Skin lesions are generally observed in 25.0–48.9% of ATL patients at the initial diagnosis,[2,38] which is consistent with the results obtained in the present study at the initial diagnosis. In the allo-SCT group, relapse with skin involvement was more likely to develop in the absence of GVHD. We previously reported that recurrent ATL with skin involvement represented a good target for DLI;[22] therefore, skin involvement needs to be accurately diagnosed. Although it is often difficult to distinguish a cutaneous lesion of ATL from other causes (including GVHD and viral infection),[38] southern blotting analysis or high-throughput DNA sequencing may be a promising tool for an accurate diagnosis by showing the clonal proliferation of HTLV-1-infected cells.[39,40]

An early and accurate diagnosis of relapse could be beneficial for selecting an appropriate treatment strategy (for example, DLI, radiation, intrathecal administration of CHT and mogamulizumab) and improving the prognosis of patients.[17,21,22,41] However, no standardized method has yet been established to detect the relapse of ATL after allo-SCT. In present clinical practices, symptoms are carefully monitored for the early detection of ATL relapse. Therefore, recognizing differences in relapse patterns between SCT and CHT, as well as in the sites of relapse likely to involve the primary lesion (that is, lymph nodes, spleen and gastrointestinal tract) and CNS as relapse only with new lesions, will be important. Diagnostic modalities for CNS involvement, such as lumbar puncture and diagnostic imaging, should be considered as soon as possible when neurological symptoms are noted in ATL patients, especially those with high-risk factors.

The present study highlighted the clinical features of relapsed ATL in a retrospective cohort. However, the present study had several limitations. The number of patients in our study was relatively small and patient characteristics were highly heterogeneous. Moreover, selection bias was unavoidable in patients who underwent allo-SCT. Therefore, these factors may have affected the results obtained; therefore, the results presented here should be interpreted carefully and need to be confirmed in a larger study.

In conclusion, we here demonstrated a lower rate of relapse in the peripheral blood and a higher rate of recurrent disease in new lesions only in post-transplant patients than in those receiving CHT. The optimal salvage treatment may be more effective, even for post-transplant patients, when the relapse of ATL is detected early and accurately; therefore, further clinical and experimental studies are needed to establish monitoring systems for patients with ATL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

HI, JT and Y Miyazaki conceived and designed the study; HI, H Taniguchi, JM and Y Miyazaki collected the data; HI and Y Miyazaki analyzed the data; HI, SH and Y Miyazaki performed the statistical analyses; HI and Y Miyazaki wrote the manuscript and created the figures and tables; and all authors critically reviewed the manuscript and read and approved the final version.

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Anti-HLA Antibodies Other than Against HLA-A, -B, -DRB1 Adversely Affect Engraftment and Nonrelapse Mortality in HLA-Mismatched Single Cord Blood Transplantation: Possible Implications of Unrecognized Donor-specific Antibodies

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The impact of anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, on engraftment was retrospectively evaluated in 175 single cord blood transplantations (CBT). Patients and donors had been typed at HLA-A, -B, and -DRB1 antigens, and anti-HLA antibodies had been screened before transplantation to avoid the use of cord blood (CB) units with corresponding antigens. The median age was 59 (range, 17 to 74) years. Overall, 61% were male, 89% had high-risk disease status, 77% received myeloablative conditioning regimens, and over 80% were heavily transfused patients. Sixty-nine of the 175 (39.4%) were positive for anti-HLA antibodies. Thirty-nine patients had antibodies only against HLA-A, -B, or -DRB1, 13 had antibodies only against HLA-C, -DP, -DQ, or -DRB3/4/5, and 17 had antibodies both against HLA-C, -DP, -DQ, or -DRB3/4/5 and against HLA-A, -B, or -DRB1. Because CB units had not been typed at HLA-C, -DP, -DQ, or -DRB3/4/5, it was possible that antibodies against them were unrecognized donor-specific antibodies. Patients with antibodies only against HLA-A, -B, or -DRB1 showed comparable neutrophil engraftment rates to those without antibodies (89.7% versus 83%, $P = .65$), whereas patients having antibodies against C, DP, DQ, or -DRB3/4/5 showed lower engraftment rate (66.7%, $P = .12$), which became statistically significant in a subgroup of HLA-mismatched donor-recipient pairs (50%, $P = .01$). Our results demonstrated that the presence of donor nonspecific anti-HLA-A, -B, -DRB1 antibodies had no significant influence on engraftment, whereas anti-HLA-C, -DP, -DQ, or -DRB3/4/5 antibodies adversely affect engraftment, possibly because of unrecognized donor-specific anti-HLA antibodies against them, especially in HLA-mismatched CBT.

INTRODUCTION

Cord blood transplantation (CBT) has become a valuable alternative for patients who require allogeneic stem cell transplantation (Allo-SCT) but who lack HLA-identical sibling or a matched unrelated donor [1]. Although the outcomes of CBT are almost comparable to those of Allo-SCT using unrelated donor, graft failure (GF), or engraftment delay, is one of the major concerns after CBT, leading to increased early nonrelapse mortality (NRM) [2-6]. The pathogenesis of GF is likely multifactorial, and to date, several factors, including cell dose infused, HLA disparities, the type of conditioning regimens, and chemo-naïve status of recipient, have been identified as risk factors associated with GF after CBT [1,7-10].

Recently, the impact of anti-HLA antibodies on engraftment in Allo-SCT has drawn increasing attention because of an increasing number of patients who undergo Allo-SCT using HLA-mismatched donors [11]. Recent clinical data demonstrated that the presence of donor-specific anti-HLA antibodies (DSA) in the recipient is significantly associated with GF in unrelated Allo-SCT and related haploidentical stem cell transplantation (Haplo-SCT) [11], as well as in CBT. In the setting of

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CBT, the impact of DSA on engraftment appears to be strong because the majority of the patients receive HLA-mismatched units with a relatively lower cell dose compared with that of unrelated allo-SCT or haploidentical-SCT recipients. Takanashi et al. clearly demonstrated that only 32% of patients with DSA achieve engraftment, compared with 83% of patients without DSA, in their retrospective analysis of 386 patients who underwent myeloablative single-unit CBT [12]. The negative impact of DSA on engraftment in CBT was also confirmed in the setting of reduced-intensity conditioning (RIC) and/or double CBT [13,14], although another study showed no significant effect of DSA on engraftment, probably because of the different thresholds for the definition of DSA positivity [15]. Based on the growing body of evidence, there has been a consensus that we should avoid selecting cord blood (CB) units when the anti-HLA antibodies are directed against the mismatched HLA of the CB unit. However, all studies performed in the CBT field have specifically focused on anti-HLA-A, -B, and -DRB1 antibodies in the recipient. So far, the impact of anti-HLA antibodies against HLA-C, -DP -DQ, or DRB3/4/5 has not entirely been evaluated. Further, clinical significance of anti-HLA antibodies not corresponding to HLA antigens expressed on CB remains to be determined. In this study, we retrospectively evaluated the impact of a presence of these anti-HLA antibodies on outcomes in CBT, with a special focus on its association with engraftment.

PATIENTS AND METHODS

Patients and Cord Blood Transplantation

This study included 175 consecutive adult patients who underwent CBT as their first Allo-SCT at Toranomon Hospital from March 2008 through July 2011. All patients received a single CB unit after either myeloablative or RIC regimens. Conditioning regimens were classified based on the report by the Center for International Blood and Marrow Transplant Research [16]. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus (TAC) plus mycophenolate mofetil (MMF) or TAC alone. Granulocyte colony-stimulating factor was administered intravenously from around day 3 until neutrophil recovery. For disease status, those with hematologic malignancies in 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, whereas those in other situations were defined as being at high risk. Details of supportive care during transplantation was performed as previously reported [17,18]. All patients gave written informed consent, and this study was approved by the institutional review board.

Cord Blood Units and Anti-HLA Antibodies

CB units were obtained from the Japanese Cord Blood Bank Network. In the Japanese Cord Blood Bank Network, CB units were serologically typed only at HLA-A, -B, and -DRB1 locus before selection. The CB unit was selected from those with no more than 2 antigen-mismatches to recipients and principally contained 2×10^7 and more total nucleated cells (TNC) counts per kilogram of recipient body weight. Since March 2008, we prospectively screened anti-HLA antibodies in recipients before CBT to select proper units of CB without corresponding HLA-A, -B, and -DRB1 antigens to DSA. In this study, CB unit with DSAs against HLA-A, -B or -DRB1 were not selected for transplantation. Anti-HLA antibodies were tested using LAB Screen PRA and Single Antigen (One Lambda, Canoga Park, CA) for class I (HLA-A/-B/-C) and class II (HLA-DR/-DP/-DQ) anti-HLA antibodies [19,20]. Median fluorescence intensity (MFI) of $\geq$ 1000 was defined to be positive. In this analysis, patients positive for anti-HLA antibodies were divided into 2 groups: group A included patients with antibodies only against HLA-A, -B, or -DRB1 without those against HLA-C, -DP, -DQ, or -DRB3/4/5, whereas group B included patients having antibodies against HLA-C, -DP, -DQ, or DRB3/4/5 with or without those against HLA-A, -B, or -DRB1. Because CB units had not been typed at HLA-C, -DP, -DQ, or -DRB3/4/5, it was possible that patients in group B harbored DSA against them.

Definitions and Statistical Analysis

Neutrophil engraftment was defined as the first of 3 consecutive days with absolute neutrophil count of at least 500 cells/mm^3 by 60 days after transplantation. *Platelet engraftment* was defined as the first day of a platelet count of 20,000/mm^3 without transfusion support by 100 days after transplantation. Chimerism was assessed using fluorescence in situ hybridization in sex-mismatched donor-recipient pairs, and in sex-matched pairs, PCR for a variable number of tandem repeats was used with donor cells detected at a sensitivity of 10%. Whole blood, CD3$^+$ cells, or bone marrow cells were assessed at the time of neutrophil engraftment and repeated as indicated, according to the patients' condition. Acute and chronic GVHD were diagnosed and graded according to the standard criteria [21,22]. A pre-transplantation hematopoietic cell transplantation-specific comorbidity index score was calculated retrospectively for each patient using previously reported scoring system [23]. For statistical analysis, categorical variables were compared by the chi-square test or Fisher exact test, whereas continuous variables were compared by Wilcoxon rank-sum test. The probability of overall survival (OS) were estimated using the Kaplan-Meier method and the groups were compared using the log-rank test. The probabilities of neutrophil and platelet engraftment, relapse, and NRM were estimated based on cumulative incidence curves [24]. Competing events were death or relapse without engraftment for neutrophil and platelet engraftment, death without relapse for relapse, and relapse for NRM. The groups were compared using Gray's test. The Cox proportional hazard model and the Fine-Gray proportional hazards model were used to determine the significance of multiple variables in determining these outcomes.

RESULTS

Patient Characteristics

The patients' characteristics are summarized in Table 1. Their median age was 59 (range, 17 to 74). One hundred and seven (61.1%) were male and 156 (89.1%) had high-risk disease status. The majority of the patients had extensive prior history of transfusion, both in red blood cell and platelet concentrate. One hundred thirty-five patients (77.1%) were conditioned with myeloablative regimens, whereas 40 patients received RIC regimens. One hundred forty-four (82.2%) received TAC plus MMF for GVHD prophylaxis. The median TNC and CD34$^+$ cells infused were 2.55 (range, 1.67 to 5.65) $\times$ 10^7/kg and .91 (.27 to 2.97) $\times$ 10^5/kg, respectively. HLA mismatch at HLA-A, -B, and -DRB1 in host-versus-graft (HVG) direction was 0/6 (n = 4), 1/6 (n = 48), and 2/6 (n = 123).

Anti-HLA Antibodies

Sixty-nine of the 175 (39.4%) patients were positive for anti-HLA antibodies. The median number of anti-HLA specificities was 2 (range, 1 to 73), and the median value of maximum MFI was 2150 (range, 1004 to 16,402). Among the antibody-positive group, 39 patients had antibodies only against HLA-A or -B, or -DRB1 (categorized as group A), 13 had antibodies only against HLA-C or -DP, or -DQ, or -DRB3/4/5, and 17 had antibodies both against HLA-C, -DP, -DQ, or -DRB3/4/5 and against HLA-A, -B, or -DRB1 (the latter 2 were categorized as group B). Among the 30 patients who were categorized as group B, 17 had antibodies against HLA-C, 5 against HLA-DP, 12 against HLA-DQ, and 6 against HLA-DRB3/4/5, including overlapping cases. Characteristics of patients with or without anti-HLA antibodies are summarized in Table 1. The antibody-positive group included older patients than the negative group ($P = .01$). Patients in the positive group received a higher TNC dose than the antibody-negative group ($P = .04$). The degree of HLA-mismatch in the HVG direction in the antibody-positive group was suggestively lower than those in antibody-negative group, although it was not statistically significant ($P = .06$). More intensive GVHD prophylaxis using TAC plus MMF and RIC regimes were used in the antibody-positive group, although it was not statistically significant ($P = .06$ in both).

Effect of Anti-HLA Antibodies on Hematopoietic Recovery

Among the 175 patients, 143 achieved neutrophil engraftment. In the 32 who did not achieve engraftment, 8 had graft failure and proceeded to second transplantation, 5 had early disease progression, and 19 had NRM. The cumulative incidences of neutrophil and platelet engraftment

Table 1
Characteristics of All Patients and Those with or without Anti-HLA Antibodies

Characteristic	All	Ab Positive	Ab Negative	*P* Value
No. of patients	n = 175	n = 69	n = 106	
Age, median (range), yr	59 (17-74)	60 (21-73)	57.5 (17-74)	.01
Gender				
Male	107 (61.1%)	39 (56.5%)	68 (64.1%)	.34
Female	68 (38.8%)	30 (43.4%)	38 (35.8%)	
Diagnosis				
AML	51 (29.1%)	18 (26%)	33 (31.1%)	.19
MDS/MPN overt AML	62 (35.4%)	32 (46.3%)	30 (28.3%)	
MDS	10 (5.7%)	3 (4.3%)	7 (6.6%)	
CML	7 (4%)	1 (1.4%)	6 (5.6%)	
ALL	11 (6.2%)	5 (7.2%)	6 (5.6%)	
ML	20 (11.4%)	4 (5.7%)	16 (15%)	
ATLL	8 (4.5%)	4 (5.7%)	4 (3.7%)	
SAA	5 (2.8%)	2 (2.8%)	3 (2.8%)	
MM	1 (.5%)		1 (.9%)	-
Disease status*				
Standard risk	19 (10.8%)	8 (11.5%)	11 (10.3%)	.80
High risk	156 (89.1%)	61 (88.4%)	95 (89.6%)	
Prior history of RBC transfusion				
≥20 times	149 (85.1%)	55 (79.7%)	94 (88.6%)	.11
< 20 times	16 (9.1%)	7 (10.1%)	9 (8.4%)	
Unknown	10 (5.7%)	7 (10.1%)	3 (2.8%)	
Prior history of PC transfusion				
≥ 20 times	155 (88.5%)	58 (84%)	97 (91.5%)	.23
< 20 times	14 (8%)	7 (10.1%)	7 (6.6%)	
Unknown	6 (3.4%)	4 (5.7%)	2 (1.8%)	
HCT-CI score				
0	33 (18.8%)	11 (15.9%)	22 (20.7%)	.41
1	25 (14.2%)	10 (14.1%)	15 (14.1%)	
2	38 (21.7%)	20 (28.9%)	18 (16.9%)	
3	37 (21.1%)	12 (17.3%)	25 (23.5%)	
≥ 4	42 (24%)	16 (23.1%)	26 (24.5%)	
Conditioning regimen				
RIC	40 (22.8%)	21 (30.4%)	19 (17.9%)	.06
Myeloablative	135 (77.1%)	48 (69.5%)	87 (82.0%)	
GVHD prophylaxis				
TAC	28 (16%)	6 (8.6%)	22 (20.7%)	.06
TAC + MMF	144 (82.2%)	62 (89.8%)	82 (77.3%)	
TAC + sMTX	3 (1.7%)	1 (1.4%)	2 (1.8%)	
Number of TNC infused				
Median (range), × 10^7/kg	2.55 (1.67-5.65)	2.70 (1.83-5.09)	2.49 (1.67-5.65)	.04
Number of $CD34^+$ cells infused				
Median (range), × 10^5/kg	.91 (.27-2.97)	.91 (.32-2.97)	.91 (.27-2.35)	.41
HLA antigen mismatch				
HVG direction				
0	4 (2.2%)	2 (2.8%)	2 (1.8%)	.06
1	48 (27.4%)	25 (36.2%)	23 (21.6%)	
2	123 (70.2%)	42 (60.8%)	81 (76.4%)	

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; ML, malignant lymphoma; ATLL, adult T cell leukemia/lymphoma; SAA, severe aplastic anemia; MM, multiple myeloma; PC, platelet concentrate; HCT-CI, Hematopoietic Cell Transplantation–Specific Comorbidity Index; RIC, reduced-intensity conditioning; TAC, tacrolimus; MMF, mycophenolate mofetil; sMTX, short-term methotrexate; TNC, total nucleated cells; HVG, host-versus-graft.
Data presented are n (%), unless otherwise indicated.

* Acute leukemia in first or second complete remission, CML in chronic phase, MDS in refractory anemia, and severe aplastic anemia were defined as standard risk. All the others were considered high risk.

were 81.7% (95% confidence interval [CI], 75.0% to 86.8%) and 53.5% (95% CI, 45.7% to 60.6%), respectively. The median time to neutrophil and platelet engraftment were 19 days (range, 11 to 60) and 43 days (range, 24 to 94) after transplantation, respectively. The presence of anti-HLA antibodies itself had no significant effect on neutrophil and platelet engraftment (positive versus negative: 79.7% versus 83.0%. $P = .44$, in neutrophil [Figure 1A]; 50.7% versus 55.2%, $P = .53$, in platelet). The number (≥5 versus < 5) and intensity (MFI ≥ 2000 versus < 2000) of anti-HLA antibodies also had no significant effect on engraftment. Patients with a higher degree of HLA-antigen mismatch in the HVG direction (2 versus 0 to 1 antigen mismatch) showed an inferior neutrophil engraftment rate (79.7% versus 86.5%, $P = .02$), whereas TNC (≥2.5 × 10^7/kg versus < 2.5 × 10^7/kg) did not affect engraftment in our CBT setting (78.3% versus 85.5%, $P = .62$). In multivariate analysis, a higher degree of HLA mismatch in the HVG direction was the only negative factor for neutrophil engraftment (hazard ratio [HR], .82; 95% CI, .68 to .9; $P = .03$), and presence of anti-HLA antibodies did not show a statistical significance. Regarding the impact of anti-HLA antibodies other than against HLA-A, -B, -DRB1 on neutrophil engraftment, group B tended to show lower engraftment rates (66.7%, n = 30) compared with group A (89.7%, n = 39) or the negative group (83%, n = 106), although the difference did not reach statistical significance (Figure 1B) ($P = .12$). In fact, among the 30 patients who were categorized as group B, only 20 patients (66%) achieved

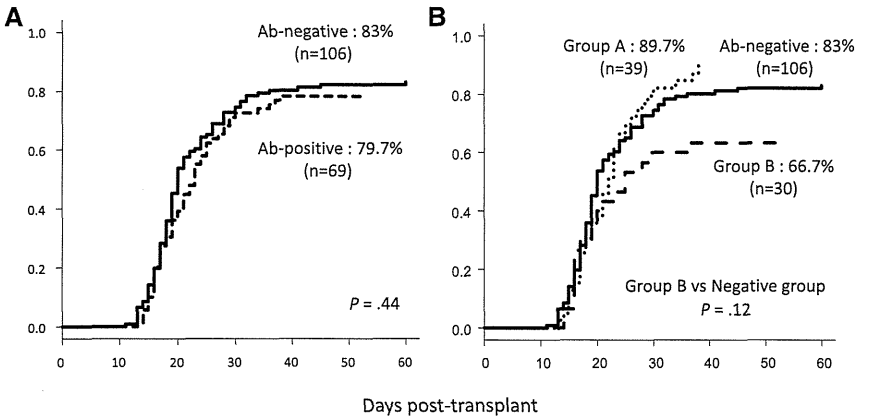

Figure 1. Cumulative incidence of neutrophil engraftment in all the studied patients (n = 175). (A) The incidence was 79.7% (95% confidence interval [CI], 67.2% to 87.9%) for the antibodies-positive group (n = 69) and 83.0% (95% CI, 73.8% to 89.2%) for the antibodies-negative group (n = 106), respectively ($P = .44$). Positive group represented by dashed line and the negative group represented by solid line. (B) The incidence according to the type of anti-HLA antibodies. Group B tended to show lower engraftment rates (66.7% [95% CI, 43.8% to 81.9%], n = 30) compared with group A (89.7% [95% CI, 70.6% to 96.7%], n = 39) or the antibodies-negative group (83.0% [95% CI, 73.8% to 89.2%], n = 106). ($P = .12$; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

engraftment. Among 13 patients who had antibodies only against HLA-C, -DP, -DQ, or DRB3/4/5, only 8 (61.5%) patients achieved engraftment.

Among 8 patients who had engraftment failure, 5 had anti-HLA antibodies (4 in group B and 1 in group A). In the chimerism analysis, 2 of the 3 patients without antibodies showed transient donor-dominant chimerism, whereas recipient cell dominance was observed consistently in all 5 patients with anti-HLA antibodies.

In the subgroup analysis in patients who received 2 antigen-mismatched CB in the HVG direction (n = 123), overall, the HLA-antibodies–positive group tended to show lower engraftment rates compared with the negative group (Figure 2A) (positive group [n = 81]: 71.4%, negative group [n = 42]: 84%, $P = .07$). In this analysis, group A showed comparable engraftment rates to the negative group, whereas group B showed extremely low engraftment rates with statistical significance (Figure 2B) (group A [n = 24]: 87.5%, negative group [n = 81]: 84%, group B [n = 18]: 50%. Group B versus negative group, $P = .01$). Multivariate analysis in this subgroup showed that group B and patients older than 55 years were identified as the negative factor for neutrophil engraftment (HR, 2.79; 95% CI, 1.39 to 5.62; $P \leq .01$ in group B; HR, .66; 95% CI, .44 to .97; $P = .03$ in elderly patients).

Effect of Anti-HLA Antibodies on Survival

At a median follow up of 18 (range, 5.1 to 46) months, the cumulative incidence of NRM at 2 years was 34.0% (95% CI, 26.8% to 41.2%). The cumulative incidence of NRM at 2 years was almost comparable between the anti-HLA antibody-positive and -negative groups (Figure 3A) (positive group [n = 69]: 38.3%, negative group [n = 106]: 31.2%; $P = .28$), whereas group B tended to show a higher incidence of NRM compared with the negative group (Figure 3B) (group B [n = 30]: 47.9%; $P = .07$). NRM in group A was almost identical to that in the antibodies-negative group (Figure 3B) (group A [n = 39]: 31.5%). Early NRM within 28 days after transplantation was significantly higher in group B compared with other groups (20% in group B, 7.7% in group A, 6.6% in negative group; group B versus negative, $P = .03$). Two-year OS for the entire study population was 45.1% (95% CI, 37.1% to 52.7%). The OS at 2 years was almost comparable between

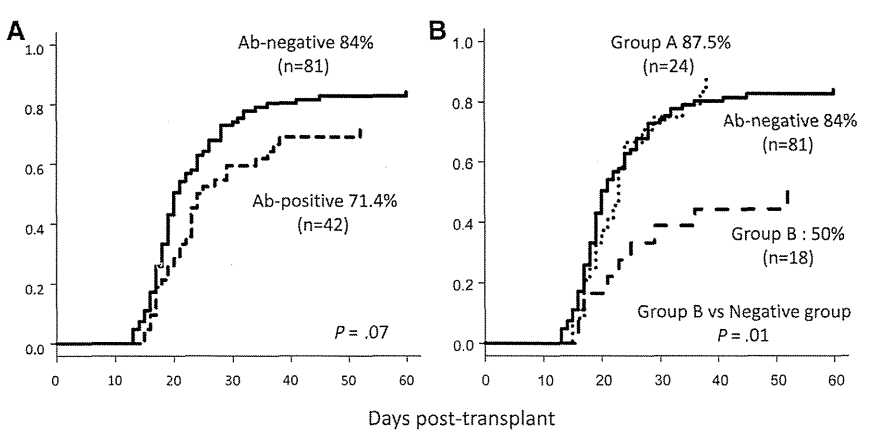

Figure 2. Cumulative incidence of neutrophil engraftment in patients who received 2 antigen-mismatched CB (n = 123). (A) The incidence was 71.4% (95% CI, 53.5% to 83.5%) for the antibodies-positive group (n = 42) and 84.0% (95% CI, 73.1% to 90.7%) for the antibodies-negative group (n = 81), respectively ($P = .07$). Positive group represented by dashed line and the negative group represented by solid line. (B) The incidence according to the type of anti-HLA antibodies. Group B showed lower engraftment rates (50% [95% CI, 21.6% to 73.1%], n = 18) compared with group A (87.5% [95% CI, 57.3% to 96.9%], n = 24) or the antibodies-negative group (84.0% [95% CI, 73.1% to 90.7%], n = 81) with statistical significance ($P = .01$; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

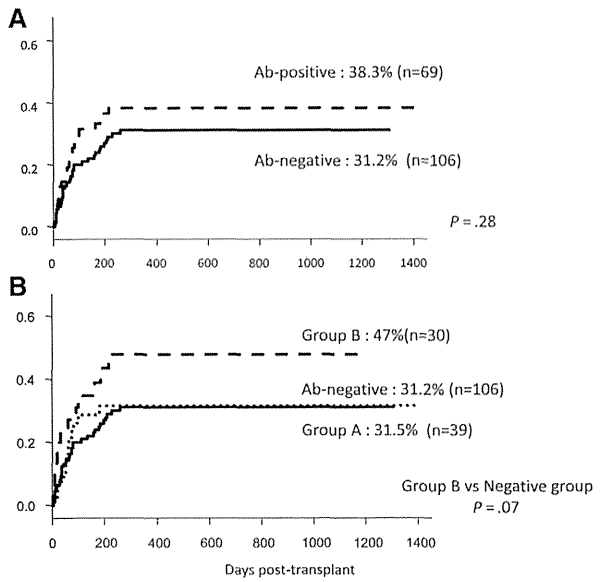

Figure 3. Cumulative incidence of nonrelapse mortality (NRM) (n = 175). (A) NRM at 2 years was 38.3% (95% CI, 26.4% to 50.0%) for the antibodies-positive group (n = 69) and 31.2% (95% CI, 22.4% to 40.3%) for the antibodies-negative group (n = 106), respectively ($P = .28$). Positive group represented by dashed line and the negative group represented by solid line. (B) NRM according to the type of anti-HLA antibodies. Group B showed a significantly higher incidence of NRM (47.9% [95% CI, 27.4% to 65.9%], n = 30) compared with group A (31.5% [95% CI, 17.5% to 46.5%], n = 39) or the antibodies-negative group (31.2% [95% CI, 22.4% to 40.3%], n = 106). ($P = .07$; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

the antibodies-positive and -negative groups (Figure 4A) (positive group [n = 69]: 42.9%, negative group [n = 106]: 46.2%; $P = .47$), although group B had a tendency of decreased OS rate compared with the negative group (Figure 4B) (group B [n = 30]: 35.1%; $P = .26$).

DISCUSSION

The aim of this study was to evaluate the effect of anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, on engraftment after single CBT. In this study, we demonstrated that the presence of non-DSA had no significant influence on engraftment, NRM, and OS after CBT. In particular, among patients with antibodies only against HLA-A, -B, or -DRB1 (group A), the results were comparable with those without antibodies. These observations strongly support that necessity to screen for DSA before the selection of CB units, and indicated that CBT is an available option.

On the other hand, patients with antibodies including HLA-C, -DP, -DQ, or -DRB3/4/5 (group B) showed a lower incidence of engraftment compared with those only having donor nonspecific anti-HLA-A, -B, -DRB1 antibodies, "true non-DSA," or those without antibodies, especially in HLA-mismatched CBT. The findings suggest that unrecognized DSA against for HLA-C, -DP, -DQ, or -DRB3/4/5 might adversely affect engraftment after CBT. All previous studies of anti-HLA antibodies performed in the CBT field have specifically looked at anti-HLA-A, -B, and -DRB1 antibodies in the recipient [12-15], and the clinical significance of anti-HLA antibodies against HLA-C, -DP, -DQ, or -DRB3/4/5 has not been investigated so far. In the largest study of anti-HLA antibodies after CBT reported by Takanashi et al., patients who had anti-HLA antibodies, except for donor-specific anti-HLA-A, -B, -DRB1 antibodies, showed significantly lower neutrophil engraftment compared with those without anti-HLA antibodies (73% versus 83%) [12]. One possible reason for the lower engraftment rate in that study might be the existence of unrecognized DSA against HLA-C, -DP, -DQ, or -DRB3/4/5, as suggested in the present study. In unrelated Allo-SCT, anti-DPB1 DSA has been recognized to be associated with increased risk for engraftment failure [25,26]. In the setting of HLA-mismatched CBT, additional mismatch in the HLA-C, -DP, -DQ, or -DRB3/4/5 antigens could likely be present because of linkage disequilibrium, especially between the HLA-B and -C locus, or the -DRB1 and -DQ locus, respectively [27,28]. In this study, there were 12 patients who had antibodies against HLA-DQ, and 7 were matched and 5 were mismatched for DRB1. Neutrophil recovery was observed in all 7 HLA-DRB1–matched and 3 of 5 in the mismatched group. The results further strengthen the association of DSA against HLA-DQ with engraftment failure. Moreover, HLA-DP and -DQ antigens are also known to be expressed on hematopoietic precursor cells [26,29-31]. Our results, combined with previous findings, strongly suggest the possibility of graft rejection associated with unrecognized DSA against HLA-C, -DP, -DQ, -DRB3/4/5 antigens of an

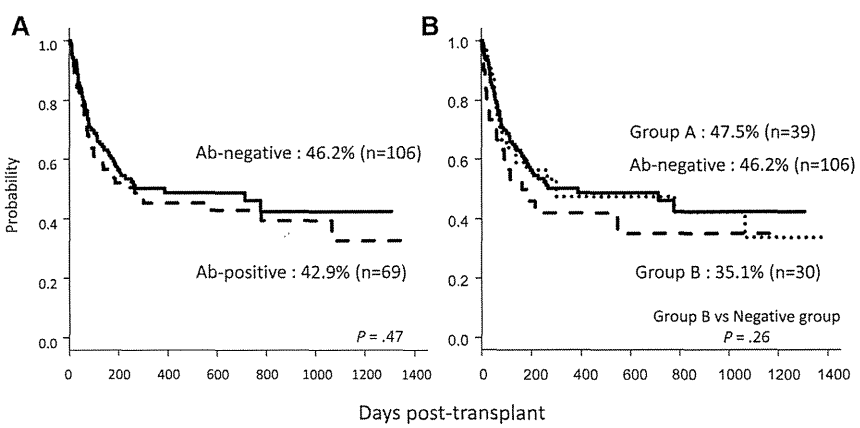

Figure 4. Probability of overall survival (OS) (n = 175). (A) OS at 2 years was 42.9% (95% CI, 30.5% to 54.7%) for the antibodies-positive group (n = 69) and 46.2% (95% CI, 35.5% to 56.3%) for the antibodies-negative group (n = 106), respectively ($P = .47$). Positive group represented by dashed line and the negative group represented by solid line. (B) OS according to the type of anti-HLA antibodies. Group B had tendency to decreased OS rate (35.1% [95% CI, 16.8% to 54.1%], n = 30) compared with group A (47.5% [95% CI, 31.0% to 62.3%], n = 39) or the antibodies-negative group (46.2% [95% CI, 35.5% to 56.3%], n = 106). ($P = .26$; group B versus negative group). Group A is represented by dotted line, group B by dashed line, and negative group by solid line.

infused CB unit. Multivariate analysis showed that a higher degree of HLA-mismatch in HVG direction had a significantly negative effect on engraftment. This result suggests that the HLA-specific cellular immunity plays a critical role in engraftment process, as previously reported [32,33]. However, in multivariate analysis for patients who received 2 antigen-mismatched CB, the presence of anti-HLA-C, -DP, -DQ, -DRB3/4/5 antibodies was the negative factor for neutrophil engraftment. Thus, in the setting of transplantation using HLA-mismatched grafts, humoral immunity could adversely affect engraftment.

Because this is a retrospective analysis, there is no information available on HLA-DP, -DQ, or -DRB3/4/5 antigens of CB units, negating direct assessment whether these antigens were DSAs. We have done HLA-C typing retrospectively in 16 patients who had anti–HLA-C antibodies. There were 2 patients who had DSA against HLA-C; 1 engrafted and the other died early before engraftment. Among the remaining 14 who did not have DSA against HLA-C, 2 developed engraftment failure, 3 died early before engraftment, and 9 engrafted, and with such a small sample size, we were not able to assess the impact of DSA against HLA-C on engraftment. It remains to be determined whether more mismatches in HLA-C, -DP, -DQ, or -DRB3/4/5 antigens, or the combined effect of mismatches and DSA, have a negative effect on engraftment. Furthermore, patients in group B showed a higher incidence of NRM before engraftment, which could be associated with the low engraftment rate. The high rate of early NRM observed in group B became clear in the subgroup analysis for patients who received 2 antigen-mismatched CB (Supplementary Table S1). In the subgroup analysis for patients who survived for 28 days or longer after transplantation and who received 2 antigen-mismatched CB ($n = 110$), group B ($n = 12$) still tended to show a lower engraftment rate compared with other groups (75% in group B, 95.5% in group A, and 89.5% in negative group; group B versus negative; $P = .30$). Lower engraftment or delayed neutrophil recovery observed in group B could have affected this higher NRM. The presence of other factors that have an impact on engraftment or early morality in group B cannot be excluded, although background characteristics among group B were almost comparable to the others (Supplementary Table S1). In this study, the frequency of patients with anti-HLA antibodies is higher compared with other previous series [11,12]. Our patient characteristics, including elderly or heavily transfused patients, might have possibly affected the result. The existence of a "natural antibody" is also one of the factors to be considered [34,35]. The recent use of more sensitive methods has resulted in the detection of low-level antibodies not contributable to allogeneic antigens exposure. When we re-evaluated our results based on cross-reactive group [36,37], some antibodies seem to be classified as natural antibodies; however, we could not show their relevance to the clinical outcomes.

In conclusion, our results demonstrate that the presence of donor nonspecific anti-HLA-A, -B, -DRB1 antibodies had no significant influence on engraftment, NRM, and OS in HLA-mismatched CBT. On the other hand, a high rate of engraftment failure was seen in the presence of anti-HLA-C, -DP, -DQ, or -DRB3/4/5 antibodies, suggesting unrecognized DSA against HLA-C, -DP, -DQ, or -DRB3/4/5 antigens adversely affect engraftment. Because this is a retrospective study including a small and heterogeneous group of patients with various characteristics, the results should be confirmed by larger scale studies involving information on HLA-C, -DP, -DQ, or -DRB3/4/5, which will establish the optimal strategies for selecting CB units.

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Authorship statement: H.Y., N.U., N.M., S.M. and S.Taniguchi designed the study; H.Y. and N.M. performed the research and extracted data; S.M. performed the Ab testing; A.Y. reviewed histopathological results; H.Y., N.U., N.M., H.O., K.K., S.W., D.K., A.N., K.Ishiwata, S.Takagi, M.T., Y.A.-M.,G.Y., K.Izutsu, K.M., A.W., and S.Taniguchi performed transplantation and reviewed patients' data. H.Y., N.U., K.Izutsu, and S.Taniguchi contributed to writing the paper.

Conflict of interest statement: There are no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.06.024.

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