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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

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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

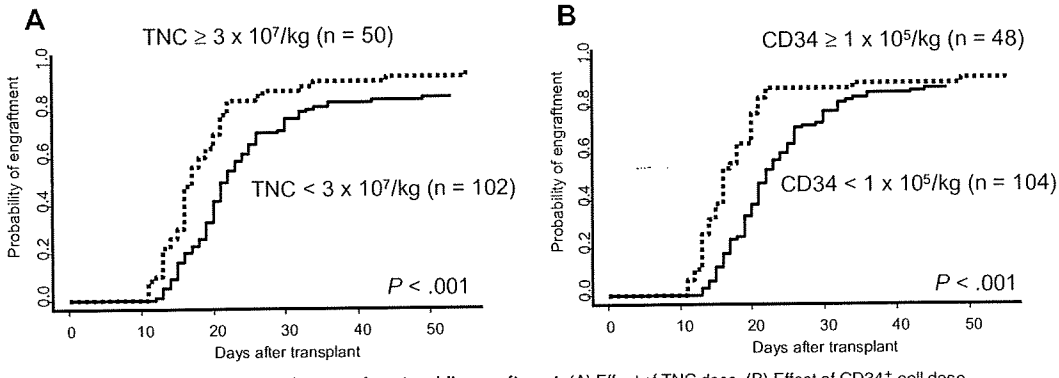

Figure 1. Cumulative incidence of neutrophil engraftment. (A) Effect of TNC dose. (B) Effect of CD34$^+$ cell dose.

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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).

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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	

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

No. of HLA mismatches	Neutrophil engraftment				
	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

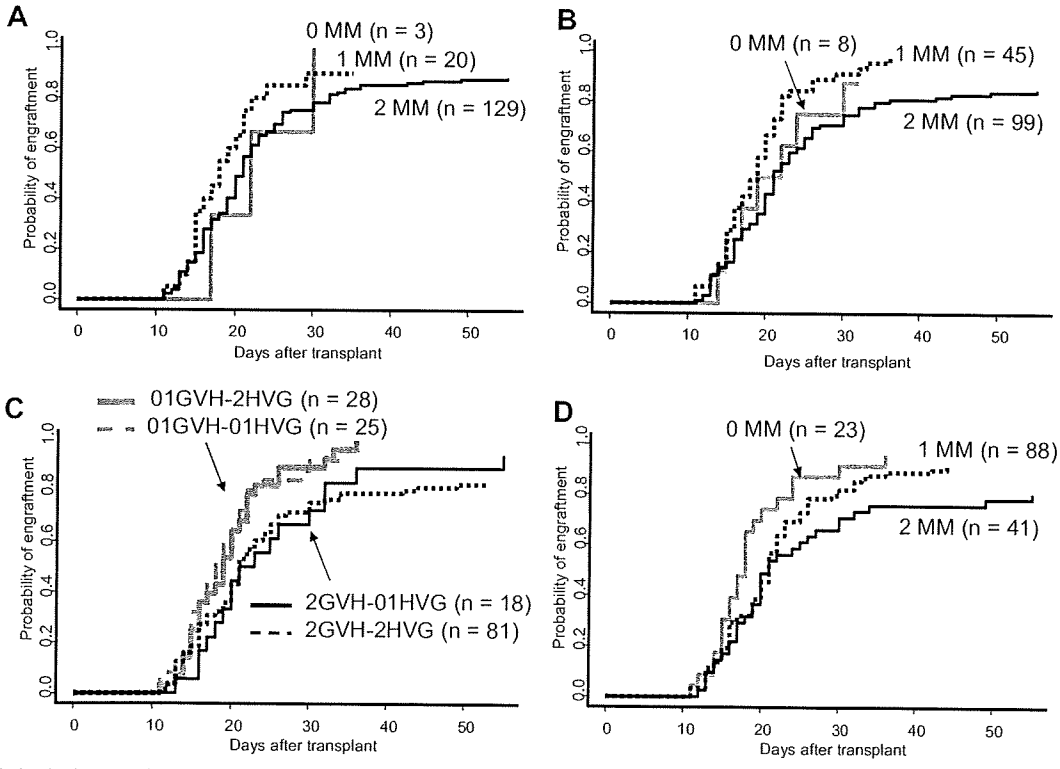

Figure 2. Cumulative incidence of neutrophil engraftment. MM indicates mismatch. (A) Effect of HLA antigen mismatch. (B) Effect of HLA antigen mismatch in the GVH direction. (C) Effect of HLA antigen mismatch according to mismatch both in the GVH and the HVG directions. 2GVH indicates 2 antigens mismatch 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.

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

No. of HLA mismatches in the GVH direction	Acute GVHD II-IV			2-year overall survival		
	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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LETTER TO THE EDITOR

T-cell post-transplant lymphoproliferative disorder in a patient with chronic idiopathic myelofibrosis following allogeneic PBSC transplantation

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Post-transplant lymphoproliferative disorder (PTLD) is a well-recognized complication after solid organ and hematopoietic SCTs (HSCTs). The majority are of B-cell origin and EBV related.[1] Most of the T-cell PTLD cases have been described as occurring after solid organ transplantations;[2] T-cell PTLD cases following HSCT are exceedingly rare. There are only three reported cases of T-cell PTLD following allogeneic HSCT[3] and four cases following autologous HSCT.[4–7] Here we report a case of T-cell PTLD after allogeneic-PBSC transplantation (allo-PBSCT) in a patient with chronic idiopathic myelofibrosis (CIMF).

A 44-year-old Japanese woman with anemia and fever was diagnosed with CIMF in November 2006. At the time of her diagnosis, her WBC count was 900/μl, Hb 6.9 g/dl, plt count 39 000/μl with no morphologically abnormal cells in her peripheral blood, and an abdominal CT scan showed mild splenomegaly without hepatomegaly, lymphadenopathy or liver tumor. A specimen of her biopsied BM showed diffuse fibrosis and a decreased number of hematopoietic cells. No abnormal cell proliferation was observed. In December 2006, she underwent allo-PBSCT from an HLA-identical brother. Neutrophil engraftment was achieved on day 17 after transplant, and BM analysis showed full hematological recovery with 100% donor-type chimerism assessed by Y chromosome-based FISH analysis. As grade II acute GVHD involving the skin and subsequently an extensive type of chronic GVHD (cGVHD) developed; continued immunosuppressive therapy with cyclosporine and prednisolone was required for several months after the transplant. At 5 months after transplant, a liver tumor, 2 cm in diameter, was detected by an abdominal CT scan. Although PTLD was raised as a differential diagnosis, biopsied liver tissue was inadequate for pathological examination. Immunosuppressive therapy was reduced, resulting in a decrease in liver tumor size to 1.6 cm in 2 months. However, a subsequent flare-up of cGVHD required more intensive immunosuppressive therapy, and the liver tumor's diameter increased twice in size. A liver tumor biopsy performed at this time showed a diffuse proliferation of atypical lymphoid cells (Figure 1a). Immunohistochemically, these tumor cells were positive for LCA, CD3, CD7 and CD8, and negative for CD4, CD5, CD34, CD79a, MPO, CD30, CD56 and TdT (Figure 1b). These pathological findings are compatible with peripheral T-cell lymphoma-unspecified (Figure 1c). EBV infection was not detected by *in situ* hybridization. Y chromosome-based FISH analysis revealed the tumor cells were of recipient origin. She suffered from fever, pancytopenia and decreased liver function, and was hospitalized for further therapy in November 2007. BM examination showed infiltration of 4% abnormal lymphoid cells and the proliferation of macrophage with hemophagocytosis, with no sign of CIMF recurrence. Chromosome analysis of the BM cells showed 44, X, der(X)t(X;7)(q13;q11.2), add(2)(q21), add(4)(p11), add(4)(p16), der(9;17)(q10;q10), −10, −13, add(15)(p11), +mar [2/20]. An abdominal CT scan showed that the liver tumor grew rapidly to a size of $12 \times 6\,cm^2$ (Figure 1d). Serological tests for HIV, HBV, HCV and HTLV-1 were negative, and the EBV VCA IgG was positive but negative for IgM. Analyses by real-time PCR were negative for human herpesvirus-6, VZV, CMV and EBV in her peripheral blood. She was diagnosed with T-cell PTLD with lymphoma-associated hemophagocytic syndrome. CHOP therapy was started, but the disease progressed within 2 weeks after this. She underwent urgent unrelated cord blood transplantation (UCBT) from an HLA two antigen-mismatched donor. Her post-transplant course was complicated by sepsis, renal failure and respiratory failure. She died on day 6 after UCBT. An autopsy was not performed.

To our knowledge, there have been only four cases of T-cell PTLD following allo-SCT, including our case (Table 1). Time to T-cell PTLD diagnosis ranges from 2 to 43 months after a transplant. Although the type of PTLD was not consistent, ranging from precursor to peripheral T-cell neoplasms, none of them were associated with EBV infection. Our case was negative for EBV, and the type was peripheral T-cell lymphoma-unspecified.

There have been a few reports describing myelofibrosis in association with T-cell lymphoma.[8] In these cases, PDGF and tumor growth factor β, which may have been secreted by neoplastic T lymphocytes, had an important role in the development of myelofibrosis. In our case, there was no clinical evidence of T-cell lymphoma at the time of CIMF diagnosis, and no sign of myelofibrosis recurrence at the onset of T-cell lymphoma. Thus, the development of T-cell lymphoma in this case was considered to be independent of the CIMF.

All three patients reported as having T-cell PTLD following allo-SCT had severe GVHD and received a heavy dose of immunosuppressive agents, suggesting some viral agents in an immunosuppressed state may have an important role in the development of T-cell PTLD. However, we were unable to find any evidence of viral

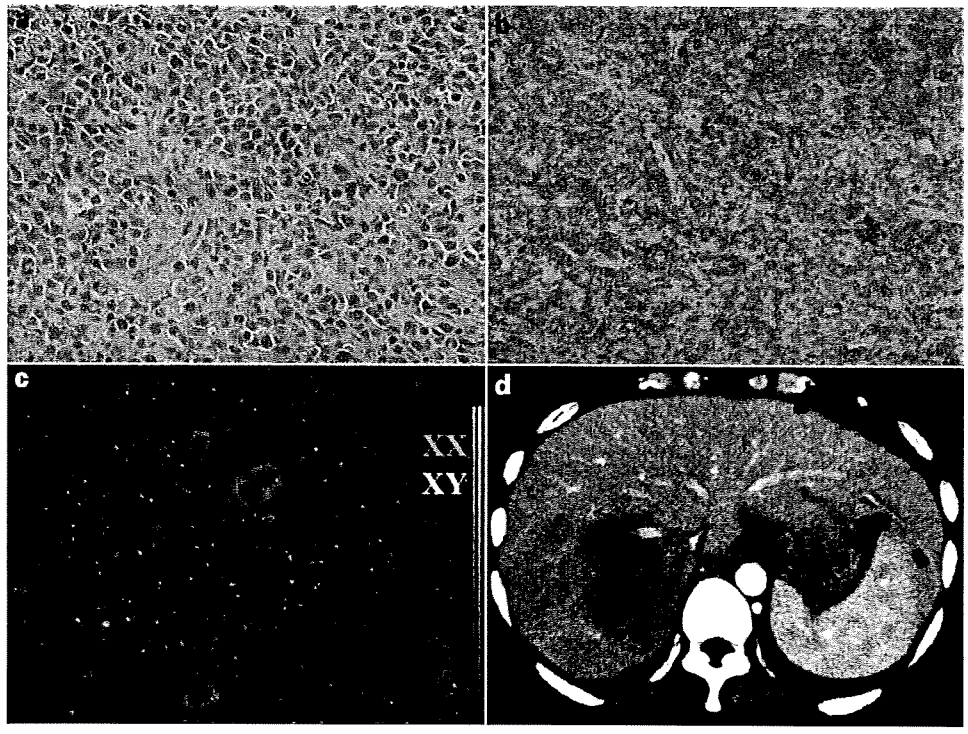

Figure 1 (a) Liver tumor biopsy shows monotonous infiltration of atypical lymphoid cells (H&E stain ×400). (b) Immunostaining for CD3 shows a large number of positive cells within the tumor. (c) Y chromosome-based FISH reveals the tumor cells are of recipient origin (XX signal). (d) Abdominal CT scan shows a low-density area with 12 cm diameter on the right side of the liver.

Table 1 T-cell post-transplant lymphoproliferative disorder after allogeneic stem cell transplantation

Authors	*Age/sex*	*Initial Dx*	*HSCT*	*Type of PTLD Dx (months after HSCT)*	*Origin*	*EBV*	*GVHD*	*Outcome (months after Dx)*
Zutter *et al.*[3]	14/M	AML	HLA-identical BM graft	T-lymphoblastic lymphoma (43)	Recipient	Neg	Mild aGVHD(S,L,Gut) Severe cGVHD(S,L,Gut)	Death (28)
	9/M	ALL	HLA-identical BM graft	T-lymphoblastic lymphoma (21)	Donor	Neg	Mild aGVHD(S) Severe cGVHD(S,L)	Death (6)
	2/F	ALL	HLA-2 mismatched BM graft	Polymorphic T-cell lymphoma (2)	Donor?	Neg	Severe aGVHD(S,L)	Death (0)
Present case	44/F	CIMF	HLA-identical allogeneic PBSC	PTCL-u (5)	Recipient	Neg	aGVHDII(S3,L0,Gut0) Extensive cGVHD(S,L)	Death (2)

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CIMF = chronic idiopathic myelofibrosis; Dx = diagnosis; F = female; Gut = gastrointestinal tract; HSCT = hematopoietic stem cell transplantation; L = liver; M = male; neg = negative; PTCL-u = peripheral T-cell lymphoma-unspecified; PTLD = post-transplant lymphoproliferative disorder; S = skin.

infection and reactivation in our case and previously reported cases. It has been reported that only 15 of 76 cases of T-cell PTLD after solid organ transplantation were EBV positive,[9] and any other viral involvement has not been clearly demonstrated. These findings suggest that not only viral infection but also other factors, such as chronic antigenic stimulation, impaired immunoregulation and genetic factors, may be associated with the development of T-cell PTLD.[10]

The outcomes of reported T-cell PTLD so far are poor. All patients died because of the progression of the disease. In our patient, a transient response was observed by reducing immunosuppression, suggesting a graft-versus-lymphoma effect, which was necessitated to increase the immunosuppression. Standard cytotoxic chemotherapy led to a poor response in our patient, similar to the other cases previously described. More intensive chemotherapy, donor lymphocyte infusion or second HSCT should be considered at an early stage of the disease.

In conclusion, T-cell PTLD rarely occurs after allo-HSCT. Further research, however, is needed to fully characterize the clinicopathological features of this condition and to investigate the optimal therapy.

Conflict of interest

The authors declare no conflict of interest.

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ELSEVIER

Extended Mycophenolate Mofetil Administration Beyond Day 30 in Allogeneic Hematopoietic Stem Cell Transplantation as Preemptive Therapy for Severe Graft-Versus-Host Disease

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ABSTRACT

To prevent acute graft-versus-host disease (GVHD), mycophenolate mofetil (MMF) combined with calcineurin inhibitors have been used in allogeneic hematopoietic stem cell transplantation (allo-SCT). Previous studies commonly utilize MMF treatment until day 30 after allo-SCT. However, the feasibility of continuous administration after day 30 has not been well evaluated. We retrospectively assessed the safety and efficacy of extended drug administration. Twenty-five patients ceased MMF at day 30 (group A); whereas, 16 patients (group B) received extended regimens depending on individual risk factors for GVHD. No severe adverse events were observed in either group. Although the cumulative incidence (CI) of grade I to IV GVHD at day 100 was comparable between the 2 groups, the CI of grade II to IV GVHD was less among group B (12.5%) compared with group A (42.3%). Extended MMF administration may be safe and beneficial as preemptive therapy to reduce the development of moderate-to-severe acute GVHD.

ACUTE graft-versus-host disease (GVHD) is the most common early complication after allogeneic hematopoietic stem cell transplantation (allo-SCT). As an immunosuppressant for acute GVHD prophylaxis, mycophenolate mofetil (MMF) has been recently suggested to be a substitute for a short course of methotrexate, which is increasingly used in allo-SCT, especially in nonmyeloablative conditioning regimens and/or cord blood transplantation.[1–11] For better clinical outcomes with MMF, a higher plasma level of its active metabolite, mycophenolic acid (MPA), has been proposed to be desirable.[2,4,5,7,12] However, recent studies showed wide individual variations in pharmacokinetic parameters, such as MPA area under the curve (AUC). The MPA plasma levels of allo-SCT patients are relatively lower than the therapeutic range recommended for solid organ transplantations.[13–15]

Recently, on the basis of real-time pharmacokinetic monitoring,[12] we demonstrated that for allo-SCT MMF should be administered every 8 hours at least until day 30, seeking to maintain higher MPA plasma levels without symptomatic adverse events. Several previous reports also confirmed a positive relationship between the clinical benefit of MMF and higher MPA exposures, which were reflected by the concentrations at steady state (Css) or the trough values (Ctrough).[2,4,5,7,12] Moreover, a recent animal study confirmed the efficacy of every 8 hour oral administration of MMF.[16]

As a next candidate protocol we examined the feasibility of continuous administration of MMF after day 30. Depending on the development of acute GVHD each individual institution allows MMF to be continued, tapered, or stopped after day 30.[1,4,5,7,10,11] Herein, we retrospectively evaluated the safety and efficacy of extended MMF administration beyond day 30 based on our single-center experience.

MATERIALS AND METHODS

Patient Characteristics

Forty-one patients, who received allo-SCT using MMF between December 2003 and March 2008, were enrolled in this study.

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Written informed consent was obtained from all patients. MMF was administered as prophylaxis for GVHD in combination with a calcineurin inhibitor, FK506 or cyclosporine (CyA). MMF was given orally from 4 to 6 hours after allo-SCT on day 0; then on the succeeding days, it was given every 8 or 12 hours up to a total daily dose of 3000 mg. In group A (n = 25), all patients stopped MMF at day 30. In group B (n = 16), MMF was continued and/or tapered after day 30, depending on the risk factors for GVHD development described below. In group A, the regimen for 92% (23/25) of patients was 15–25 mg/kg every 12 hours and the remaining 2 patients were given 1000 mg every 8 hours. In group B, the regimen for 87% (14/16) of patients was 1000 mg every 8 hours, and the remaining 2 patients were given 15–25 mg/kg every 12 hours. Then, MMF was continued beyond day 30 for the following reasons: (1) allo-SCT from at least a single HLA class I or II allele mismatched donor, (2) greater than grade I acute GVHD, (3) eosinophilia of $>0.5 \times 10^9$/L, or (4) fever 37.5°C without infection. The median extended dosing period of MMF in group B was 64.5 days (range, 50–94 days). FK506 was administered to 37 patients (24 in group A, 13 in group B) at a daily dose of 0.03 mg/kg by continuous intravenous infusion from day −1, and then converted to oral therapy when tolerated. The other 4 patients (1 in group A, 3 in group B) received CyA at a daily dose of 3 mg/kg by continuous intravenous infusion from day −1.

The patient and treatment characteristics are summarized in Table 1. The median $CD34^+$ cell doses were as follows: related peripheral blood stem cells (PBSC) were 3.7×10^6/kg (range, $2.8–5.5 \times 10^6$/kg), related bone marrow (BM) were 3.9×10^6/kg, unrelated BM were 1.6×10^6/kg (range, $0.7–10.9 \times 10^6$/kg), and umbilical cord blood (UCB) were 1.3×10^5/kg (range, $0.3–7.4 \times 10^5$/kg).

Table 1. Patient Characteristics

Variable	Group A	Group B
No. of patients	25	16
Median age, y (range)	43 (21–65)	53 (28–66)
Gender, male/female	8/17	9/7
Diagnosis at allo-SCT		
AML	4	3
ALL	9	4
MDS	7	6
NHL	2	2
Others	3	1
Conditioning regimen		
Myeloablative, with/without TBI	15/1	8/0
Nonmyeloablative, with/without TBI	4/5	7/1
Donor type & HLA typing		
Related PBSC		
4/6		
5/6	0	3
6/6	0	3
Related BM		
5/6	0	1
Unrelated BM		
5/6	2	1
6/6	6	0
Unrelated UCB		
3/6	3	2
4/6	8	6
5/6	3	0
6/6	1	0

Note: Group A received MMF until day 30. Group B continued MMF past day 30.
Abbreviations: AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; ATLL, adult T-cell leukemia lymphoma; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; AA, aplastic anemia; TBI, total body irradiation; PBSC, peripheral blood stem cell; BM, bone marrow; UCB, umbilical cord blood.

Evaluation of Toxicities and Acute GVHD

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 2.0 for descriptive purposes. We assessed the occurrences of mucositis and gastrointestinal toxicities, which were greater than grade III after allo-SCT, and monitored cytomegalovirus (CMV) reactivation through measurement of CMV pp65 antigen. Relapse and disease progression were defined as recurrent disease after complete remission and progression of persistent disease, respectively. Acute GVHD was graded according to the consensus grading scale.[17] The median follow-up period for all patients was 273 days (range, 21–1694 days).

Statistical Analysis

We evaluated the correlation between the number of patients who experienced adverse events and CMV reactivation in the 2 groups using the chi-square test. The cumulative incidence (CI) of acute GVHD was calculated by treating death as a competing event. Hazard ratios were estimated from Cox regression models.

RESULTS AND DISCUSSION

Safety of Extended MMF Administration Beyond Day 30

The toxicities are summarized in Table 2. Grade III oral mucositis until day 30 developed in only 1 group A patient. Gastrointestinal events greater than grade III, most of which were diarrhea, occurred in 11 group A versus 10 group B patients to day 30 (44% vs 62.5%; $P = .25$), in whom they were transient, resolving without MMF withdrawal. In addition, none of the patients in either group experienced mucositis or diarrhea after day 30. Therefore, the gastrointestinal events seemed unlikely to be adverse effects of MMF, but rather to be caused by the preparative regimens.

All patients were CMV sero-positive before allo-SCT. The high incidence of CMV reactivation was observed similarly in both groups regardless of the dosing period of MMF (76.0% vs 68.8%; $P = .94$). It might have been due to the stem cell source, because more than half of group A (n = 15; 60%) and group B (n = 8; 50%) used UCB (Table 1).

Regarding the incidence of relapse, there was no significant difference between the 2 groups (20.0 vs 18.8%; $P = .92$). Thus, the continuous administration of MMF over day 30 was well tolerated.

Efficacy for Prevention of Acute GVHD

As shown in Figure 1A, the CI of grade I to IV acute GVHD showed no difference between groups A and B at day 30 (45.5% vs 43.8%; hazard ratio, 1.05; $P = .92$) or at

day 100 (72.1% vs 62.5%; hazard ratio, 1.20; P = .63). In contrast, the CI of grade II to IV acute GVHD at day 100 was less in group B (12.5%) compared with group A (42.3%; hazard ratio, 0.24; P = .045; Fig 1B). Among the patients who experienced greater than grade I acute GVHD until day 30, 8 of 11 group A patients developed grade II to IV acute GVHD; whereas, only 1 of 8 suffered it among group B (P = .017). There was no grade IV acute GVHD in either group.

In addition to the extended MMF administration beyond day 30, the favorable outcome of group B in the reduction of grade II to IV acute GVHD at day 100 might have been due to MMF dosing until day 30. Most group B patients (87%) received MMF doses every 8 hours (3000 mg/d), whereas most group subjects (92%) were prescribed every 12 hour regimens (15–25 mg/kg/dose; maximum 3000 mg/d). It is of interest whether MMF dosing in the early acute phase affects the onset of late or chronic GVHD.

To date, the dosing period of MMF has not been well established. Taken together with previous reports,[5,7,11] our limited data with a median dosing period of 64.5 days (range, 50–94 days) suggest that the proper duration is until day 100. We should pay careful attention to the incidence of severe viral infections as well as the relapse rate by prolonged MMF administration.

In this study, we have proposed a new schema of MMF administration to reduce moderate-to-severe acute GVHD. Two therapeutic strategies could be combined after day 30 as shown in Figure 1C. First, the MMF regimen should be extended when used as prophylaxis for high-risk patients who have received allo-SCT from HLA allele–mismatched donors or who have experienced clinical graft-versus-host reactions before day 30, such as eosinophilia and noninfectious fever. Second, the extended MMF regimen is a preemptive therapy to prevent the development of moderate-to-severe acute GVHD from grade I. This strategy may be useful for double UCB transplantation under nonmyeloablative conditioning because this transplantation has been reported to frequently develop severe acute GVHD.[10]

To establish GVHD prophylaxis with MMF as a gold standard, a prospective randomized study would be required to clarify whether 3 times daily dosing until day 30 is critical, or whether the same protocol should be followed by an extended administration, depending on GVHD risk factors.

Table 2. Possible Toxities of MMF

Variable	Group A (%)	Group B (%)
No. of patients	25	16
Mucositis (≥grade 3)		
Until d 30	1 (4.0)	0 (0)
After d 30	0 (0)	0 (0)
GI toxicities (≥grade 3)		
Until d 30	11 (44.0)	10 (62.5)
After d 30	0 (0)	0 (0)
CMV reactivation	19 (76.0)	11 (68.8)

Abbreviation: GI, gastrointestinal.

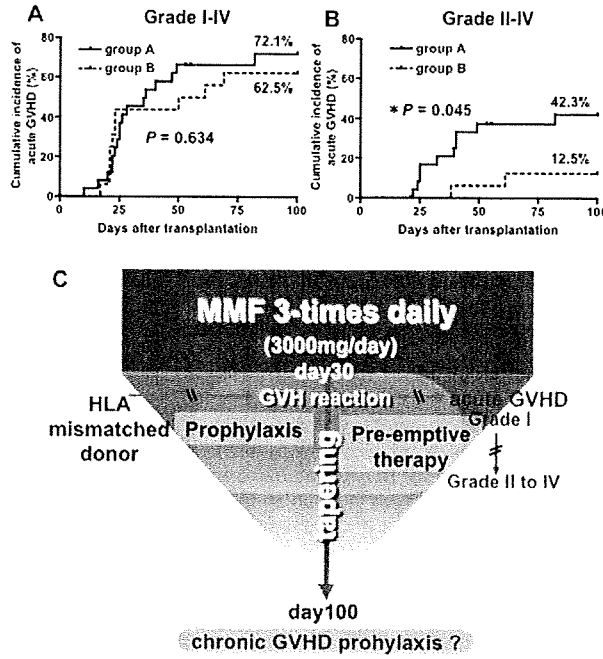

Fig 1. Effective prevention of moderate-to-severe acute GVHD by the extended MMF administration. **(A)** Cumulative incidences (%) of grade I to IV acute GVHD and (B) cumulative incidences of grades II to IV acute GVHD at day 100 after allo-SCT. Group A and group B are indicated by solid and dotted lines, respectively. Significant difference on the incidence of grade II to IV acute GVHD at day 100 **(B)** was observed between group A and the 2 groups (hazard ratio, 0.24; P = .045). **(C)** A comprehensive schema of a promising dosing strategy of MMF before/after day 30 after allo-SCT. The extended MMF administration might play a role not only as GVHD prophylaxis, but also as preemptive GVHD therapy.

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CASE REPORT

Cord blood transplantation using minimum conditioning regimens for patients with hematologic malignancies complicated by severe infections

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Abstract Patients with severe infections are thought to be ineligible for cord blood stem cell transplantation (CBT) because the conventional 5–6 day-conditioning regimens potentially makes them susceptible to fatal infections by the time neutrophil engraftment occurs. Two patients were treated with minimum conditioning regimens consisting of 30 mg/m^2 fludarabin (Flu) and 2 g/m^2 cyclophosphamide (CY) on day-1 and total body irradiation (TBI) of 2 or 4 Gy on day −1 or 0 followed by single unit CBT. The reasons for adopting such weak regimen were febrile neutropenia due to the rejection of the first cord blood (CB) graft given to a patient with follicular lymphoma resistant to chemotherapy and pulmonary aspergillosis in another patient with AML who relapsed after CBT. The AML patient received 40 mg/m^2 of melphalan on day-2 to reduce the leukemia burden. Both patients achieved 100% donor chimerism by day 19 and day 20 after CBT without an apparent exacerbation of the infections and remained in remission at 23 and 18 months after the CBT. These findings suggest that the 1–2 day regimens excluding antihuman thymocyte globulin may be sufficiently potent to ensure engraftment of CB in immunocompromised patients and safely administered even when patients are complicated by active infections.

Keywords Cord blood transplantation · Active infection · Minimum intensity conditioning regimen

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1 Introduction

Cord blood (CB) is becoming a major source of allogeneic hematopoietic stem cell transplantation [1, 2]. The success of reduced intensity CB transplantation has accelerated the use of CB for treatment of aged patients with hematologic malignancies [3]. However, patients complicated by severe documented infections are still considered ineligible for cord blood transplantation (CBT) even if reduced intensity regimens are adopted because the preconditioning causes severe neutropenia which usually lasts until day 20 after transplantation [2, 3] and exacerbates infections leading to treatment related-death. As a result, some patients with hematologic malignancies who failed to achieve remission after chemotherapy or those who failed to engraft after allogeneic stem cell transplantation cannot benefit from CBT.

One possible measure to solve this problem is to shorten the time for preconditioning in addition to reducing the intensity. Since most conventional preconditioning regimens take more than 4 days, they need to be started at least 5 days prior to the day of transplantation [4]. Shortening the time for preconditioning to 1 or 2 days may help patients to survive a neutopenic period from the start of preconditioning to neutrophil engraftment. Goggins et al. used a 1-day conditioning regimen consisting of fludarabin (Flu), alemtuzumab and cyclophosphamide (CY) to treat five leukemia patients with allogeneic peripheral blood stem cell transplantation (PBSCT) and observed stable engraftment in three patients. A similar 1-day regimen consisting of Flu, CY and antihuman thymocyte globulin (ATG) was used to treat a myelodysplastic syndrome (MDS) patient with a second allogeneic PBSCT (K. Mochizuki et al., in preparation). The patient suffered from a high fever suggestive of bacteremia due to

persistent neutropenia following the rejection of the first PBSC graft. The second PBSC of another HLA-identical sibling from the original donor successfully engrafted and the patient has been in remission for more than 4 years. However, all of these cases used PBSC grafts containing a high number of hematopoietic stem cells as well as T cells which are thought to be helpful to accelerate the engraftment of donor stem cells and rapid neutrophil recovery. It is still unclear whether CB can engraft after such a very weak regimen and eventually rescue neutropenic patients complicated by severe infections.

This report describes two patients with a devastating condition who were successfully treated with a minimum intensity regimen of 1–2 days followed by single unit CBT.

2 Patients

2.1 Patient 1

In January 2005, a 56-year-old man was diagnosed to have a clinical stage IV follicular lymphoma. He achieved only PR after standard chemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone and was refractory to other chemotherapy regimens for salvage. He underwent CBT following a reduced conditioning regimen consisting of cladribine, CY and 4 Gy of total body irradiation (TBI). His neutrophil count remained at 0 on day 21 after the CBT. A chimerism analysis of the bone marrow cells preformed on the same day revealed 100% cells to be recipient-type, thus indicating graft rejection. There was no sign of autologous hematologic recovery and a high fever persisted. There was no sign of an autologous hematologic recovery and a high fever persisted despite the administration of meropenem 1.0 g twice daily and micafungin 300 mg daily. The patient's CRP rose to 25.9 mg/dl on day 25. On day 27 after CBT, he received 30 mg/m^2 Flu and 2 g/m^2 CY followed by 2 Gy of TBI in the morning of the next day. HLA 2 locus-mismatched CB containing 2.6 $\times$ 10^7/kg cells and 9.8 $\times$ 10^6 CD34$^+$ cells/kg was infused 13 h after the completion of CY infusion. Clinical data including HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. The high fever started abating on day 16 after the second CBT and his neutrophil count surpassed 0.5 $\times$ 10^9/l on day 19. A chimerism analysis performed on day 26 revealed the 100% of the peripheral blood leukocytes were donor-type. Although grade I GVHD occurred, it resolved without treatment. CT scanning on day 33 after the second CBT showed a marked reduction of cervical lymph node swelling in comparison to that at 29 days before the first CBT (Fig. 1). He remains well in partial remission 30 months after the second CBT.

Table 1 Clinical data and HLA alleles of the patients and cord blood donors

	Sex	Blood type	HLA-A	HLA-B	HLA-DR
Patient 1	M	O+	0206/3303	3901/4403	1302/1501
First CB for patient 1	M	O+	0201/3303	3501/4403	1302/1501
Second CB for patient 1	M	A+	1101/3303	3901/4403	0803/1501
Patient 2	F	A+	2402/–	3501/4001	0901/1302
First CB for patient 2	M	AB+	0201/2402	3501/4006	0901/1302
Second CB for patient 2	M	B+	2402/–	4001/4006	0901/1501

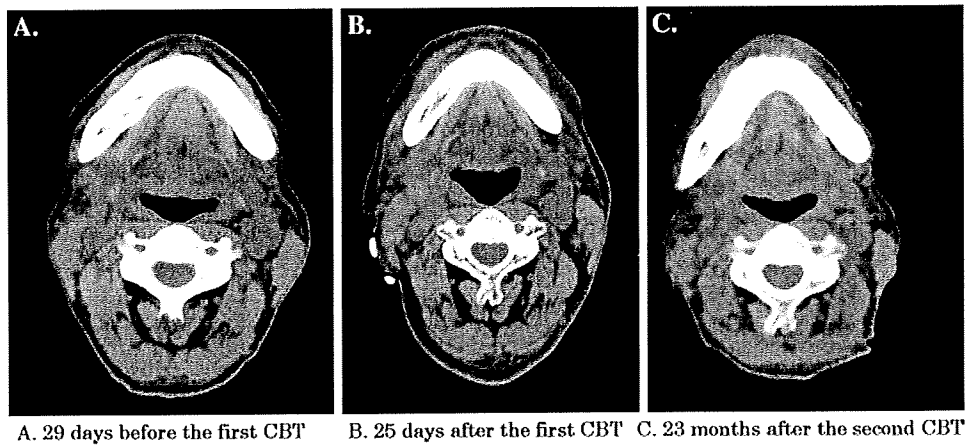

Fig. 1 Changes in the cervical lymphoma lesions after CBT in patient 1. CT scan on 23 months after the second CBT showed a marked reduction in size of the cervical lymph nodes in comparison to those before the first and the second CBT

A. 29 days before the first CBT B. 25 days after the first CBT C. 23 months after the second CBT

2.2 Patient 2

In April 2005, a 66-year-old female was diagnosed to have AML evolving from MDS. Chemotherapy consisting of idarubicin (IDA) and cytosine arabinoside (Ara-C) failed to induce remission and severe pancytopenia persisted. She underwent CBT following a conditioning regimen with fludarabine, melphalan, rabbit ATG and 4 Gy of TBI. The CB was 2-loci mismatched and contained 2.9×10^7/kg cells. Engraftment was confirmed on day 18 and she achieved complete remission. However, the AML relapsed in 18 months after the CBT. Remission induction with IDA and Ara-C only induced marrow hypoplasia with 33% residual leukemia cells. On day 18 of the chemotherapy, invasive aspergillosis developed in the left lung. Liposomal amphotericin B, 2.5 mg/kg daily, was administered from the same day without any appreciable effects. The neutrophil count remained at 0 on day 22 of the chemotherapy. She received melphalan 40 mg/m^2 to reduce leukemic cell burden, followed by 30 mg/m^2 Flu and 2 g/m^2 CY on the next day. In the morning of the following day, she received 4 Gy of TBI and underwent a second CBT 12 h after the completion of CY infusion. The CB was 2-loci mismatched, and contained 2.9×10^7/kg cells and 1.9×10^6 CD34$^+$ cells/kg. HLA alleles of the patient, the first CB donor, and the second CB donor are shown in Table 1. Tacrolimus was given from day-1 for prophylaxis of GVHD. Liposomal amphotericin B was switched to voliconazole, 4.0 mg/kg daily, on day 48 after the second CBT due to a rise in the creatinine level. Although her pulmonary aspergillosis was transiently exacerbated on day 6 after the second CBT, the high fever abated on day 17 and engraftment of donor cells was confirmed on the same day. The aspergillosis lesion was encapsulated with time after the second CBT (Fig. 2).

She underwent a left upper lobectomy on day 113 and presently remains in CR at 24 months after the second CBT.

3 Discussion

Treatment of the patients with hematologic malignancies complicated by severe neutropenic infections with no hope of prompt hematologic recovery is challenging. Although immunoablative conditioning followed by allogeneic stem cell transplantation is the only measure to rescue patients with such devastating conditions, this treatment may also tend to sometimes hasten the patients' death by aggravating the preexisting infections. Even if reduced intensity regimens are adopted, severe neutropenia which lasts from the day of preconditioning until 2–3 weeks after SCT greatly increases the risk of infectious death [3, 5, 6]. In order to solve this dilemma, Goggins et al. pioneered a very weak conditioning regimen, known as the 1-day regimen [7]. They treated five infirmed patients with 30 mg/m^2 Flu, 2 g/m^2 CY, 20 mg/kg alemtuzumab, TBI 2 Gy on day-1 and infused PBSC from family donors who were HLA 1–3 loci mismatched. Engraftment occurred in three patients, two of whom achieved long-term remission. According to their protocol, an MDS patient who suffered febrile neutropenia due to rejection of the first PBSCT was treated with Flu (30 mg/m^2), CY (2 g/m^2), horse ATG (15 mg/kg) and TBI (2 Gy) followed by PBSCT from a second HLA-identical sibling donor. The neutrophil count promptly recovered and the patient achieved complete donor chimerism. This experience indicated that the alemtuzumab in the 1 day regimen can be replaced with low dose ATG and that the minimum conditioning regimen coupled with PBSCT from a second donor can overcome the rejection after SCT.

Cord blood transplantation is associated with a higher incidence of engraftment failure [8–12] and a slower neutrophil recovery [2, 9, 13] than BMT or PBSCT due to the low number of hematopoietic stem cells and mature T cells in the CB graft. The disadvantages of CBT has precluded the use of CB for treatment of patients with very low intensity regimens for allogeneic stem cell transplantation such as 2 Gy TBI alone [14] or ATG + total lymphoid

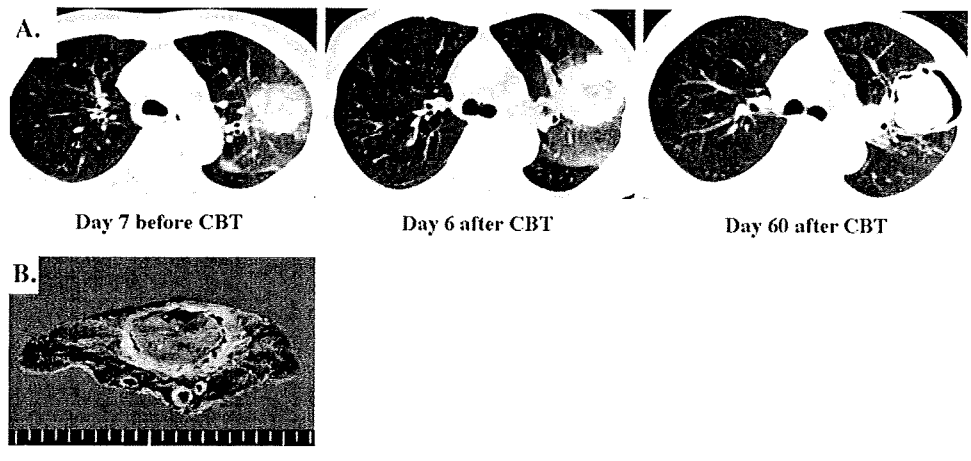

Fig. 2 Pulmonary aspergillosis lesion of patient 2. **a** Changes in the CT findings before and after CBT. **b** *Left upper* lung resected on day113 after CBT

irradiation regimens [15]. However, there were no options other than CBT for the two current patients because they did not have matched family donors and could not afford to wait until an HLA-matched unrelated donor was available. ATG was not included in the conditioning regimen for those patients because they could have succumbed to their infections which became exacerbated by the administration of ATG. Despite their devastating conditions and the elimination of ATG from the conditioning regimen, both patients achieved engraftment of CB without any apparent exacerbation of their infections or the development of severe GVHD. Therefore, in vivo purging of T cells using anti-T cell antibodies may not be a prerequisite for engraftment of CB after the 1–2-day regimen. However, it should be noted that both patients had been previously treated with conditioning regimens for allo-SCT. Prior conditioning regimens used for the first CBT may therefore be necessary for patients to take CB following such a minimum conditioning regimen. Other reduced-intensity regimens have been successfully used as preconditioning for a second transplantation using CB to treat graft rejection after allo-SCT [16–20]. However, all such regimens were administered for over 5 days and were not as weak as the regimens we used for the above described two patients.

Sustained engraftment of CB after the weak regimen in the current patients may therefore have important implications in the management of patients with hematologic malignancies refractory to chemotherapy. Patients who fail chemotherapy often suffer from severe infections due to persistent neutropenia and are therefore excluded as candidates for hematopoietic stem cell transplantation, particularly CBT, which is associated with delayed neutrophil recovery. Following very weak preconditioning, the patients not only circumvented life threatening infections but also achieved hematologic remission possibly with the help of the graft-versus-leukemia/lymphoma effects of CBT. CB can be utilized for patients with severe complications because of its easy accessibility and prompt availability [21]. Therefore, CBT following the minimum intensity conditioning may provide a chance to achieve complete chimerism in patients suffering from severe infections associated with profound neutropenia due to graft rejection or chemotherapy for leukemic relapse after the first allo-SCT.

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Relationship Between Tumor-infiltrating T Lymphocytes and Clinical Response After Reduced-intensity Allogeneic Hematopoietic Stem Cell Transplantation for Advanced Renal Cell Carcinoma: A Single Center Prospective Study

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Objective: Renal cell carcinoma (RCC) is refractory to conventional therapy, including chemotherapy and radiation. However, because RCC is sensitive to cytokine therapy, an immunotherapeutic approach such as hematopoietic stem cell transplantation (HSCT) might lead to a cure. We performed an institutional clinical study of HSCT for refractory RCC patients.
Methods: RCC patients aged 50 years or over, refractory to therapy, were eligible for the study. HSCT was performed after reduced-intensity conditioning. Primary endpoint was defined as the survival at day 100 after HSCT with complete donor chimerism, and secondary endpoint was the effectiveness of HSCT.
Results: Seven patients, provided with written informed consent, were enrolled in the study. Six of the seven patients achieved complete donor chimera at day 30 after HSCT, but one patient received second HSCT because of graft rejection. Four patients achieved a partial response (PR) and stable disease was observed in another patient, but these responses were temporary. The disease of the other two patients became progressive. Autopsy findings revealed an accumulation of $CD8^+$ lymphocytes and degenerative changes in the local RCC lesion in three of six patients who responded clinically. An autopsy of a patient who had obtained a PR revealed lymphocyte involvement with a cytotoxic T cell (CTL) phenotype in the metastasis of RCC.
Conclusions: Our results demonstrate the efficacy of HSCT for RCC and suggest that the graft-versus-tumor effect elicited by CTLs is induced *in vivo*. HSCT should be further explored as a potential curative treatment for RCC.

Key words: renal cell carcinoma – hematopoietic stem cell transplantation – cytotoxic T cells – graft-versus-tumor effect

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INTRODUCTION

Most renal cell carcinoma (RCC) patients at an advanced stage are refractory to conventional cytotoxic agents and are not amenable to complete resection, which currently offers the only chance of a cure. The estimated overall survival (OS) curve for RCC patients receiving various treatments does not attain a plateau (1), indicating the need for more effective therapies. In contrast, better outcomes are seen with interleukin-2 (IL-2) treatment, showing that RCC responds to immunotherapy (2). However, most cytokine therapies, such as dendritic cell therapy and lymphokine-activated killer therapy, produce a

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