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Impact of highly conserved HLA haplotype on acute graft-versus-host disease

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Although the effects of human leukocyte antigen (HLA) locus matching on clinical outcome in unrelated hematopoietic stem cell transplantations have been characterized, the biologic implications of HLA haplotypes have not been defined. We demonstrated the genetic fixity of Japanese conserved extended haplotypes by multi–single nucleotide polymorphism analysis in 1810 Japanese donor-recipient pairs matching with HLA-A, -B, -C, -DRB1, and -DQB1 alleles. Three major Japanese conserved extended haplotypes (named HP-P1, HP-P2, and HP-P3) were essentially completely conserved at least in the 3.3-Mb HLA region from HLA-A to -DPB1, and extended far beyond HLA-A. The risk of acute graft-versus-host disease (GVHD) of these HLA haplotypes was assessed with multivariate Cox regression in 712 patients transplanted from HLA fully (HLA-A, B, C, DRB1, DQB1, and DPB1) matched unrelated donors. HP-P2 itself reduced the risk of grade 2 to 4 acute GVHD (hazard ratio [HR] = 0.63; $P = .032$ compared with HP-P2-negative), whereas HP-P3 tended to increase the risk (HR = 1.38; $P = .07$). Among 381 patients with HP-P1, HP-P1/P3 (HR = 3.35; $P = .024$) significantly increased the risk of acute GVHD compared with homozygous HP-P1. This study is the first to demonstrate that a genetic difference derived from HLA haplotype itself is associated with acute GVHD in allogeneic hematopoietic stem cell transplantation. (*Blood*. 2010;115(23): 4664-4670)

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)–matched unrelated (UR) donor has been established as a mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders, when an HLA-identical sibling donor is unavailable. Although the effect of donor and recipient HLA locus matching on the clinical outcome of UR-HSCT has been well elucidated,[1-4] the biologic implications of HLA haplotype itself have not been explored for HSCT.

HLA-identical sibling shares 2 identical major histocompatibility complex (MHC) haplotypes by descent, including non-HLA polymorphic genes, and it has been generally accepted that transplantation between these related pairs provides a superior outcome. On the other hand, there is no guarantee of matching for non-HLA genes between HLA-allele matched UR donor and recipient pairs, and mismatching of haplotype block in MHC has been suggested to lead to severe acute GVHD[5] and an inferior outcome[6,7] in UR-HSCT.

In human population, multiple DNA blocks in the MHC are strongly associated with each other, and these relatively long stretches of conserved DNA sequence in the MHC have been named conserved extended haplotypes (CEHs) or ancestral haplotypes.[8,9] CEHs are often population-specific and have been investigated as markers for disease susceptibility, particularly in autoimmune diseases.[10] However, the relation between CEHs and clinical outcome of UR-HSCT has not been yet reported.

Using the large-scale Japan Marrow Donor Program (JMDP) data, we evaluated the conservation of common HLA haplotypes among a Japanese population and elucidated its impact on acute graft-versus-host disease (GVHD) and other clinical outcomes in UR-HSCT.

Methods

Study population

A total of 5210 donor-recipient pairs who underwent transplantation through the JMDP between January 1993 and January 2006 were retrospectively genotyped for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles as described elsewhere.[4] For the genome-wide association studies, 1810 pairs (3620 persons) who matched HLA-A, -B, -C, -DRB1, and -DQB1 alleles and were available for DNA sample were selected from these 5210 pairs. For the analysis of acute GVHD, 712 patients who had received T cell–replete bone marrow from an HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 allele-matched donor were selected from these 5210 pairs. The characteristics of these 712 patients are shown in Table 1. A final clinical survey of the patients was completed by June 2007. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval of the study was obtained from the Institutional Review Board of Aichi Cancer Center and JMDP.

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Table 1. Clinical characteristics of patients according to HLA haplotype

Characteristic	Haplotype P1		Haplotype P2		Haplotype P3	
	Negative	Positive	Negative	Positive	Negative	Positive
No. of patients	381	331	601	111	608	104
Median patient age, y (range)	33 (0-70)	33 (1-68)	33 (0-70)	35 (1-65)	33 (1-68)	36 (0-70)
Sex (donor/ patient)						
Male/male	160	152	250	62	274	38
Male/female	71	63	117	17	112	22
Female/male	66	49	103	12	100	15
Female/female	84	67	131	20	122	29
Disease						
ALL	91	71	138	24	144	18
ANLL	90	106	167	29	166	30
CML	65	54	95	24	101	18
Hereditary disease	5	9	11	3	12	2
MDS	52	39	76	15	78	13
Malignant lymphoma	39	30	61	8	52	17
Multiple myeloma	4	4	7	1	7	1
Severe aplastic anemia	24	9	28	5	31	2
Other	11	9	18	2	17	3
Risk of leukemia relapse*						
Standard	137	112	211	38	214	35
High	109	119	189	39	197	31
Disease other than leukemia	135	100	201	34	197	38
GVHD prophylaxis						
Cyclosporine-based	199	203	345	57	339	63
Tacrolimus-based	182	128	256	54	269	41
ATG						
ATG	25	23	38	10	40	8
Non-ATG	356	308	563	101	568	96
Preconditioning						
TBI regimen	298	241	456	83	463	76
Non-TBI regimen	83	90	145	28	145	28

HLA indicates human leukocyte antigen; ALL, acute lymphoblastic leukemia; ANLL, acute nonlymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; ATG, anti–human thymocyte globulin; and TBI, total body irradiation.

*Standard risk for leukemia relapse was defined as the status of the first complete remission of ALL and ANLL and the first chronic phase of CML at transplantation. High risk was defined as a more advanced status than standard risk in AML, ANLL, and CML. Disease other than leukemia was defined as other than ALL, ANLL, and CML.

SNP typing and HLA haplotype analysis

The single nucleotide polymorphism (SNP) array experiments were performed according to the standard protocol of Affymetrix GeneChip Mapping 500K Array (Affymetrix). After excluding those SNPs showing less than 95% call rate and deviation from Hardy-Weinberg equilibrium ($P < .001$), 10.8% of SNPs for the HLA region failed. And 4761 SNPs in the region spanning the MHC (20-46 Mb from the telomere in chromosome 6p) were analyzed to evaluate the conservation of common HLA haplotypes.

Persons who were homozygous for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 were isolated, and the 3 major HLA haplotypes were named HP-P1, HP-P2, and HP-P3. The homozygosity of consecutive SNPs of these HLA haplotypes was analyzed to assess the region of conservation. SNP alleles of the extended homozygous region in each HLA haplotype were analyzed to determine allele frequencies, and a consensus sequence of major alleles in each haplotype was established. Then, the SNP sequence of persons who carried at least one copy of HLA haplotype (shared the same HLA alleles as common HLA haplotype at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci) was compared with this consensus sequence. Missing alleles in a sample were not accounted for in this analysis.

Statistical analysis

Cumulative incidences of acute GVHD and relapse were assessed by the method described elsewhere to eliminate the effect of competing risk.[4] Overall survival was calculated using the Kaplan-Meier method. The competing event regarding acute GVHD was defined as death without acute GVHD. A log-rank test was applied to assess the impact by the factor of interest. Multivariable Cox regression analyses were conducted to evaluate the impact of the specific haplotype on acute GVHD, leukemia relapse, and mortality after transplantation. Confounders considered were sex (donor-recipient pair), patient age (linear), donor age (linear), transplantation year, type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (cyclosporine-based regimen vs tacrolimus-based regimen), anti–thymocyte globulin (anti–thymocyte globulin vs no anti–thymocyte globulin), and preconditioning (total body irradiation vs non–total body irradiation).

Results

Highly conserved common HLA haplotypes among Japanese

To evaluate for conservation of Japanese common HLA haplotypes, persons who were homozygous HLA haplotype (having homozygous alleles in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci) were selected from a total 3620 persons (1810 donor-recipient pairs) for whom genome-wide association study was performed. Among those, 72 persons were homozygous HLA-A*2402 -Cw*1202 -B*5201 -DRB1*1502 -DQB1*0601 -DPB1*0901 (named HP-P1), 10 persons were homozygous HLA-A*3303 -Cw*1403 -B*4403 -DRB1*1302 -DQB1*0604 -DPB1*0401 (named HP-P2), and 8 persons were homozygous HLA-A*2402 -Cw*0702 -B*0702 -DRB1*0101 -DQB1*0501 -DPB1*0402 (named HP-P3).

Homozygosity at consecutive SNP loci of persons with homozygous HLA haplotype was shown in Figure 1. The extended

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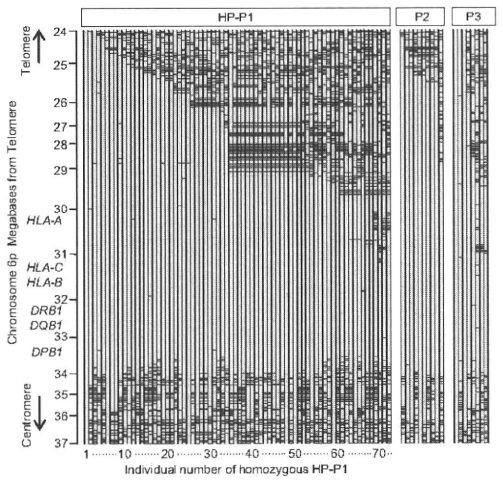

Figure 1. Representation of genotypes in persons with homozygous HLA haplotype. Data from chromosome 6p (24-37 Mb) of persons with homozygous HLA haplotype are shown. Each column indicates 1 person (72 persons with homozygous HP-P1, 10 persons with homozygous HP-P2, and 8 persons with homozygous HP-P3). Each of the 2389 evenly spaced rows represents 1 SNP locus. Blue row represents homozygous genotype; and red row, heterozygous genotype. Missing genotypes were not counted.

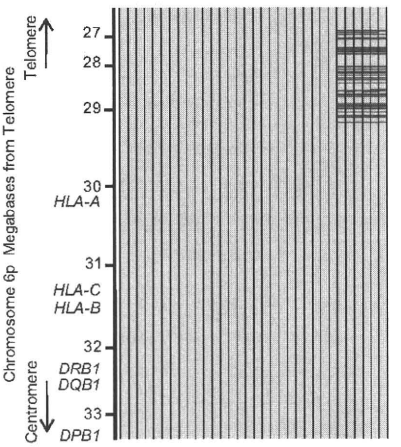

Figure 2. Subtypes of HLA haplotype P1. Data from chromosome 6p (from nucleotides 26252770-33187790) of 32 persons with consecutive homozygous SNPs throughout the 6.9-Mb region. The SNP sequence of persons was compared with consensus sequence across the 6.9-Mb region. Each column indicates 1 person. Each of 1395 evenly spaced rows represents 1 SNP locus. Gray row represents SNPs identical to the consensus alleles; and red row, SNPs different from the consensus alleles. Missing SNPs were not counted. A total of 26 of 32 persons had alleles identical to consensus alleles across 6.9 Mb (subtype A of HP-P1), whereas the remaining 6 persons had apparently different alleles in the telomeric region from nucleotide 29414635 (subtype B of HP-P1). These data indicated that the telomeric region of HP-P1 was clearly divided into 2 different haplotypes.

homozygous region of HP-P1 gradually broke up the region of HLA-A. The longest homozygous region in persons with HP-P1 was 18.7 Mb. Of 72 persons with homozygous HP-P1, 32 persons (nos. 1-32 of HP-P1 in Figure 1) had more than 99.0% of 1395 consecutive homozygous SNPs throughout the 6.9-Mb region from rs806971 to rs6937061 (nucleotides 26252770-33187790). Although haplotypes of all those 32 persons were identical centromeric rs9257745 (nucleotide 29414635), the telomeric region was clearly divided into 2 different haplotypes (Figure 2). A total of 26 of 32 persons had one of the homozygous haplotypes (named subtype A of HP-P1), and the remaining 6 persons (nos. 3, 9, 19, 23, 24, and 25 of HP-P1 in Figure 1) had another homozygous haplotype (subtype B of HP-P1). A total of 65 of 72 persons with homozygous HP-P1 (nos. 1-65 of HP-P1 in Figure 1) had more than 99.0% homozygous alleles for 888 consecutive SNPs throughout the 3.3-Mb region from rs1610630 to rs6937061 (nucleotides 29837265-33187790). Seven other persons (nos. 66-72 in Figure 1) had an apparently lower conserved region, with 1.0% to 10.0% heterozygous alleles within the 3.3-Mb region.

All 10 persons with homozygous HP-P2 had more than 99.0% homozygous alleles for consecutive SNPs throughout the 3.3-Mb region. Furthermore, 9 of 10 persons with homozygous HP-P2 showed homozygosity extending across the 7.7-Mb region from rs6912426 to rs6937061 (nucleotides 25517764-33187790), and those 9 persons had identical genotypes in almost all the 1540 consecutive SNPs throughout the 7.7-Mb region.

Among 8 persons with homozygous HP-P3, 5 persons had more than 99.0% homozygous alleles throughout the 3.3-Mb region, and the other 3 persons had 1.7% to 8.0% heterozygous alleles within that region. One person with homozygous HP-P3 showed an extraordinary long stretch of homozygosity across the 25.4-Mb region (nucleotides 20162518-45595922).

Consensus sequence of each haplotype was determined using analysis of persons who were homozygous for almost all the SNPs, ie, 6.9-Mb region in HP-P1 (subtypes A and B), 7.7-Mb region in HP-P2, and 3.3-Mb region HP-P3. The person with the longest homozygous telomeric region of HLA-A served to determine a further extended haplotype (Table 2). These persons had alleles identical to the consensus sequence of major alleles in each haplotype described above in this section.

We ascertained whether the consensus sequence of each HLA haplotype was present in the persons carrying at least one copy of HLA haplotype, that is, sharing the same HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles of common HLA haplotypes (Figure 3; supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Among 3620 persons analyzed for multi-SNP, 1000 of 1045 persons (95%) with HP-P1 had identical alleles for more than 99.5% of consecutive 888 SNPs as a consensus sequence across 3.3-Mb region from 181 kb telomeric HLA-A to 25 kb centromeric HLA-DPB1.

Table 2. Longest homozygous region of common HLA haplotype

	Start of homozygous region		End of homozygous region		
Type	SNP	Position, kb	SNP	Position, kb	Region length, Mb
HP-P1					
Subtype A	rs199026	23441.813	rs2395801	42231.646	18.8
Subtype B	rs573863	24080.365	rs1536501	33835.863	9.7
HP-P2	rs1175427	24387.055	rs1873254	34134.467	9.7
HP-P3	rs1688325	20162.518	rs6905847	45595.922	25.4

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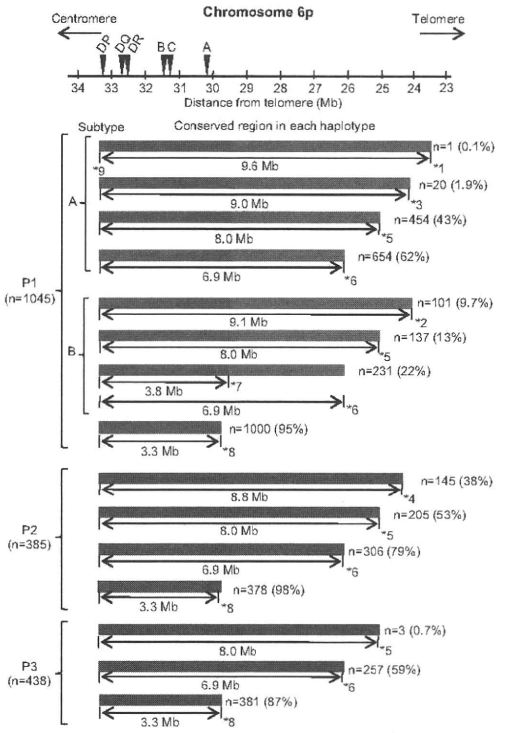

Figure 3. Conservation of common HLA haplotypes. The SNP sequence of persons who carried at least 1 copy of HLA haplotype (shared the same HLA alleles as common HLA haplotype) was compared with consensus sequence of common HLA haplotypes, and conserved regions in each HLA haplotype were illustrated schematically. The majority of persons who share the same HLA alleles in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 as common HLA haplotypes possess at least a 3.3-Mb conserved region from HLA-A to DPB1. HP-P1 splits into the A and B subtypes. The length of conservation in HP-P1, HP-P2, and HP-P3 is more extensive to the telomeric region of HLA-A. ID and position of SNPs are: *1 rs199026 (nucleotide 23551813), *2 rs573863 (nucleotide 24080365), *3 rs1397843 (nucleotide 24111433), *4 rs11754278 (nucleotide 24387055), *5 rs303031 (nucleotide 25144959), *6 rs806971 (nucleotide 26252770), *7 rs9257745 (nucleotide 29414635), *8 rs1610630 (nucleotide 29837265), and *9 rs6937061 (nucleotide 33187790).

Furthermore, 654 of 1045 (62%) persons with HP-P1 had identical alleles for more than 99.5% of consecutive 1395 SNPs as subtype A of HP-P1 across the 6.9-Mb region, and 231 persons (22%) had identical alleles as subtype B of HP-P1 across the 6.9-Mb region. Fewer persons showed the conserved region extending up to 9.0 Mb. Among 385 persons with HP-P2, 378 (98%) had identical alleles for 888 consecutive SNPs across the 3.3-Mb region. Furthermore, 305 (79%) had identical alleles for more than 99.5% of 1395 consecutive SNPs as a consensus sequence across the 6.9-Mb region, and 205 (53%) also did across the 8.0-Mb region (nucleotides 24111433-33187790). Among 438 persons with HP-P3, 381 (87%) had identical alleles across the 3.3-Mb region, and 257 (59%) had identical alleles for consecutive SNPs as a consensus sequence of HP-P3 across the 6.9-Mb region.

These results indicate that most of the persons with a common HLA haplotype had a conserved region at least 3.3 Mb from HLA-A to HLA-DPB1. Furthermore, a considerable number of unrelated persons with common HLA haplotype had a more extended conserved telomeric region of HLA-A.

Effect of HLA haplotype on acute GVHD

To elucidate the effect of specific HLA haplotype on acute GVHD, we analyzed 712 patients who underwent transplantation from HLA fully matched (12 of 12 HLA alleles) donor with T cell-replete marrow (Table 1). We excluded HLA-mismatched transplantation to avoid obscuring the relationship between HLA haplotype itself and acute GVHD by powerful allogeneic immune responses caused by HLA allele disparities. Among those patients, 331 (46.4%) had HP-P1, 111 (15.0%) had HP-P2, and 104 (14.6%) had HP-P3.

At first, grade 2 to 4 acute GVHD in patients with specific haplotype was compared with those without specific haplotype using multivariate analysis (Table 3). There was no significant difference in the hazard ratio (HR) in grades 2 to 4 acute GVHD between HP-P1-positive and -negative patients, and also no significant difference in the cumulative incidence of acute GVHD between HP-P1-positive and HP-P1-negative patients (31.5% vs 30.7%; Figure 4). Of note, HR of grades 2 to 4 acute GVHD in HP-P2-positive patients was 0.63 (95% confidence interval [CI], 0.41-0.96, $P = .032$) compared with HP-P2-negative patients, and the cumulative incidence of acute GVHD in patients with HP-P2 was significantly lower than HP-P2-negative patients (22.3% vs 33.7%, $P = .031$; Figure 4). On the other hand, a trend of increasing risk of acute GVHD was observed in HP-P3-positive patients (HR = 1.38; 95% CI, 0.97-1.95; $P = .07$). The cumulative incidence of acute GVHD in HP-P3-positive patients was 39.2% and HP-P3-negative patients 29.5% ($P = .064$; Figure 4).

A total of 331 patients with HP-P1 in HLA fully matched transplantation made it possible to elucidate the effect of another HLA haplotype on acute GVHD (supplemental Table 1 for patient characteristics). We did not determine unique haplotypes other than HP-P1, HP-P2, and HP-P3 in this study, so all the persons with HP-P1 and unknown haplotype were lumped together with HP-P1/other. The incidence of grade 2 to 4 acute GVHD in patients with HP-P1/P3 (49.9%) was significantly higher than those with homozygous HP-P1 (16.0%), and there was no significant difference between patients with homozygous HP-P1 and those with HP-P1/P2 (12.0%; Figure 5). Multivariate analyses of HR for acute GVHD showed the same results (Table 3). There was no significant difference in the risk of acute GVHD between patients with homozygous HP-P1 and those with HP-P1/P2 ($P = .64$). Compared with patients with homozygous HP-P1, patients with HP-P1/P3 (HR = 3.35; 95% CI, 1.18-9.55; $P = .024$) had a significantly higher risk of acute GVHD.

As for grade 3 to 4 acute GVHD, there were no significant differences between HP-positive and -negative patients. When 331 patients with HP-P1 in HLA fully match transplantation

Table 3. Hazard ratio of HLA haplotype on acute GVHD (grade 2-4)

HLA haplotype	Negative/positive	No.	Hazard ratio (95% CI)	P
P1	Negative	381	Referent 1.00	
P1	Positive	331	1.06 (0.81-1.39)	.665
P2	Negative	601	Referent 1.00	
P2	Positive	111	0.63 (0.41-0.96)	.032
P3	Negative	608	Referent 1.00	
P3	Positive	104	1.38 (0.97-1.95)	.07
P1/P1		36	Referent 1.00	
P1/P2		25	0.71 (0.17-2.93)	.64
P1/P3		19	3.35 (1.18-9.55)	.024
P1/other		251	2.49 (1.06-5.85)	.036

Multivariate analysis adjusted by clinical factors (see Table 2 and supplemental Table 1).

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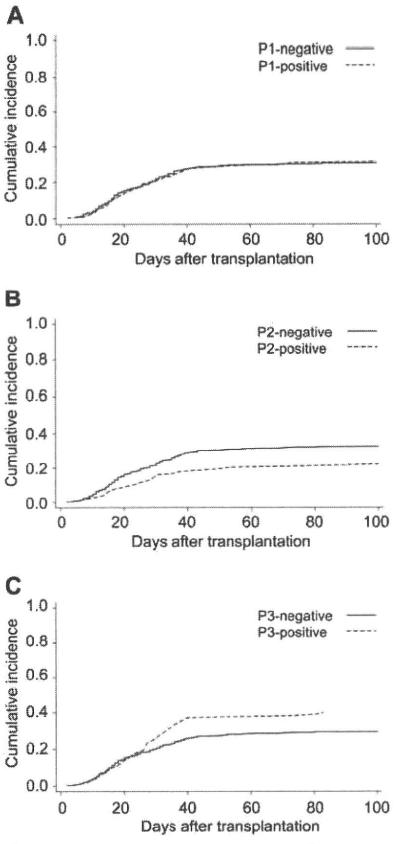

Figure 4. Cumulative incidence of grade 2 to 4 acute GVHD by common HLA haplotype. The endpoint was the time to diagnosis of grade 2 to 4 acute GVHD with censoring of date of death until 100 days after transplantation. *P* value was calculated with the log-rank test. (A) Patients with or without HP-P1. (B) Patients with or without HP-P2. (C) Patients with or without HP-P3.

were analyzed, grade 3 to 4 acute GVHD showed the same tendency with grade 2 to 4 acute GVHD. Incidence in patients with homozygous HP-P1 was 2.7%, HP-P1/P2 8.0%, and HP-P1/P3 23.6%.

The cumulative incidence of relapse showed a higher trend in HP-P2-positive patients compared with -negative patients (37.3% vs 29.6%, $P = .051$), and HR was 1.34 (95% CI, 0.94-1.91, $P = .108$). There were no significant differences in the relapse rate between HP-P1-positive and -negative patients, and also between HP-P3 -positive and -negative patients.

Overall survival showed no significant differences between HP-positive and -negative patients. When 331 patients with HP-P1 in HLA fully matched transplantation were analyzed, HR of mortality in HP-P1/P3 was 2.03 (95% CI, 0.92-4.49 $P = .08$) compared with patients with homozygous HP-P1, and HR of HP-P1/P2 1.69 (95% CI, 0.78-3.70, $P = .186$).

Discussion

First, we demonstrated that Japanese common HLA haplotypes were extraordinarily conserved. Preferential selection of HLA-A–, -B–, and -DR–matched donor through JMDP made it easy to identify a considerable number of persons with homozygous common HLA haplotype extending HLA-DPB1. HP-P1, HP-P2, and HP-P3 have been previously reported as common HLA haplotypes in the Japanese population.[11-13] The haplotype frequency of HP-P1 was 0.054 to 0.062, that of HP-P2 was 0.029 to 0.036, and that of HP-P3 was 0.016 to 0.040.

CEHs have been mainly identified by blocks of fragment in the MHC region, such as complement genes, alleles of HLA class I /II gene, and tumor necrosis factor-α gene. Recently, high-density SNP analysis in the HLA region made it possible to confirm the genetic fixity of HLA haplotype.[14-16]

We determined the consensus sequence of these HLA haplotypes using multi-SNP data of unrelated persons with homozygous HLA haplotype, and the majority of persons who share the same HLA alleles in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 as common HLA haplotypes possess at least a 3.3-Mb conserved region from HLA-A to -DPB1. Furthermore, we showed that those Japanese common HLA haplotypes extend far beyond the HLA-A. We also found, for the first time, that HP-P1 was divided into 2 subtypes based on the telomeric region from nucleotide 29414635 (subtypes A and B). The A1-B8-DR3 CEH, which is one of the most frequent haplotypes in northern European populations, showed that the region of conservation extended 6 Mb telomeric to HLA-A.[16,17] Caucasian common HLA haplotypes have often reportedly shown a lack of nonrandom association between HLA-DR, -DQ, and -DP,[18] whereas Japanese common HLA haplotypes have been subdivided into haplotypes with only a very limited number of HLA-DPB1.[11,12] The highly conserved HLA haplotype that extends to HLA-DPB1 might be attributable to ethnic isolation in the Japanese.

For the analysis of comparison between haplotype-positive and -negative patients (Figure 4; Table 3), patients with HP-P2 significantly reduced the risk of acute GVHD. On the other hand, patients with HP-P3 showed a tendency to increase the risk. Although the relapse rate showed a higher trend in patients with HP-P2 compared with HP-P2-negative patients, the grade 3 to 4 acute GVHD and overall survival did not differ between HP-P2-positive and -negative patients, nor between HP-P3-positive and -negative patients. We suspected the differences with weak power might be attributed to the effects of various other haplotypes combined with a particular haplotype. To confirm the differences in the effects on acute GVHD among a particular HLA haplotype, we analyzed the effect of another

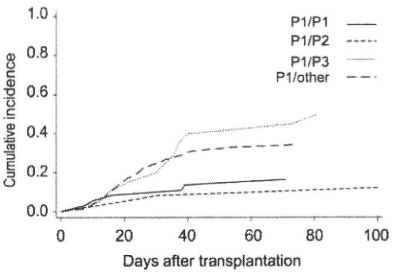

Figure 5. Cumulative incidence of grade 2 to 4 acute GVHD by common HLA haplotype in patients with HP-P1. The endpoint was the time to the diagnosis of grade 2 to 4 acute GVHD with censoring of date of death until 100 days after transplantation. *P* value was calculated with the log-rank test. A total of 331 patients with HP-P1 were analyzed for the effect of another HLA haplotype by HP-P1, HP-P2, HP-P3, and the other haplotypes, which were lumped together.

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haplotype on acute GVHD among patients with HP-P1 (Figure 5). Patients with HP-P1/P3 showed a significantly higher risk of grade 2 to 4 acute GVHD compared with patients with homozygous HP-P1, a tendency to increase the incidence of grade 3 to 4, and also a tendency to decrease overall survival. Patients with homozygous HP-P1 and patients with HP-P1/P2 showed an extremely lower incidence of grade 2 to 4 acute GVHD (16.2% and 12.0%). Therefore, we could not detect any difference between HP-P1 and HP-P2.

Thus, we demonstrated, for the first time, that the HLA haplotype itself affected the occurrence of acute GVHD. These findings suggest that the genetic factor of specific haplotypes would contribute to reducing or increasing the risk of acute GVHD. Alternatively, we should consider the effect of donor and recipient mismatch SNPs because HP-P3 showed more variation than HP-P1 or HP-P2.

There are several possible explanations for the reduced or increased risk of acute GVHD in patients with the specific haplotype. In HSCT, GVHD has been known to result mainly from donor T cells recognizing minor histocompatibility antigens presented on HLA molecules of a recipient's organs.[19] Different HLA haplotypes possess a different combination of HLA alleles. Therefore, various HLA alleles in each haplotype might present different immunodominant peptides to T cells and evoke different alloreactivity in HLA-matched UR-HSCT. Presumably, the critical but as yet unidentified minor histocompatibility antigens linked to major histocompatibility antigen should be explored based on HLA haplotype, such as common gene deletion polymorphisms.[20] Distinct forms of GVHD were found in different MHC haplotypes in mice, and it has been argued that genes in the MHC locus can dominantly determine the forms of GVHD, probably through MHC-based selection of immunodominant antigens.[21]

In human retrospective analysis, several single-center studies have shown a reduced incidence of acute GVHD,[22] reduced relapse rate,[23] and improved overall survival[24] for HLA-DR15-positive patients transplanted from HLA-matched donors. HLA-DR15 has been known to be a marker of disease susceptibility and clinical response to immunosuppressive therapy in autoimmune-mediated bone marrow failure,[25,26] and it is speculated that immune responses specific to HLA-DR15 are induced. However, our analysis of the effect of HLA-DR15 using the same database showed no effect on acute GVHD (data not shown).

HLA haplotype serves as a model system for studies of disease association, especially in autoimmune disease or infection, and several candidate genes in the HLA region associated with specific haplotype have the potential to modulate immune or inflammatory responses.[10] The observed effect of HLA haplotypes on GVHD development could be explained by particular SNPs that are closely associated with those HLA haplotypes. Within the region of conserved HLA haplotypes, there exist several candidate genes whose SNPs may be related to immune responses. Genetic variants of tumor necrosis factor-α gene located in the HLA region might influence the risk of developing GVHD.[27,28] In addition, *TAP1*/*TAP2* and *LMP2*/*LMP7* genes encode subunit components of the proteasomes implicated in the processing of class I HLA-bound peptides,[29,30] and polymorphisms of these genes may affect antigen presentation on recipient tissues, leading to different susceptibility to GVHD. However, they probably do not explain the observed effects of haplotypes; correlations of each haplotype with known SNPs in these genes are generally weak, although D' among these alleles is high (> 0.98). Moreover, currently no haplotype-specific non-HLA polymorphisms have been identified in our series, although we could not exclude the possibility that there may exist some nonobserved SNPs that are closely associated with relevant HLA haplotypes.

We showed that Japanese common HLA haplotypes were conserved from HLA-DPB1 to extensively telomeric HLA-A, so it might be possible that the responsible gene is located in the telomeric region of the classic HLA. Interestingly, HP-P1 was divided into 2 subtypes based on the telomeric region from nucleotide 29414635 (subtypes A and B). Although we analyzed the effect of those subtypes on grade 2 to 4 acute GVHD among patients with HP-P1, we could not detect significant differences between patients with HP-P1 subtype A and subtype B. We also could not detect the differences between patients transplanted from a donor with HP-P1 subtype A and subtype B (data not shown).

In conclusion, in the present study, we demonstrated that highly conserved HLA haplotype might contribute to the occurrence of acute GVHD in HSCT. Although the clinical implications of our results should be considered cautiously, these results imply the proof of principle for an association between one or another HLA haplotype and GVHD. Our findings on the conservation of the MHC region may also provide background for exploring not only genetic factors associated with acute GVHD but also genetic disease susceptibility in our population. More extensive studies are warranted to identify specific genes associated with a particular haplotype contributing to acute GVHD.

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Authorship

Contribution: S.M., Y.M., S.O., H.S., M.S., H.I., and T.S. participated in the design of this study; S.O., K.K., A.M., and Y.N. performed histocompatibility analysis; Y.M., S.K., and Y.K. organized data collection for transplantation; T.K. performed statistical data analysis; S.M. and Y.M. performed analysis and wrote this paper; and all authors checked the final version of the paper.

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ORIGINAL ARTICLE

Peripheral blood stem cell versus bone marrow transplantation from HLA-identical sibling donors in patients with leukemia: a propensity score-based comparison from the Japan Society for Hematopoietic Stem Cell Transplantation registry

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Abstract We retrospectively analyzed the results of 707 adult patients who underwent myeloablative peripheral blood stem cell transplantation (PBSCT) ($n = 365$) and myeloablative bone marrow transplantation (BMT) ($n = 342$) for leukemia from HLA-identical sibling donors between 2000 and 2005 using the propensity score method. The results were obtained from the Japan Society for Hematopoietic Cell Transplantation registry. Multivariate Cox analysis showed that PBSCT was associated with lower overall survival (OS) in standard-risk patients [adjusted hazard ratio (aHR) = 1.83; 95% confidence interval (CI) 1.04–3.23; $P = 0.036$], but not in high-risk patients (aHR = 1.11; 95% CI 0.76–1.61; $P = 0.599$). Hematopoietic recovery was significantly faster after

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PBSCT. The risk of acquiring grade III–IV acute graft-versus-host disease (GVHD) (aHR = 2.23; $P = 0.040$) and extensive chronic GVHD (aHR = 1.93; $P = 0.001$) were significantly higher after PBSCT. PBSCT was associated with higher non-relapse mortality in standard-risk patients (aHR = 2.30; 95% CI 1.08–4.88; $P = 0.030$), but not in high-risk patients (aHR = 1.29; 95% CI 0.65–2.54; $P = 0.468$). Relapse after transplantation did not differ between PBSCT and BMT either in standard-risk group or in high-risk group (aHR = 1.17; 95% CI 0.55–2.52; $P = 0.684$ and aHR = 0.81; 95% CI 0.52–1.28; $P = 0.370$, respectively). In this retrospective analysis, OS was significantly lower after PBSCT in standard-risk patients, but not in high-risk patients. PBSCT was associated with significant risks of grade III–IV acute GVHD and extensive chronic GVHD.

Keywords Bone marrow transplantation · Peripheral blood stem cell transplantation · Allogeneic · Graft-versus-host disease

1 Introduction

During the past decade, allogeneic peripheral blood stem cell transplantation (allo-PBSCT) has been increasingly used as an alternative to allogeneic bone marrow transplantation (allo-BMT) [1]. Furthermore, allo-PBSCT is associated with rapid hematopoietic recovery. Several prospective randomized controlled trials conducted in Western countries have shown an increased incidence of chronic graft-versus-host disease (GVHD) [2–11]. Nevertheless, there is still substantial controversy regarding survival, acute GVHD, non-relapse mortality (NRM), and relapse [12–14].

Ethnicity has been reported to affect the incidence and severity of GVHD [15]. Japanese patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) were found to have a lower incidence of acute GVHD than those from Western patients [16, 17]. Therefore, the outcome of allo-PBSCT compared with that of allo-BMT may differ according to the ethnic background.

Using the propensity score method, we retrospectively analyzed the clinical outcomes of 707 adult Japanese leukemia patients who received allogeneic HSCT with myeloablative conditioning from HLA-identical sibling donors. These data were obtained from the Japan Society for Hematopoietic Cell Transplantation (JSHCT) registry. A propensity scoring system was devised to estimate the effects of treatments by comparing outcomes of those subjects who were not randomly assigned to experimental or control groups in an observational study [18]. A randomized control trial is superior in eliminating the confounding factors of known and unknown covariates by random treatment assignment. The propensity score expresses the likelihood of being assigned to experimental or control treatments, and is calculated using logistic regression models, including variables measured prior to treatment as much as possible. Considering the propensity score in this analysis, we expected that a hypothetical evaluation of an experimental trial in an observational study would give results similar to those of an evaluation in a randomized controlled trial.

2 Patients and methods

2.1 Study population

Using a standardized reporting form, JSHCT collects data on individual transplant patients from each transplant center, and follow-up reports are submitted annually after transplantation. A total of 1,426 patients, who underwent allogeneic HSCT between 2000 and 2005 for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML), have been reported to JSHCT. Patients were excluded from the study if their data were incomplete ($n = 205$), if they received a non-myeloablative or reduced-intensity conditioning regimen ($n = 223$), if they received grafts from other than HLA-identical siblings ($n = 217$), if they were less than 18 years of age ($n = 38$), if they had a previous history of HSCT ($n = 10$), and if they had non-allo-PBSCT or non-allo-BMT ($n = 16$). In Japan, most

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allo-HSCT patients have received granulocyte-colony stimulating factor (G-CSF) post-transplant [19]. The August 2006 data of the remaining 707 patients were analyzed. This study was approved by the Data Management Committee for the Nationwide Survey of JSHCT.

2.2 Definitions

Risk status at transplantation was categorized as either standard or high. Standard-risk diseases included acute leukemia in first complete remission (CR) and CML in first chronic phase (CP). Other disease status was categorized as high-risk disease [11]. The day of neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count (ANC) of more than 0.5×10^9/L. The day of platelet engraftment was defined as the first of seven consecutive days with a platelet count of more than 20×10^9/L without platelet transfusion. Acute GVHD was graded according to the standard criteria [20]. All patients who had no evidence of graft failure and survived beyond day 28 were considered to be evaluable for acute GVHD. GVHD persisting beyond day 100 and de novo GVHD occurring after day 100 were classified as chronic GVHD. The incidence of chronic GVHD was calculated in patients followed for more than 100 days, and the disease was classified as none, limited, or extensive [21]. Overall survival (OS) was defined as the duration of survival between transplantation and either death or the last follow-up.

Relapse was defined as disease progression with censored NRM. NRM included all causes of death other than relapse occurring at any time after transplantation. All deaths were considered in the estimation of OS.

2.2.1 Endpoints

The primary endpoint of comparison was OS. Secondary endpoints were hematopoietic recovery, acute GVHD (grade II–IV and III–IV), chronic GVHD (overall and extensive), NRM, and relapse.

2.2.2 Propensity score calculation

We calculated the propensity score using the pscore command in STATA version 10.1. (STATA, College Station, TX, USA) [22]. Factors included in the propensity score were as follows: age at HSCT in categories (<40, 40–49, and 50+) as an ordinal variable; sex (male/female) as an indicator variable; year of transplantation as a continuous variable; performance status at transplantation as an ordinal variable; risk status (CR1/CP1, CR2/CP2, or more advanced) as an indicator variable; a cumulative number of HSCT from related donors at an institution between 2000 and 2005 (1: 1–4, 62 institutions; 2: 5–11, 58 institutions; 3: 12 or more, 52 institutions) as an ordinal variable; and the percentage of allo-PBSCT out of total HSCT from HLA-identical siblings in tertile (1: <56%, 59 institutions; 2: 56–90%, 56 institutions; 3: 91% or more, 57 institutions) as an ordinal variable. We utilized as many variables as possible in the propensity score to evaluate the effects of known and unknown factors on the choice of treatment. After calculating the propensity score, the subjects were divided into four groups according to quartile. The numbers of subjects in quartiles 1–4 (allo-PBSCT/allo-BMT) were 23/154, 58/120, 126/50, and 158/18, respectively.

2.2.3 Statistical analysis

Patient characteristics and therapeutic outcomes were compared between allo-PBSCT and allo-BMT groups. OS was assessed using the Kaplan–Meier product limit method [23, 24]. Cumulative incidences of acute GVHD, chronic GVHD, NRM, and relapse were evaluated as 1 − (Kaplan–Meier estimate) instead of applying methods considering competing risks [25, 26] to maintain statistical consistency between logrank tests and methods of cumulative incidence estimation. Allo-PBSCT and allo-BMT groups were compared using the propensity score in quartiles [1–4], a stratified logrank test, and a stratified Cox proportional hazards model. Diagnosis (AML, ALL, and CML) and quartile of the propensity score were stratification factors. Confounders considered in the Cox proportional hazards model were as follows: year of diagnosis as a continuous variable; year of transplantation as a continuous variable; age at transplantation as a continuous variable; sex (male/female); sex matching (match/male to female/female to male/unknown); performance status (0, 1, 2, 3–4, and unknown); risk status (standard/high); GVHD prophylaxis [cyclosporin (CsA) + methotrexate (MTX), tacrolimus (TAC) + MTX, and others]; and conditioning regimen [total body irradiation (TBI)-containing regimen, busulfan and cyclophosphamide (BU/CY), and others]. All analyses were performed using STATA version 10.1, and P values less than 0.05 were considered statistically significant.

3 Results

3.1 Patient characteristics

The characteristics of patients are summarized in Table 1. The number of patients who underwent allo-PBSCT was 365, and that who underwent allo-BMT was 342. The median age at HSCT was 39 years (range 18–64 years) in the allo-PBSCT group and 39 years (range 18–59 years) in the allo-BMT group. The allo-PBSCT group included significantly more male patients from female donors than

Table 1 Characteristics of patients

	PBSCT *n* (%)	BMT *n* (%)	*P* value (Mann–Whitney test)
No. of patients	365	342	
Median patients age, years (range)	39, 18–64	39, 18–59	0.962
Patients sex (male/female)	210/155	189/153	0.543
Sex matching			
Matched	176 (48.2)	185 (54.1)	
Male to female	70 (19.2)	78 (22.8)	
Female to male	106 (29.0)	71 (20.8)	
Unknown	13 (3.6)	8 (2.3)	0.043
Risk group			
Standard-risk	149 (40.8)	202 (59.1)	
High-risk	216 (59.2)	140 (40.9)	<0.001
Diagnosis			
Standard-risk			
AML	58 (38.9)	76 (37.6)	
ALL	46 (30.9)	51 (25.2)	
CML	45 (30.2)	75 (37.2)	0.322
High-risk			
AML	128 (59.3)	75 (53.6)	
ALL	58 (26.9)	28 (20.0)	
CML	30 (13.8)	37 (26.4)	0.026
Performance status			
0	185 (50.7)	138 (40.4)	
1	73 (20.0)	55 (16.1)	
2	24 (6.6)	16 (4.7)	
3 or 4	12 (3.3)	2 (0.6)	
Unknown	71 (19.5)	131 (38.3)	<0.001
Conditioning regimen			
TBI-based	225 (61.6)	205 (59.9)	
Bu/CY	110 (30.1)	118 (34.5)	
Others	30 (8.3)	19 (5.6)	0.23
GVHD prophylaxis			
CsA + MTX	308 (84.4)	300 (87.7)	
TAC + MTX	12 (3.3)	14 (4.1)	
Others	45 (12.3)	28 (8.2)	0.176

Standard-risk diseases: acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase; other disease status was categorized as high-risk diseases

PBSCT peripheral blood stem cell transplantation, *BMT* bone marrow transplantation, *AML* acute myelogenous leukemia, *ALL* acute lymphoblastic leukemia, *CML* chronic myelogenous leukemia, *TBI* total body irradiation, *Bu* busulfan, *CY* cyclophosphamide, *GVHD* graft-versus-host disease, *CsA* cyclosporin, *MTX* methotrexate, *TAC* tacrolimus

the allo-BMT group (Mann–Whitney test, $P = 0.043$). AML, ALL, and CML were diagnosed in 337, 183, and 187 patients, respectively. The allo-PBSCT group included significantly more high-risk patients than the allo-BMT group ($P < 0.001$). Among the high-risk patients, the allo-BMT group had significantly more CML patients than the allo-PBSCT group ($P = 0.026$). Conditioning regimen and GVHD prophylaxis were performed according to the protocol of each institution, and there were no differences between the two groups. The most frequently used conditioning regimens were BU/CY (busulfan 1 mg/kg × 4/day × 4 days with cyclophosphamide 60 mg/kg/day × 2 days) and CY/TBI (cyclophosphamide 60 mg/kg/day × 2 days with total body irradiation 10–12 Gy). CsA plus MTX was used most frequently for GVHD prophylaxis. Median follow-up period for the surviving patients at the time of analysis was 33 months (1.8–55 months) in the PBSCT group and 31 months (1–53 months) in the BMT group.

3.2 Primary endpoint

3.2.1 Overall survival

Three-year OS in standard-risk patients was 68% [95% confidence interval (CI) 59–75] after allo-PBSCT and 77%

(95% CI 70–82) after allo-BMT (by disease and quartile in the propensity-score stratified logrank test; $P = 0.023$). Three-year OS in high-risk patients after allo-PBSCT and allo-BMT was 38% (95% CI 31–45) and 54% (95% CI 44–62), respectively ($P = 0.587$) (Fig. 1). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for lower OS in the population with standard-risk [adjusted hazard ratio (aHR) = 1.83; 95% CI 1.04–3.23; $P = 0.036$], but not that with high-risk (aHR = 1.11; 95% CI 0.76–1.61; $P = 0.599$).

3.3 Secondary endpoints

3.3.1 Hematopoietic recovery

Engraftment occurred in all patients receiving allo-PBSCT and allo-BMT (allo-PBSCT, $n = 324$; allo-BMT, $n = 305$) surviving for more than 28 days. Allo-PBSCT patients showed significantly faster neutrophil and platelet recovery compared with allo-BMT patients. The median time of recovery to ANC $> 0.5 \times 10^9$/L was 14 days for the allo-PBSCT group and 16 days for the allo-BMT group, respectively (stratified logrank test, $P < 0.0001$). The median time of recovery to a platelet count $>20 \times 10^9$/L was 15 days for the allo-PBSCT group and 21 days for the allo-BMT group, respectively ($P < 0.0001$). In the multivariate Cox analysis, allo-PBSCT was a significant factor for faster neutrophil (aHR = 0.57; 95% CI 0.45–0.71; $P < 0.001$) and platelet (aHR = 0.56; 95% CI 0.44–0.71; $P < 0.001$) recovery compared with allo-BMT.

3.3.2 Acute GVHD

The cumulative incidence of grade II–IV acute GVHD was 31% (95% CI 27–35) in all patients, whereas that in allo-PBSCT and allo-BMT groups was 35% (95% CI 30–41) and 26% (95% CI 22–32) (stratified logrank test, $P = 0.221$), respectively. The aHR for grade II–IV acute GVHD after allo-PBSCT was 1.25 (95% CI 0.85–1.84; $P = 0.260$) by multivariate Cox analysis. The cumulative incidence of grade III–IV acute GVHD was 14% (95% CI 10–18) and 5.4% (95% CI 3.3–8.8) in the allo-PBSCT and allo-BMT groups, respectively ($P = 0.021$). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for the development of grade III–IV acute GVHD (aHR = 2.23; 95% CI 1.04–4.78; $P = 0.040$; Fig. 2).

3.3.3 Chronic GVHD

The risk of chronic GVHD in the first year after transplantation was significantly higher after allo-PBSCT than after allo-BMT (cumulative incidence at 1 year, 51%; 95% CI 44–58 after allo-PBSCT vs. 34%; 95% CI 28–41 after allo-BMT; $P = 0.0005$ with stratified logrank test). The extensive form of chronic GVHD was more prevalent in the allo-PBSCT group than in the allo-BMT group (26%; 95% CI 21–33 with allo-PBSCT and 15%; 95% CI 11–20 with allo-BMT; $P = 0.0017$). Multivariate Cox analysis showed that allo-PBSCT was a significant factor for the development of extensive chronic GVHD (aHR = 1.93; 95% CI 1.32–2.84; $P = 0.001$; Fig. 3).

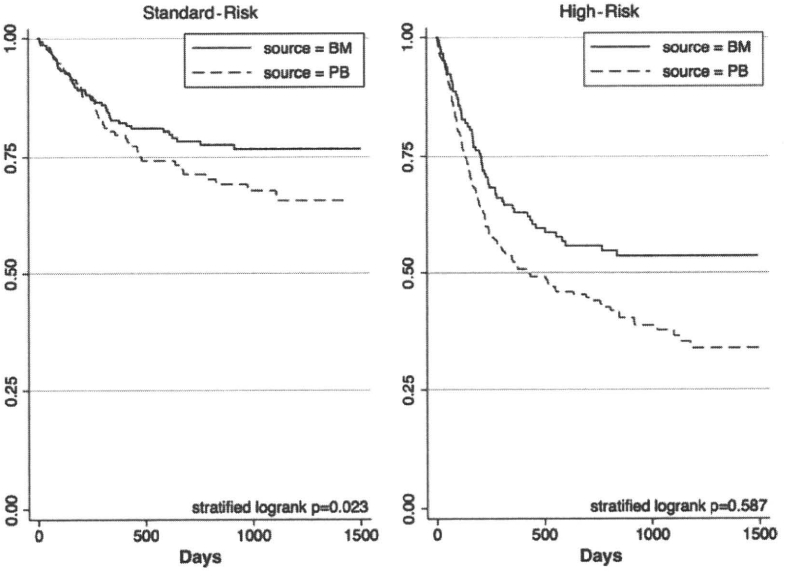

Fig. 1 Probabilities of overall survival after peripheral blood stem cell transplantation compared with bone marrow transplantation. Standard-risk diseases included acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase. Other diseases were categorized as high-risk diseases

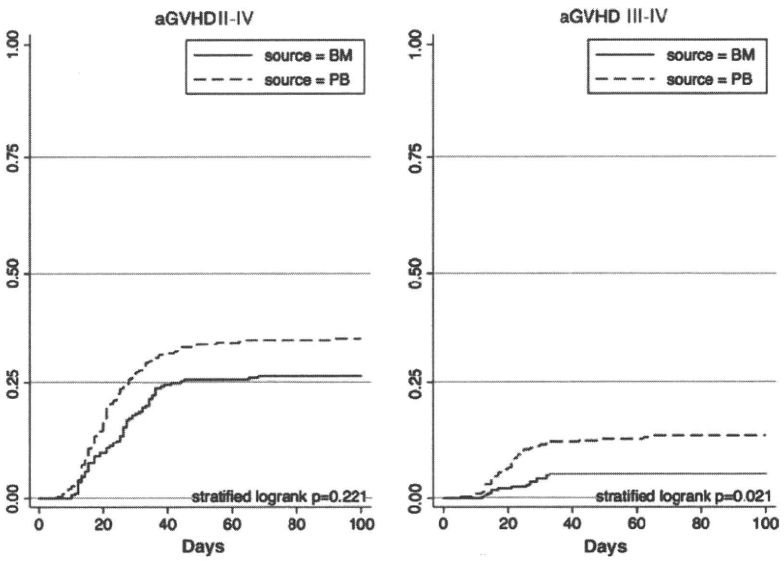

Fig. 2 Probabilities for grade II–IV and III–IV acute graft-versus-host disease (GVHD) after peripheral blood stem cell transplantation compared with bone marrow transplantation

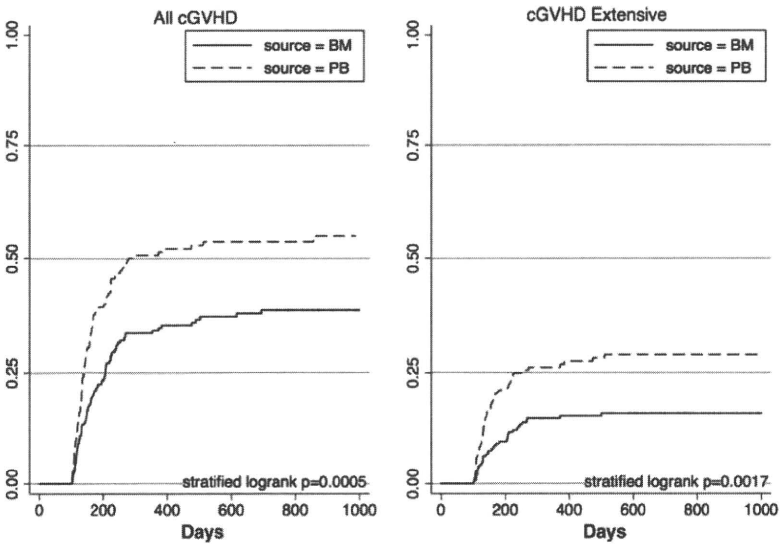

Fig. 3 Probabilities for chronic GVHD and extensive chronic GVHD after peripheral blood stem cell transplantation compared with bone marrow transplantation

3.3.4 Non-relapse mortality

The cumulative incidence of NRM for the standard-risk group at day 100 was 4.7% (95% CI 2.3–9.7) after allo-PBSCT and 6.0% (95% CI 3.4–10.2) after allo-BMT, and that at 1 year was 14.2% (95% CI 9.4–21.1) after allo-PBSCT and 11.2% (95% CI 8.0–17.2) after allo-BMT (stratified logrank test, $P = 0.047$). The cumulative incidence of NRM for the high-risk group at day 100 was 11.2% (95% CI 7.6–16.4) after allo-PBSCT and 8.9% (95% CI 5.1–15.1) after allo-BMT, and that at 1 year was 24.4% (95% CI 18.7–31.4) after allo-PBSCT and 14.7% (95% CI 9.6–22.2) after allo-BMT (stratified logrank test, $P = 0.221$) (Fig. 4).

Multivariate Cox analysis showed that allo-PBSCT was a significant factor for higher NRM in the standard-risk (aHR = 2.30; 95% CI 1.08–4.88; $P = 0.030$), but not in the high-risk (aHR = 1.29; 95% CI 0.65–2.54; $P = 0.468$).

3.3.5 Relapse

The cumulative incidence of relapse at 1 year for the standard-risk group was similar for allo-PBSCT (13.8%; 95% CI 8.9–21.0) and allo-BMT (9.7%; 95% CI 6.1–15.2) ($P = 0.518$ by stratified logrank test). Similarly, in the high-risk group the incidence was 32.4% (95% CI 25.6–40.3) for allo-PBSCT and 31.5% (95% CI 23.7–41.1) for allo-BMT ($P = 0.200$) (Fig. 5).

Multivariate Cox analysis showed no significant difference in the risk of relapse after allo-PBSCT and allo-BMT either in the standard-risk group or in the high-risk group (aHR = 1.17; 95% CI 0.55–2.52; $P = 0.684$ and aHR = 0.81; 95% CI 0.52–1.28; $P = 0.370$, respectively).

4 Discussion

In the present study, we analyzed results for 707 patients who underwent myeloablative HSCT for leukemia from HLA-identical sibling donors between 2000 and 2005. These data were obtained from the JSHCT registry. Health insurance coverage of allo-PBSCT was approved in Japan in 2000, and since then the number of allo-PBSCTs rapidly increased and exceeded the number of allo-BMTs between 2000 and 2003. Subsequently, the number of allo-PBSCTs decreased, and the numbers of allo-PBSCTs and allo-BMTs became equivalent in 2005. Thus, this analysis indicates the rather immature status of allo-PBSCT in Japan.

The Stem Cell Trialists' Collaborative Group [27] reported an individual patient data meta-analysis of nine randomized trials by comparing outcomes of allo-PBSCT versus allo-BMT from HLA-matched related donors for the treatment of hematologic malignancies. Allo-PBSCT was associated with a higher probability of 5-year OS in the subset analysis of patients with late disease due to decreased relapse. International Bone Marrow Transplant registry/European Group for Blood and Marrow Transplantation (IBMTR/EBMT) registry data of 398 adult allo-BMT and 208 allo-PBSCT patients with leukemia were analyzed using information on 6 or more years of follow-up [28]. OS in patients with early and advanced leukemia did not differ significantly between the two groups. The IBMTR report comparing outcomes after allo-PBSCT and allo-BMT for acute leukemia in children and adolescents showed that OS was lower after allo-PBSCT [29]. These controversial data indicate that the difference in stem cell source can affect OS depending on the underlying disease, disease status, and the patients' age.

In our study, OS was lower after allo-PBSCT than after allo-BMT in the standard-risk patients, but not in the high-risk patients. Considering the difference in stem cell source, factors affecting OS include hematopoietic and

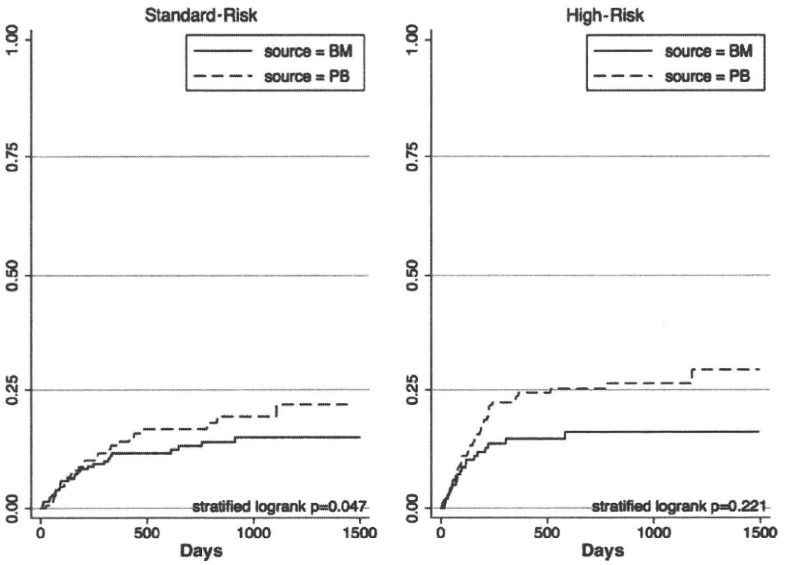

Fig. 4 Cumulative incidences of non-relapse mortality (NRM) after peripheral blood stem cell transplantation compared with bone marrow transplantation. Standard-risk diseases included acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase. Other diseases were categorized as high-risk diseases

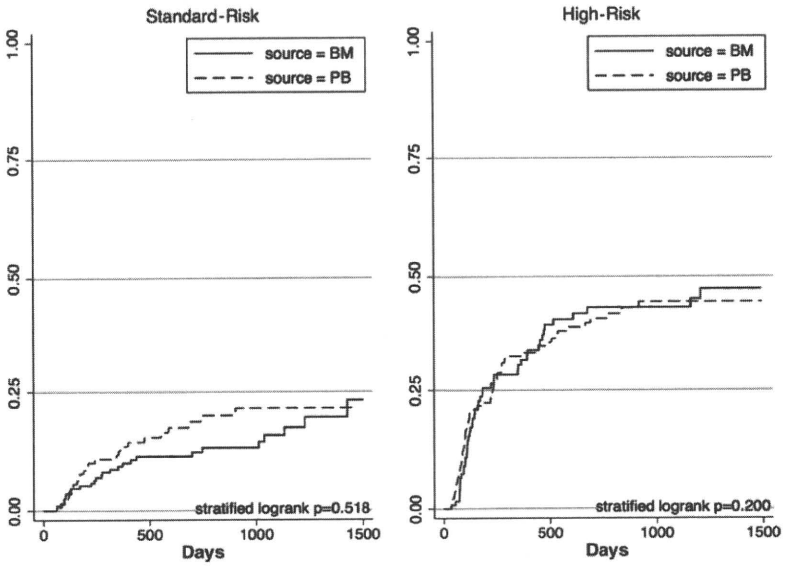

Fig. 5 Cumulative incidences of relapse after peripheral blood stem cell transplantation compared with bone marrow transplantation. Standard-risk diseases included acute leukemia in first complete remission and chronic myelogenous leukemia in first chronic phase. Other diseases were categorized as high-risk diseases

immune recovery, acute and chronic GVHD, and graft-versus-leukemia (GVL) effect or relapse [30].

In our analysis, allo-PBSCT was associated with more rapid hematopoietic recovery than allo-BMT as has been shown in most previous studies [4, 5, 11, 31]. Most randomized trials demonstrated that neutrophil recovery generally occurs 5–7 days earlier after allo-PBSCT compared with allo-BMT without G-CSF post-transplant [27, 32]. The EBMT study reported by Schmitz et al. [5] showed that neutrophil recovery was achieved 3 days earlier after allo-PBSCT than after allo-BMT with G-CSF post-transplant, and transplantation-related mortality did not differ between allo-PBSCT and allo-BMT groups. In Japan, most allo-HSCT patients receive G-CSF post-transplant, and in our study neutrophil recovery was observed 2 days earlier after allo-PBSCT than after allo-BMT. Accordingly, infectious complications may not decrease after allo-PBSCT compared to allo-BMT.

With regard to acute GVHD, the meta-analysis showed that allo-PBSCT was associated with a significant increase in the development of grade III–IV acute GVHD, but not grade II–IV acute GVHD [27]. In the present analysis, allo-PBSCT was also a significant factor in the incidence of grade III–IV acute GVHD. The increased incidence of grade III–IV acute GVHD in allo-PBSCT would have a negative effect on OS [33].

Extensive chronic GVHD was more frequent after allo-PBSCT than after allo-BMT in our study. This finding is in line with those of previous reports [5, 9, 11, 19, 31, 34].

In our analysis, NRM was higher after allo-PBSCT in the standard-risk patients, but not in the high-risk patients. The higher NRM after allo-PBSCT in the standard-risk group was likely due to increased grade III–IV acute GVHD and extensive chronic GVHD. Increased NRM after allo-PBSCT has been reported from children and adolescents suffering with acute leukemia [29]. A higher risk of mortality due to acute and chronic GVHD may counteract any benefit of more rapid hematopoietic recovery in the early transplant period.

In the allo-BMT setting, the development of both acute and chronic GVHD is associated with decreased relapse of leukemia, whereas the effect of GVHD on OS appears to be different depending on the study population [33, 35, 36]. The meta-analysis showed that allo-PBSCT was associated with a significant decrease in relapse in both early and late-stage disease patients [27]. On the contrary, increased extensive chronic GVHD in the allo-PBSCT group did not lead to a decrease in relapse in our analysis. We do not have a good explanation for this, but a similar observation was reported from the IBMTR/EBMT [28] registry data of adult patients with leukemia and the IBMTR [29] study in children and adolescents with acute leukemia. The advantage in term of the GVL effect with the cost of increased GVHD after allo-PBSCT relative to after allo-BMT remains controversial [27–29]. The allogeneic GVL effect varies from one disease to another, with the stage of the disease, and with donor histocompatibility. The GVL effect is believed to act while the leukemic burden is relatively

low [37]. Thus, to investigate the relationship between GVHD and relapse, subgroups differing in underlying disease and disease status would be needed.

We used the propensity score method to minimize selection bias. However, retrospective analysis has limitations. We could not exclude the possibility of unidentified confounding variables affecting the transplant outcomes and the inability to adjust the data for unknown or unmeasured factors. For example, we did not have data regarding pre-transplant infectious complications. Since allo-PBSCT is associated with more rapid hematopoietic recovery than allo-BMT, patients with serious infectious problems may have a tendency to undergo allo-PBSCT rather than allo-BMT. In this analysis, standard-risk diseases included acute leukemia in first CR and CML in first CP, while high-risk diseases included other diseases [11]. However, even in first CR acute leukemia patients, cytogenetic and molecular markers affect the prognosis with respect to survival in the allo-HSCT setting [38, 39]. We cannot deny the possibility that higher-risk patients in first CR tended to undergo allo-PBSCT. Thus, the results presented here should be interpreted with caution. It is also important to realize that our analysis was based on matched sibling myeloablative HSCT not on non-myeloablative HSCT. However, contrary to the result of the meta-analysis [27], multivariate Cox analysis showed that the allo-PBSCT group was associated with a lower OS in the populations with standard-risk. Prospective randomized trials are necessary to elucidate the advantages and disadvantages of allo-PBSCT in comparison with allo-BMT from HLA-identical sibling donors for the treatment of adult Japanese patients with leukemia.

Conflict of interest statement The authors declare no financial conflict of interest.

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Acute Lymphoblastic Leukemia of Male-Recipient Origin Demonstrating Female Karyotype After Cord Blood Transplantation

A 12-year-old boy was diagnosed with French-American-British classification L2 acute lymphoblastic leukemia (ALL). He was treated according to the pediatric ALL protocol and achieved a complete remission (CR). When the patient was 22 years old, he presented with dyspnea. A CBC revealed a WBC of 1.2 × 10^9/L (blasts, 2%), a hemoglobin level of 5.8 g/dL, and a platelet count of 160 × 10^9/L. Bone marrow aspiration showed 88% blasts that were cytochemically negative to myeloperoxidase staining (Fig 1A). Flow cytometric immunophenotyping showed that the blasts were CD10−, CD19+, CD13−, CD33+, CD34+, and HLA-DR+. Cytogenetic analysis showed a normal male karyotype, 46,XY, in each of the 20 metaphase cells analyzed (Fig 2A).

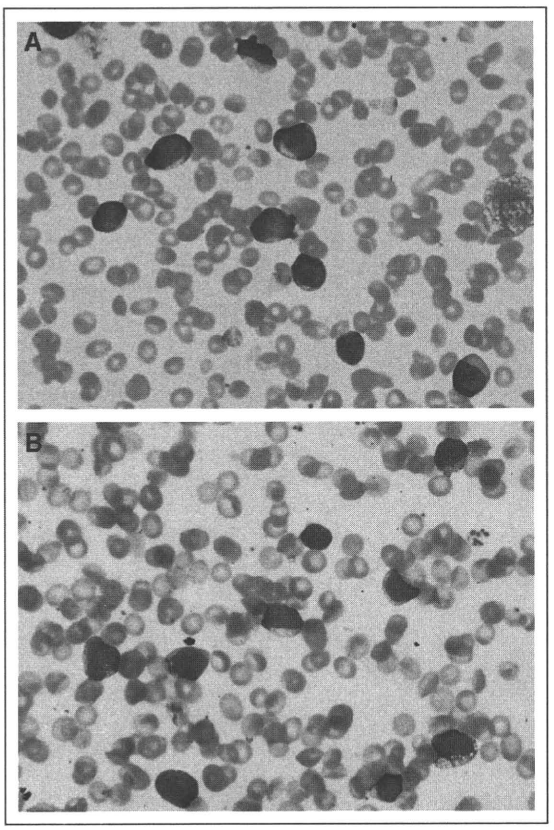

Fig 1.

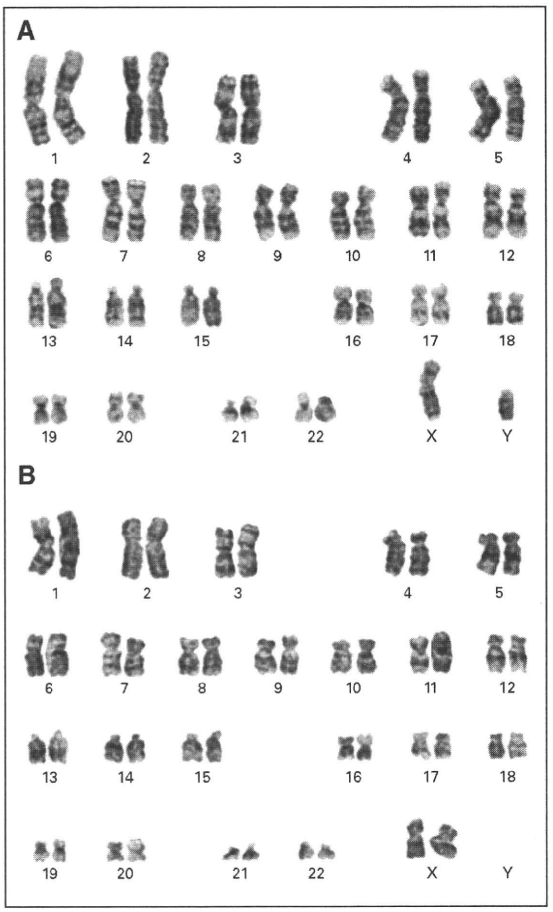

Fig 2.

On the basis of these results, the patient was diagnosed with a relapse of ALL (first relapse). After the patient achieved CR with intensive chemotherapy, three courses of consolidation therapy were administered. Seven months after diagnosis of the first relapse, an unrelated cord blood transplantation (CBT) from a human leukocyte antigen (HLA)–mismatched female donor was performed with a preparative regimen of cytarabine, cyclophosphamide, and total-body irradiation. Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and short-term methotrexate. Neutrophil engraftment occurred on day 25. The patient developed skin-only, grade II acute GVHD, which resolved with topical corticosteroids. Tacrolimus was discontinued 6 months after the transplantation with no evidence of chronic

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