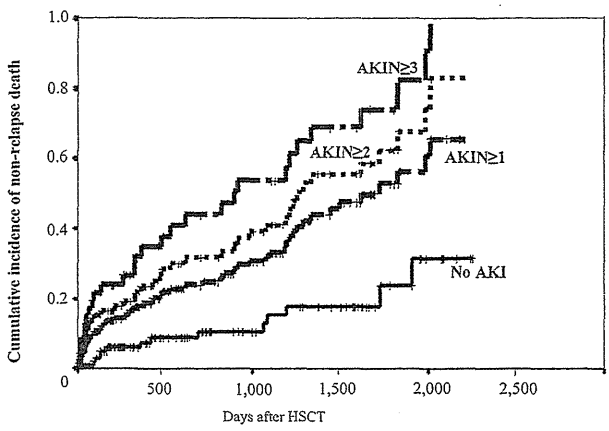

Figure. Cumulative incidence of non-relapse death in each group of AKIN stage (no AKI, AKIN stage≥1, AKIN stage≥2, AKIN stage≥3). Severity of AKI contributed to not only early death but also long-term non-relapse death.

Table 5. Cause of Death

	AKIN stage				
Cause of death	0	Stage ≥1	Stage ≥2	Stage ≥3	Total
Pneumonia		16	10	6	16
Sepsis	3	7	7	5	10
Viral encephalitis	1	4	2	2	5
Infection*		3	1	1	3
Graft failure		5	5	3	5
GVHD	3	5	3	2	8
TMA		3	2	1	3
SOS		4	4	3	4
Non-infectious lung complication		3	1	1	3
Heart failure	1	2	0		3
Renal failure		1	1		1
Other malignancy	1	2	1	1	3
Relapse	19	27	16	8	46
Total deaths	28	82	53	33	110

Abbreviations: GVHD: graft-versus-host disease, TMA: thrombotic microangiopathy, SOS: sinusoidal obstruction syndrome. *Infection includes infectious episodes without documentation of causative organisms.

relapse death in each AKIN stage (Figure), patients with a more severe stage were likely to be followed by more early and late death and causes of death in each stage were summarized in Table 5. Thus, severity of AKI might contribute to non-relapse death all the way through the transplantation. Although the precise mechanism by which AKI can contribute to long-term poor prognosis is not fully understood, the volume overload, coagulation abnormalities, and cytokine or immune-mediated organ dysfunction may also account for decreased long-term survival of patients who developed AKI (5). Moreover, chronic kidney disease might ensue as a consequence of previous AKI event (13), therefore, subsequent hypertension, proteinuria and increased cardiovascular disease could be a possible cause of poor long-term outcome among AKI patients (5). Specifically, in the setting of allo HSCT, AKI may interfere with dosing of calcineurin inhibitors, and may lead to the development of graft-versus host disease (GVHD) (5).

In conclusion, on the basis of our analysis, sepsis, hemorrhagic cystitis, and acute GVHD were associated with severe AKI, and SOS was associated with any stage of AKI. However, in view of the retrospective nature of the study and the relatively small number of patients, further investigation is necessary to confirm these correlations in analyses of large patient populations.

The authors state that they have no Conflict of Interest (COI).

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ORIGINAL ARTICLE

Clinical significance of hemophagocytosis in BM clot sections during the peri-engraftment period following allogeneic hematopoietic SCT

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The effects of macrophage activation on the outcome of allogeneic hematopoietic SCT (allo-HSCT) have yet to be fully examined. A total of 70 adult patients who received a first allo-HSCT for hematological diseases were studied. We counted the number of hemophagocytic cells in BM clot sections on day +14 ± 7, and analyzed its impact on subsequent outcome. In all, 23 patients were diagnosed as having increased numbers of hemophagocytic cells (HP group), whereas 47 were not (non-HP group). The HP group was not associated with an increased incidence of acute or chronic GVHD, but was associated with worse hematopoietic recovery than the non-HP group. The 2-year OS for the HP group and the non-HP group was 30 and 65% ($P<0.01$), respectively, and 2-year non-relapse mortality was 48% and 27% ($P<0.01$), respectively. Multivariate analysis confirmed that the HP group was associated with a lower OS (hazard ratio (HR) = 2.3; 95% confidence interval (CI), 1.0–5.4; $P=0.048$) and higher non-relapse mortality (HR = 4.0; 95% CI, 1.6–9.9; $P<0.01$). The HP group had higher incidences of death due to graft failure ($P<0.01$) and endothelial complications, such as sinusoidal obstruction syndrome and transplant-associated microangiopathy ($P=0.01$). Macrophage activation is a previously unrecognized complication with negative impact on outcome of allo-HSCT.

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Keywords: hemophagocytosis; macrophages; SCT; non-relapse mortality

Introduction

Macrophages have an indispensable role in both innate and acquired immunity and they have at least 3 major functions: antigen presentation, phagocytosis and immunomodulation.[1,2] Following allogeneic hematopoietic SCT (allo-HSCT), macrophages contribute to the development of acute GVHD by producing pro-inflammatory cytokines.[3] In addition to aGVHD, pro-inflammatory cytokine release is implicated in the pathogenesis of various early complications after allo-HSCT, such as sinusoidal obstruction syndrome, engraftment syndrome (ES) and capillary leakage syndrome.[4–7] Although the role of macrophages in these complications is undetermined, macrophages have an ability to secrete significant amounts of pro-inflammatory cytokines.[2] Furthermore, fatal outcomes of hemophagocytic syndrome after allo-HSCT have been described in case reports.[8] This evidence suggests that activation of macrophages has a significant impact on post-transplantation outcome. However, there are only a few clinical studies that have analyzed the effects of macrophage activation on outcome of allo-HSCT.[9]

Measuring the levels of cytokines or chemokines produced by activated macrophages, such as IL-1, IL-6, IL-12, TNF-α and macrophage inflammatory protein-1, may be a possible method to evaluate the activation of macrophages.[1,2,10] However, as these cytokines and chemokines are produced by many cell types, their elevated levels are not specific to macrophage activation.[1,10–12] An alternative method for evaluating the activation of macrophages is to assess the morphological change associated with macrophage activation, namely phagocytosis. Although phagocytosis reflects only a part of macrophage activation, the increased number of phagocytic cells provides direct evidence that macrophages are activated.[2] In addition, assessment of hemophagocytosis can be carried out easily using BM clot sections. Thus, hemophagocytosis serves as a specific and simple marker of macrophage activation. We assessed hemophagocytosis in BM clot sections during the early post-transplantation period, and analyzed its impact on subsequent outcome.

Patients and methods

Patients

We reviewed 96 consecutive adult patients who received their first allo-BM or PBSCT between December 2005 and December 2008 at the Japanese Red Cross Nagoya First Hospital. As our purpose was to examine the impact of

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hemophagocytosis in BM clot sections on day +14±7 on subsequent outcome, two patients who died within the first 21 days after transplantation were excluded. Although BM aspiration is routinely performed on day +14±7 in our institution, it was not performed in 17 patients. In addition, specimens were insufficient for evaluation in seven patients. As a result, 70 patients were included in the analysis, all of whom received T cell-replete grafts. Standard risk diseases were defined as AML in first or second CR, ALL in first CR, CML in first chronic phase, myelodysplastic syndrome as refractory anemia, malignant lymphoma in CR, chronic active EB-virus infection, aplastic anemia and paroxysmal nocturnal hemoglobinuria, whereas high-risk diseases were defined as the others. This study was approved by the institutional review board. All patients provided written informed consent.

Transplantation procedure

Conditioning included myeloablative and reduced-intensity regimens. The myeloablative regimens were mainly CY/TBI based, whereas the reduced-intensity conditioning regimens were mainly fludarabine 125 mg/m^2 plus melphalan 135–180 mg/m^2. Antithymocyte globulin was added in two patients who received HLA-mismatched transplants, and alemtuzumab was added in one patient with aplastic anemia. For GVHD prophylaxis, CYA and short-term MTX were used for allo-HSCT from a related donor, and tacrolimus and short-term MTX for allo-HSCT from an unrelated donor. All patients were cared for in laminar airflow units and received oral gut decontamination. Standard prophylaxis against *Pneumocystis carinii*, fungal infections and herpes simplex virus was given. G-CSF was administered after transplantation in all patients until engraftment was confirmed. Engraftment was defined as an ANC of more than 500/μL for 3 consecutive days. Primary graft failure was said to have occurred when engraftment was not seen in patients surviving more than 21 days after transplantation.[13] Secondary graft failure was defined as loss of neutrophil engraftment as determined by an ANC of less than 500/μL for 3 consecutive days after having achieved neutrophil engraftment, and no evidence of disease progression in the marrow.[14] ES was diagnosed, if patients presented with two or more of the following symptoms within 96 h of the start of neutrophil recovery (ANC >100/μL): (1) fever (temperature >=38.5°C) without an identifiable infectious cause; (2) weight gain >=5% over the pre-transplantation baseline weight; (3) erythematous rash not attributable to a medication; and (4) hypoxia, pulmonary infiltrates or both not attributable to infection or cardiac disease.[15,16] Acute GVHD was evaluated by established criteria.[17] Chronic GVHD was evaluated in patients who survived beyond day +100 without a relapse according to the traditional Seattle criteria.[18]

BM examination

BM aspiration was routinely performed on day +14±7. All specimens were fixed in formalin solution, embedded in paraffin and stained with hematoxylin-eosin. BM clot sections were reviewed retrospectively and the total number of hemophagocytic cells in three fields at a 200-fold magnification was counted (Figure 1).

Statistical considerations

Chi-square, Fisher's exact and Mann–Whitney tests were used to compare clinical and patient characteristics. The probability of survival was calculated using the Kaplan–Meier method, and the differences between groups were compared using log-rank statistics. Probabilities of non-relapse mortality (NRM) and relapse were calculated using the cumulative incidence function.[19] For NRM, relapse was the competing event, and for relapse, death in the absence of persistent or recurrent disease was the competing event. As our purpose was to examine the impact of hemophagocytosis in BM clot sections on day +14±7 on subsequent outcomes, all time-to-event comparisons were made from day +21 after transplantation. The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis

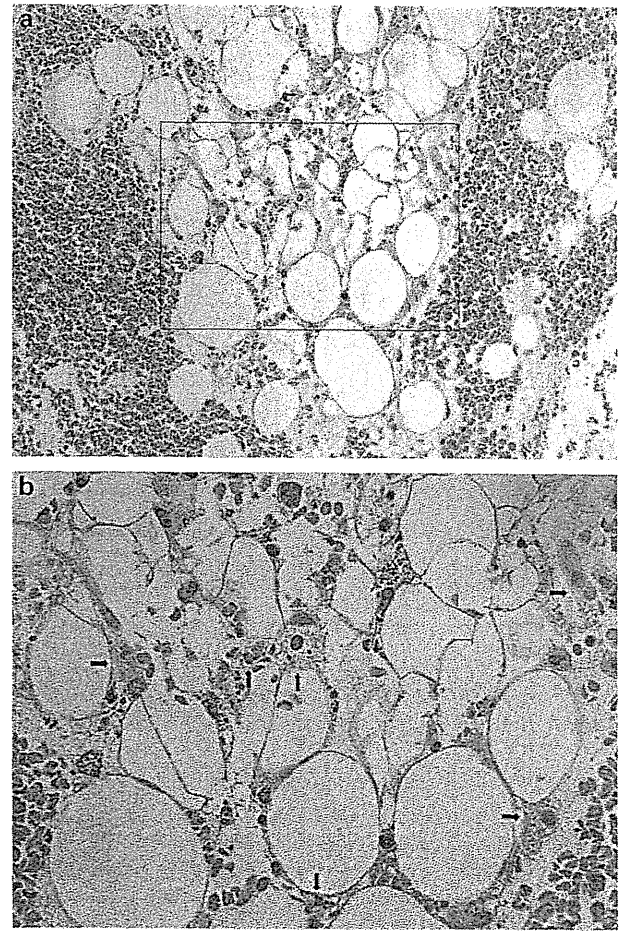

Figure 1 BM clot section stained with hematoxylin-eosin. (a) The specimen is from a representative patient who had an increased number of hemophagocytic cells. The indicated region is magnified in (b). Original magnification ×200. (b) Arrow indicates hemophagocytosis. Original magnification ×400.

were entered into multivariable models and sequentially eliminated in a stepwise backward manner. Potential prognostic factors considered in the analysis were recipient age, disease risk, hematopoietic cell transplantation-specific comorbidity index,[20] HLA disparity in the GVH direction in HLA-A, -B, -C, -DRB1 alleles, donor type (related or unrelated), donor-recipient sex-match, conditioning regimen, SC source, GVHD prophylaxis, total number of hemophagocytic cells in BM clot sections on day $+14\pm7$ and presence or absence of documented infections between day +7 and day +21. ES, sinusoidal obstruction syndrome, intestinal transplant-associated microangiopathy (intestinal TAM),[21,22] and posterior reversible encephalopathy syndrome were categorized as endothelial complications, as they are caused by vascular endothelial damage.[4,5,21–25] *P*-values of less than 0.05 were considered statistically significant.

Results

Patient characteristics

The median age of patients was 43 (range, 17–61) years. Diagnoses included AML ($n=24$), ALL ($n=13$), CML ($n=2$), myelodysplastic syndrome ($n=11$), malignant lymphoma ($n=9$), T-cell prolymphocytic leukemia ($n=1$), chronic active EB-virus infection ($n=1$), aplastic anemia ($n=7$) and paroxysmal nocturnal hemoglobinuria ($n=2$). Disease risk was standard in 40 patients and high in 30 patients. HLA was matched in the GVH direction in 47 patients, and matched in the host-vs-graft direction in 45 patients. Myeloablative conditioning regimens were used in 40 patients, and reduced-intensity conditioning regimens in 30. GVHD prophylaxis consisted of a combination of CYA and short-term MTX ($n=21$) or that of tacrolimus and short-term MTX ($n=49$). The median follow-up period of survivors was 556 (range, 236–1272) days.

The median total number of hemophagocytic cells in three fields was two (range, 0–30). The patients were divided into two groups on the basis of nearly bimodal distribution of total numbers of hemophagocytic cells (Figure 2): HP group (total number of hemophagocytic cells $>=5$, median 8, $n=23$) and non-HP group (total number of hemophagocytic cells <5, median 1, $n=47$). Patient characteristics are summarized in Table 1. There were no significant differences between the HP and non-HP groups.

Clinical and laboratory features

Clinical and laboratory features from day +7 to day +21 were compared between the HP and non-HP groups (Table 2). Compared with the non-HP group, fever, neurological symptoms, body weight gain, hypoxia, elevated total bilirubin, elevated serum creatinine and elevated C-reactive protein were more frequent in the HP group. In contrast, there were no significant differences in the incidence of skin rash and diarrhea between the two groups.

Among the 23 patients in the HP group, 14 (61%) developed infections between day +7 and +21. The causes

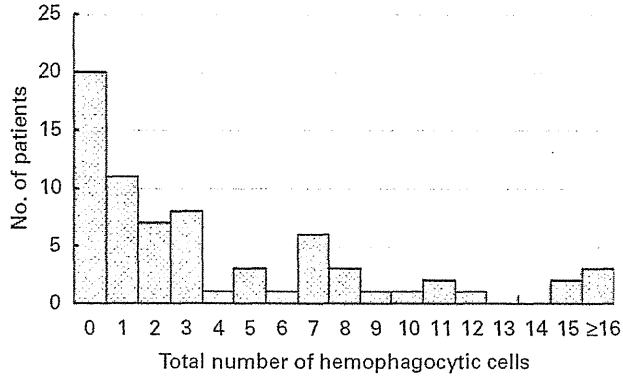

Figure 2 Distribution of numbers of hemophagocytic cells.

of these infections were bacteria ($n=9$), adenovirus and BK virus ($n=1$), CMV ($n=1$), human herpesvirus-6 ($n=1$), aspergillus ($n=1$) and bacteria and aspergillus ($n=1$). Of the 47 patients in the non-HP group, 8 (17%) developed infections. The causes of these infections were bacteria ($n=5$), adenovirus ($n=2$) and *Pneumocystis carinii* ($n=1$). The incidence of infections between day +7 and +21 was significantly higher in the HP group ($P<0.01$).

Hematopoietic recovery

Five patients (22%) in the HP group had graft failure, whereas one patient (2%) in the non-HP group had graft failure ($P=0.01$). Of these six patients, four had primary graft failure and two in the HP group had secondary graft failure. The median time to ANC 500/μL was 18 (range, 12–32) days in the HP group and 15 (range, 11–23) days in the non-HP group ($P<0.01$). Median time to platelet 50 000/μL was 31 (range, 18–214) days and 24.5 (range, 11–94) days, respectively ($P=0.04$), and the median time to reticulocyte 1% was 24.5 (range, 17–38) days and 20 (range, 13–38) days, respectively ($P<0.01$).

We next analyzed hematopoietic recovery after excluding 22 patients who had concomitant infections because the HP group included significantly more patients who had concomitant infections, and infections are known to interfere with effective and sustained reconstitution of hematopoiesis.[14] One (11%) out of nine patients in the HP group had graft failure, whereas one (3%) out of the 39 patients in the non-HP group had graft failure ($P=0.34$). The HP group was associated with significantly slower neutrophil, platelet and reticulocyte recovery than the non-HP group (18 days vs 14.5 days, $P<0.01$ for neutrophil recovery; 36 days vs 24 days, $P=0.01$ for platelet recovery; 24 days vs 19.5 days, $P=0.02$ for reticulocyte recovery). To eliminate the effect of PBSC use on hematopoietic recovery, we carried out subgroup analysis that included only those patients who received BMT. This subgroup analysis also showed that the HP group was associated with worse hematopoietic recovery than the non-HP group (data not shown).

Engraftment syndrome and GVHD

Among those patients who engrafted, there was no significant difference in the incidence of ES between the

Table 1 Patient characteristics

Characteristics	*HP group*	*Non-HP group*	P-*value*
No. of patients	23	47	
Median age, years (range)	49 (17–61)	41 (19–60)	0.33
Female, *n* (%)/male, *n* (%)	11 (48)/12 (52)	22 (47)/25 (53)	0.94
Donor/patient sex, *n* (%)			0.53
Femal/male	7 (30)	11 (23)	
Others	16 (70)	36 (77)	
Diagnosis, *n* (%)			0.25
Myeloid malignancy	12 (52)	25 (53)	
Lymphoid malignancy	6 (26)	18 (38)	
Benign hematologic disease	5 (22)	4 (9)	
Disease risk, *n* (%)			0.27
Standard	11 (48)	29 (62)	
High	12 (52)	18 (38)	
HCT-CI, *n* (%)			>0.99
<3	21 (91)	41 (87)	
>=3	2 (9)	6 (13)	
SC source, *n* (%)			0.35
BM	20 (87)	35 (74)	
PB	3 (13)	12 (26)	
Cell dose[a]			0.79
>=median	13 (57)	25 (53)	
<median	10 (43)	22 (47)	
Donor type, *n* (%)			0.68
Related	11 (48)	20 (43)	
Unrelated	12 (52)	27 (57)	
HLA disparity in GVH direction, *n* (%)			0.43
Match	14 (61)	33 (70)	
Mismatch	9 (39)	14 (30)	
HLA disparity in HVG direction, *n* (%)			0.68
Match	14 (61)	31 (66)	
Mismatch	9 (39)	16 (34)	
Conditioning regimen, *n* (%)			0.11
Myeloablative	10 (43)	30 (64)	
Reduced-intensity	13 (57)	17 (36)	
GVHD prophylaxis, *n* (%)			0.54
CsA+MTX	8 (35)	13 (28)	
FK+MTX	15 (65)	34 (72)	
ABO compatibility, *n* (%)			0.56
Match	11 (48)	26 (55)	
Mismatch	12 (52)	21 (45)	

Abbreviations: FK=tacrolimus; HCT-CI=hematopoietic cell transplantation-specific comorbidity index; HVG=host versus graft.
[a]The median nucleated cell dose was 2.83×10^8/kg (range, 1.21–5.03) in BM recipients, and the median CD34+ cell dose was 3.50×10^6/kg (range, 2.03–5.95) in PBSC recipients.

HP group and the non-HP group (20% in the HP group vs 20% in the non-HP group, $P>0.99$). In all, 10 patients (43%) in the HP group and 22 patients (47%) in the non-HP group developed aGVHD ($P=0.79$). Chronic GVHD developed in four of nine evaluable patients (44%) in the HP group, whereas it developed in 22 of 41 evaluable patients (54%) in the non-HP group ($P=0.72$).

OS, non-relapse mortality and relapse

When patients were divided into quartiles according to the total number of hemophagocytic cells, 20 patients were in the first quartile (no hemophagocytic cells), 18 were in the second quartile (1 to 2 hemophagocytic cells), 19 were in the third quartile (3 to 7 hemophagocytic cells) and 13 were in the fourth quartile (8 or more hemophagocytic cells). As the cut-off point of 5 or more hemophagocytic cells was used to define the HP group, the third quartile was further divided into two groups (3 to 4 and 5 to 7 hemophagocytic cells). A 2-year cumulative incidences of NRM for patients with 0 to 4 hemophagocytic cells were 18–33%, whereas those for patients with 5 or more hemophagocytic cells were as high as approximately 50% (Table 3).

OS rates for the HP group and non-HP group were 52 and 94% at day +100, and 30 and 65% at 2 years ($P<0.01$), respectively (Figure 3). Cumulative incidences of NRM for the HP group and non-HP group were 43 and 4% at day +100, and 48 and 27% at 2 years ($P<0.01$), respectively (Figure 4a). The cumulative incidences of relapse for the HP group and non-HP group were 22 and 13% at 2 years ($P=0.31$), respectively (Figure 4b).

Results of univariate and multivariate analysis of factors affecting post-transplantation outcomes are summarized in Table 4. Multivariate analysis showed that the prognostic factors for lower OS were HP group (hazard ratio (HR)=2.3; 95% confidence interval (CI), 1.0–5.4; $P=0.048$), high-risk disease (HR=3.8; 95% CI, 1.6–9.1; $P<0.01$), and presence of infections (HR=3.2; 95% CI, 1.2–8.3; $P=0.02$). Similarly, the prognostic factors for higher NRM were HP group (HR=4.0; 95% CI, 1.6–9.9; $P<0.01$), and patient age >=50 years (HR=4.5; 95% CI, 1.7–12; $P<0.01$). Furthermore, HP group was associated with an increased NRM at day +100 on multivariate analysis (HR=11; 95% CI, 2.4–52; $P<0.01$). Multivariate analysis showed that the prognostic factors for higher relapse rates were HP group (HR=3.6; 95% CI, 1.1–12; $P=0.04$), and high-risk disease (HR=4.9; 95% CI, 1.0–23; $P=0.047$).

Causes of death

Of 23 patients in the HP group, 16 died. Their causes of death were relapse ($n=5$), graft failure ($n=4$), ES ($n=1$), posterior reversible encephalopathy syndrome ($n=1$), sinusoidal obstruction syndrome ($n=1$), intestinal TAM[21,22] ($n=2$), and infection ($n=2$). Of 47 patients in the non-HP group, 14 died. Their causes of death were relapse ($n=5$), interstitial pneumonia ($n=3$), infection ($n=2$), cryptogenic organizing pneumonia ($n=1$), chronic GVHD ($n=1$), intestinal TAM ($n=1$), and secondary cancer ($n=1$). The incidence of death due to graft failure was significantly higher in the HP group than in the non-HP group (17 vs 0%, $P<0.01$), and that of death due to endothelial complications (ie, ES, sinusoidal obstruction syndrome, intestinal TAM, and posterior reversible encephalopathy syndrome) was significantly higher in the HP group than in the non-HP group (22 vs 2%, $P=0.01$).

Discussion

This study demonstrated that activation of macrophages in the BM early in the post-transplant period was associated

Table 2 Features during day +7 to day +21

	HP group n=*23*	*Non-HP group* n=*47*	P-*value*
Fever (>=38.3 °C for 3 consecutive days), *n* (%)	15 (65)	15 (32)	<0.01
Skin rash, *n* (%)	3 (13)	9 (19)	0.74
Diarrhea, *n* (%)[a]	6 (26)	8 (17)	0.37
Neurological symptoms, *n* (%)	3 (13)	0 (0)	0.03
Body weight gain (>=5% of baseline), *n* (%)[b]	16 (73)	14 (30)	<0.01
Hypoxia (SpO2 <=95%), *n* (%)	9 (39)	6 (13)	0.01
Total bilirubin >=2 mg/dL, *n* (%)	17 (74)	5 (11)	<0.01
AST >=twice the UNL, *n* (%)	6 (26)	6 (13)	0.16
ALT >=twice the UNL, *n* (%)	12 (52)	22 (47)	0.67
LDH >=twice the UNL, *n* (%)	6 (26)	10 (21)	0.65
Creatinine >=twice the baseline, *n* (%)	5 (22)	1 (2)	0.01
CRP >=10 mg/dL, *n* (%)	13 (57)	7 (15)	<0.01
Infection, *n* (%)	14 (61)	8 (17)	<0.01

Abbreviations: AST=aspartate aminotransferase; ALT=alanine aminotransferase; CRP=C-reactive protein; LDH=lactate dehydrogenase.
[a]Diarrhea, which is grade 3 or 4 according to the National Cancer Institute common toxicity criteria.
[b]Body weight of one patient in the HP group and one patient in the non-HP group was not evaluable because of poor performance status.

Table 3 Non-relapse mortality and OS according to the total number of hemophagocytic cells

Total no. of hemophagocytic cells	*2-year NRM*	*2-year OS*
0 (*n*=20)	18	68
1–2 (*n*=18)	30	64
3–4 (*n*=9)	33	67
5–7 (*n*=10)	50	20
>=8 (*n*=13)	46	38

Abbreviation: NRM=non-relapse mortality.

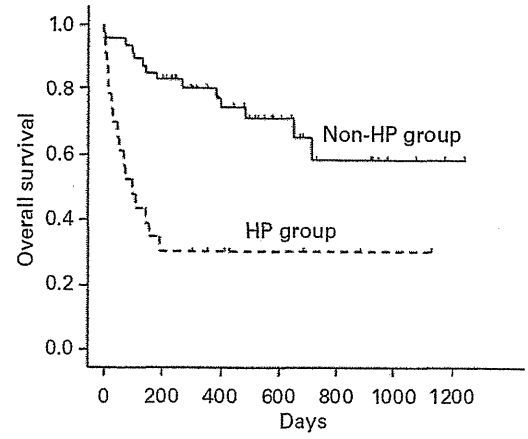

Figure 3 Probabilities of OS were significantly lower in the HP group than in the non-HP group (HP group: 52% at day +100 and 30% at 2 years; non-HP group: 94% at day +100 and 65% at 2 years; $P<0.01$).

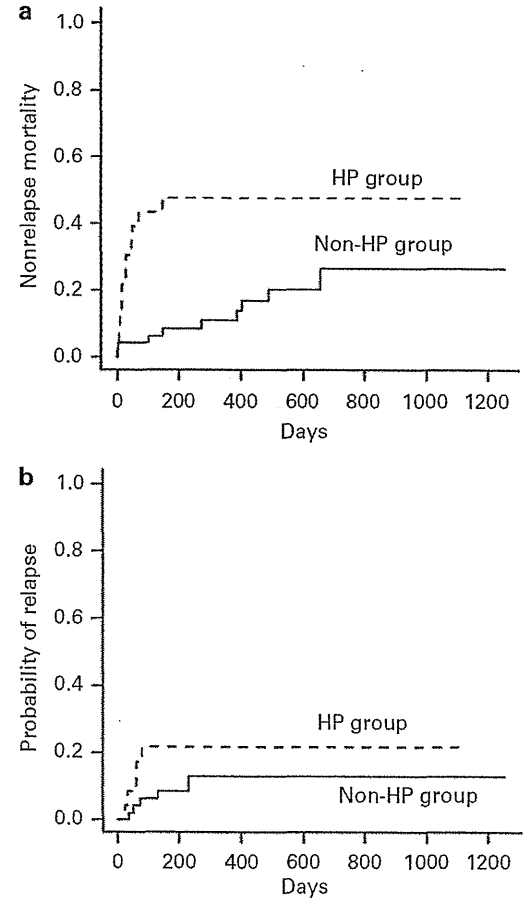

Figure 4 (a) Cumulative incidences of NRM were significantly higher in the HP group than in the non-HP group (HP group: 43% at day +100 and 48% at 2 years; non-HP group: 4% at day +100 and 27% at 2 years; $P<0.01$). (b) Cumulative incidences of relapse did not differ significantly between the HP group and the non-HP group (HP group: 22% at 2 years; non-HP group: 13% at 2 years; $P=0.31$).

with impaired hematopoietic recovery, distinctive clinical and laboratory features, higher NRM rates and lower OS rates. The results of this study revealed that early macrophage activation is an important complication, which has a significant impact on outcomes of allo-HSCT.

In this study, 23 out of 70 patients (33%) were diagnosed as having hemophagocytosis. This suggests that early macrophage activation is a relatively common but unrecognized complication. Even if none of the 26 patients excluded from the analysis had hemophagocytosis, the incidence of hemophagocytosis would be still as high as 24% (23/96).

Factors known to influence hematopoietic recovery after allo-HSCT include intensity of conditioning, cell dose and HLA compatibility.[14] Although there were no significant differences in these factors between the HP group and the non-HP group, the incidence of graft failure was higher and hematopoietic recovery was slower in the HP group than in the non-HP group. The HP group was associated with slower hematopoietic recovery than the non-HP group when excluding from analysis those patients who had concomitant infections, which are known to interfere with reconstitution of hematopoiesis.[14] Furthermore, among those patients who received BM as a SC source, the HP group had inferior hematopoietic recovery to the non-HP group. Thus, the HP group is an independent factor affecting hematopoietic recovery.

Analysis of clinical features demonstrated that fever, neurological symptoms, weight gain, hypoxia, elevated

Table 4 Factors affecting (a) overall mortality, (b) non-relapse mortality and (c) relapse

Variables	*Adverse factors*	*Univariate*		*Multivariate*	
		HR (95% CI)	P-*value*	*HR (95% CI)*	P-*value*
(a)					
Hemophagocytosis	HP group	3.9 (1.9–8.2)	<0.01	2.3 (1.0–5.4)	0.048
Patients age	> = 50 years	2.4 (1.2–5.0)	0.02		
Disease risk	High	2.8 (1.3–5.8)	<0.01	3.8 (1.6–9.1)	<0.01
HCT-CI	> = 3	0.43 (0.10–1.8)	0.25		
HLA disparity in GVH direction	Mismatch	1.7 (0.80–3.4)	0.18		
Donor type	Related	1.9 (0.92–3.9)	0.08		
Donor/patient sex	Femal/male	2.1 (0.97–4.4)	0.06		
Conditioning regimen	RIC	2.2 (1.1–4.6)	0.03		
SC source	PB	1.8 (0.80–3.9)	0.16		
GVHD prophylaxis	FK + MTX	0.59 (0.28–1.2)	0.15		
Infections during day +7 ± 21	Yes	2.7 (1.3–5.6)	<0.01	3.2 (1.2–8.3)	0.02
(b)					
Hemophagocytosis	HP group	4.1 (1.7–10)	<0.01	4.0 (1.6–9.9)	<0.01
Patients age	> = 50 years	4.6 (1.8–12)	<0.01	4.5 (1.7–12)	<0.01
Disease risk	High	1.9 (0.80–4.7)	0.14		
HCT-CI	> = 3	0.66 (0.15–2.9)	0.58		
HLA disparity in GVH direction	Mismatch	1.6 (0.64–3.8)	0.33		
Donor type	Related	1.8 (0.73–4.3)	0.21		
Donor/patient sex	Femal/male	2.4 (0.98–6.0)	0.06		
Conditioning regimen	RIC	3.0 (1.2–7.4)	0.02		
SC source	PB	1.4 (0.50–3.9)	0.52		
GVHD prophylaxis	FK + MTX	0.48 (0.20–1.2)	0.10		
Infections during day +7 ± 21	Yes	4.0 (1.6–9.9)	<0.01		
(c)					
Hemophagocytosis	HP group	5.1 (1.5–17)	<0.01	3.6 (1.1–12)	0.04
Patients age	> = 50 years	0.41 (0.09–1.9)	0.26		
Disease risk	High	6.2 (1.3–29)	0.02	4.9 (1.0–23)	0.047
HCT-CI	> = 3	UE	0.23[a]		
HLA disparity in GVH direction	Mismatch	1.8 (0.51–6.0)	0.37		
Donor type	Related	2.0 (0.62–6.7)	0.24		
Donor/patient sex	Femal/male	1.3 (0.33–4.7)	0.74		
Conditioning regimen	RIC	1.5 (0.45–4.8)	0.53		
SC source	PB	2.4 (0.69–8.1)	0.17		
GVHD prophylaxis	FK + MTX	1.3 (0.34–4.9)	0.70		
Infections during day +7 ± 21	Yes	1.3 (0.28–6.1)	0.73		

Abbreviation: CI = confidence interval; FK = tacrolimus; HR = hazard ratio; HCT-CI = hematopoietic cell transplantation-specific comorbidity index; RIC = reduced-intensity conditioning; UE = unevaluable.
[a]None of the six patients with HCT-CI > = 3 relapsed ($P = 0.23$; log-rank test).

total bilirubin and elevated serum creatinine were more frequently observed among the HP group than the non-HP group. These abnormalities are similar to those observed in ES. Furthermore, macrophage activation, ES and aGVHD share the common feature of being associated with elevated levels of pro-inflammatory cytokines.[2,3,5] Therefore, we speculated that there might be an overlap between early macrophage activation following allo-HSCT, ES and aGVHD, but there were no statistically significant differences in the incidence of ES and that of aGVHD between the HP group and the non-HP group. These results suggest that early macrophage activation was rather an independent complication from ES and aGVHD, although we could not draw a definite conclusion because of insufficient statistical power of this analysis.

The HP group had significantly higher NRM rates, resulting in significantly worse OS compared with the non-HP group. Although the incidence of concomitant infections was significantly higher in the HP group over the non-HP group, multivariate analysis demonstrated that the HP group and concomitant infections were independent risk factors for OS. Therefore, early macrophage activation seems to be an independent complication affecting transplant outcome. Of note, the incidence of death due to graft failure and endothelial complications was significantly higher in the HP group than in the non-HP group. Elevated levels of pro-inflammatory cytokines associated with activated macrophages such as TNF-α and macrophage inflammatory protein-1α might have contributed to the development of graft failure and exacerbation of endothelial complications, resulting in higher NRM rates in the HP group.[6,7,10,26,27] That C-reactive protein values were found to be higher in the HP group than in the non-HP group suggested that early macrophage activation was associated with a hyperinflammatory state. Future studies to measure the serum cytokine levels are warranted.

The results of our study suggest that early identification of patients at high risk of NRM might be possible by simply performing BM aspiration on day $+14\pm7$. This may have important implications for future therapeutic strategies because we could potentially lower the NRM rates by administering macrophage-targeted therapies in those patients who have an increased number of hemophagocytic cells in BM on day $+14\pm7$. The potential therapeutic options targeting macrophages could include anti-TNFα agents, etoposide or liposomal corticosteroids.[28–31] As we cannot rule out the possibility that early macrophage activation is the result rather than the cause of inflammatory processes, prospective trials are warranted to examine whether macrophage-targeted therapies for early macrophage activation can lower NRM rates.

Unexpectedly, the HP group was a risk factor for relapse in multivariate Cox regression analysis. However, this result should be interpreted with caution because of the relatively small sample size of our study. The impact of macrophage activation on relapse needs to be confirmed by larger studies.

Many types of cells decrease conspicuously in number following conditioning therapy, whereas macrophages do not.[32] Accordingly, the 'proportion' of hemophagocytic cells is likely to increase in hypocellular BM, but the 'absolute number' of hemophagocytic cells counted in BM clot sections would be little affected by the cellularity of BM. Additionally, it is difficult to distinguish between hemophagocytic cells and macrophages covered with other cells in BM smears, whereas overlapping of cells is virtually negligible in clot sections because clot sections are very thin (2 μm). For these reasons, we counted the absolute number of hemophagocytic cells in BM clot sections in this study.

We tested CD163 immunostaining in an effort to increase objectivity. CD163 is a specific marker for cells of the monocyte/macrophage lineage.[33] There was a good, but not perfect, correlation between the total number of $CD163^+$ macrophages and that of hemophagocytic cells identified by hematoxylin-eosin staining (Spearman $r=0.70$, $P<0.01$), which could be explained by the fact that hemophagocytic macrophages identified by hematoxylin-eosin staining are a sub-population of $CD163^+$ macrophages.[34–36] Although an increased number of $CD163^+$ macrophages was not a statistically significant factor for NRM (adjusted HR 2.1; 95% CI, 0.85–5.0; $P=0.11$), limited statistical power precluded us from excluding a clinically meaningful effect of it. Further studies are warranted.

In conclusion, the activation of macrophages in the BM early in the post-transplantation period is a relatively common but unrecognized complication with a negative impact on outcomes of allo-HSCT. The results of our study indicate the clinical usefulness of BM examination during the early post-transplantation period for the prediction of outcome.

Conflict of interest

The authors declare no conflict of interest.

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Related transplantation with HLA-1 Ag mismatch in the GVH direction and HLA-8/8 allele-matched unrelated transplantation: a nationwide retrospective study

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To clarify which is preferable, a related donor with an HLA-1 Ag mismatch at the HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) or an HLA 8/8-allele (HLA-A, HLA-B, HLA-C, and HLA-DRB1)–matched unrelated donor (8/8-MUD), we evaluated 779 patients with acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome who received a T cell–replete graft from an RD/1AG-MM-GVH or 8/8-MUD. The use of an RD/1AG-MM-GVH donor was significantly associated with a higher overall mortality rate than the use of an 8/8-MUD in a multivariate analysis (hazard ratio, 1.49; $P < .001$), and this impact was statistically significant only in patients with standard-risk diseases ($P = .001$). Among patients with standard-risk diseases who received transplantation from an RD/1AG-MM-GVH donor, the presence of an HLA-B Ag mismatch was significantly associated with a lower overall survival rate than an HLA-DR Ag mismatch because of an increased risk of treatment-related mortality. The HLA-C Ag mismatch or multiple allelic mismatches were frequently observed in the HLA-B Ag-mismatched group, and were possibly associated with the poor outcome. In conclusion, an 8/8-MUD should be prioritized over an RD/1AG-MM-GVH donor during donor selection. In particular, an HLA-B Ag mismatch in the GVH direction has an adverse effect on overall survival and treatment-related mortality in patients with standard-risk diseases. (*Blood.* 2012;119(10):2409-2416)

Introduction

An HLA-matched unrelated donor (MUD) is considered to be an alternative donor in hematopoietic stem cell transplantation (SCT) for patients who lack an HLA-identical sibling. However, it is difficult to find an MUD for patients with rare HLA haplotypes. SCT from a related donor with 1 Ag mismatch at HLA-A, HLA-B, or HLA-DR loci in the graft-versus-host (GVH) direction results in a higher but acceptable incidence of acute GVHD and outcomes comparable to that of SCT from a matched related donor (MRD) in patients with high-risk diseases because it reduces the risk of relapse via a graft-versus-leukemia (GVL) effect.[1-3] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with higher graft failure and lower overall survival (OS).[1,2,4] However, strategies to reduce the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress recipient immune system.[5] Therefore, in current clinical practice in Japan, SCT from a related donor with 1 Ag mismatch in the GVH direction and accepting multiple Ag mismatches in the HVG direction without specific stem cell manipulation is being performed,[1,2] although such an approach has not yet been evaluated in a large cohort.

Our previous study showed that SCT from an HLA-1 Ag-mismatched donor in the GVH or HVG direction is comparable to that from an HLA-A, HLA-B, or HLA-DR Ag-MUD.[1] However, this study is relatively old (1991-2000) and may not reflect current practice. Furthermore, the analysis was mainly performed based on serological matching, because information on HLA allele matching in unrelated transplantation was insufficient at that time. The importance of allele matching at the HLA-A, HLA-B, and HLA-DRB1 loci in unrelated donor transplantation has been established previously.[6-8] In addition, the importance of allele matching at the HLA-C locus has been highlighted in several recent studies of unrelated transplantation, although HLA-C matching is, in general,

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still not considered in related transplantation.[9-12] Therefore, we conducted a nationwide retrospective study to compare the clinical outcomes of transplantation from a related donor with an HLA-1 Ag mismatch at the HLA-A, HLA-B, or HLA-DR loci in the GVH direction (RD/1AG-MM-GVH) with an HLA 8/8-allele (HLA-A, HLA-B, HLA-C, and HLA-DRB1)–MUD (8/8-MUD).

Methods

Data collection

Data for patients 16-70 years of age with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), or chronic myelogenous leukemia (CML) who received a first allogeneic transplantation from a related donor or HLA-6/6–Ag-MUD between January 1, 2001 and December 31, 2008 were obtained from the Transplant Registry Unified Management Program,[13] which includes data from the Japan Society for Hematopoietic Cell Transplantation and the Japan Marrow Donor Program. Our analysis included 344 patients who received a graft from an RD/1AG-MM-GVH donor and 453 patients who received a graft from an 8/8-MUD. The following patients were excluded: 11 patients who lacked data on survival status, survival date, sex of recipient and donor, stem cell source, GVHD prophylaxis, or performance status; 2 patients who received both BM and peripheral blood in related transplantation; and 5 patients who received stem cells manipulated by ex vivo T-cell depletion or CD34 selection. Finally, 327 patients who received a graft from an RD/1AG-MM-GVH donor and 452 patients who received a graft from an 8/8-MUD fulfilled the criteria. The data on 2318 patients who received transplantation from an MRD were also collected on the basis of similar inclusion and exclusion criteria to compare the OS rate. The study was approved by the data management committees of Transplant Registry Unified Management Program and by the institutional review board of Saitama Medical Center (Jichi Medical University, Saitama, Japan), where this study was organized.

Histocompatibility

Histocompatibility data for serological and genomic typing for the HLA-A, HLA-B, HLA-C, and HLA-DR loci were obtained from reports obtained from the institution at which the transplantation was performed. To reflect current practice in Japan, HLA matching in RD/1AG-MM-GVH donors was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci, whereas that in 8/8-MUD was assessed by genomic data for HLA-A, HLA-B, HLA-C, and HLA-DR loci. When the recipient's Ags or alleles were not shared by the donor, this was considered an HLA mismatch in the GVH direction; when the donor's Ags or alleles were not shared by the recipient, this was considered a mismatch in the HVG direction. SCT from a related donor with 1 Ag mismatch in the GVH direction has been performed by accepting multiple Ag mismatches in the HVG direction,[1,2] and therefore was included in this study.

End points and statistical analyses

The primary end point of the study was to compare OS rates between the RD/1AG-MM-GVH and 8/8-MUD groups. For exploratory purposes, OS, treatment-related mortality (TRM), relapse, acute and chronic GVHD, and cumulative incidences of neutrophil engraftment were analyzed in a subset of cohorts. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to standard criteria.[14,15] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days. Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/L for 3 consecutive days after transplantation.

Descriptive statistics were used to summarize variables related to patient characteristics. Comparisons between groups were performed with the χ^2 statistic or extended Fisher exact test as appropriate for categorical variables and the Mann-Whitney U test or the Kruskal-Wallis test as appropriate for continuous variables. The probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared with the log-rank test. The probabilities of TRM, relapse, acute and chronic GVHD, and neutrophil engraftment were estimated on the basis of cumulative incidence curves to accommodate the following competing events[16]: death for relapse, relapse for TRM, death without GVHD for acute and chronic GVHD, and death without engraftment for neutrophil engraftment; the groups were compared with a Gray test.[17] Cox proportional-hazards regression was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazard model was used to evaluate variables that may affect TRM, relapse, acute and chronic GVHD, and neutrophil engraftment.[18] For patients for whom conditioning intensity (myeloablative or reduced-intensity) was not reported, we reclassified the conditioning regimen as either myeloablative or reduced-intensity according to the National Marrow Donor Program/Center for International Blood and Marrow Transplant Research operational definitions.[19] To be consistent with our previous study, acute leukemia in the first or second remission, CML in the first or second chronic phase, and MDS without leukemic transformation were defined as standard-risk diseases, and others were defined as high-risk diseases.[1] The following variables were considered: the recipient's age group (≤ 50 years or > 50 years at transplantation), recipient's sex, presence of female (donor) to male (recipient) sex mismatch, performance status (0-1 or 2-4), disease (AML, ALL, CML, or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced-intensity), type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other), use of antithymocyte globulin or alemtuzumab, and the time from diagnosis to transplantation (< 6 months or ≥ 6 months). In addition, a variable of graft source (BM or peripheral blood) was also considered in the analysis specific to related donors. Factors with $P < .10$ in the univariate analysis were used in the first multivariate model without donor type and deleted in a stepwise manner from the model by backward selection. We added donor type to the final model. All tests were 2-sided, and $P < .05$ was considered to indicate statistical significance. All statistical analyses were performed with STATA Version 11 software (StataCorp) and R Version 2.12.0 software (The R Foundation for Statistical Computing).

Results

Patient characteristics

Compared with recipients of an 8/8-MUD, recipients of an RD/1AG-MM-GVH were more likely to be younger, to be male receiving a transplantation from a female donor, to have a shorter duration from diagnosis to transplantation, to have a high-risk disease, to receive cyclosporine for GVHD prophylaxis, to receive antithymocyte globulin or alemtuzumab, and to have a longer follow-up period (Table 1). Approximately half of the recipients in the RD/1AG-MM-GVH group received peripheral blood stem cells, whereas during this period in Japan, the source of transplantation from an MUD was restricted to BM. In the RD/1AG-MM-GVH group, the number of Ag mismatches in the HVG direction was 0 in 11%, 1 in 67%, 2 in 20%, and 3 in 2%. HLA-A, HLA-B, and HLA-DRB1 allelic information in both recipients and donors was available in 148 of 327 transplantations from an RD/1AG-MM-GVH donor and information on HLA-C Ag mismatch in either the GVH or HVG direction was available in 123 of 327.

OS

The 2-year OS rates in the 8/8-MUD and RD/1AG-MM-GVH groups were 0.59 (95% confidence interval [CI], 0.53-0.64) and 0.44 (95% CI, 0.38-0.49), respectively (log-rank test; $P < .001$; Figure 1A). Multivariate analysis revealed that, compared with the use of an 8/8-MUD, the use of an RD/1AG-MM-GVH was a significant adverse factor for OS (hazard ratio [HR], 1.49; 95% CI,

Table 1. Patient characteristics

Variable	RD/1AG-MM-GVH (n = 327)	8/8 MUD (n = 452)	P
Median age at transplantation, y (range)	45 (16-69)	48 (16-68)	.043
Recipient sex, n (%)			
Male	184 (56%)	267 (59%)	.434
Female	143 (44%)	185 (41%)	
Sex combination of donors and recipients, n (%)			
Female to male	91 (28%)	73 (16%)	< .001
Other combinations	236 (72%)	379 (84%)	
Performance status, n (%)			
0/1	298 (91%)	415 (92%)	.736
2/3/4	29 (9%)	37 (8%)	
Disease, n (%)			
AML	167 (51%)	249 (55%)	.512
ALL	90 (28%)	107 (24%)	
CML	19 (6%)	21 (5%)	
MDS	51 (16%)	75 (17%)	
Duration from diagnosis to transplantation, n (%)			
< 6 mo	124 (38%)	102 (23%)	< .001
≥ 6 mo	191 (58%)	350 (77%)	
Unknown	12 (4%)	0 (0%)	
Disease risk, n (%)			
Standard	175 (54%)	317 (70%)	< .001
High	133 (41%)	129 (29%)	
Unknown	19 (6%)	6 (1%)	
Source of stem cells, n (%)			
BM	142 (43%)	452 (100%)	< .001
Peripheral blood	185 (57%)		
HLA compatibility in the HVG direction, n (%)*			
Matched	36 (11%)	452 (100%)	< .001
1-antigen mismatch	218 (67%)		
2-antigen mismatch	65 (20%)		
3-antigen mismatch	8 (2%)		
HLA compatibility in the GVH direction, n (%)*			
Matched	0 (0%)	452 (100%)	< .001
1-allele mismatch	111 (34%)		
2-allele mismatch	36 (11%)		
3-allele mismatch	1 (0%)		
Uncertain/missing	179 (55%)		
Conditioning regimen, n (%)			
Myeloablative	243 (74%)	338 (75%)	.883
Reduced-intensity	84 (26%)	114 (25%)	
GVHD prophylaxis, n (%)			
Cyclosporine-based	113 (35%)	108 (24%)	0.004
Tacrolimus-based	209 (64%)	338 (75%)	
Others	5 (2%)	6 (1%)	
Use of ATG/alemtuzumab, n (%)			
Yes	33 (10%)	13 (3%)	< .001
No	294 (90%)	439 (97%)	
Median follow-up of survivors, mo (range)	36.2 (3.0-95.7)	13.5 (1.7-62.8)	< .001

*HLA compatibility was defined according to the HLA-A, HLA-B, and HLA-DR loci.

1.19-1.86; $P < .001$; Table 2). Age > 50 years, performance status ≥ 2, and high-risk disease were also found to be significant adverse factors, whereas other variables, such as the time from diagnosis to transplantation, were not.

Because our previous study showed that the impact of an HLA-1 Ag mismatch in a related transplantation on OS differed according to whether patients had standard-risk or high-risk diseases,[1] the survival rates were compared separately in each disease-risk group. The OS rates of patients with standard-risk diseases in the 8/8-MUD group were significantly higher than those in the RD/1AG-MM-GVH group ($P = .003$), whereas there was no significant difference in high-risk patients ($P = .090$; Figure 1B-C). Although the interaction between the donor type and disease risk did not reach statistical significance ($P = .140$), multivariate analyses in each disease-risk group showed that the adverse impact of the use of an RD/1AG-MM-GVH donor was significant in standard-risk patients (HR, 1.72; 95% CI, 1.24-2.39; $P = .001$), but not in high-risk patients (Table 2).

To visually compare MRDs and other stem-cell sources, the OS rate for MRDs was layered on those for MUDs and RD/1AG-MM-GVHs (Figure 1). The OS curve of transplantation from an MRD

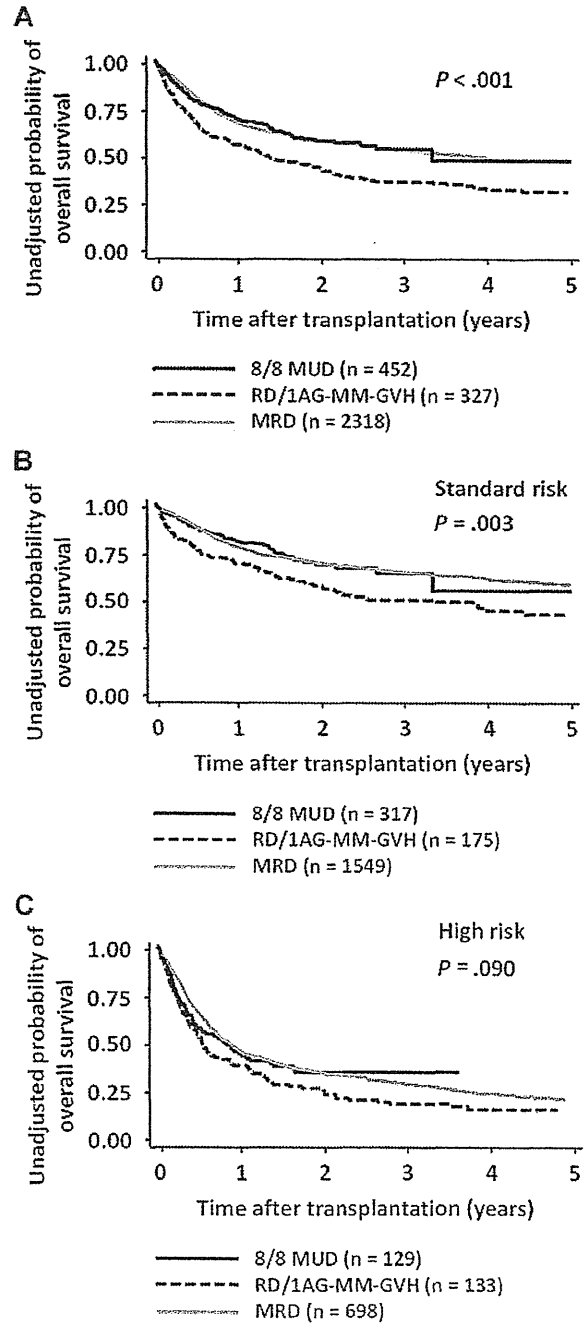

Figure 1. OS according to donor type and risk of disease. OS after transplantation from an RD/1AG-MM-GVH donor, an 8/8-MUD, and HLA-MRD in patients with both-risk (A), standard-risk (B), or high-risk diseases (C). Survival rates in the 8/8-MUD and RD/1AG-MM-GVH groups were compared with the log-rank test.

Table 2. Multivariate analysis of OS

	Total (n = 779)		Standard-risk (n = 492)		High-risk (n = 262)	
Variable	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Donor type						
8/8 MUD	1.00		1.00		1.00	
RD/1AG-MM-GVH	1.49 (1.19-1.86)	< .001	1.72 (1.24-2.39)	.001	1.30 (0.96-1.76)	.095
Age, y						
≤ 50	1.00		1.00			
> 50	1.44 (1.16-1.79)	.001	1.55 (1.13-2.15)	.007		
Performance status						
0/1	1.00				1.00	
2/3/4	1.79 (1.30-2.48)	< .001			1.76 (1.24-2.52)	.002
Disease risk						
Standard	1.00					
High	2.41 (1.92-3.03)	< .001				
Unknown	1.38 (0.82-2.33)	.227				

Only variables that remained after backward selection in the multivariate analysis are shown.

was superimposed on that from an MUD in both standard- and high-risk patients (MRD vs MUD: standard-risk group, $P = .895$, and high-risk group, $P = .581$). Multivariate analysis confirmed that OS in the MRD group was comparable to the MUD group (MRD vs MUD: standard-risk group, HR, 1.02; 95% CI, 0.79-1.32; $P = .878$; high-risk group, HR, 0.98; 95% CI, 0.76-1.26; $P = .865$).

Effect of HLA mismatches on OS

To identify factors that may contribute to the inferior OS in standard-risk patients in the RD/1AG-MM-GVH group compared with those in the 8/8-MUD group, we evaluated the impact of each HLA-A, HLA-B, or HLA-DR Ag mismatch in the GVH direction and the number of Ag mismatches in the HVG direction on OS rates in the RD/1AG-MM-GVH group.

In the RD/1AG-MM-GVH group, the OS rate for patients who received a transplantation from a related donor with an HLA-B Ag mismatch in the GVH direction and that from a donor with 2 or 3 Ag mismatches in the HVG direction were significantly lower than those in other groups (log-rank test for HLA-A Ag mismatch vs HLA-B Ag mismatch vs HLA-DR Ag mismatch in the GVH direction, $P < .001$, and 0-1 mismatches vs 2-3 mismatches in the HVG direction, $P = .003$; Figure 2). However, multivariate analysis revealed that only the presence of an HLA-B Ag mismatch in the GVH direction (HR, 1.57; 95% CI, 1.13-2.18; $P = .007$) was significantly associated with a lower OS (Table 3).

The OS rates were also compared separately in the standard-risk and high-risk disease groups (Figure 2). Although the interaction between the presence of an HLA-B Ag mismatch and disease risk did not reach statistical difference ($P = .232$), the adverse impact of an HLA-B Ag mismatch in the GVH direction was observed in the standard-risk group (HR, 1.86 95% CI, 1.14-3.01; $P = .012$), but not in the high-risk group (Table 3). Conversely, the survival curve for the HLA-A Ag or HLA-DR Ag-mismatched group was almost superimposed on that for 8/8-MUDs (Figure 2; standard-risk group: for the HLA-A Ag-mismatched group vs the 8/8-MUD group, HR, 1.26; 95% CI, 0.73-2.19; $P = .411$; for the HLA-DR Ag-mismatched group vs the 8/8-MUD group, HR, 1.37; 95% CI, 0.89-2.11; $P = .154$; high-risk group: for the HLA-A Ag-mismatched group vs the 8/8-MUD group, HR, 1.26; 95% CI, 0.80-2.00; $P = .320$; and for the HLA-DR Ag-mismatched group vs the 8/8-MUD group, HR, 1.03; 95% CI, 0.67-1.59]; $P = .880$). The impact of 2 or 3 Ag mismatches in the HVG direction was not significant in either the standard-risk or high-risk group (Table 3).

Effect of an HLA-B mismatch on TRM, relapse, GVHD, and neutrophil engraftment in patients with standard-risk diseases

Our findings showed that an HLA-B Ag mismatch in the GVH direction strongly contributed to the low survival rate in standard-risk patients, which can explain the inferior survival rates in the RD/1AG-MM-GVH group compared with the 8/8-MUD group. Therefore, we evaluated the impact of an HLA-B Ag mismatch in the GVH direction on other outcomes in patients with standard-risk diseases in the RD/1AG-MM-GVH group.

First, we compared the characteristics of patients with standard-risk diseases who received transplantation from a related donor with an HLA-A, HLA-B, and HLA-DR Ag mismatch in the GVH direction (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Two or 3 Ag mismatches in the HVG direction were observed more frequently in the HLA-B Ag-mismatched group (28%) than in the HLA-A Ag-mismatched group (2%) or the HLA-DR Ag-mismatched group (17%). Although there was no information available on allelic mismatch or HLA-C Ag mismatch in more than half of the patients, an HLA-C Ag mismatch in either the GVH or HVG direction was observed more frequently in the HLA-B Ag-mismatched group (61% among the available data) than in the HLA-A Ag-mismatched group (25%) or the HLA-DR Ag-mismatched group (17%).

The incidence of TRM was higher in the HLA-B Ag-mismatched group (3-year mortality rate: HR, 0.47; 95% CI, 0.32-0.60) than in the HLA-A Ag-mismatched group (HR, 0.28; 95% CI, 0.14-0.44) or the HLA-DR Ag-mismatched group (HR, 0.27; 95% CI, 0.17-0.38; Figure 3A; log-rank test, $P = .030$). The presence of an HLA-B Ag mismatch in the GVH direction was an independent significant adverse factor that affected TRM in the RD/1AG-MM-GVH group (Table 4). Conversely, the incidence of relapse did not significantly differ among the 3 groups (Figure 3B and Table 4).

The incidence of grade 2-4 acute GVHD in the HLA-B Ag-mismatched group was higher than that in the HLA-A Ag-mismatched group, but comparable to that in the HLA-DR Ag-mismatched group (supplemental Figure 1 and supplemental Table 2). There was no significant difference in the incidence of grade 3-4 acute GVHD among the 3 groups. Regarding neutrophil engraftment, multivariate analysis showed that an HLA-B Ag mismatch was significantly associated with inferior neutrophil engraftment and 2 or 3 Ag mismatches in the HVG direction were

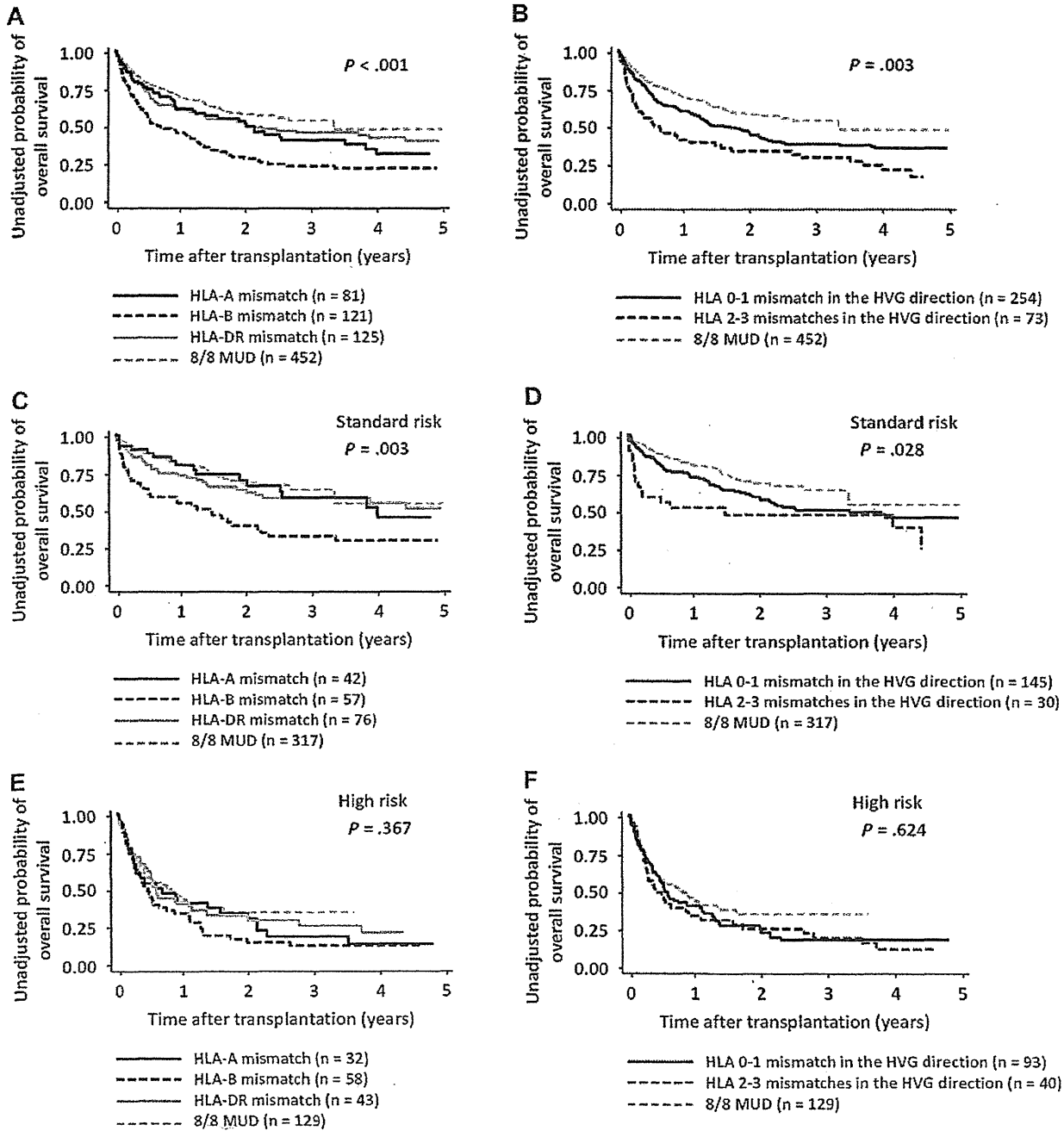

Figure 2. OS in patients with both-risk, standard-risk, or high-risk diseases according to the locus of HLA mismatch in the GVH direction and the number of mismatches in the HVG direction. Survival rates in patients with HLA-A, HLA-B, and HLA-DR Ag mismatches in the GVH direction were compared with the log-rank test (A,C,E). Survival rates in patients with 0-1 and 2-3 mismatches in the HVG direction were compared with the log-rank test (B,D,F). Survival rates of the 8/8-MUD group are shown for visual comparison.

associated with inferior neutrophil engraftment, with marginal significance (supplemental Table 2).

Discussion

In this nationwide retrospective study, we found that the survival rate of the RD/1AG-MM-GVH group was significantly inferior to that of the 8/8-MUD group, and this significant difference was observed only in patients with standard-risk diseases, although the interaction between donor type and disease risk did not reach statistical significance. We reported previously that transplantation from a related donor with 1 Ag mismatch in the GVH or HVG direction gave a clinical outcome comparable to that of transplantation from a 6/6-Ag-MUD in patients with either standard-risk or high-risk diseases.[1] However, because HLA matching at the allelic level in unrelated transplantation significantly reduces the risk of GVHD, in the present study, the survival curve of transplantation from an 8/8-MUD was substantially improved, and could be superimposed on a curve corresponding to that from an MRD.

Table 3. Multivariate analysis of OS in patients receiving transplantation from a related donor with a 1-antigen mismatch in the GVH direction

	Total (n = 327)		Standard-risk (n = 175)		High-risk (n = 133)	
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
HLA mismatch in the GVH direction						
HLA-DR mismatch	1.00		1.00		1.00	
HLA-A mismatch	1.07 (0.73-1.56)	.737	0.98 (0.54-1.81)	.966	1.11 (0.65-1.89)	.701
HLA-B mismatch	1.57 (1.13-2.18)	.007	1.86 (1.14-3.01)	.012	1.36 (0.86-2.17)	.193
HLA mismatch in the HVG direction						
0-1 mismatches	1.00		1.00		1.00	
2-3 mismatches	1.27 (0.91-1.76)	.154	1.67 (0.98-2.85)	.061	1.06 (0.69-1.61)	.799
Age, y						
≤ 50	1.00		1.00			
> 50	1.52 (1.14-2.03)	.004	1.87 (1.21-2.91)	.005		
Disease risk						
Standard	1.00					
High	2.06 (1.53-2.78)	< .001				
Unknown	1.00 (0.53-1.89)	.989				

Only variables that remained after backward selection in the multivariate analysis are shown.

Consistent with our findings, several studies have shown that the clinical outcomes of transplantation from an 8/8-10/10 MUD are comparable to those from an MRD.[20,21] The significant difference in survival rates between transplantation from an RD/1AG-MM-GVH donor and an 8/8-MUD disappeared in patients with high-risk diseases, probably because the adverse impact of acute GVHD on survival might be offset by the potential GVL effect in transplantation from an RD/1AG-MM-GVH donor.[1,2,22]

We evaluated factors that may contribute to the inferior OS in patients with standard-risk diseases in the RD/1AG-MM-GVH group and found that, compared with the presence of an HLA-DR Ag mismatch, the presence of an HLA-B Ag mismatch in the GVH direction was significantly associated with lower OS and higher TRM. Conversely, the rates of OS and TRM in the HLA-A Ag- or HLA-DR Ag-mismatched group were superimposed on those in the MUD group. However, HLA-A, HLA-B, and HLA-DR Ag mismatches had similar effects on the incidence of severe acute GVHD; consequently, the causal relationship between an HLA-B Ag mismatch in the GVH direction and higher TRM remains unknown. In contrast to our findings, Valcarcel et al reported that there was no significant difference in OS between the use of 1-Ag–mismatched related donors (n = 89) and 8/8-MUDs (n = 700) in transplantation for AML and ALL during the first or second complete remission.[23] This difference from our results can be partly

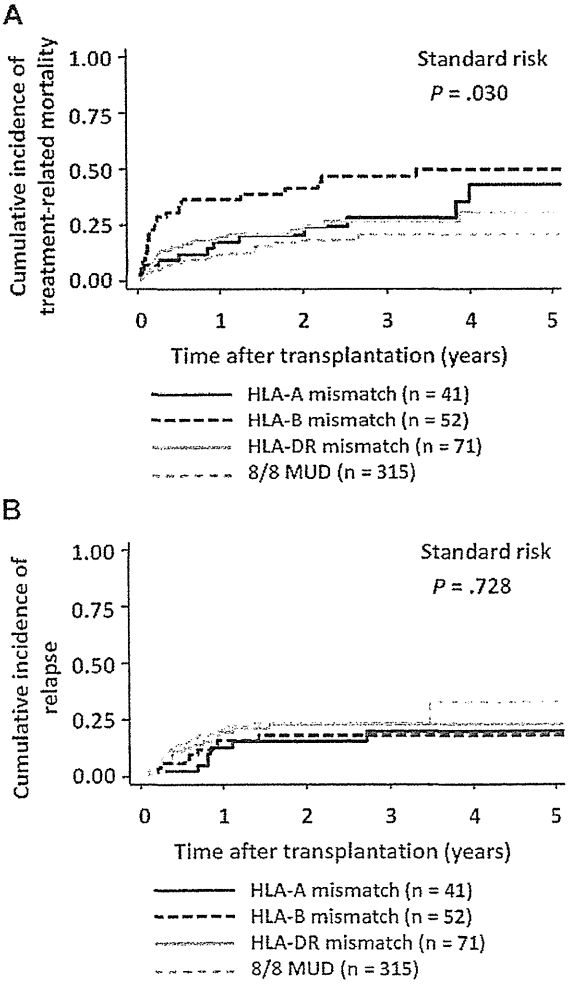

Figure 3. Cumulative incidence according to the locus of HLA mismatch in the GVH direction in patients with standard-risk diseases. Cumulative incidences in the related transplantation groups were compared with the Gray test. (A) TRM. (B) Relapse.

Table 4. Multivariate analysis of TRM and relapse in patients with standard-risk diseases receiving transplantations from a related donor with a 1-antigen mismatch in the GVH direction

	TRM (n = 164)		Relapse (n = 164)	
Variable	HR (95% CI)	P	HR (95% CI)	P
HLA mismatch in the GVH direction				
HLA-DR mismatch	1.00		1.00	
HLA-A mismatch	1.22 (0.59-2.52)	.587	0.70 (0.29-1.67)	.418
HLA-B mismatch	2.00 (1.09-3.65)	.025	0.80 (0.34-1.87)	.605
HLA mismatch in the HVG direction				
0-1 mismatches	1.00		1.00	
2-3 mismatches	2.21 (1.14-4.28)	.019	0.67 (0.23-1.98)	.467
Age, y				
≤ 50	1.00			
> 50	2.08 (1.18-3.65)	.011		
Duration from diagnosis to transplantation				
< 6 mo	1.00			
≥ 6 mo	2.40 (1.19-4.82)	.014		
Unknown	2.23 (0.77-6.48)	.140		

Only variables that remained after backward selection in the multivariate analysis are shown.

explained by the fact that the MUD group in their study included a significantly smaller number of ALL patients with low-risk cytogenetics. In addition, in our study, genetic homogeneity in the Japanese population might affect the lower incidence of severe acute GVHD in MUD transplantation because of the less frequent mismatches in minor histocompatibility Ags.[24,25]

The frequency of an HLA-C Ag mismatch was substantially higher in the HLA-B Ag-mismatched group than in the HLA-A or HLA-DR Ag-mismatched groups. This finding may represent linkage disequilibrium between the HLA-B and HLA-C genes, which are located at a very close physical proximity within the major histocompatibility complex.[26,27] Therefore, the impact of HLA-B-Ag might be affected by the co-presence of HLA-C Ag mismatch. We could not evaluate the impact of HLA-C Ag mismatch on OS rates because of the limited information on HLA-C Ag mismatch; therefore, an analysis with larger cohorts with complete HLA-C Ag information will be needed to evaluate the impact of HLA-C and/or HLA-B mismatch in transplantation from an RD/1AG-MM-GVH donor. Accordingly, we could not evaluate the impact of the KIR ligand mismatch. Although the impact of KIR ligand mismatch is still controversial, several studies analyzing T cell–replete transplantation showed that KIR ligand mismatch is associated with lower OS.[12,28,29] The analysis of KIR matching would be helpful in elucidating the mechanism underlying the adverse effect of HLA-B mismatch in T cell–replete transplantation from an RD/1AG-MM-GVH donor.

Whether the presence of allelic mismatches in addition to the 1-Ag mismatch (2 or more allelic mismatches in total) affects transplantation outcome is also an important clinical question in transplantation from an RD/1AG-MM-GVH donor. A high frequency of 2-allele mismatches in the GVH direction was seen in the HLA-B Ag-mismatched group, suggesting a possible association between 2-allele mismatches and low OS. However, we did not observe a significant effect of the number of allelic mismatches on OS after transplantation from an RD/1AG-MM-GVH donor, possibly because of the small sample size.

Our study has several limitations. First, because several months are required to arrange unrelated transplantations, patients at low risk for relapse may more often be selected for these procedures. To minimize this bias, we included the duration from diagnosis to transplantation in the multivariate analysis; however, this variable did not have a significant effect in the multivariate analysis. Second, heterogeneous backgrounds may have resulted in a bias. In particular, the stem-cell source in unrelated transplantation was exclusively BM. However, the analysis of OS in the subgroup of patients who received a BM graft from an RD/1AG-MM-GVH donor or an 8/8-MUD showed similar results. Third, because we have incomplete Ag and allele information on the HLA-C and -DQB1 loci, we may have underestimated the degree of mismatching in transplantation from an RD/1AG-MM-GVH donor. Fourth, the difference in the impact of donor type between standard- and high-risk diseases should be cautiously interpreted, because the interaction between the donor type and disease risk did not reach statistical significance. This may be partly because of the lower statistical power to detect the interaction than the main effect.

In conclusion, our findings suggest that an 8/8-MUD, if available, should be prioritized over an RD/1AG-MM-GVH donor for patients without an MRD if an immediate transplantation is not necessary. In particular, the presence of an HLA-B Ag mismatch in the GVH direction has an adverse effect on OS because of treatment-related complications. This may be because of the high frequencies of additional mismatches of HLA-C Ag or allele in the HLA-B Ag-mismatched group. To elucidate the mechanism of the adverse outcomes in RD/1AG-MM-GVH donors with an HLA-B Ag mismatch, HLA Ag/allele matching including HLA-C should be performed in transplantations from an RD/1AG-MM-GVH donor.

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Authorship

Contribution: Y.K. designed the research and organized the project; J.K., H. Saji, and Y.K. reviewed and analyzed the data and wrote the manuscript; J.K. and Y.K. performed the statistical analysis; H. Sakamaki, J.T., R.S., and Y.A. collected data from Japan Society for Hematopoietic Cell Transplantation; K.K. and Y.M. collected data from Japan Marrow Donor Program; and all authors interpreted the data and reviewed and approved the final manuscript.

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Impact of a donor source on adult Philadelphia chromosome-negative acute lymphoblastic leukemia: a retrospective analysis from the Adult Acute Lymphoblastic Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation

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Background: We aimed to clarify the impact of the donor source of allogeneic stem cell transplantation (allo-SCT) on Philadelphia chromosome-negative acute lymphoblastic leukemia [Ph(–) ALL] with focus on cord blood (CB).

Patients and methods: We retrospectively analyzed data of 1726 patients who underwent myeloablative allo-SCT for adult Ph(–) ALL. The sources of the allo-SCT were related donors (RD; $N = 684$), unrelated donors (URD; $N = 809$), and CB ($N = 233$).

Results: Overall survival (OS) in patients after CB allo-SCT in first complete remission (CR1) was comparable with that after RD or URD allo-SCT (RD: 65%, URD: 64% and CB: 57% at 4 years, $P = 0.11$). CB was not a significant risk factor for relapse or non-relapse mortality as well as for OS in multivariate analyses. Similarly, the donor source was not a significant risk factor for OS in subsequent CR or non-CR (RD: 47%, URD: 39% and CB: 48% in subsequent CR, $P = 0.33$; RD: 15%, URD: 21% and CB: 18% in non-CR, $P = 0.20$ at 4 years).

Conclusion: Allo-SCT using CB led to OS similar to those of RD or URD in any disease status. To avoid missing the appropriate timing, CB is a favorable alternative source for adult Ph(–) ALL patients without a suitable RD or URD.

Key words: allogeneic stem cell transplantation, cord blood, donor source, Philadelphia chromosome-negative acute lymphoblastic leukemia

introduction

The prognosis of adult acute lymphoblastic leukemia (ALL) is still unsatisfactory; long-term survival has been achieved in only ~30%–40% of patients despite a considerably high complete remission (CR) rate (78%–93% in major clinical trials) [1–8]. Allogeneic stem cell transplantation (allo-SCT) is the most potent post-consolidation therapy and curative option for ALL. It may be better to treat Philadelphia chromosome-positive [Ph(+)] ALL and Philadelphia chromosome-negative [Ph(–)] ALL as different diseases, since their treatment would differ in an era of tyrosine kinase inhibitors (TKIs) [9]. Therefore, it would be more practical here to discuss data only from patients with Ph(–) ALL [10–14].

From the results of a large prospective donor versus no donor study by the UK Medical Research Council UKALL /Eastern

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Cooperative Oncology Group (MRC/ECOG), it was concluded that related donor (RD) allo-SCT for Ph(−) ALL in CR1 could achieve significantly better overall survival (OS) than that without a suitable RD. As for unrelated donor (URD) allo-SCT, OS was reported to be comparable with that with RD allo-SCT for Ph(−) ALL in any disease status [12]. Since there has been no large-scale study on unrelated cord blood (CB) allo-SCT for Ph(−) ALL, further investigation is needed to find the position of CB when a suitable RD or URD cannot be found.

In this study, we analyzed the impact of a donor source on Ph(−) ALL, particularly the role of CB allo-SCT in each disease status, and we obtained data which would be useful to clinicians. Since how to handle CR1 patients would be the most important to improve the outcome [10–12], we mainly focused on the analyses of Ph(−) ALL in CR1.

patients and methods

collection of data and data source

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT), which collected recipients' clinical data at 100 days after allo-SCT. Data on survival, disease status, and long-term complications, such as chronic graft-versus-host disease (GVHD), are renewed annually by follow-up forms. This study was approved by the data management committees of JSHCT as a study of the adult ALL Working Group of JSHCT. Informed consent was obtained from both recipients and donors in accordance with the Declaration of Helsinki.

patients

Data for 2314 patients of at least 16 years of age who underwent their first allo-SCT for Ph(−) ALL between 1998 and 2009 were available in the registration database of JSHCT. After excluding 388 patients without data for HLA or disease status, 25 patients who received double CB, and 175 patients who received reduced intensity conditioning regimens, we analyzed data of 1726 adult Ph(−) ALL patients (684 RD, 809 URD, and 233 single CB) with focus on data of 917 patients transplanted in CR1, according to the donor types (RD, URD, and CB). RD peripheral blood (PB) was used for 299 of 684 allo-SCTs (43.7%). Only bone marrow grafts were used in URD allo-SCT because PB stem cell donation from URD was approved in 2010 in Japan.

definition

Neutrophil recovery was defined by an absolute neutrophil count of at least 0.5×10^9/l for 3 consecutive days, and platelet recovery was defined by a count of at least 50×10^9/l without transfusion support. Acute GVHD and chronic GVHD were diagnosed and graded according to the consensus criteria [15, 16]. Relapse was defined as hematologic leukemia recurrence. Non-relapse mortality (NRM) was defined as death during continuous remission. For analyses of OS, failure was death from any cause, and surviving patients were censored at the date of last contact. The date of allo-SCT was the starting time point for calculating all outcomes. Patients were classified at diagnosis by the Japan Adult Leukemia Study Group (JALSG) risk stratification: low risk was defined as <30 years at diagnosis and white blood cell (WBC) count of <30 000/μl at diagnosis, high risk was defined as ≥30 years at diagnosis and WBC count of ≥30 000/μl at diagnosis, and intermediate risk was defined as other [8]. HLA matching of CB was carried out using low-resolution typing for HLA-A, -B, and -C and high-resolution molecular typing for HLA-DRB1. HLA matching of URD was carried out using high-resolution typing for HLA-A, -B, -C, and HLA-DRB1 [17–19]. For RD or URD, 'well-matched' was defined as no known disparity at HLA-A, -B, -C, or -DRB1, 'partially matched' was defined as one locus disparity with their donors, and 'mismatched' was defined as two or more locus disparities. For CB, 'well-matched' was defined as no known disparity at HLA-A, -B, -C, or -DRB1, 'partially matched' was defined as at least four locus matches, and 'mismatched' was defined as less than three locus matches since CB of at least four of six HLA-matched and total nucleated cells $\geq 2 \times 10^7$/kg or CD34+ cells $\geq 1 \times 10^5$/kg is preferably selected in Japan [20, 21].

statistical analysis

The two-sided χ^2 test was used for categorical variables. OS rates were estimated by the Kaplan–Meier method and P values were calculated using a log-rank test [22, 23]. Cumulative incidences of relapse, NRM, and GVHD were calculated by Gray's method [24, 25]. Death without relapse was considered as a competing event for relapse, and relapse was considered as a competing event for NRM. Univariate and multivariate analyses were carried out using a Cox proportional hazard regression model [26]. A significance level of $P < 0.05$ was used for all analyses. Covariates included in the multivariate analyses were age at allo-SCT, WBC counts at diagnosis, sex mismatch, phenotypes, cytogenetics, JALSG risk, disease status, HLA disparity, time from diagnosis to allo-SCT, preparative regimens, GVHD prophylaxis, and year of allo-SCT.

results

patient characteristics

Of 1726 patients, 917 received allo-SCT in CR1 (388 RD, 434 URD and 95 CB), 300 received allo-SCT in subsequent CR, and 509 received allo-SCT in non-CR. The characteristics of the patients are shown in Table 1. The frequencies of HLA partially mismatched donor and those transplanted between 2005 and 2009 and age at allo-SCT were higher among patients receiving CB allo-SCT in CR1, whereas the frequency of tacrolimus-based GVHD prophylaxis was higher and the interval from diagnosis to allo-SCT was longer among patients receiving URD allo-SCT in CR1. Among 233 CB recipients, anti-thymocyte globulin (ATG) was used for three patients (two in CR1 and one in non-CR). The median CB dose of infused nucleated cells and CD34-positive cells were 2.46×10^7/kg (range 0.03–24.77×10^7/kg) and 0.83×10^5/kg (range 0.22–5.66×10^5/kg).

survival among patients transplanted in CR1

The median follow-up period for survivors was 44 months (range 4.7–153 months). OS rates at 4 years were 65% in CR1, 44% in subsequent CR, and 18% in non-CR ($P < 0.0001$). Since the disease status at allo-SCT was an obvious factor for the outcome of allo-SCT, we carried out analyses according to the disease status. Among 917 patients transplanted in CR1, there were no significant differences in OS between RD, URD, and CB allo-SCTs (65% in RD, 64% in URD, and 57% in CB at 4 years; $P = 0.11$) (Figure 1A). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, JALSG intermediate or high risk, HLA partially matched or mismatched, non-TBI preparative regimens, transplantation between 1998 and 2004, and <6 months from diagnosis to allo-SCT were significant risk factors for OS (Table 2). The donor source was not a significant risk factor [URD: hazard ratio (HR) 1.09 (95% CI

Table 1. Characteristics of Ph(−) ALL patients undergoing myeloablative allo-SCT, according to the disease status

	CR1							Subsequent CR		non-CR	
	Related		Unrelated BM		Cord blood		*P*				
No. of patients	388	(%)	434	(%)	95	(%)		300	(%)	509	(%)
Median white blood cell (WBC) count at diagnosis /μl (range)	11 400 (400–801 000)		13 235 (500–892 000)		10 400 (400–771 300)		0.48	9000 (500–706 000)		19 400 (500–840 200)	
Median patient age at allo-SCT, year (range)	31 (16–66)		34 (16–59)		37 (16–70)		0.01	25 (16–65)		30 (16–65)	
Patient sex, *n* (%)							0.47				
Male	207	53	240	55	46	48		166	55	295	58
Female	181	47	194	45	49	52		134	45	214	42
Lineage							<0.0001				
T	66	17	74	17	17	18		53	18	118	23
B	276	71	325	75	57	60		226	75	346	68
Other	28	7	17	4	17	18		12	4	35	7
Unknown	18	5	18	4	4	4		9	3	10	2
Cytogenetics							0.65				
Normal	226	58	255	59	53	56		195	65	243	48
t(4;11)	9	2	12	3	4	4		2	1	20	4
t(8;14)	4	1	3	1	0	0					
Hypodiploid	3	1	3	1	2	2		3	1	6	1
Hyperdiploid	11	3	15	3	0	0		15	5	26	5
Other [no *t*(9;22)]	135	35	146	34	36	38		85	28	214	42
JALSG risk stratification							0.09				
Low	112	29	96	22	22	23		121	40	149	29
Intermediate	213	55	253	58	57	60		151	50	227	45
High	54	14	84	19	16	17		23	8	116	23
Unknown	9	2	1	0	0	0		5	2	17	3
Source											
Related								89	30	207	41
Unrelated BM								158	53	217	43
Cord blood								53	18	85	17
HLA matching							<0.0001				
Well matched	351	90	278	64	4	4		153	51	246	48
Partially matched	27	7	102	24	71	75		93	31	168	33
Mismatched	10	3	54	12	20	21		54	18	95	19
Time from diagnosis to transplantation, month (range)	6 (2–64)		9 (3–48)		6 (2–16)		<0.0001	23 (3–272)		10 (1–261)	
<6	189	49	35	8	40	42		13	4	88	17
6 ≤, <10	150	39	222	51	44	46		40	13	159	31
10≤	49	13	177	41	11	12		247	82	262	51
Preparative regimen							<0.0001				
CY + TBI (8–13.2 Gy)	216	56	228	53	36	38		114	38	145	28
CA + CY + TBI	58	15	88	20	27	28		58	19	125	25
Other TBI (8–13.2 Gy) regimens	89	23	105	24	28	29		112	37	203	40
BU + CY	17	4	13	3	3	3		9	3	15	3
Other non-TBI regimens	8	2	0	0	1	1		7	2	21	4
GVHD prophylaxis							<0.0001				
Cyclosporine A ± other	338	87	172	40	59	62		157	52	250	49
Tacrolimus ± other	40	10	253	58	36	38		135	45	245	48
Other	10	3	9	2	0	0		8	3	14	3
Year of allo-SCT							<0.0001				
1998–2004	203	52	229	53	27	28		181	60	259	51
2005–2009	185	48	205	47	68	72		119	40	250	49

CR, complete remission; PIF, primary induction failure; BM, bone marrow; allo-SCT, allogeneic stem cell transplantation; CY, cyclophosphamide; TBI, total body irradiation; CA, cytarabine; BU, busulfan; GVHD, graft-versus-host disease.