation time immediately after the accident.

Analysis of chimerism

FISH, using X- and Y-sex chromosome-specific probes, showed that almost 60% of BMMNCs were donor CB-derived XX cells in the sternal bone marrow, thought to be irradiated at a relatively high dose. Almost 40% of BMMNCs showed donor-derived XX signals in the iliac bone marrow, which had had a lower estimated irradiation dose due to attenuation of neutrons through the trunk. Almost half of all PBMNCs exhibited donor-derived XX signals, the rest XY signals. Donor-derived XX signals gradually decreased in intensity in both BMMNCs and PBMNCs with a reduction in CYA (Figure 2a–c).

Chromosomal analysis of sternal BM, iliac BM, and PB MNCs, showed complex chromosomal abnormalities, which were mainly non-clonal complex single cell abnormalities (SCA) (Figure 3a–c). We examined the fractional chromosome analysis to compare chimerisms of the mononuclear cell and granulocyte fractions. The granulocyte-enriched fraction indicated that 91.8% of granulocytes had recipient origin 46, XY chromosomes, and only 7.8% of granulocytes had the donor-derived 46, XX, chromosome. However, the lymphocyte and monocyte-enriched fraction possessed 24.3% and 26.8% of donor-derived 46, XX chromosome, respectively. These data indicated that only a small fraction of granulocytes was differentiated from donor-derived myeloid progenitor cells, but a quarter of

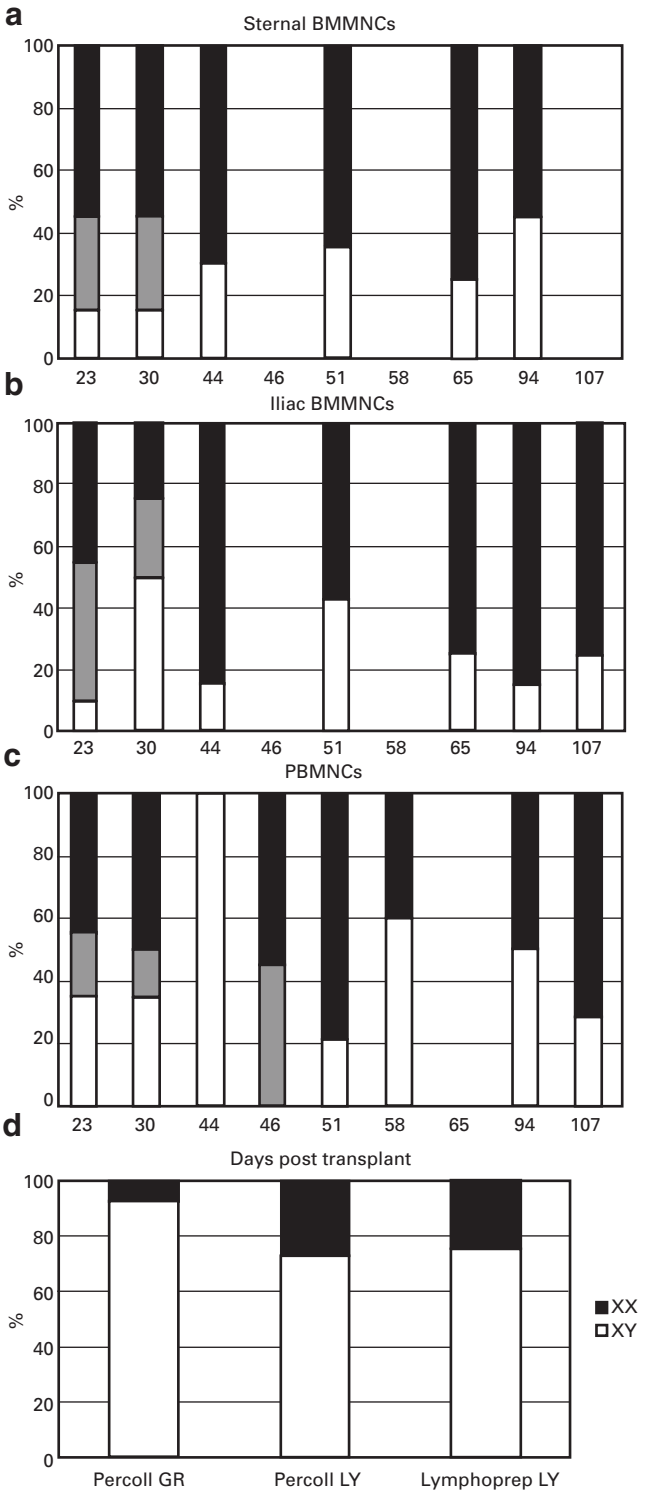

Figure 3 Chromosomal analysis of patient-derived BM or PBMNCs: open columns, percentages of normal karyotype cells; striped columns, percentages of cells with 46,XY, inv (9), thought to be a normal variant type chromosomal translocation; solid columns, percentages of cells possessing the non-clonal complex chromosomal abnormalities, which are so called single cell abnormalities (SCA). (**a**) Analysis of sternal bone marrow (sternal), (**b**) iliac bone marrow (iliac), (**c**) peripheral blood mononuclear cells (PBMNCs), and (**d**) analysis of day 20 PBMNCs and granulocytes.

lymphocytes and monocytes were of donor origin (Figure 3d).

Clonal mutations including chromosomal translocation t(4;11)(q23;q24) were detected only in the PB- and iliac BM-derived PHA blasts on day 107 after CBT (Table 3).

Monitoring of immunological reconstitution

The number of T cells after transplantation in this patient is shown in Figure 4. The number of $CD3^+CD4^+$ cells rose to above 1.0×10^9/l about 80 days after transplantation. In contrast, the number of $CD3^+CD8^+$ cells began to progressively increase in spite of the administration of CYA. After termination of CYA treatment, the number of both $CD3^+CD4^+$ and $CD3^+CD8^+$ cells further increased and that of $CD3^+CD8^+$ cells reached as high as 3.0×10^9/l. During treatment, CMV reactivation was detected by PCR and antigen detection methods using C9 and C10 monoclonal antibodies. However, the CMV rapidly disappeared after administration of ganciclovir. Concurrently, the number of $CD3^+CD8^+$ T cells abruptly increased.

We detected transcriptional expressions of GM-CSF, IL-4, IL-6, IL-12p40, IL-12p35, IFN-γ, and TNF-α mRNA after stimulation with PMA and IoM using the RT-PCR method. However, the expression levels did not differ from those of the healthy volunteer donor (data not shown). Thus, there were no obvious differences in cytokine mRNA expression of PBMNCs between the patient and healthy donor.

To clarify the functional activation and maturation of the patient-derived T cells, we examined the mitogenic responses of these T cells *in vitro* (data not shown). Patient-derived T cells did not respond to mitogens including PHA or ConA, suggesting that the patient's immune responses were severely impaired. Even more surprisingly, an allogeneic mixed leukocyte reaction with third-party HLA-DRB1 disparate unrelated donors showed the patient-derived PBMNCs to be unresponsive to allogeneic antigen-presenting cells, and to have impaired antigen-presenting capacities against allogeneic responder cells (data not shown). Endogenous immunoglobulin producing capacities were also suppressed until 120 days after the accident (data not shown). These results suggest that the patient's immune system was fatally impaired in spite of the number of autologous hematopoietic cells which had recovered.

Discussion

Here, we present the case of an adult male, who was lethally irradiated with neutrons from a critical fission reaction.[11] The accidents at the Chernobyl and Soreq nuclear power plants[1,2] suggest that bone marrow transplantation has only a limited role in the treatment of victims of radiation. The limitation stems from a lack of HLA-matched donors, a requirement for additional immunosuppression and the risk of GVHD. Given these experiences, transplants should probably be considered only for victims who have received doses in the range of 8–12 Gy without serious skin injuries, severe internal contamination or conventional injuries.[2] We selected umbilical cord blood as the hemato-

Table 3 Chromosomal analysis of BM and PBMNCs after PHA blastoid formation

Iliac BMMNCs					
Days post-transplant					107
SCA					10
46,XY,t(4;11)(q23;q24) or 46,XY,der(4)t(4;11)(q23;q24),der(11)(t(4;11)add(4)(q31)					5
46,XY,add(2)(p11),−5,−7,add(12)(q24),−17,+mar4,+mar5,+mar6					3
46,XY,t(3;3)(p14;p23)					2
Total					20
PBMNCs					
Days post-transplant	46	51	58	95	7
SCA		1	4		11
46,XY	3	2	9		3
46,XY,t(4;11)(q23;q24) or 46,XY,der(4)t(4;11)(q23;q24),der(11)(t(4;11)add(4)(q31)					2
46,Y,t(X;13)(p22;q12),t(7;14)(p13;q32)					2
46,XY,t(1;3)(q11;p11),del(6)(q23q25),add(7)(q11),−8,add(10)(p11),−14,−17,−19,+mar1,+mar2,+mar3,+mar4					2
Total	3	3	13	0	20

SCA = single cell abnormalities.

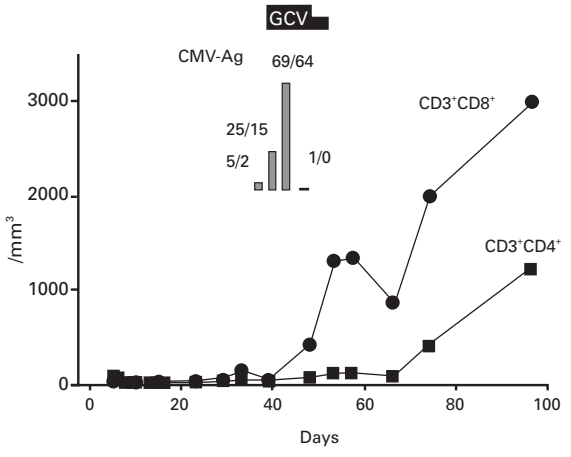

Figure 4 Absolute T cell number before and after CBT: ●, absolute number of $CD3^+CD8^+$ cells; ■, absolute number of $CD3^+CD4^+$ cells; CMV-Ag, CMV antigenemia; GCV, ganciclovir. Bars and numbers in the inset show the results of CMV-Ag.

poietic stem cell source to avoid donor acquisition delay and fatal GVHD. During the optimum time for stem cell transplantation, we closely observed our patient as there was no hope of autologous hematopoietic recovery according to the results of dosimetry and the clinical manifestations.

Umbilical cord blood T cells contain a relatively large number of naive T cells and produce fewer inflammatory cytokines, resulting in an amelioration of acute GVHD.[6] In clinical studies, a low incidence of and less acute GVHD with CBT have been reported.[4,5,7–9] Rocha *et al*[9] described a statistically significant reduction in the incidence of aGVHD and cGVHD in CBT recipients from HLA-matched siblings, compared to that seen with bone marrow. We previously described that the lower incidence of acute GVHD was related to the reduced post-membrane phosphorylation activities and Fas ligand (FasL) cell surface expression on cord blood T cells compared to that on adult PB T cells.[12] The absence of acute GVHD in our patient may be related not only to these characteristics of umbilical cord blood T cells but also to the donor/recipient mixed chimerism.[13,14] Storb *et al* in Seattle established the ideal stable mixed chimeric state between donor and recipient using a canine model.[15–19] Storb *et al* also described the use of stable mixed chimerism in the treatment of non-malignant hematological disorders, including hemoglobinopathies (β-thalassemia), inherited T cell deficiencies,[18] and autoimmune disorders.[19] In the non-malignant settings as with bone marrow failures and ARS, donor-derived hematopoiesis sufficiently compensates the defective host-derived hematopoiesis with mixed chimerism, without a risk of GVHD.

Dysfunctional regulation of FasL[12] may also induce a less potent graft-protecting effect, destroying recipient residual bone marrow cells and allowing autologous reconstitution. Rubinstein *et al*[8] reported 13 recipients with autologous reconstitution among 562 unrelated umbilical cord blood recipients (2.3%) after myeloablative conditioning. Decreased cytotoxic properties of CB T cells may contribute to autologous reconstitution after CBT.

In our case, ARS recrudesced after IS taper and granulocyte recovery. Further continuation of IS would be needed for the treatment of ARS and for the maintenance of stable mixed chimerism. We discussed the indication for granulocyte transfusions until engraftment after CBT in this case, but we did not undertake this, because of the danger of worsening the ARS. Only intensive antibiotic therapy, using vancomycin and arbekacin was given. The worsening of the cutaneous ARS after neutrophil recovery supports the integrity of this decision.

Many studies show a correlation between transfused cell numbers and the time to engraftment of the CBT.[4,7–9] The time required to reach an absolute neutrophil count greater than 0.5×10^9/l ranged from 25 to 42 days among recipients who were transplanted with 2×10^7 nucleated cells/kg of umbilical cord blood in the New York experience.[8] In the present case, recovery was faster than we had predicted. This recovery may be attributed to hematopoietic growth factors (G-CSF/EPO/TPO) although the effectiveness of this combination of growth factors has not yet been established.

Although several studies have examined T cell recovery after CBT,[4,10,20–22] little is known about immune reconstitution after myeloablative doses of irradiation. We documented the early immune reconstitution after a two loci HLA-mismatched CBT in an adult recipient compared to that seen with bone marrow, avoiding the use of ATG and mPSL in the conditioning and GVHD prophylaxis. A lower incidence of, and reduced GVHD may permit early termination of immunosuppressive therapy, resulting in early immune reconstitution after CBT.[10] Thomson *et al*[20] examined immune recovery after unrelated umbilical cord blood transplantation in childhood. They reported similar immune recovery after CBT, without delayed $CD8^+$ T cell recovery, compared to that reported for other stem cell sources. However, there are some reports of delayed immune recovery after CBT, in which transplant procedures involved ATG and mPSL for conditioning and GVHD prophylaxis.[21,22] In the present case, hematopoesis was supported by autologous hematopoietic recovery, and not comparable to previous studies that attained donor-derived complete chimerism. However, dysfunctional immunological reconstitution was observed soon after CBT, concurrently with irradiated autologous hematopoietic recovery. The profile of immunological reconstitution is summarized as follows: relatively normal numbers of $CD3^+CD4^+$, $CD3^+CD8^+$, normal cytokine mRNA expression in PBMNCs, defective mitogenic responses and impaired reactivity against third-party allogeneic PBMNCs of patient-derived PBMNCs. Immunoglobulin-producing capacities were suppressed until 4 months after irradiation. These results indicated severe impairment of immune function although autologous hematopoiesis recovered after ARS.

Skin grafts were obtained from a single cadaver donor not tested for HLA alleles. However, these grafts survived until the patient's death. The engraftment rate of cultured allografts is usually more than 40% in our experience, so we were able to obtain better results than was our prediction. The successful skin engraftment may be ascribed to post-radiation immunosuppression including a lack of mitogenic or allo-MLR responses of T cells.

In summary, CB is a useful stem cell source because of the potential for rapid acquisition, and the low incidence of GVHD associated with its use.[3–10,23] The relatively weak cord blood T cell-derived effect via down-regulating FasL[12] may induce donor/recipient mixed chimeric tolerance, which allows autologous hematopoietic reconstitution and ameliorates the risk of GVHD. Avoidance of transplant-related mortality including GVHD, and protection from opportunistic infections are the purposes of clinical intervention for ARS. We discontinued further IS therapy as we were afraid of infection, but the subsequent graft rejection was complicated by a severely immunosuppressed state, because the recovered autologous hematopoietic cells had aberrant chromosomes, and severely impaired immune functions, as the result of the fatal neutron irradiation. Donor-specific tolerance without non-specific immunosuppression established by stable mixed chimerism is the ideal platform for intervention against ARS. Antigen-specific immunosuppression is desirable for the suppression of HVG reaction.[24]

We were able to temporarily rescue the hematopoietic and dermal stem cells by allogeneic transplantation. However, the targeted organs of ARS are not limited to only bone marrow and skin, but the lungs and GI tract are also susceptible to ARS. To overcome the limitations of transplantation procedures, an improvement in 'regenerative medicine' that may compensate for the defective stem cells is desirable. Further evaluation is needed to improve stem cell rescue for nuclear accident victims.

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