engraftment failure after CBT and HPS might be one of the relevant mechanisms. HLA disparity in the graft-*versus*-host direction may augment allo-immune reactions, which evoke hypercytokinaemia, macrophage activation, and occasionally result in establishment of HPS. Indeed, most of our patients received cord blood units with an HLA mismatch due to the limited availability of cord blood units with a sufficient cell dose, and received relatively less intensive GVHD prophylaxis using calcineurin inhibitor alone. Thus, the donor T cells in the grafts were more susceptible to stimuli of cytokines triggered by infections and tissue damage from preparative regimens. In most of the other reported series, methotrexate (MTX), anti-thymocyte globulin (ATG), steroid, or MMF was used along with calcineurin inhibitor for GVHD prophylaxis and there are little reports about HPS. More intensive immunosuppression may have a positive effect on preventing post-transplant immune reactions (Narimatsu *et al*, 2007b) and the development of HPS.

An optimal strategy has not been established to treat HPS, especially after HSCT. Although CS was administered at the discretion of the primary physician to 13 HPS patients to reduce macrophage activation, HPS was resolved in only three patients and all four who could tolerate a second rescue CBT achieved durable engraftment.

In conclusion, HPS is a significant complication associated with engraftment delay and failure following CBT. The development of HPS increased mortality rates after CBT, worsening the prognosis. The precise mechanism of HPS development after HSCT remains unknown, although several lines of evidence suggest that donor immune cells are critically involved and therefore a key. The identification of high risk patients, more intensified GVHD prophylaxis, close and careful follow-up and prompt differential diagnosis are important for managing HSCT–HPS and avoiding engraftment failure. More detailed data from patients who have undergone CBT as well as other types of transplantation are warranted to further understand the mechanisms behind the development of HSCT–HPS and to develop more effective prophylaxis and treatment for this complication.

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Authors' contribution

S. Takagi and K.M. performed research and extracted data; Y.O., K.O. and A.Y. reviewed histopathological findings; N.M. and S. Takagi performed statistical analysis; N.U. and S. Taniguchi reviewed study design and methods; K.I., A.H., M.T., H.Y., D.K., Y.M., E.K., S.S., T.M., S. Miyakoshi and S. Makino contributed to writing the paper.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation

Naofumi Matsuno,[1] Atsushi Wake,[1] Naoyuki Uchida,[1] Kazuya Ishiwata,[1] Hideki Araoka,[2] Shinsuke Takagi,[1] Masanori Tsuji,[1] Hisashi Yamamoto,[1] Daisuke Kato,[1] Yoshiko Matsuhashi,[1] Sachiko Seo,[1] Kazuhiro Masuoka,[1] Shigesaburo Miyakoshi,[1] Shigeyoshi Makino,[3] Akiko Yoneyama,[2] Yoshinobu Kanda,[4] and Shuichi Taniguchi[1]

[1]Department of Hematology, Toranomon Hospital, Tokyo; [2]Department of Infectious Diseases, Toranomon Hospital, Tokyo; [3]Department of Transfusion Medicine, Toranomon Hospital, Tokyo; and [4]Division of Hematology, Saitama Medical Center, Jichi Medical School, Saitama, Japan

Delayed engraftment or graft failure is one of the major complications after cord blood transplantation (CBT). To investigate factors impacting engraftment, we conducted a retrospective analysis of adult patients who underwent reduced-intensity CBT at our institute, in which preparative regimens mainly consisted of fludarabine, melphalan, and total body irradiation with graft-versus-host (GVH) disease prophylaxis using single calcineurin inhibitors. Among 152 evaluable patients, the cumulative incidence of neutrophil engraftment was 89%. High total nucleated cell and CD34$^+$ cell dose were associated with the faster speed and higher probability of engraftment. In addition, the degree of human leukocyte antigen (HLA) mismatch in the GVH direction was inversely associated with engraftment kinetics, whereas no statistically significant association was observed with the degree of HLA mismatch in the host-versus-graft direction. Similarly, the number of HLA class I antigens mismatched in the GVH direction, but not in the host-versus-graft direction, showed a negative correlation with engraftment kinetics. HLA disparity did not have significant impact on the development of GVH disease or survival. This result indicates the significant role of HLA disparity in the GVH direction in the successful engraftment, raising the novel mechanism responsible for graft failure in CBT. (Blood. 2009;114: 1689-1695)

Introduction

Recent studies have demonstrated cord blood transplantation (CBT) as a safe and feasible alternative to bone marrow (BM) or peripheral blood (PB) stem cell transplantation (SCT) in adults when no suitable related donor is available.[1-4] The incidence and severity of acute graft-versus-host disease (GVHD) after CBT have been low compared with those after unrelated donor BM transplantation,[1-4] permitting use of a mismatched unit as a graft. The use of CBT has also been increasing because of the potential advantage of rapid availability and the lower risk to donors. The development of reduced-intensity (RI) conditioning regimens for transplantation, which results in less toxicity and depends largely on graft-versus-tumor effects rather than high-dose therapy to eliminate malignant cells, has been shown to allow elderly patients to undergo allogeneic transplantation.[5,6] We and other groups have reported the feasibility of RI-CBT for adult patients with advanced hematologic diseases.[7-12]

Despite the obvious advantage of CBT, high treatment-related toxicity has been observed, which precludes the application of CBT as a primary graft source. One of the major complications of CBT is delayed engraftment or graft failure. Thus far, several factors have been found to impact engraftment, including total nucleated cell (TNC) dose, CD34$^+$ cell dose, and human leukocyte antigen (HLA) disparity.[13-15] Here, we report the results of a retrospective analysis of 163 adult patients who underwent RI-CBT at our institute, which revealed, for the first time, the importance of HLA disparity in the graft-versus-host (GVH) direction, adding a new viable factor in choosing cord blood (CB) units as transplantable grafts.

Methods

Study patients

This study included adult patients with hematologic malignancies who underwent RI-CBT as their first allogeneic SCT at Toranomon Hospital between January 2002 and December 2006 consecutively. Twenty-nine patients who had active serious infection or showed an Eastern Cooperative Oncology Group performance status of 3 or 4 before transplantation were not eligible for this study because of differences in transplantation procedures or supportive care resulting from serious organ dysfunction and active infection. Then, the remaining 163 consecutive patients were reviewed. All patients had diseases that were incurable with conventional treatments, lacked suitable sibling or unrelated donors, and were considered inappropriate for conventional allo-SCT as they were older than 50 years and/or had organ dysfunction (often attributable to previous intense chemotherapy and/or radiotherapy). Characteristics of the 163 patients are summarized in Table 1.

For disease status, those with hematologic malignancies in the first or second complete remission at the time of transplantation, those in the chronic phase or accelerated phase of chronic myeloid leukemia, and those with refractory anemia of myelodysplastic syndrome were defined as being at standard risk (n = 32), whereas those in other situations were defined as being at high risk (n = 131). All patients received a single CB unit. All patients provided written informed consent in accordance with the Declaration of Helsinki, and the study was conducted in accordance with the requirements of the Institutional Review Board of Toranomon Hospital.

Donor selection

CB units were obtained from the Japanese Cord Blood Bank Network. All CB samples, as well as the patient's blood samples, were serologically typed for HLA-A, -B and -DR antigens before transplantation. Alleles at the HLA-A, -B,

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Table 1. Patient and cord blood characteristics

Variable	Value
No. of patients	163
Median age, y (range)	55 (17-79)
Sex: male/female, no. of patients	98/65
Primary diseases, no. of patients	
Acute lymphoblastic leukemia	20
Acute myeloid leukemia	63
Chronic myelogenous leukemia	5
Myelodysplastic syndrome	12
Malignant lymphoma	39
Adult T-cell leukemia/lymphoma	18
Multiple myeloma	2
Others	4
Risk of underlying disease, no. of patients: standard/high	32/131
Preparative regimens, no. of patients	
Flu + Mel + TBI 2-8 Gy	135
Flu + BU + TBI 4-8 Gy	18
Flu + Mel	6
Flu + BU	4
Median no. of infused nucleated cells, 10^7/kg (range)	2.68 (1.82-4.83)
Median no. of infused CD34$^+$ cells, 10^5/kg (range)	0.76 (0.05-4.40)
Blood-type mismatch, no. of patients: match/mismatch	47/116
HLA antigen mismatch, no. of patients	
0	3
1	24
2	136
GVHD prophylaxis, no. of patients	
Cyclosporine A alone	73
Tacrolimus alone	90

Flu indicates fludarabine; Mel, melphalan; TBI, total body irradiation; and BU, busulfan.

and -DRB1 loci were identified by high-resolution DNA typing in 107 pairs because HLA typing of alleles was not routinely performed in Japanese CB banks. In 127 pairs, HLA-A and -B antigens were identified by serologic typing and HLA-DRB1 alleles were determined by high-resolution DNA typing. CB grafts had at most 2 mismatches for HLA-A, -B, and -DR antigens and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. Mismatch was counted separately in the GVH and host-versus-graft (HVG) direction, respectively. HLA mismatch in the GVH direction was defined when the recipient's antigens or alleles were not shared by the donor, whereas HLA mismatch in the HVG direction was defined when the donor's antigens or alleles were not shared by the recipient.

Transplantation procedures

Pretransplantation conditioning regimens varied and were determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. All patients received purine analog-based preparative regimens. The majority of patients (n = 119) received preparative regimens consisting of fludarabine 125 mg/m^2, melphalan 80 mg/m^2, and 4 Gy total body irradiation (TBI). Patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of melphalan. GVHD prophylaxis was carried out using a continuous infusion of cyclosporine A 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patients could tolerate oral administration.

Supportive care

All patients were treated in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis. Fluoroquinolone, azole, and acyclovir were administered to prevent bacterial, fungal, and herpes virus infection, respectively. Cytomegalovirus pp65 antigenemia was monitored weekly. Hemoglobin and platelet counts were maintained at more than 7 g/dL and at 10×10^9/L, respectively. Granulocyte colony-stimulating factor was administered intravenously from day 1 until neutrophil recovery became durable.

Definition of engraftment, GVHD, and survival

Date of engraftment was defined as the first of 3 consecutive days when the neutrophil counts exceeded 0.5×10^9/L. Patients who did not achieve this criterion at any time after transplantation were considered as primary graft failure. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10%. Acute and chronic GVHD was diagnosed and graded according to standard criteria.[16,17] Overall survival was calculated from the day of transplantation until death from any cause or last follow-up. Event-free survival was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. Final follow-up was conducted in December 2007, with a median follow-up of surviving patients being 29.0 months (range, 3.7-58.9 months).

Statistical methods

Cumulative incidence of neutrophil engraftment was calculated using the Gray method, treating death before engraftment or second transplantation as competing events.[18] Similarly, in the analysis of GVHD, death resulting from other causes or relapse leading to early withdrawal of immune suppression was considered competing risk. The probabilities of survival were estimated using the Kaplan-Meier method. Multivariate analysis was performed using the proportional hazards model. P values $< .05$ were considered statistically significant.

Results

Engraftment

Eleven of the 163 patients reviewed were not evaluable for the analyses of donor engraftment resulting from early death (before 28 days after

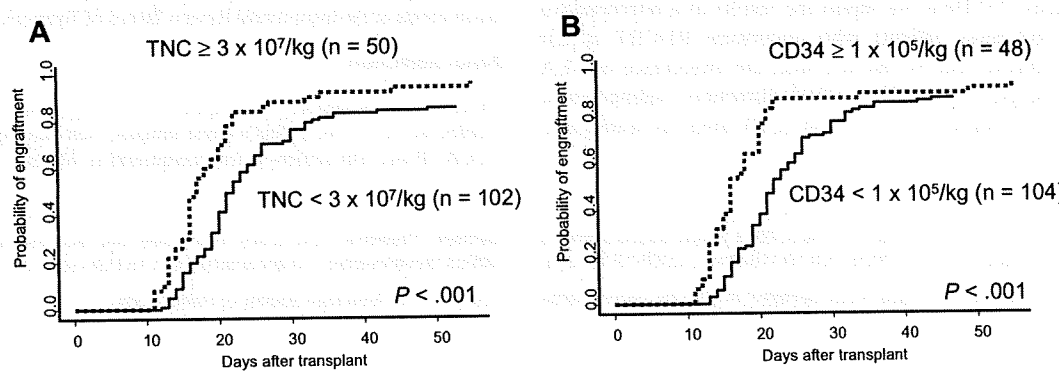

Figure 1. Cumulative incidence of neutrophil engraftment. (A) Effect of TNC dose. (B) Effect of CD34$^+$ cell dose.

transplantation) from disease progression (n = 1), infection (n = 6), and multiple organ failure (n = 4). Of 152 evaluable patients, 135 patients achieved neutrophil engraftment. The cumulative incidence of engraftment at day 60 was 89%, and the median time to engraftment was 20 days (range, 11-55 days). Chimerism analyses were performed in 125 of 135 patients who achieved engraftment using either PB or BM samples at the time of neutrophil recovery. All patients except for one who had residual leukemic cells in PB at the time of engraftment showed complete donor chimerism (> 90%). The median length of time required to donor chimerism was 22 days (range, 11-55 days).

Age, recipient sex, risk of underlying disease, blood type mismatch, and GVHD prophylaxis did not affect engraftment kinetics (data not shown). TNC more than or equal to 3×10^7/kg was associated with a significantly higher probability of engraftment ($P < .001$), with the median time to engraftment of 16.5 days (range, 11-55 days) compared with 21 days (range, 12-49 days) for those who received less than 3×10^7/kg (Figure 1A). Similarly, $CD34^+$ cell dose more than or equal to 10^5/kg was associated with a significantly faster engraftment ($P < .001$) than those who received less than 10^5/kg (Figure 1B).

The cumulative incidence of engraftment and the time to engraftment according to the degree of HLA mismatch are shown in Table 2. Patients who had 0 and 1 antigen mismatch with the grafts were combined, considering the small number of patients in 0 mismatch group and comparable rate of engraftment and time to neutrophil recovery between 0 and 1 antigen-mismatched group (Figure 2A-B), and were compared with those of 2 antigens mismatched. Although patients with 0 or 1 antigen mismatch showed a trend toward superior engraftment kinetics compared with patients with 2 antigens mismatched, the differences did not reach statistical significance (Figure 2A; Table 2). We further analyzed the influence of HLA disparity on engraftment in both the HVG and GVH direction. In the HVG direction, the cumulative incidence of engraftment at day 60 was 93% in 0 or 1 antigen mismatch and 87% in 2 antigens mismatched ($P = .4$, Table 2). In the GVH direction, however, the cumulative incidence of engraftment was 96% in 0 or 1 antigen mismatch and 85% in 2 antigens mismatched ($P < .001$, Figure 2B; Table 2), demonstrating that HLA antigen disparity in the GVH direction was significantly associated with engraftment kinetics. As shown in Figure 2C, HLA antigen disparity in the HVG direction did not contribute to engraftment kinetics in patients with 0 or 1 antigen mismatch in the GVH direction, as was also observed in those with 2 antigens mismatched in the GVH direction. Although the number of patients in each group was small, patients with 0 or 1 mismatch in the GVH direction but 2 mismatches in the HVG direction (n = 28) showed a trend toward superior engraftment kinetics compared with patients with 0 or 1 mismatch in the HVG direction but 2 mismatches in the GVH direction (n = 18; $P = .07$). This finding may indicate that HLA disparity in the GVH direction plays a greater role in engraftment than that in the HVG direction.

In addition to the degree of mismatch, we analyzed the significance of class I (HLA-A, -B) or class II (HLA-DR) mismatch (Table 2). The number of class I antigens mismatched in the GVH direction showed a negative correlation with the probability and the speed of engraftment ($P = .006$, Figure 2D), but not in the HVG or both directions. More specifically, the presence of HLA-B antigens mismatched in the GVH direction was significantly associated with inferior engraftment kinetics ($P = .04$). To the contrary, HLA-DR antigen mismatch did not influence engraftment kinetics in either the HVG or the GVH direction.

The cumulative incidence of engraftment was also assessed using 120 pairs who had HLA-A, -B antigens and -DRB1 allele information available (Table 2). Patients with 0 or 1 mismatch showed better engraftment kinetics compared with those with 2, 3, or 4 mismatches in the GVH direction, which was about to be significant statistically ($P = .05$), whereas HLA mismatch in the HVG direction did not show significant impact on engraftment.

HLA allele mismatch at the HLA-A, -B, and -DR was examined in 102 pairs. In the GVH direction, the cumulative incidence of engraftment was 94% in 0 or 1 allele mismatch, 88% in 2 alleles mismatched, and 80% in 3 to 5 alleles mismatched ($P = .05$), showing that alleles mismatched in the GVH direction could be inversely associated with engraftment kinetics (Table 2). In contrast, allele disparity in the HVG direction did not affect engraftment (Table 2). When HLA-A, -B, and -DR alleles were analyzed independently, no statistically significant differences were observed in any allele tested in either the GVH or HVG direction (data not shown).

Multivariate analyses revealed that low TNC dose ($< 3 \times 10^7$/kg) and HLA antigens mismatched in the GVH direction (0 or 1 vs 2 antigens mismatched) were significantly associated with inferior engraftment kinetics, when age, recipient sex, risk of underlying disease, GVHD prophylaxis, and blood type mismatch were included as covariates ($P = .002$ and $P = .004$, respectively).

Clinical features of graft failure

There were 17 patients who failed to achieve engraftment: 8 males and 9 females, median age of 55 years (range, 17-68 years), high-risk diseases in 12 patients. Median TNC dose of CB grafts was 2.36×10^7/kg (range, $2.01\text{-}3.40 \times 10^7$/kg), and median $CD34^+$ cell dose was 0.59×10^5/kg (range, $0.30\text{-}1.38 \times 10^5$/kg). Nine of them died before engraftment because of disease progression (n = 2), infection (n = 5), multiple organ failure (n = 1), and idiopathic pneumonia syndrome (n = 1). The remaining 8 patients received a second RI-CBT at a median of 34 days (range, 28-49 days) after first RI-CBT, and 3 of them were alive in remission.

Among those who did not achieve engraftment, chimerism analyses in the BM early after transplantation were performed on 8 patients (median, 12 days; range, 10-17 days). Of those, 4 achieved complete donor chimerism, one had mixed chimerism (60% donor type), and 3 patients showed recipient chimerism. Four of 5 patients with donor dominant chimerism showed hemophagocytosis in the BM. On the other hand, all 3 patients with recipient chimerism did not show hemophagocytosis.

GVHD and survival

Among 134 evaluable patients, the cumulative incidence of acute GVHD of grade II to IV was 43%. The incidence of acute GVHD according to HLA disparity in the GVH direction was summarized in Table 3. Patients with 2 antigens mismatched showed a trend toward higher incidence of acute GVHD II-IV ($P = .08$). The number of class I or class II antigens mismatched had no correlation with the incidence of acute GVHD. Similarly, HLA disparity in the allele level was not significantly associated with the incidence of acute GVHD. Among 66 evaluable patients, the cumulative incidence of chronic GVHD was 51%. The degree of HLA mismatch was not significantly associated with the incidence of chronic GVHD (data not shown). Other pretransplantation factors, including age, infused cells, and GVHD prophylaxis, did not affect the incidence of GVHD. Overall survival and event-free survival at 2 years were 35% and 30%, respectively. HLA disparity in the GVH direction, as well as in the HVG direction, did not influence overall survival and event-free survival (Table 3; and data not shown).

Table 2. Univariate analyses of engraftment kinetics according to HLA disparity

	Neutrophil engraftment				
No. of HLA mismatches	n	Cumulative incidence, %	Median day	Range	*P*
HLA-A, -B, -DR (antigen)					.09
0 + 1	23	91	17	11-30	
2	129	89	20	11-55	
HLA-A, -B, -DR (antigen, HVG)					.4
0 + 1	43	93	19	11-55	
2	109	87	20	11-49	
HLA-A, -B, -DR (antigen, GVH)					< .001
0 + 1	53	96	19	11-36	
2	99	85	20	11-55	
HLA-A, -B (class I antigen)					.1
0	13	92	17	12-30	
1	86	91	20	11-44	
2	53	85	20	11-55	
HLA-A, -B (class I antigen, HVG)					.4
0	22	96	18	12-36	
1	86	89	20	11-55	
2	44	84	20	11-49	
HLA-A, -B (class I antigen, GVH)					.006
0	23	95	17.5	11-36	
1	88	91	20.5	11-44	
2	41	81	20	12-55	
HLA-A (antigen)					.7
0	87	89	19	11-44	
1 + 2	65	89	20	11-55	
HLA-A (antigen, HVG)					.8
0	96	89	20	11-55	
1 + 2	56	89	20	11-49	
HLA-A (antigen, GVH)					.2
0	103	90	19	11-44	
1 + 2	49	86	20	13-55	
HLA-B (antigen)					.07
0	36	94	19	12-34	
1 + 2	116	87	20	11-55	
HLA-B (antigen, HVG)					.06
0	45	95	19	12-36	
1 + 2	107	86	20	11-55	
HLA-B (antigen, GVH)					.04
0	42	95	18.5	11-36	
1 + 2	110	86	20	11-55	
HLA-DR (antigen)					.4
0	70	87	20	11-55	
1 + 2	82	90	19.5	11-44	
HLA-DR (antigen, HVG)					.7
0	76	88	20	11-55	
1 + 2	76	89	20	11-44	
HLA-DR (antigen, GVH)					.8
0	83	88	20	11-55	
1 + 2	69	90	20	11-44	
HLA-A, -B (antigen), -DR (allele)					.5
0 + 1	13	92	18	14-30	
2	63	84	20	11-47	
3 + 4	44	86	20	11-49	
HLA-A, -B (antigen, HVG), -DR (allele, HVG)					.2
0 + 1	25	96	18	11-32	
2	54	80	20	11-44	
3 + 4	41	90	20	11-49	
HLA-A, -B (antigen, GVH), -DR (allele, GVH)					.05
0 + 1	26	96	18	11-36	
2	57	84	19.5	11-49	
3 + 4	37	84	20	11-34	

Table 2. Univariate analyses of engraftment kinetics according to HLA disparity (Continued)

	Neutrophil engraftment				
No. of HLA mismatches	n	Cumulative incidence, %	Median day	Range	*P*
HLA-A, -B, -DR (allele)					.4
0 + 1	10	90	18	14-30	
2	36	86	20	11-44	
3 + 4 + 5	56	84	19	11-49	
HLA-A, -B, -DR (allele, HVG)					.3
0 + 1	19	94	19	11-32	
2	34	79	20	13-44	
3 + 4 + 5	49	86	21	11-49	
HLA-A, -B, -DR (allele, GVH)					.05
0 + 1	16	94	17	11-30	
2	40	88	20	11-44	
3 + 4 + 5	46	80	20	11-49	

Discussion

Delayed hematopoietic recovery and graft failure are significant concerns in adult CBT. In the present study, median time to engraftment was 20 days, which was comparable with that reported in previous studies.[1,4,7,19] These data indicate that our pretransplantation conditioning regimens, consisting mainly of fludarabine, melphalan, and 4 Gy TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert reasonable immunosuppressive effects that allow rapid hematopoietic recovery after CBT. The engraftment was durable except for disease progression.

Almost all reports on CBT have demonstrated the profound impact of infused cell dose on engraftment.[13,14,20] We showed that both high numbers of TNCs and $CD34^+$ cells were favorably associated with time to engraftment and the probability of engraftment, confirming previous findings on the association of cell dose with neutrophil recovery. Considering that $CD34^+$ cell dose reflects stem cell contents in the CB unit, stem cell dose is one of the major determinants of successful engraftment, as has been observed in the xenogeneic transplantation model.[21-23]

Although our results, demonstrating that HLA disparity in the GVH direction affected engraftment kinetics more than HLA disparity in the HVG direction, may seem paradoxical to the former notion of graft failure that results from graft rejection in most cases, they suggest a novel mechanism of graft failure in CBT. Previously, we have reported that a high incidence of noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9 in more than 50% of the patients receiving CBT.[8,24] We regarded this reaction as early onset of acute

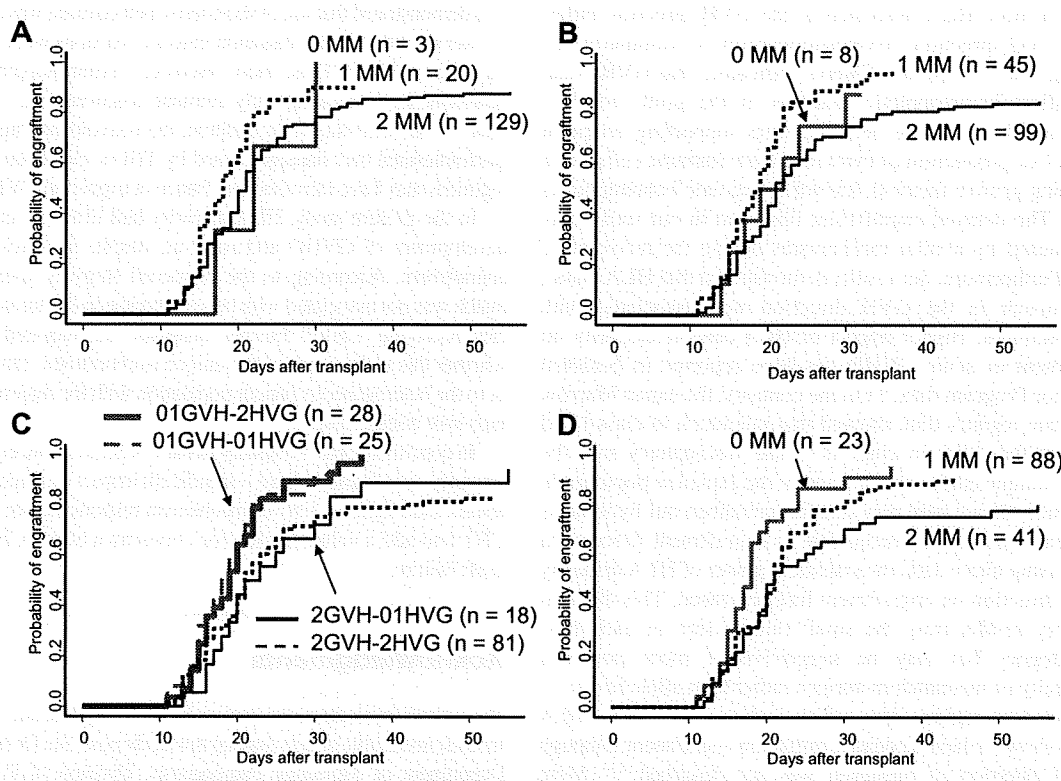

Figure 2. Cumulative incidence of neutrophil engraftment. MM indicates mismatch. (A) Effect of HLA antigen mismatch. (B) Effect of HLA antigen mismatch in the GVH direction. (C) Effect of HLA antigen mismatch according to mismatch both in the GVH and the HVG directions. 2GVH indicates 2 antigens mismatch in the GVH direction; 2HVG, 2 antigens mismatch in the HVG direction; 01GVH, 0 or 1 antigen mismatch in the GVH direction; 01HVG, 0 or 1 antigen mismatch in the HVG direction. (D) Effect of HLA class I antigen mismatch in the GVH direction.

Table 3. Univariate analyses of acute GVHD and survival according to HLA disparity in the GVH direction

	Acute GVHD II-IV			2-year overall survival		
No. of HLA mismatches in the GVH direction	n	Cumulative incidence, %	*P*	n	Survival rate, %	*P*
HLA-A, -B, -DR (antigen)			.08			.5
0 + 1	50	33		59	36	
2	84	48		104	35	
HLA-A, -B (class I antigen)			.5			.2
0	22	36		24	54	
1	80	42		96	32	
2	32	46		43	32	
HLA-DR (class II antigen)			.5			.9
0	71	38		91	32	
1 + 2	63	47		72	38	
HLA-A, -B (antigen), -DR (allele)			.4			1.0
0 + 1	25	32		29	38	
2	48	51		60	38	
3 + 4	30	44		38	39	
HLA-A, -B, -DR (allele)			.3			.4
0 + 1	15	27		16	56	
2	35	49		41	37	
3 + 4 + 5	36	51		50	35	

GVHD in which activated donor T cells secreted various cytokines.[25] HLA disparity in the GVH direction may augment alloimmune reactions, which evoke hypercytokinemia and macrophage activation and occasionally result in establishment of hemophagocytic syndrome, one of the major complications directly related to graft failure in recipients.[26-28] Indeed, a considerable number of patients showed hemophagocytosis in the BM with donor dominancy, leading to graft failure, even though we cannot exclude the possibility of graft rejection caused by recipient lymphocytes in some cases. In addition, among those who achieved donor cell engraftment, delayed neutrophil recovery was prominent for those with more HLA mismatch in the GVH direction rather than in the HVG direction. Myelosuppression is commonly observed during acute or chronic GVHD, indicating that GVHD can negatively affect hematopoietic function of the graft, possibly because of an attack on the hematopoiesis-supporting recipient stromal cells[29] or production of cytokines from immune cells, such as transforming growth factor-β, known to regulate hematopoiesis negatively.[30] The delayed engraftment observed in our study may have been caused by similar mechanisms during the recovery of donor cells. Furthermore, our results demonstrated that HLA class I antigen mismatch in the GVH direction was associated with inferior engraftment. Higher impact of HLA class II disparity on the development of acute GVHD has been reported in National Marrow Donor Program data.[31] On the contrary, the Japan Marrow Donor Program registry data showed that mismatch in class I had higher impact than that in class II.[32] The discrepancy may be explained by unique ethnic background of the Japanese population. The observation shown here may further strengthen our hypothesis that GVH reactions play a crucial role in engraftment process. In the analysis using allele data, the statistical power of HLA disparity in the GVH direction on engraftment had decreased. This discrepancy probably results from the small sample size in each mismatched category but may be suggestive of more powerful immunogenicity of mismatch in antigen rather than allele level.

In the Eurocord registry data, which includes 550 CBTs, HLA disparity was shown to have a negative impact on engraftment, although the effect of direction of mismatch was not described.[14,33] More specifically, it was reported from the Düsseldorf Cord Blood Bank and Eurocord-Netcord Registry that HLA-A locus high-resolution typing in the HVG direction was associated with reduced cumulative incidence of engraftment in 122 patients receiving CBT.[34] Several reasons may explain this discrepancy from our observations. First, patients included in our study received relatively uniform pretransplantation conditioning regimens consisting mainly of fludarabine, melphalan, and TBI, whereas those in the Eurocord database had more variable pretransplantation conditioning regimens. Second, all of our patients had GVHD prophylaxis using single calcineurin inhibitors, whereas most of those in the Eurocord Registry received additional chemicals or anti-thymocyte globulin. Many institutes use methotrexate,[35,36] mycophenolate mofetil,[19,37] corticosteroids,[13] or anti-thymocyte globulin[38,39] in combination with a calcineurin inhibitor as GVHD prophylaxis in CBT. Narimatsu et al demonstrated that use of short-term methotrexate was associated with a lower rate of posttransplantation immune reactions without compromising engraftment.[36] Thus, more intensive immunosuppression may be beneficial for controlling early immune reactions and overcoming the issue of HLA mismatch. In addition, the unavoidable high incidence of gastrointestinal tract damage caused by TBI or melphalan in preparative regimens may have increased the chance of triggering GVH reactions.[40]

In the present study, HLA disparity had little association with the development of GVHD and survival, despite its obvious impact on engraftment. According to the Eurocord Registry data, better HLA match was not associated with better outcome in hematologic malignancies receiving CBT.[20] Further analyses are required to determine whether this is the result of the unique immunologic immaturity of CB or to the heterogeneous patient population with the majority being in the high-risk disease status.

In conclusion, HLA disparity in the GVH direction, especially class I disparity, was found to have a significant impact on engraftment. These results shed light on a novel mechanism responsible for graft failure in CBT and add a valuable clue for choosing a better CB unit to avoid graft failure.

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Authorship

Contribution: N.M. and A.W. performed research and extracted data; A.Y. reviewed histopathologic methods; N.M. and Y.K. performed statistical analysis; N.U. and S. Taniguchi reviewed study design and methods; and K.I., H.A., S. Takagi, M.T., H.Y., D.K., Y.M., S.S., K.M., S. Miyakoshi, and S. Makino contributed to the writing of the paper.

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Correspondence: Naoyuki Uchida, 2-2-2 Toranomon, Minato-Ku, Tokyo 105-8470; e-mail: nuchida@toranomon.gr.jp.

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Umbilical Cord Blood Transplantation after Reduced-Intensity Conditioning for Elderly Patients with Hematologic Diseases

Naoyuki Uchida, Atsushi Wake, Shinsuke Takagi, Hisashi Yamamoto, Daisuke Kato, Yoshiko Matsuhashi, Tomoko Matsumura, Sachiko Seo, Naofumi Matsuno, Kazuhiro Masuoka, Eiji Kusumi, Koichiro Yuji, Shigesaburo Miyakoshi, Michio Matsuzaki, Akiko Yoneyama, Shuichi Taniguchi

Department of Hematology, Toranomon Hospital, Tokyo, Japan

Correspondence and reprint requests: Naoyuki Uchida, MD, 2-2-2 Toranomon, Minato-Ku, Tokyo 105-8470, Japan (e-mail: nuchida@toranomon.gr.jp).

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ABSTRACT

Although allogeneic hematopoietic stem cell transplantation is a potentially curative approach for advanced hematologic diseases, its application to elderly people is limited because of their comorbid physical conditions and lower chance of finding suitable related donors. Umbilical cord blood transplantation with reduced-intensity pretransplant conditioning (RI-UCBT) is 1 way to avoid these obstacles. We analyzed elderly patients aged 55 years and older with hematologic diseases who underwent RI-UCBT at our institute to assess feasibility and effectiveness of this treatment approach. Among the 70 patients included, 50 died, 74% of them from nonrelapse causes. Infection was the primary cause of death. Estimated overall survival and progression-free survival at 2 years were both 23%. In multivariate analyses, standard-risk diseases, age younger than 61 years, grade 0-II acute graft-versus-host disease, and the absence of preengraftment immune reaction were significantly associated with better overall survival. RI-UCBT is a potentially curative and applicable approach for elderly patients. Higher mortality, especially from nonrelapse causes, is the biggest problem to be solved to increase the feasibility of this approach.

KEY WORDS

Cord blood transplantation • Reduced intensity • Elderly patients • Hematologic diseases

INTRODUCTION

Although morbidity associated with hematologic malignant diseases in elderly patients is higher than that in younger patients [1], elderly patients are less likely to be candidates for allogeneic stem cell transplantation, because of the fact that they are more likely to have comorbid organ conditions, either clinically or subclinically, which result in a higher rate of procedure-related mortality [2], and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity conditioning (RIC) for transplants, which results in less toxicity and depends largely on graft-versus-tumor effects rather than high-dose therapy to eliminate malignant cells, has been shown to allow elderly patients to undergo allogeneic transplants [3-5]. The use of umbilical cord blood transplantation (UCBT) has been increasing because of the potential advantage of rapid availability and the lower risk of graft-versus-host disease (GVHD), thus permitting less stringent HLA matching [6,7]. The outcome of UCBT has been reported to be similar to unrelated bone marrow in the myeloablative setting [8-10]. UCBT with reduced-intensity pretransplant conditioning (RI-UCBT) for adults, mostly younger than 55 years old, has been increasingly reported, and has been shown to be applicable even in patients with a relatively low number of nucleated cells for their body weight [11-16]. However, little information has been available on whether elderly patients can tolerate slower engraftment, more infectious complications [17], and the unique preengraftment immune reaction (PIR) associated with UCBT [18,19]. PIR has been described by us and others [18,19], characterized

by the symptoms induced possibly by hypercytokinemia, which sometimes cause severe organ damage and fatal outcome. We therefore retrospectively evaluated the use of the RI-UCBT in patients aged 55 and older by analyzing engraftment, nonrelapse mortality (NRM), GVHD, progression-free (PFS), and overall survival (OS) to address the feasibility and effectiveness of this method in older patients.

PATIENTS, MATERIALS, AND METHODS

Patients

This study included patients aged 55 and older who underwent RI-UCBT at our institute from July 18, 2002 through October 28, 2005. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia (AA) refractory to standard immunosuppressive therapy, as well as if they were unable to find suitable related or unrelated bone marrow (BM)/peripheral blood (PB) donors within reasonable time periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse because of adverse cytogenetic abnormalities, have a prior hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia with excess of blasts or chronic myelomonocytic leukemia, or have refractory anemia with transfusion dependency and/or severe neutropenia. Patients with chronic myeloid leukemia (CML) had to be beyond the first chronic phase. Lymphoma patients had to be beyond the first remission except those with acute or lymphoma type adult T cell leukemia. Patients who had end-stage organ dysfunction (DLco <30% predicted or LVEF <35%), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent, and the study was approved by the appropriate institutional review boards.

Donor Selection

UCB units were obtained from Japanese Cord Blood Bank Network. HLA-A and HLA-B antigens were identified by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using polymerase chain reaction (PCR) sequence-specific primers. UCB grafts had at least 4 of 6 HLA-A, B antigens, and DRB1 alleles that were matched to the recipient and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. The median total nucleated cell number and median $CD34^+$ cell number were 2.8 (range: 1.8–5.2) $\times 10^7$/kg and 0.84 (0.11-3.28) $\times 10^5$/kg, respectively.

Patient Characteristics

Seventy consecutive patients were included in this study. Their characteristics are shown in Table 1.

Table 1. *Patient and Donor Umbilical Cord Blood Characteristics*

Characteristic	No. (%) of Patients
Sex	
Male	45 (64)
Female	25 (36)
Age (years)	
Median (range)	61 (55-79)
Age distribution (years)	
55 to 59	31 (44)
60 to 64	16 (23)
65 to 69	17 (24)
At least 70	6 (9)
Diagnosis	
AML	28 (40)
MDS	3 (4)
CML	4 (6)
ALL	11 (16)
NHL	8 (11)
ATL	12 (17)
MM	1 (1)
PCL	1 (1)
AA	2 (3)
HCT-CI	
0	24 (34)
1	25 (36)
2	11 (16)
3 or greater	10 (14)
History of prior chemotherapy	
Yes	59 (84)
No	11 (16)
History of prior documented infections	
Yes	15 (21)
No	55 (79)
Disease status	
Standard risk	15 (21)
High risk	55 (79)
Conditioning regimen	
Flu/Mel/TBI	65 (93)
Flu/Bu/TBI	4 (6)
Others	1 (1)
GVHD prophylaxis	
Cyclosporine A alone	37 (53)
Tacrolimus alone	33 (47)
HLA disparity to UCB	
5/6	9 (13)
4/6	61 (87)
Sex mismatch to UCB	
Yes	51 (73)
No	19 (27)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; MM, multiple myeloma; PCL, plasma cell leukemia; AA, aplastic anemia; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; UCB, umbilical cord blood: HCT-CI, hematopoietic cell transplantation-specific comorbidity index.

Of these 70 patients, 25 were women and 45 were men. Their median age was 61 years (range: 55-79 years). The patients' diagnoses included acute myeloid leukemia (AML; n = 28), acute lymphoblastic leukemia (ALL; n = 11), MDS (n = 3), CML (n = 4), non-Hodgkin lymphoma (NHL; n = 8), adult T cell

leukemia (n = 12), plasma cell leukemia (n = 1), multiple myeloma (n = 1), and AA (n = 2). Three patients had previous autologous hematopoietic cell transplantation. For disease status, those with hematologic malignancies in first or second complete remission at the time of transplant, those in the chronic phase or accelerated phase of CML, those with refractory anemia of MDS, and those with nonmalignant diseases were defined as being at standard risk (n = 15), whereas those in other situations were defined as being at high risk (n = 55). Patients were assessed for their comorbidity by the previously reported scoring system [20].

Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditioning varied, and was determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. Sixty-five patients underwent conditioning regimens with 125-180 mg/m^2 of fludarabine (Flu; 25 mg/m^2 for 5 days or 30 mg/m^2 for 6 days), along with 80 mg/m^2 of melphalan (Mel; 40 mg/m^2 for 2 days) and total-body irradiation (TBI) at a total dose of 4 Gy for 63 and 2 Gy for 2. Four patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of Mel. One patient underwent a conditioning regimen with thiotepa (5 mg/kg for 2 days) in addition to 125 mg/m^2 of Flu and 80 mg/m^2 of Mel, because of the urgent transplant schedule that did not allow access to TBI. Valproate sodium (300 mg/day) was administered to all patients who received Bu. Immunosuppressive therapy with cyclosporine A (CsA, 3 mg/kg continuous infusion, aiming for a serum concentration of 250-400 ng/mL) or tacrolimus (Tac, 0.03 mg/kg continuous infusion, aiming for 12-17 ng/mL) was started on day −1. CsA was used for patients in the early phase of this study, and, based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA.

Supportive Care

Prophylactic antibiotics, including fluorquinolone, fluconazole, and acyclovir, were used routinely. Patients received ganciclovir or foscarnet for any sign of a cytomegalovirus reactivation, such as isolation of CMV or detection of viral proteins (pp65) or nucleic acid in any body fluid or tissue specimen. *Pneumocystis jiroveci* prophylaxis included trimethoprim-sulfamethoxazole as first-line therapy.

Definition of Engraftment, Preengraftment Immune Reaction, and End Points

OS ands PFS were computed from the date of transplantation. Engraftment was defined as absolute neutrophil count >0.5 × 10^9/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-mismatched pairs, PCR for variable number of tandem repeats was used with donor cells detected at a sensitivity of 10%. Whole blood or BM cells were assessed at the time of granulocyte engraftment. PIR was characterized by the presence of at least 2 of the following symptoms with no direct consequences of infection or adverse effects of medication 6 or more days before engraftment, as described previously [12,18]; a high fever (>38.5°C), skin eruptions, diarrhea, jaundice (serum levels of total bilirubin >2.0 mg/dL), or body weight gain >10% of baseline. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for AML, ALL, CML, or MDS was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for NHL was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD (aGVHD, cGVHD) were defined and graded by standard criteria [21]. The following factors were considered potential predictors of outcomes: recipient's age, disease risk (standard versus high), ECOG performance status, HCT-specific comorbidity index score, history of prior chemotherapy (all cytoreductive chemotherapy excluding hydroxyurea and imatinib mesylate), history of prior documented infections (infectious episode with positive culture results for bacterial or yeast infections, and at least probable diagnosis of mold infection by EORTC/NIH-MSG criteria [22]), number of total nucleated cord blood cells, number of CD34$^+$ cells, HLA disparity, conditioning regimen, GVHD prophylaxis, grade of aGVHD, and the presence or absence of PIR.

Statistical Methods

OS was calculated from the day of transplantation until death from any cause or last follow-up. Disease-free survival (DFS) was calculated from the day of transplantation until relapse or death from any cause or last follow-up. The probabilities of survival and DFS were estimated and plotted using the Kaplan-Meier method [23]. Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks [24]. Similarly, in the analysis of GVHD rates, death because of other causes or relapse leading to early withdrawal of immune suppression were considered competing risks. The effect

of various patient and disease categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. Cumulative incidence curves were drawn using Gray's method [25].

RESULTS

Engraftment

Ten of the 70 patients were not evaluable for donor engraftment because of early death (before 28 days posttransplant) from disease progression (n = 1), infection (n = 7), and complications of central nervous system (n = 2). Of the 60 evaluable patients, the cumulative incidence of primary donor engraftment was 92% at a median of 18 days after transplantation (range: 11-53 days). Platelet recovery $>20 \times 10^9$/L was observed in 38 patients (63%), at a median of 35 days (range: 25-95 days). All patients required transfusions of platelets and red blood cells. Recovery of neutrophil counts $>0.5 \times 10^9$/L did not occur in 5 patients who survived beyond 28 days posttransplant; these patients were classified as primary graft failures. Two of these patients received secondary RI-UCBT and died of infection. The remaining 3 patients died of infection. All engrafting patients without BM relapse were complete donor chimeras beyond 1 month after transplantation (data not shown). Remarkably, all 3 evaluated patients of 10 who died before day 28 showed complete donor chimerism (94%, 100%, and 94.6% on days 12, 15, and 20 posttransplant, respectively).

PIR and GVHD

Forty-three patients experienced clinical symptoms defined as PIR, as described previously [12,18]. Patients who received Tac as GVHD prophylaxis tended to have a lower chance of experiencing PIR compared with those who received CsA, although differences were not statistically significant (53% versus 72%, respectively; $P = .1$).

Among 54 evaluable patients, 33 patients (61%) developed aGVHD of grade II or higher, including 23 patients (43%) who developed that of grade III or IV. Of the 30 patients who survived longer than 100 days posttransplant, 12 (40%) developed cGVHD, including 7 with limited and 5 with extensive form (Table 2).

Survival, Disease Progression, and NRM

At the time of analysis, 20 of 70 patients survived a median of 512 days (range: 103-1213 days) after transplantation. The Kaplan-Meier estimates of OS and PFS at 2 years were both 23% (Figure 1). The median OS time was 114 days (range: 7–1213 days), and the median PFS time was 92 days (range: 7–1213 days).

Table 2. *The Incidence and Severity of Graft-versus-Host Disease (GVHD)*

	Patients (n = 54)
	No. (%)
Acute GVHD	45 (83)
Grade II-IV	33 (61)
Grade III-IV	23 (43)
	Patients (n = 30)
	No. (%)
Chronic GVHD	12 (40)
Limited	7 (23)
Extensive	5 (17)

Eighteen patients (26%) showed progression of the underlying disease at a median of 134 days (range: 13–785 days) after transplantation, and 15 of these patients died of their disease.

Thirty-seven patients died of nonrelapse causes (Table 3). Nineteen of them were from infections, which was the leading cause of NRM. Among 33 deaths observed before day 100 posttransplant, 30 were from nonrelapse causes and 3 from disease progression. The cumulative incidences curves of NRM and disease progression are shown in Figure 2.

Factors Contributing to OS and NRM

In univariate analyses, survival was associated with recipient's age ($P = .01$), disease risk ($P < .01$), aGVHD ($P < .01$), and PIR ($P < .01$), with favorable outcomes in younger recipients (<61 years), those with standard risk, those with lower grade aGVHD (grade 0-II), and those without PIR (Figure 3A-D). Potential risk factors such as ECOG performance status, HCT-specific comorbidity index score, history of prior documented infection, history of prior chemotherapy, HLA disparity,

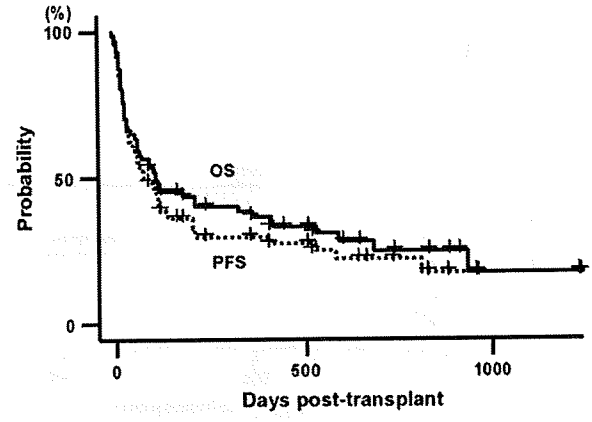

Figure 1. OS and PFS estimates for 70 patients with hematologic diseases treated with RI-UCBT.

Table 3. *Causes of Death*

	Patients (n = 70)
	No. (%)
NRM	37 (53)
Infection	19 (27)
GVHD	9 (12)
IP	4 (6)
TMA	3 (4)
Others	2 (3)
Relapse	13 (19)
Total	50 (71)

NRM indicates nonrelapse mortality; GVHD, graft-versus-host disease; IP, interstitial pneumonia; TMA, thrombotic microangiopathy.

sex mismatch, number of infused cells, number of infused $CD34^+$ cells, and cGVHD did not reach statistical significance.

In the Cox regression analyses, recipient's age equal to or older than 61 (hazard ratio [HR] = 3.33; 95% confidence interval [CI] = 1.39-7.14; P = .006), high risk disease (HR = 3.33; 95% CI = 1.01; 8.33 P = .049), grade III-IV aGVHD (HR = 2.5; 95% CI = 1.28; 5.88 P = .0002), and the presence of PIR (HR = 2.5; 95% CI = 1.14; 6.25 P = .023) were associated with statistically worse OS (Table 4). No other factors were significantly or suggestively associated with OS.

Regarding toxicity, multivariate analyses revealed that GVHD prophylaxis (HR = 3.9, 95% CI = 1.3-11.6 for CsA versus Tac; P = .01) and aGVHD (HR = 5.7, 95% CI = 2.1-15.7 for grade III–IV versus 0-II; P = .001) were associated with NRM.

DISCUSSION

This study was undertaken to evaluate engraftment and toxicities in elderly patients with advanced hematologic diseases who received UCBT matched for at least 4 loci of HLA-A, -B, and –DRB1 using a nonmyeloablative regimen.

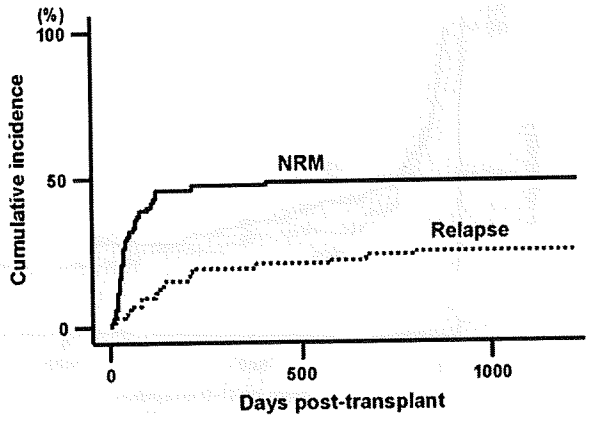

Figure 2. NRM and disease progression. Cumulative incidence estimates of NRM and disease progression for all 70 patients.

Several observations were made. First and foremost, RI-UCBT was a feasible treatment strategy for elderly patients with a successful engraftment rate of 92% without secondary graft failure except disease progression. The average interval between transplant and neurtophil recovery to 500/μL was 18 days, which is comparable to previously reported in RIC [11,12]. The chimerism study confirmed rapid engraftment of donor cells in all engrafted patients. Together with the fact that all 3 evaluated patients who died before day 28 already achieved complete donor chimerism, these data indicate that our pretransplant conditioning regimens, mainly consisting of Flu, Mel, and TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert sufficient immunosuppressive effects that allow engraftment of CB cells. Compared to the conditioning regimen containing cyclophosphamide reported from Minnesota group [11], which allow mixed chimeric state especially for myeloid lineages during the early period of posttransplant, our conditioning is more powerful in eradicating host myeloid cells as well, which may have beneficial effect for rapid control of myeloid malignancies. The OS and PFS were estimated as both 23% at 2 years posttransplant, almost comparable to or slightly less than the data reported previously [15,16,26], which can be reasonably explained by higher age range and poor disease status before transplant in this study cohort, which can be further supported by the result of subgroup analysis indicating those with standard disease status showed much better outcome (Figure 3B).

UCBT has been associated with lower incidence of aGVHD, possibly because of the immunologic naïvety of transplanted lymphocytes; however, this naïvety raises a concern about whether transplanted cells will have sufficient antimalignant activity. Several reports indicate the in vivo antimalignancy effect of cord blood cells [27-30]. Cumulative incidence of disease progression at 2 years posttransplant in our series was 24%, which is comparable to those previously reported [15,16,26]. It plateaued later than 795 days, indicating that our RI-UCBT treatment protocol offered fairly good disease control.

The incidence of GVHD was higher than previous reports in RIC [11,12], and was almost comparable to those of BM transplants, PB cell transplants, or UCBT with conventional conditioning [8-10,31-36]. Because of the poor disease status of the majority of patients included in this study, GVHD prophylaxis was initially planned to be less intensive with single calcineurin inhibitors. Older patients' age [37] or high incidence of infectious complications, which possibly induced excessive inflammatory cytokine secretions, could have been relevant to this result [38].

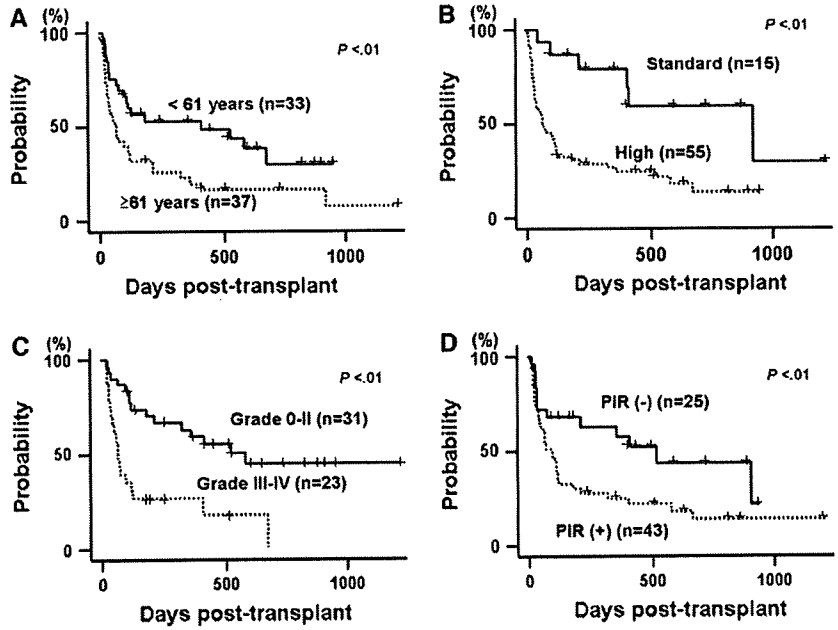

Figure 3. OS estimates after RI-UCBT (n = 70). (A) Effect of age. (B) Effect of disease status. (C) Effect of severity of aGVHD. (D) Effect of PIR.

Although RI-UCBT has been a feasible approach in terms of engraftment, a significant number of patients died from treatment-related complications. NRM was close to 3 times higher than mortality from relapse or disease progression, and most NRM occurred within 100 days posttransplant. Of 37 deaths because of NRM, 19 were from infection. Delayed engraftment relative to other stem cell sources such as BM or PB cells has been suggested to account for the higher rate of infectious complications after UCBT [32,39,40], but the time to engraftment in our series of patients was not delayed. Higher grade of aGVHD and the presence of PIR were found to be significantly associated with poor OS in multivariate analysis, indicating that immune-mediated events have strong impact on patients' outcome (Table 4). PIR is the syndrome observed in our setting of RI-UCBT. Although the mechanism behind PIR has not been investigated extensively yet, it is assumed to be reflecting alloimmune event, given our experience that more intensive GVHD prophylaxis with Tac had tendency to decrease the incidence of PIR. Moreover, development of PIR may have been suppressed in reported cases from other institutes that utilized additional agents to calcineurin inhibitors, such as methotrexate [10,19], antithymocyte globulin [31], or mycophenolate mofetil [16]. There has been a similar early immune reaction-like syndrome reported as "hyperacute GVHD" observed following BM or PBSC transplant, and responded poorly to corticosteroids compared to traditional aGVHD [41,42]. The incidence of PIR was higher than that of hyperacute GVHD, and further investigation on biologic mechanisms may help us define PIR more precisely relative to other immune-mediated diagnosis and develop optimal treatment approach. The presence of PIR was shown to cause more NRM than the absence in univariate analysis (P = .02), although it did not reach statistical significance in multivariate analysis. Thus, better management of immune-mediated complications will be the key to reduce NRM and improve OS. Based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA, because Tac was shown to be more potent than CsA in BM transplant [43-45]. Patients who received Tac as GVHD prophylaxis had less chance of experiencing PIR compared with those who received CsA and had less NRM, indicating the potential benefit of using Tac as a standard agent for GVHD prophylaxis. Adding methotrexate, mycophenolate mofetile, or sirolimus to the calcineurin inhibitor may further improve the final outcome [10,11,46,47]. Older age was another factor that influenced OS with statistical significance, even within the age range studied (Figure 3A and Table 4). Patients aged 61 years and older experienced more NRM than patients younger than 61 years (65% versus 39%), whereas their death rate because of disease progression was comparable (19% versus 18%), suggesting the vulnerability of higher aged population to procedure toxicity. Although the possible impact of slight variation in conditioning regimen to the outcome cannot be excluded, it is unlikely, because the great majority (93%) were conditioned with Flu/Mel/TBI regimen fairly uniformly, and comparison between Flu/Mel/TBI and others did not reach statistical significance.

Table 4. *Cox Regression Analyses of Factors Potentially Associated with OS and NRM after RI-UCBT*

Variables	HR	95% CI	P
OS			
Age			
Less than 61 years (n = 33)	0.3	0.14-0.72	.006
At least 61 years (n = 37)	1.0		
Disease risk			
Standard (n = 15)	0.3	0.12-0.995	.049
High (n = 55)	1.0		
PIR			
No (n = 25)	0.4	0.16-0.88	.023
Yes (n = 43)	1.0		
Acute GVHD			
Grade 0-II (n = 31)	0.4	0.17-0.78	.0002
Grade III-IV (n = 23)	1.0		
NRM			
GVHD prophylaxis			
CsA (n = 37)	3.9	1.3-11.6	.01
Tac (n = 33)	1.0		
Acute GVHD			
Grade 0-II (n = 31)	1.0		
Grade III-IV (n = 43)	5.7	2.1-15.7	.001

GVHD indicates graft-versus-host disease; CsA, cyclosporine A; Tac, tacrolimus; NRM, nonrelapse mortality; CI, confidence interval; HR, hazard ratio; OS, overall survival.

In conclusion, this is the first study specifically focusing on elderly patients aged 55 years and older with advanced hematologic diseases to show the feasibility of RI-UCBT. Older age per se cannot be considered to be contraindication to RI-UCBT, although a high NRM has been observed. Further optimization of the treatment protocol, such as immunosuppressive therapy for GVHD prophylaxis, is warranted to establish the safety of this promising treatment strategy for elderly patients with advanced hematologic diseases.

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Tacrolimus as Prophylaxis for Acute Graft-Versus-Host Disease in Reduced Intensity Cord Blood Transplantation for Adult Patients With Advanced Hematologic Diseases

Shigesaburo Miyakoshi,[1,6] Masahiro Kami,[2] Tetsuya Tanimoto,[3] Takuhiro Yamaguchi,[4] Hiroto Narimatsu,[1] Eiji Kusumi,[1] Tomoko Matsumura,[2] Shinsuke Takagi,[1] Daisuke Kato,[1] Yukiko Kishi,[1] Naoko Murashige,[5] Koichiro Yuji,[1] Naoyuki Uchida,[1] Kazuhiro Masuoka,[1] Atsushi Wake,[1] and Shuichi Taniguchi[1]

Background. Myeloablative cord blood transplantation (CBT) for adult patients offers a 90% chance of engraftment with a 50% rate of transplant-related mortality, mostly attributable to infection. We have demonstrated the feasibility of reduced-intensity CBT (RI-CBT) for adult patients, in which cyclosporine was used for acute graft-versus-host disease (GVHD) prophylaxis. Transplantation-related mortality (TRM) was 27% within 100 days. Therefore our objective was to evaluate the feasibility of RI-CBT with tacrolimus as GVHD prophylaxis for adult patients with hematologic malignancies.

Methods. Thirty-four patients with a median age of 56.5 years (range; 22–68) with hematologic diseases underwent RI-CBT at Toranomon Hospital between November 2003 and September 2004. Preparative regimen comprised fludarabine 25 mg/m2 on days −7 to −3, melphalan 80 mg/m2 on day −2, and 4 Gy total body irradiation on day −1. GVHD prophylaxis was continuous intravenous infusion of tacrolimus 0.03 mg/kg, starting on day −1.

Results. Thirty-one patients achieved neutrophil engraftment at a median of day 20. Median infused total cell dose was 2.4×10E7/kg (range; 1.6–4.8). Thirty-two patients achieved complete donor chimerism at day 60. Grade II–IV acute GVHD occurred in 45% of patients, with a median onset of day 26. Primary disease recurred in five patients, and TRM within 100 days was 12%. Estimated 1-year overall survival was 70%.

Conclusion. This study demonstrated the possible improvement in transplant-related mortality by tacrolimus as GVHD prophylaxis in adult RI-CBT recipients.

Keywords: Tacrolimus, Acute graft-versus-host disease, Reduced intensity cord blood transplantation.

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Cord blood transplantation (CBT) represents an attractive alternative for patients with hematologic diseases who lack matched related or unrelated donors. The value of CBT using myeloablative preparative regimens has been confirmed for pediatric and adult patients (*1–5*). Myeloablative CBT for adult patients offers a 90% chance of engraftment with a 50% rate of transplant-related mortality, mostly attributable to infection (*1–4, 6*).

We and other groups have demonstrated the feasibility of reduced-intensity CBT (RI-CBT) for adult patients, in which cyclosporine was used for acute graft-versus-host disease (GVHD) prophylaxis (*7, 8*). Transplantation-related mortality (TRM) was 27% within 100 days (*7*). Posttransplant immune disorders including pre-engraftment immune reactions (PIR) and acute GVHD were problematic in RI-CBT for adult patients (*7, 9*). These reactions and/or additional immunosuppressive therapy might have increased the risk of infection and/or organ dysfunction, leading to a high TRM (*10, 11*).

We employed tacrolimus for acute GVHD prophylaxis in place of cyclosporine from November 2003 in RI-CBT. It might reduce the incidence and severity of PIR and acute GVHD. We will summarize the results of RI-CBT using tacrolimus to investigate its safety and efficacy for acute GVHD prophylaxis after RI-CBT.

[1] Department of Hematology, Toranomon Hospital, Tokyo, Japan.
[2] Division of Exploratory Research, Institute of Medical Science, the University of Tokyo, Tokyo, Japan.
[3] Division of Clinical Laboratory Medicine, Faculty of Medicine, Tottori University, Yonago, Japan.
[4] Department of Biostatistics, School of Health Sciences and Nursing, University of Tokyo, Tokyo, Japan.
[5] Office for Life-Style Related Diseases Control, Ministry of Health, Labour and Welfare, Tokyo, Japan.
[6] Address correspondence to: Shigesaburo Miyakoshi, M.D., Ph.D., Department of Hematology, Toranomon Hospital, 35-2, 2-2-2 Toranomon, Minato-ku, Tokyo, Japan.
E-mail: s-miyakoshi@dance.ocn.ne.jp
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MATERIALS AND METHODS

Study Patients

The study population consisted of 34 adult patients with hematologic disorders, who underwent a protocol of RI-CBT with GVHD prophylaxis using tacrolimus alone at Toranomon Hospital between November 2003 and September 2004. All of the patients were incurable with conventional treatments and were considered inappropriate for conventional allogeneic stem-cell transplantation (allo-SCT) due to the lack of an human leukocyte antigen (HLA)-identical sibling or a suitable unrelated donor, age >50 years old, and/or organ dysfunction. Patients with no suitable HLA-matched related donor were eligible for this protocol if a matched unrelated bone marrow donor was unavailable as a first treatment option. If there was insufficient time for an unrelated bone marrow donor search due to rapidly progressive disease or if the preliminary search indicated a low possibility of obtaining a matched unrelated bone marrow donor, we attempted to locate cord blood grafts. Patients who received RI-CBT as second allo-SCT were excluded from this study. The clinical protocol was approved by the Institutional Review Board of Toranomon Hospital, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

HLA Typing and Donor Matching

HLA-A and HLA-B antigens were determined by serologic typing. HLA-DRB1 alleles were identified by high-resolution molecular typing using polymerase chain reaction method (PCR) with sequence-specific primers (SSP). All cord blood grafts were evaluated by HLA-A, HLA-B, and HLA-DRB1 typing and nucleated cell counts. Preferred cord blood units were those matched to 4 or 6 of 6 HLA loci and contained at least a cell count of 2×10E7 nucleated cells/kg of recipient body weight before freezing. All cord blood units came from cord blood bank in the Japan Cord Blood Bank Network (*12*).

Preparative Regimen and GVHD Prophylaxis

Preparative regimen comprised fludarabine 25 mg/m^2 on days –7 to –3, melphalan 80 mg/m^2 on day –2, and 4 Gy total body irradiation (TBI) in two fractions on day –1 (*7*). GVHD prophylaxis was continuous intravenous infusion of tacrolimus 0.03 mg/kg, starting on day –1. Once oral intake could be tolerated, patients were administered oral tacrolimus at a dose ratio of 1:2–3, in two divided doses based on the latest intravenous dose. In the absence of GVHD, it was tapered from day 100 until day 150. Tacrolimus was reduced when serum creatinine levels were elevated above 1.5 times baseline or other serious adverse effects occurred. If grade II–IV acute GVHD developed, methylprednisolone at a dose of 1 to 2 mg/kg was added to tacrolimus.

Supportive Cares

All of the patients were managed in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis. Fluoroquinolone and fluconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir was also given. Neutropenic fever was managed according to the guidelines (*13*). Cytomegalovirus (CMV) pp65 antigenemia was monitored once a week. If positive results were identified, preemptive therapy with foscarnet was initiated. Hemoglobin and platelet counts were maintained at >7 g/dL and 10×10E9/L, respectively, with in-line filtered and irradiated blood transfusions. All the patients received granulocyte colony stimulating factor (G-CSF) at a dose of 5 μg/kg intravenously, starting on day 1 until durable neutrophil recovery was achieved.

Assessment of Engraftment, GVHD, Regimen-Related Toxicity (RRT) and Survival

Engraftment was defined as the first of two consecutive days in which white blood cell counts >1.0×10E9/L or the absolute neutrophil counts (ANC) >0.5×10E9/L. The date of platelet recovery was defined as the first of seven consecutive days during which the nontransfused platelet count was at least 20×10E9/L.

Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, PCR for variable number of tandem repeats was used with donor cells detected at a sensitivity of 10% (*14*). Whole blood and CD3-positive cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed at least once during life.

Primary graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, without detection of donor markers by cytogenetic and/or molecular techniques (*15*). Late graft failure was defined among the patients who attained neutrophil engraftment as a decline of ANC to less than 0.5×10E9/L for at least seven consecutive days with evidence of severe hypocellularity of bone marrow confirmed by histopathological examination. PIR was diagnosed as reported previously (*9*). When febrile patients (body temperature ≥38 °C) with no evidence of infection or adverse effects of medication exhibited skin eruption, diarrhea, jaundice (serum total bilirubin >2.0 mg/dL) or body weight gain >10% of baseline, these changes were defined as immune reactions. Immune reactions developing ≥6 days before engraftment were defined as PIR. Acute and chronic GVHD were diagnosed and graded according to standard criteria (*16*, *17*). GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. All the patients who had evidence of donor cell engraftment were considered to be evaluable for acute GVHD. Response to corticosteroid was evaluated according to the report by Martin et al. (*18*). Chronic GVHD was evaluated in patients who survived without relapse or disease progression for at least 100 days after transplantation. Patients with hematologic malignancies in complete remission (CR) at the time of transplant, in chronic phase of chronic myelogenous leukemia (CML), with refractory anemia (RA), or refractory anemia with ringed sideroblasts (RARS) of myelodysplastic syndrome (MDS) and with nonmalignant disease were defined as being at standard risk. The other patients were defined as being at high risk. Chemotherapy resistance was defined as relapse after initial cytotoxic chemotherapy or failure to achieve remission.

Overall survival (OS) was applied to all the patients and