The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors

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To improve the clinical outcome of allogeneic hematopoietic stem cell transplantation from an unrelated donor, the identification of human leukocyte antigen (HLA) alleles responsible for immunologic events such as graft-versus-host disease (GVHD), engraftment failure, and graft-versus-leukemia effect is essential. Genomic typing of HLA-A, -B, -C, -DRB1, and -DQB1 was retrospectively performed in 1298 donor-patient pairs in cases where marrow was donated from serologically HLA-A, -B, and -DR compatible donors. Single disparities of the HLA-A, -B, -C, or -DRB1 allele were independent risk factors for acute GVHD, and the synergistic effect of the HLA-C allele mismatch with other HLA allele mismatches on acute GVHD was remarkable. HLA-A and/or HLA-B allele mismatch was found to be a significant factor for the occurrence of chronic GVHD. HLA class I (A, B, and/or C) allele mismatch caused a significantly higher incidence of engraftment failure than HLA match. Significant association of HLA-C allele mismatch with leukemia relapse was not observed. As the result of these events, HLA-A and/or HLA-B allele mismatch reduced overall survival remarkably in both standard-risk and high-risk leukemia cases, whereas the HLA-C mismatch or HLA-class II (DRB1 and/or DQB1) mismatch did not. Furthermore, multiple mismatch of the HLA locus was found to reduce survival in leukemia cases. Thus, the role of the HLA class I allele in unrelated bone marrow transplantation was elucidated. Notably, HLA-C alleles had a different mode from HLA-A or -B alleles for acute GVHD and survival. (Blood. 2002;99:4200-4206)

Introduction

Hematopoietic stem cell transplantation from a human leukocyte antigen (HLA)–matched unrelated donor (UR-HSCT) has been established as one mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders.[1-3] The high mortality after UR-HSCT due to severe acute graft-versus-host disease (GVHD) and its related complications is still a barrier to the improvement of patient survival and a cure. The induction of the graft-versus-leukemia (GVL) effect to reduce leukemia relapse is considered one of the advantages of allogeneic HSCT.[4,5] These transplant-related immunologic events are affected by the disparities of major and/or minor histocompatibility of antigens between donor and recipient. In UR-HSCT, it has become evident that some cases have a difference of HLAs at the allele (genotypical) level among serologically HLA-A, -B, and -DR identical pairs.[6-10] Therefore, the identification of single HLA alleles responsible for these immunologic events is important in order to optimize HLA matching and minimize GVHD and engraftment failure, as well as increase GVL effects.[7,11-16] There exists some controversy over the HLA alleles responsible. Our previous report[7] through the Japan Marrow Donor Program (JMDP) indicated the effect of matching of HLA class I alleles (A, B, and C) on acute GVHD and a possible role for HLA-C in the GVL effect. Petersdorf et al[14,16] have reported the importance of HLA class II matching for GVHD and the effect of HLA-C matching on graft rejection.

In this report, we extended the analysis of JMDP and identified the genotype of HLA-A, -B, -C, -DRB1, and -DQB1 in 1298 pairs of HLA-A, -B and -DR serologically matched UR-HSCTs performed through JMDP, and analyzed the effects of HLA compatibilitiy on these immunologic events and on overall survival, focusing on the role of the HLA class I allele as a transplantation antigen.

Patients, materials, and methods

Patients

According to the donor selection criteria of JMDP, patients received marrow transplants from serologically HLA-A, -B, and -DR antigen

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completely matched donors. The donor-recipient pairs (1298) were sequentially identified for genotypes of HLA-A, -B, -C, -DRB1, and -DQB1 retrospectively, and entered in this study. Transplantations were performed between January 1993 and April 1998, and a final clinical survey of these patients was done on June 30, 2000. Informed concent was obtained from patient and donor according to the Declaration of Helsinki, and an approval was obtained from the institutional review board at Aichi Cancer Center for this study.

Characteristics of patients are listed in Table 1. Patients ranged from 0 to 51 (median 23) years old, and donors ranged from 20 to 51 (median 34) years old. There were 304 patients with acute myeloid leukemia (AML), of whom 99 received transplants in the first complete remission (CR), 116 in the second or greater CR, and 89 in non-CR. There were 353 patients with acute lymphoblastic leukemia (ALL), of whom 122 were in the first CR, 133 in the second or greater CR, and 98 in non-CR. There were 367 patients with chronic myeloid leukemia (CML), of whom 259 were in the first chronic phase (CP), 23 in the second or greater CP, 59 in accelerated phase (AP), and 26 in blastic phase (BP). There were 99 patients with myelodysplastic syndrome (MDS); 39 with malignant lymphoma; 101 with severe aplastic anemia; and 35 with hereditary disorders in children. Standard risk for leukemia relapse was defined as the status of the first CR of AML and ALL, and the first CP of CML at transplantation, whereas high risk was defined as a more advanced status than standard risk.

Identified HLA alleles

Serologic typing for HLA-A, -B, and -DR was performed with a standard 2-stage complement-dependent test of microcytotoxicity or with low-resolution DNA typing in part. Alleles at the HLA-A, -B, -C, -DRB1, and -DQB1 loci were identified by high-resolution DNA typing as described previously.[7,17,18] The identified genotype of each allele and its number (patient, donor) were as follows for HLA-A: 0101 (8,8); 0201 (256,259); 0206 (245,245); 0207 (89,90); 0210 (9,6); 0301 (6,6); 1101 (184,185); 1102 (2,2); 2402 (906,906); 2601 (150,134); 2602 (32,31); 2603 (45,64); 2605 (0, 1); 3001 (1,1); 3101 (183,183); 3303 (205,205). For HLA-B: 0702 (173,173); 1301 (28,28); 1501 (182,183); 1502 (1,1); 1507 (22,24); 1511 (6,6); 1518 (19,19); 1527 (3,1); 3501 (193,193); 3701 (8,8); 3901 (69,67); 3902 (2,5); 3904 (4,3); 4001 (99,73); 4002 (187,196); 4003 (8,8); 4006 (146,141); 4402 (10,10); 4403 (200,201); 4601 (119,119); 4801 (45,45); 5101 (200,202); 5102 (2,0); 5201 (405,405); 5401 (217,217); 5502 (42,42); 5601 (17,17); 5801 (12,12); 5901 (52,52); 6701 (21,21). For HLA-C: 0102 (419,421); 0301 (1,1); 0302 (8,7); 0303 (278,272); 0304 (267,272); 0401 (98,106); 0501 (8,4); 0602 (8,8); 0702 (290,279); 0704 (20,15); 0801 (178,182); 0803 (32,31); 1201 (1,0); 1202 (404,406); 1203 (1,1); 1301 (2,1); 1402 (171,162); 1403 (203,206); 1502 (64,55); 1503 (0,1); 1504 (1,1). For HLA-DRB1: 0101 (171,171); 0301 (1,1); 0401 (21,15); 0403 (72,71); 0404 (1,2); 0405 (356,351); 0406 (85,84); 0407 (9,16); 0410 (34,43); 0802 (90,93); 0803 (192,191); 0901 (379,378); 1001 (8,8); 1101 (32,32); 1201 (58,57); 1202 (35,36); 1301 (10,10); 1302 (193,195); 1329 (1,0); 1401 (66,73); 1403 (27,33); 1405 (30,31); 1406 (27,12); 1407 (1,1); 1412 (1,1); 1501 (180,182); 1502 (387,385); 1602 (11,11). For HLA-DQB1: 0201 (1,1); 0301 (185,166); 0302 (210,218); 0303 (394,394); 0401 (350,346); 0402 (92,88); 0406 (1,0); 0501 (178,180); 0502 (45,38); 0503 (61,76); 0601 (546,547); 0602 (167,178); 0603 (9,8); 0604 (185,189); 0605 (6,1); 0609 (1,1); 0613 (0,1).

Matching of the HLA allele between patient and donor

In the analysis of acute GVHD and chronic GVHD, an HLA allele mismatch among the donor-recipient pair was scored when the recipient's alleles were not shared by the donor (GVHD vector), and engraftment failure when the donor's alleles were not shared by the recipient (rejection vector). In the analysis of nonrelapse mortality, relapse rate, and survival, the mismatch was defined as that of either the GVHD vector or the rejection vector.

Table 1. Patient characteristics

			HLA mismatch locus*						
	Total	Match	A/B	C	DR/DQ	A/B+C	A/B+DR/DQ	C+DR/DQ	A/B+C+DR/DQ
Number of cases	1298	566	118	156	141	124	45	90	58
Patient age (median, y)	23	23	25	22	24	25	24	24	21
Sex (Donor/patient)									
Male/male	494	214	40	61	44	55	20	41	19
Male/female	268	112	30	32	28	22	10	22	12
Female/male	298	138	20	38	41	27	8	15	11
Female/female	238	102	28	25	28	20	7	12	16
Disease									
AML	304	150	24	34	34	28	12	14	8
ALL	353	151	31	46	45	34	14	23	9
CML	367	141	39	42	39	36	15	29	26
MDS	99	43	12	15	8	9	1	7	4
Malignant lymphoma	39	23	1	3	6	0	0	3	3
Severe aplastic anemia	101	38	7	11	8	16	3	11	7
Hereditary disease	35	20	4	5	1	1	0	3	1
Risk of leukemia relapse									
Standard	480	218	45	60	51	40	13	34	19
High	544	224	49	62	67	58	28	32	24
GVHD prophylaxis									
Cyclosporine based	964	425	98	123	109	89	30	52	38
Tacrolimus based	141	65	9	14	18	10	5	13	7
ATG based	176	70	11	18	11	23	7	23	13
T-cell depletion	16	6	0	0	3	2	3	2	0
Preconditioning									
TBI regimen	1027	447	99	121	109	98	34	73	46
Non-TBI regimen	271	119	19	35	32	26	11	17	12

AML indicates acute myelocytic leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; GVHD, graft-versus-host disease; ATG, antithymocyte globulin; TBI, total body irradiation.

*Match: HLA-A, -B, -C, -DR, and -DQ allele match. A/B: HLA-A and/or HLA-B allele mismatch. C: HLA-C allele mismatch. DR/DQ: HLA-DRB1 and/or HLA-DQB1 mismatch in GVHD vector.

Table 2. Multivariate analysis for factors affecting acute GVHD, chronic GVHD, engraftment, leukemia relapse, and mortality

Outcome and significant factor*	Hazard risk (95% CI)	*P*
Acute GVHD (grade III or IV)†		
HLA-C allele (match vs mismatch)	1.85 (1.42-2.41)	< .001
HLA-A allele (match vs mismatch)	1.58 (1.20-2.09)	.001
HLA-B allele (match vs mismatch)	1.43 (1.01-2.01)	.041
HLA-DRB1 allele (match vs mismatch)	1.42 (1.07-1.90)	.017
Chronic GVHD†		
HLA-A/B allele‡ (match vs mismatch)	1.45 (1.13-1.85)	.003
Patient age (linear)	1.014 (1.003-1.024)	.006
Engraftment†		
HLA class I allele‖ (match vs mismatch)	0.86 (0.76-0.96)	.011
Transplanted cell number (linear)	1.00047 (1.00007-1.00088)	.023
Disease (ALL vs CML)	0.79 (0.69-0.90)	.001
Leukemia relapse§		
Risk (standard risk vs high risk)	3.40 (2.43-4.76)	< .001
Mortality§		
HLA-A allele (match vs mismatch)	1.63 (1.35-1.97)	< .001
HLA-B allele (match vs mismatch)	1.33 (1.04-1.70)	.022
Patient age (linear)	1.019 (1.012-1.026)	< .001
Risk of leukemia relapse (standard vs high)	2.05 (1.72-2.45)	< .001
GVHD prophylaxis (cyclosporine vs ATG)	1.39 (1.03-1.89)	.030

For abbreviations, see Table 1.

*The variables entered in each stepwise analysis were sex (donor-recipient pairs), patient age, donor age, diagnosis, risk of leukemia relapse, graft-versus-host disease prophylaxis, preconditioning, transplanted cell dose, and matching of HLA-A, -B, -C, -DRB1, -DQB1 alleles (see Table 1 and the matching of HLA allele between patient and donor in "Patients, materials, and methods"). The significant level of HLA matching in each event is shown in this table.

†Analyzed in all cases.

‡HLA-A and/or HLA-B allele.

§Analyzed in leukemia cases.

‖HLA-A, HLA-B, and/or HLA-C allele.

Of 1298 pairs, the number and frequency of a one-allele and 2-allele mismatch in either GVHD or rejection vector in each HLA allele were 246 (19.0%) and 18 (1.4%) in HLA-A; 142 (10.9%) and 3 (0.2%) in HLA-B; 422 (32.5%) and 36 (2.8%) in HLA-C; 224 (17.3%) and 21 (1.6%) in DRB1; and 290 (22.3%) and 23 (1.8%) in HLA-DQB1, respectively.

There were 566 (43.6%) HLA allele (HLA-A, -B, -C, -DR, and -DQ) matched pairs in GVHD vector and 567 (43.7%) in rejection vector. A single allele mismatch in GVHD vector was found in 95 pairs for HLA-A; 14 in HLA-B; 156 for HLA-C; 14 for HLA-DRB1; and 41 for HLA-DQB1. As the number of single allele mismatch pairs of HLA-B, -DRB1, or -DQB1 was too small for the analysis, HLA-A and -B were grouped into the mismatch of the HLA-A and/or HLA-B allele (A/B), and HLA-DR and -DQ into the mismatch of the HLA-DRB1 and/or HLA-DQB1 allele (DR/DQ). Thus, 1298 pairs were classified into 8 HLA matching groups, that is, complete match of HLA-A, -B, -C, -DR, and -DQ, and either mismatch of A/B, C, DR/DQ, A/B+C, A/B+DR/DQ, C+DR/DQ, or A/B+C+DR/DQ. Patient characteristics of each HLA matching group are listed in Table 1. These 8 HLA matching groups were applied for the univariate analysis for clinical events. The 3 kinds of HLA locus allele matching levels were as follows: (1) HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ; (2) HLA-A and/or B (A/B), HLA-C, HLA-DR and/or DQ (DR/DQ); and (3) HLA-A, B, and/or C (HLA-class I), HLA-DR and/or DQ (HLA-class II), and the result of a significant level for HLA matching is shown in Table 2.

Transplantation procedures and the definition of transplant-related complications

Prophylaxis of GVHD. Among 1282 patients who received non–T-cell–depleted marrow transplants, a cyclosporine-based regimen was administered in 964 cases, a tacrolimus-based regimen in 141 cases, and an antithymocyte globulin (ATG)–based regimen in 176 cases for the prophylaxis of GVHD. T-cell depletion from bone marrow was performed in 16 cases. The occurrence of acute GVHD was evaluated according to grading criteria[1,19] in patients who survived more than 7 days after transplantation. The occurrence of chronic GVHD was evaluated according to the criteria[20] in patients who survived more than 100 days after transplantation.

Preconditioning regimen. There were 1027 patients who received total body irradiation (TBI)–containing regimens, and 271 patients who received non-TBI regimens.

Engraftment. Engraftment was defined as a peripheral granulocyte count of more than 500/μL for 3 successive days. A primary engraftment failure was defined as when engraftment was not obtained in patients who survived more than 21 days after transplantation. A secondary engraftment failure (rejection) was defined as when a peripheral granulocyte count became less than 500/μL with the finding of severe hypoplastic marrow in engrafted cases. Kaplan-Meier curve of engraftment was made by plotting the engrafted day of each patient, and patients who had secondary engraftment failure were censored at the rejected day. The rate of engraftment failure was calculated as (100 − engraftment rate [%]) at day 100 after transplantation. The number of nucleated bone marrow cells before the manipulation of bone marrow was replaced with the transplanted cell number.

Statistical analysis

Incidences of acute GVHD, chronic GVHD, engraftment, nonrelapse mortality, leukemia relapse, and survival were calculated by the Kaplan-Meier method,[21] and assessed by the log-rank test. The Cox proportional hazard model[22] was applied for multivariate analysis using the computer program STATA Version 7 (STATA Corporation, College Station, TX). Selection of significant factors was based on a forward stepwise procedure. The variables entered in each stepwise analysis were sex (donor-recipient pairs), patient age (linear), donor age (linear), disease, risk of leukemia relapse (standard vs high), GVHD prophylaxis, preconditioning, transplanted cell dose (linear), and HLA matching as described above and in Table 1.

Results

Effect of HLA allele mismatch on the occurrence of acute GVHD

To see the effect of the HLA allele itself in inducing acute GVHD with grade II to IV or grade III to IV, we analyzed the incidence of acute GVHD in each HLA matching group (Table 3). HLA-A/B mismatch had a close association with the occurrence of acute GVHD compared with the HLA match. Severe acute GVHD (grade III and IV) occurred in 27.8% of HLA-A/B mismatches and 11.8% of HLA matches ($P < .001$). Although a single HLA-C mismatch increased severe acute GVHD (20.6%) more than the HLA match (11.8%) ($P < .005$), the incidence of acute GVHD in HLA-C mismatches was lower than that in HLA-A/B mismatches. HLA-DR/DQ mismatches showed a less-potent association with acute GVHD than other HLA locus mismatches.

Table 3. Effect of HLA allele mismatch on acute graft-versus-host disease

		Incidence of acute GVHD			
HLA mismatch locus	No. of cases	Grade II-IV	*P**	Grade III-IV	*P**
Match	561	34.5	—	11.8	—
A/B	115	54.9	< .001	27.8	< .001
C	156	42.7	.030	20.6	.005
DR/DQ	141	34.4	.764	16.1	.139
A/B + C	123	60.9	< .001	37.0	< .001
A/B + DR/DQ	45	38.4	.391	18.3	.166
C + DR/DQ	90	55.7	< .001	30.9	< .001
A/B + C + DR/DQ	57	64.3	< .001	42.1	< .001

**P* value compared with matched cases by univariate analyses.

Risk factors for the occurrence of severe acute GVHD, including each HLA allele matching and clinical factors, were analyzed by multivariate analysis (Table 2). HLA-C matching, HLA-A matching, HLA-B matching, and HLA-DRB1 matching were elucidated to be significant risk factors. Other factors such as HLA-DQB1, patient age, donor age, sex matching, disease, risk of leukemia relapse, prophylaxis of GVHD, and TBI were not significant.

Of interest, the HLA-C mismatch was found to have a synergistic effect on the occurrence of acute GVHD, when another HLA locus mismatch was combined. HLA-C mismatch with the combination of HLA-A/B mismatch reached to 37.0% of severe acute GVHD, and HLA-C mismatch with HLA-DR/DQ mismatch reached to 30.9%. In contrast to HLA-C, no synergistic effect of HLA-A/B mismatch was observed in the combination of HLA-DR/DQ mismatch. The mismatch of HLA-A/B+C+DR/DQ induced the highest incidence of acute GVHD.

Effect of HLA allele mismatch on chronic GVHD

The incidence of chronic GVHD (limited type and extensive type) was analyzed in patients who survived more than 100 days after transplantation (Table 4). It became evident that HLA-A/B mismatch induced a significantly higher incidence (59.6%) of chronic GVHD than did HLA match (44.8%). HLA-DR/DQ mismatch showed no association with the occurrence of chronic GVHD. No synergistic effects between HLA 2-locus mismatches were observed. The mismatch of HLA-A/B+C+DR/DQ showed the highest incidence of chronic GVHD.

The severity of acute GVHD was also found to significantly correlate with the incidence of chronic GVHD ($P < .0001$) (ie, 30.7% in 213 cases with no acute GVHD; 48.3% in 224 cases with grade I acute GVHD; 63.7% in 163 cases with grade II acute GVHD; 75.1% in 68 cases with grade III acute GVHD; and 89.1% in 25 cases with grade IV acute GVHD).

The multivariate analysis elucidated 2 significant factors: HLA-A/B matching and patient age (Table 2).

Effect of HLA allele mismatch on engraftment failure

The overall incidence of engraftment failure was estimated at 4.4%, when primary and secondary failure cases were combined. Primary engraftment failure occurred in 54 of 1251 evaluated cases, and secondary engraftment failure occurred in 30 of 1197 engrafted cases. The engraftment failure rate was 1.1% in ALL (n = 340); 2.6% in AML (n = 290); 5.0% in CML (n = 357); 2.3% in MDS; 4.4% in malignant lymphoma (n = 39); 15.9% in severe aplastic anemia (n = 94); and 15.6% in hereditary disease (n = 34).

The incidence of engraftment failure tended to be higher with the increase of mismatch locus: 1.7% in HLA match; 4.8%, 4.1%, and 4.8% in single HLA mismatch of A/B, C, or DR/DQ, respectively; 10.4%, 8.9%, and 6.0% in HLA locus mismatch of A/B+C, A/B+DR/DQ, or C+DR/DQ, respectively; and 10.6% in HLA locus mismatch of A/B+C+DR/DQ (Table 5).

Multivariate analysis for engraftment failure elucidated 3 significant factors: transplanted bone marrow nuclear cell number, CML compared with ALL, and HLA class I mismatch. Other factors such as HLA class II mismatch, TBI, patient age, donor age, sex matching, and GVHD prophylaxis were not significant independent factors (Table 2).

Table 4. Effect of HLA allele mismatch on chronic graft-versus-host disease

HLA mismatch locus	No. of cases	Incidence (%) of chronic GVHD			
		LD + EX	*P**	EX	*P**
Match	310	44.8	—	29.9	—
A/B	67	59.6	.004	40.2	.015
C	86	50.5	.178	37.6	.146
DR/DQ	60	40.5	.960	31.5	.546
A/B + C	74	51.0	.163	33.2	.429
A/B + DR/DQ	26	55.2	.135	39.7	.188
C + DR/DQ	39	52.5	.084	37.8	.103
A/B + C + DR/DQ	32	76.8	.010	53.3	.021

GVHD indicates graft-versus-host disease; LD, limited type; EX, extensive type.
**P* value compared with matched cases by univariate analyses.

Table 5. Effect of HLA allele mismatch on engraftment failure

HLA mismatch locus*	No. of cases	Incidence (%) of engraftment failure			
		Primary + secondary	*P*†	Primary	*P*†
Match	554	1.7	—	0.7	—
A/B	114	4.8	.226	3.5	.171
C	141	4.1	.085	1.3	.216
DR/DQ	125	4.8	.226	2.5	.225
A/B + C	114	10.4	.009	6.2	.063
A/B + DR/DQ	45	8.9	.021	7.1	.014
C + DR/DQ	89	6.0	.472	4.7	.491
A/B + C + DR/DQ	57	10.6	.022	8.9	.009

*Rejection vector.
†*P* value compared with matched cases by univariate analyses.

Influence of HLA compatibility to survival in leukemia cases

As the overall survival was influenced by transplant-related immunologic events and the status of leukemia at transplantation, not only survival but also nonrelapse mortality rate and relapse rate were assessed by HLA compatibility in standard-risk leukemia (AML first CR, ALL first CR, and CML first CP) and in high-risk leukemia (more advanced status of disease than standard risk) (Table 6).

In standard-risk leukemia, HLA-A/B mismatch demonstrated a lower overall survival rate (39.9% at 3 years) than HLA match (65.4%). In contrast, single HLA-C mismatch and HLA-DR/DQ mismatch had no significant differences of overall survival (68.9% and 70.9% at 3 years, respectively) from HLA match (65.4%). The 2-HLA-locus mismatch also tended to show lower survival rate (50.0%-51.5% at 3 years) than HLA match (65.4%), and the 3-HLA-locus mismatch had the lowest survival rate (39.1%). It was evident that survival rates were mainly attributed to the incidence of nonrelapse mortality in each HLA matching group. Low incidence of nonrelapse mortality in HLA-C mismatch (28.8%) and HLA-DR/DQ mismatch (27.5%) reflected better survival rates than other HLA mismatch groups. The leukemia relapse rate of the HLA mismatch was not significantly different from the HLA match in each HLA matching group. Thus, the impact of HLA compatibility on leukemia relapse was less potent, and not attributed to survival collectively.

In high-risk leukemia, the impact of HLA compatibility on nonrelapse mortality was the same as that in standard-risk leukemia and was attributed to survival. As for HLA-C mismatch, slightly increased nonrelapse mortality (49.8%) was compensated for by a lower relapse rate (34.4%), and no significant difference of survival (36.1%) from HLA match (43.1%) was observed. The combination of HLA locus mismatch of HLA-A/B+C or C+DR/DQ showed a significantly lower survival rate than HLA match. The multiple HLA locus mismatch of A/B+C+DR/DQ had the worst survival rate and the highest nonrelapse mortality.

Table 6. Effect of HLA allele mismatch on nonrelapse mortality, relapse, and survival in patients with leukemia

HLA mismatch locus*	No. of cases	3-year nonrelapse mortality (%)	P†	3-year relapse rate (%)	P	3-year survival (%)	P
Standard risk							
Match	210	27.7	—	12.6	—	65.4	—
A/B	47	54.6	< .001	20.8	.364	39.9	< .001
C	61	28.2	.672	11.0	.707	68.9	.969
DR/DQ	52	27.5	.953	4.9	.162	70.9	.631
A/B + C	38	43.2	< .001	19.0	.460	51.5	.101
A/B + DR/DQ	14	55.6	.048	22.9	.425	50.0	.092
C + DR/DQ	35	50.8	.022	10.5	.953	50.6	.062
A/B + C + DR/DQ	23	58.8	< .001	15.6	.758	39.1	< .001
High risk							
Match	214	38.0	—	40.1	—	43.1	—
A/B	44	61.9	< .001	49.2	.632	23.9	.002
C	62	49.8	.077	34.4	.518	36.1	.225
DR/DQ	64	42.4	.578	39.3	.880	35.5	.362
A/B + C	64	64.1	< .001	37.7	.767	21.2	< .001
A/B + DR/DQ	30	71.1	.048	24.9	.329	32.6	.145
C + DR/DQ	35	61.8	.006	32.5	.874	25.7	.018
A/B + C + DR/DQ	31	78.0	.001	27.5	.952	15.9	.003

*Graft-versus-host disease vector and/or rejection vector.
†*P* value compared with HLA matched cases by univariate analyses.

These survival results were compatible with those obtained by multivariate analysis. Patient age, HLA-A matching, risk of leukemia relapse, HLA-B matching, and GVHD prophylaxis were elucidated as independent factors by the stepwise method. As for leukemia relapse, only the status of disease (standard risk vs high risk) was a significant factor (Table 2).

Discussion

Our previous analysis of the initial 440 donor-recipient pairs who received marrow transplants from serologically HLA-A, -B, -DR complete-match unrelated donors through JMDP have demonstrated (1) that not only HLA-A allele mismatch but also HLA-C mismatch induced severe acute GVHD; (2) HLA-C allele mismatch tended to reduce the incidence of leukemia relapse; (3) HLA-DPB1 matching had no association to the occurrence of severe GVHD; and (4) HLA-A allele matching affected patient survival remarkably, whereas HLA-DRB1 matching, HLA-DPB1 matching, and HLA-C allele matching had no effect on patient survival.[7]

We extended the analysis to 1298 pairs who had serologically HLA-A, -B, and -DR complete-match donors, and identified the genotype of HLA-A, -B, -C, -DRB1, and -DQB1. We decided not to type HLA-DPB1, because our previous study[7] clearly demonstrated no association of HLA-DPB1 matching with acute GVHD and survival. Among 1298 pairs, considerable numbers of single HLA-allele mismatch pairs and multiple HLA-locus mismatch pairs were found, which made it possible to analyze the effect of single and multiple HLA-allele mismatches on transplant-related clinical events. Furthermore, we could newly analyze the pairs for chronic GVHD and engraftment failure in addition to acute GVHD, leukemia relapse, and survival after transplantation.

As for acute GVHD, we added 2 new findings to the previous report.[7] First, not only the disparity of HLA-C and HLA-A but also that of HLA-B and HLA-DRB1 appeared to be independent significant factors for the occurrence of severe acute GVHD by multivariate analysis. Increased numbers of analyzed pairs compared with our previous study would simply make HLA-B and HLA-DRB1 significant factors. As HLA-DQB1 was cross-linked to HLA-DRB1, it was impossible to analyze the effect of single DQB1-allele mismatch on acute GVHD. It should also be pointed out that the effect of HLA-DRB1 disparity for acute GVHD was weaker than that of HLA class I alleles. The finding of an association between HLA class I mismatch and acute GVHD conflict with the findings of Petersdorf et al[16] showing the importance of HLA class II matching in CML. The difference of the disease analyzed in both studies would not be the reason, because 480 CML cases provided the same results as 1298 cases for acute GVHD in our study (data not shown). A preliminary trial comparing the combination of mismatch genotypes in HLA class I antigens between our initial study and the findings of Petersdorf et al showed considerable differences of genotypes in each HLA allele which came from ethnic backgrounds. The site of HLA-peptide ligand on each HLA allele might be important for the induction of acute GVHD reaction. International collaborative studies are warranted to solve these questions.

Second, a synergistic effect of HLA-C mismatch with other HLA locus allele mismatches for acute GVHD was observed, although single HLA-C mismatch had less ability to induce severe acute GVHD than single HLA-A/B allele mismatch. In contrast, multiple mismatch of HLA-A/B and HLA-DR/DQ allele mismatch showed no synergistic effects for acute GVHD. This evidence indicates that the mechanism of acute GVHD caused by the disparity of HLA-C might be different from that of the HLA-A or -B allele.

Natural killer (NK) cells express killer inhibitory receptors (KIRs) such as CD158a, CD158b, and CD94, and killer activity of NK cells is reported to be suppressed by the bindings of KIRs on NK cells.[23,24] As an epitope of HLA-C shared by some HLA-C types binds to CD158a or CD158b on NK cells, the cytotoxic mechanism of NK cells via KIRs might have the potential to be partly involved in the induction of GVHD in HLA-C mismatched cases. In order to prove the attribution of this mechanism in our JMDP study, more KIR mismatched pairs among HLA-C mismatched ones in either rejection vector or GVHD vector are

required. Identification of HLA-E allospecificities is in progress to elucidate the GVHD mechanism in conjunction with the inhibition of NK cells by CD94 KIR.[25]

For the first time, the significant correlation of HLA-A/B allele disparity to chronic GVHD by both univariate analysis and multivariate analysis was demonstrated. HLA-C mismatch had a tendency to increase the incidence of chronic GVHD. HLA-DR/DQ mismatch showed no effect on chronic GVHD. Even in patients with no acute GVHD, HLA-A and/or HLA-B mismatch had a correlation to chronic GVHD (data not shown). Although chronic GVHD appears to be a syndrome of immune dysfunction, resulting in immunodeficiency and autoimmunity, the mechanism of chronic GVHD is unclear.[26] Our data indicated that the effector mechanism of chronic GVHD that might be different from that of acute GVHD would be regulated by the disparity of HLA class I alleles.

We could analyze the engraftment failure and its association with HLA allele disparities. The incidence of engraftment failure in patients who received transplants of non–T-cell–depleted marrow with HLA-A, -B, -C, -DR, and -DQ allele complete-match donors remained at a very low level. Although a significant effect of any single HLA-allele mismatch could not be elucidated, HLA class I antigen (A, B and/or C) mismatch showed a higher incidence of engraftment failure than HLA complete match. HLA class II (DR and/or DQ) mismatch also tended to increase engraftment failure. Petersdorf et al[14] showed that HLA-C mismatch in UR-HSCT was a risk factor for engraftment failure by matched-pair analysis. Our analysis by the Kaplan-Meier method and log-rank test could not determine single HLA-C mismatch in either GVHD vector or rejection vector as a significant factor for engraftment failure. Expectedly, severe aplastic anemia and hereditary disease showed higher incidences of engraftment failure than acute leukemia, although not significant by the stepwise multivariate analysis due to the small number of cases examined. The reason why CML showed an independent significant factor for engraftment failure compared with ALL is not clear at present.

In this report, the association between HLA allele disparity and leukemia relapse could be analyzed more precisely using a greater increased number of cases and a longer observation period than the previous study.[7] As a result, HLA disparity, including HLA-C, did not significantly affect leukemia relapse. Subset analyses with increased cases in AML, ALL, and CML are needed to prove the GVL effect.

HLA-A/B allele mismatch greatly influenced the overall survival in patients with high-risk and standard-risk leukemia. The high incidence of severe GVHD in HLA-A and/or HLA-B mismatch worsened the nonrelapse mortality, and resulted in poor overall survival. The overall survival rate of single HLA-C mismatch did not significantly differ from that of HLA match in both standard-risk leukemia and high-risk leukemia. The favorable survival rate in single HLA-C mismatch is mainly influenced by the low incidence of nonrelapse mortality, although relatively high incidence of severe GVHD occurred in HLA-C mismatch compared with HLA match (20.0% vs 11.2% in standard risk and 21.9% vs 13.0% in high risk, respectively). Good response to the therapy for severe GVHD in this subset group might be one of the reasons. Multiple mismatch, including HLA-C, tended to worsen the survival rate, which might be due to the synergistic effect of HLA-C to induce severe acute GVHD. Therefore, HLA-C genotyping for the selection of a most suitable donor should be considered.

Finally, we emphasize that the selection of donor based on HLA allele matching is essential to improve the outcome of UR-HSCT. Patients with standard-risk leukemia, especially, should receive transplants from HLA-A and -B allele match unrelated donors, and not from multiple HLA-locus mismatch donors.

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Appendix

The following centers in Japan participated in this study: Hokkaido University Hospital, Sapporo University Hospital, Sapporo Hokuyu Hospital, Japanese Red Cross Asahikawa Hospital, Asahikawa Medical College Hospital, Hirosaki University Hospital, Tohoku University Hospital, Yamagata University Hospital, Akita University Hospital, Fukushima Medical College, National Cancer Center Central Hospital, Institute of Medical Science at the University of Tokyo, Toho University Hospital, Omori Hospital, Tokyo Metropolitan Komagome Hospital, Nihon University Hospital, Itabashi Hospital, Jikei University Hospital, Keio University Hospital, Tokyo Medical College Hospital, Tokyo Medical and Dental University Hospital, Tokyo University Hospital, Yokohama City University Hospital, Kanagawa Children's Medical Center, Kanagawa Cancer Center, Tokai University Hospital, St Marianna University Hospital, Chiba University Hospital, Chiba Children's Hospital, Matsudo Municipal Hospital, Kameda General Hospital, Saitama Children's Medical Center, Saitama Cancer Center Hospital, Saitama Medical School Hospital, Ibaraki Children's Hospital, Jichi Medical School Hospital, Dokkyo University Hospital, Fukaya Red Cross Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Niigata University Hospital, Niigata Cancer Center Hospital, Shinshu University Hospital, Saku Central Hospital, Hamamatsu University Hospital, Hamamatsu Medical Center, Shizuoka General Hospital, Shizuoka Children's Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, Nagoya University Hospital, Nagoya Ekisaikai Hospital, National Nagoya Hospital, Aichi Medical School Hospital, Nagoya City University Hospital, Showa Hospital, Anjo Kousei Hospital, Fujita Health University Hospital, Mie University Hospital, Kanazawa University Hospital, Kanazawa Medical University Hospital, Toyama Prefectural Central Hospital, Fukui Medical School Hospital, Shiga University of Medical Science, Center for Adult Disease in Osaka, Kinki University Hospital, Osaka University Hospital, Osaka Medical Center and Research Institute for Maternal and Child Health, Matsushita Memorial Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Kobe City General Hospital, Kobe University Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Yamaguchi University Hospital, Ehime Prefectural Central Hospital, Okayama National Hospital, Kurashiki Central Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Center, St Mary's Hospital, Kokura Memorial Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, Miyazaki Prefectural Hospital, Kumamoto National Hospital, Kumamoto University Hospital, Oita Medical University Hospital, and Kagoshima University Hospital.