Patients

Data of 1976 patients who underwent their first allo-SCT for Ph⁻ ALL between 1993 and 2007 were available in the registration database of JSHCT and JMDP. Excluding 662 patients whose age was 15 years or younger, 67 patients without data of GVHD prophylaxis and the interval from diagnosis to allo-SCT, 22 patients who underwent 2 or more human leukocyte antigen (HLA) loci mismatched related allo-SCT, and 86 patients who received reduced-intensity conditioning regimens, we analyzed 1139 adult Ph⁻ ALL patients (499 related and 640 unrelated). We particularly analyzed details of 641 patients transplanted in CR1, according to donor types (310 related and 331 unrelated). All but 4 patients were donated from Japanese donors harvested in Japanese harvest centers. Only bone marrow grafts were used in unrelated allo-SCT because peripheral blood stem cell donation from unrelated donors is not yet approved in Japan. HLA high-resolution molecular typing methods were performed for HLA-A, -B, -Cw, and -DRB1 for all patients in JMDP. Donor and recipient pairs were considered matched when HLA was matched at -A, -B, and -DRB1 loci in related allo-SCT and at -A, -B, -Cw, and -DRB1 loci in unrelated allo-SCT. Mismatches were defined as at least one disparity of these loci.

Definition

Neutrophil recovery was defined by an absolute neutrophil count of at least 0.5×10^9/L for 3 consecutive days; platelet recovery was defined by a count of at least 50×10^9/L without transfusion support. Acute and chronic GVHD was diagnosed and graded according to consensus criteria.[6,7] Relapse was defined as hematologic leukemia recurrence. NRM was defined as death during continuous remission. For analyses of OS, failure was death from any cause, and surviving patients were censored at the date of last contact. The date of allo-SCT was the starting time point for calculating all outcomes. Patients were classified at diagnosis by the Japan Adult leukemia Study Group (JALSG) risk stratification: low risk was defined as less than 30 years at diagnosis and white blood cell count less than 30 000/μL at diagnosis, high risk as 30 years or more at diagnosis and white blood cell count 30 000/μL or more at diagnosis, and intermediate risk as other.[8] To determine the cut-off for the upper limit of tolerability by age, we analyzed the cumulative incidence of NRM by categorizing the patients' age every 5 years. Because NRM rates of 45- to 49-year-old and 50-year-old or older categories showed higher incidences compared with other categories, we determined the best cut-off point as 45 years old.

Statistical analysis

The 2-sided χ^2 test was used for categorical variables. OS rates were estimated by the Kaplan-Meier method, and P values were calculated using a log-rank test.[9,10] Cumulative incidences of relapse, NRM, and GVHD were calculated by the Gray method.[11,12] Death without relapse was considered as a competing event for relapse, and relapse as a competing event for NRM. Univariate and multivariate analyses were performed using Cox proportional hazard regression model.[13] A significance level of P less than .05 was used for all analyses.

Results

Patient characteristics

Of 1139 patients, 641 received allo-SCT in CR1 (310 related and 331 unrelated), 199 in subsequent remission (56 related and 143 unrelated), and 299 in nonremission (133 related and 166 unrelated). The characteristics of the patients transplanted in CR1 are shown in Table 1. The frequencies of HLA mismatched donor and tacrolimus-based GVHD prophylaxis were higher, and the interval from diagnosis to allo-SCT was longer among patients who underwent an unrelated allo-SCT than among those who underwent a related allo-SCT. There was no significant difference in the age at allo-SCT, the white blood cell counts at diagnosis, JALSG risk stratification, and year of allo-SCT between related and unrelated allo-SCTs.

Survival

Median follow-up periods among survivors were 47.7 months (range, 1.3-162 months). OS rates at 4 years were 64% in CR1, 39% in subsequent CR, and 16% in non-remission ($P < .0001$). Although OS rates were significantly different among disease stages at allo-SCT, there were no significant differences in OS rates at 4 years between related and unrelated allo-SCTs in any disease stage (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in non-remission, $P = .59$; respectively; Figure 1). There was no statistical difference in OS rates and NRM rates over transplantation years (data not shown). Among 641 patients transplanted in CR1, JALSG risk stratification did not have a significant impact on the OS after allo-SCT (68% in low risk, 62% in intermediate risk, and 58% in high risk, at 4 years, respectively; $P = .31$). To address our main issue, we performed the following analyses among patients transplanted in CR1 according to donor types.

Among 310 patients who underwent a related allo-SCT in CR1, multivariate analysis showed that age at allo-SCT and less than 6 months from diagnosis to allo-SCT were significant risk factors for OS. Among 331 patients who underwent an unrelated allo-SCT in CR1, multivariate analysis showed that abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were significant risk factors for OS (Table 2).

Relapse and NRM among patients transplanted in CR1

The cumulative incidence of relapse was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$; Figure 2A). Multivariate analyses according to donor type showed that less than 6 months from diagnosis to allo-SCT alone was associated with relapse among 310 patients who underwent a related allo-SCT in CR1, whereas only abnormal karyotype [except for t(4;11) and t(1;19)] was associated with relapse among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 3).

The cumulative incidence of NRM was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$; Figure 2B). Multivariate analyses according to donor type showed that age only 45 years or older at allo-SCT was associated with NRM among 310 patients who underwent a related allo-SCT in CR1, whereas abnormal karyotype [except for t(4;11) and t(1;19)], HLA mismatch, and 10 months or longer from diagnosis to allo-SCT were associated with NRM among 331 patients who underwent an unrelated allo-SCT in CR1 (Table 4).

Acute and chronic GVHD among patients transplanted in CR1

The cumulative incidence of grade II-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 30% vs 42% at day 100; $P = .0003$). The cumulative incidence of grade III-IV acute GVHD was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 7% vs 16% at day 100; $P = .0006$).

Table 1. Characteristics of patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
No. of patients	**No.**	**%**	**No.**	**%**	***P***
Median WBC count at diagnosis/μL (range)	10 250 (109-328 000)		11 000 (700-892 000)		.43
Median patient age at allo-SCT, y (range)	30 (16-66)		31 (16-59)		.95
16-20	66	21.3	77	23.3	
21-30	93	30.0	82	24.8	
31-40	71	22.9	86	26.0	
41-50	58	18.7	68	20.5	
51 or older	22	7.1	18	5.4	
Sex					.09
Male	157	50.6	190	57.4	
Female	153	49.4	141	42.6	
Source					< .0001
BM	212	68.4	331	100.0	
PB	98	31.6	0	0.0	
Lineage					.01
T	50	16.1	54	16.3	
B	218	70.3	203	61.3	
Other	42	13.5	74	22.4	
Cytogenetics					.07
Normal	193	62.3	208	62.8	
t(4;11)	11	3.5	5	1.5	
Other MLL/11q23 translocations	1	0.3	3	0.9	
t(1;19)	10	3.2	6	1.8	
t(8;14)	3	1.0	3	0.9	
14q32 translocations	1	0.3	0	0.0	
del(6q)	3	1.0	1	0.3	
del(7p)	2	0.6	1	0.3	
−7*	5	1.6	2	0.6	
+8*	2	0.6	0	0.0	
+X*	0	0.0	1	0.3	
del(9p)	3	1.0	9	2.7	
abnormality of 11q	0	0.0	3	0.9	
del(12p)	2	0.6	1	0.3	
del(13q)/−13	1	0.3	2	0.6	
del(17p)	0	0.0	1	0.3	
Complex	10	3.2	15	4.5	
Low hypodiploidy/near triploidy	2	0.6	0	0.0	
High hyperdiploidy	16	5.2	12	3.6	
Other abnormality (no t(9;22))†	45	14.5	58	17.5	
JALSG risk stratification					.21
Low	39	12.6	45	13.6	
Intermediate	163	52.6	192	58.0	
High	108	34.8	94	28.4	
HLA matching					< .0001
Match	285	91.9	192	58.0	
Class I 1 locus-mismatch	18	5.8	53	16.0	
Class II 1 locus-mismatch	7	2.3	32	9.7	
2 or more loci mismatch	0	0.0	54	16.3	
Time from diagnosis to transplantation, mo (range)	5.7 (1.9-36.6)		10.0 (4.0-43.0)		< .0001
< 6	169	54.5	23	6.9	
6-9	109	35.2	143	43.2	
10 or longer	32	10.3	165	49.8	
Preparative regimen					.004
CY + TBI	140	45.2	156	47.1	
CA + CY + TBI	66	21.3	84	25.4	
BU + CY + TBI	17	5.5	15	4.5	
VP + CY + TBI	23	7.4	32	9.7	
Other TBI myeloablative regimens	39	12.6	32	9.7	
BU + CY	17	5.5	12	3.6	
Other non-TBI myeloablative regimens	8	2.6	0	0.0	
GVHD prophylaxis					< .0001
Cyclosporine A with or without other	283	91.3	171	51.7	
Tacrolimus with or without other	27	8.7	160	48.3	

Table 1. Characteristics of patients transplanted in CR1, according to donor type (continued)

No. of patients	Related (n = 310) No.	%	Unrelated (n = 331) No.	%	P
Years of allo-SCT					.26
1993-1997	48	15.5	55	16.6	
1998-2002	132	42.6	120	36.3	
2003-2007	130	41.9	156	47.1	

WBC indicates white blood cell; BM, bone marrow; PB, peripheral blood; related HLA match, identical HLA-A, -B, and -DRB1 loci; unrelated HLA match, HLA-A, -B, -Cw, and -DRB1 loci; HLA mismatch, at least one disparity at one of these loci; CY, cyclophosphamide; TBI, total body irradiation; CA, cytarabine; BU, busulfan; and VP, etoposide.
*These groups exclude cases with low hypodiploidy and high hyperdiploidy.
†Abnormal karyotypes excluding those with any of the aforementioned abnormalities.

Among evaluable patients who survived at least 100 days after allo-SCT, no significant difference was observed between related and unrelated allo-SCTs in the incidence of chronic GVHD (related vs unrelated: 41% vs 41% at 2 years; $P = .76$). Extensive disease was observed in 60 (55%) of 109 with chronic GVHD after related allo-SCT and in 80 (74%) of 118 after unrelated allo-SCT ($P = .048$).

Causes of death among patients transplanted in CR1

Although relapse was the leading cause of death in both related and unrelated allo-SCTs, the proportion of relapse was significantly lower in those transplanted from unrelated donors ($P = .01$). Infection, GVHD, and organ failure were the major causes of NRM, and the incidence of interstitial pneumonia was higher in patients transplanted from unrelated donors ($P = .06$; Table 5).

Discussion

This study reports the largest series of adult Ph⁻ ALL patients who underwent allo-SCT. There was no significant survival difference between related and unrelated allo-SCTs in any disease stage. Among patients who underwent a related allo-SCT in CR1, a shorter interval from diagnosis to allo-SCT was associated with relapse, and age at allo-SCT was associated with NRM. On the other hand, among patients who underwent an unrelated allo-SCT, abnormal karyotype was associated with both relapse and NRM, and a longer interval from diagnosis to allo-SCT and HLA mismatch were associated with NRM. These results indicated that factors affecting transplantation outcomes were different according to donor type.

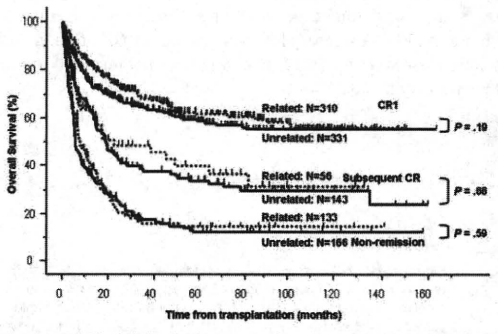

Figure 1. OS according to disease status and donor type. OS rates were significantly superior among patients transplanted in CR1. There was no significant difference between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in nonremission, $P = .59$; respectively).

In our study, unrelated allo-SCT resulted in OS rates similar to those from related allo-SCT, which was compatible with the result of one prospective study for standard-risk hematologic malignancies.[14] The rates of OS, relapse, and NRM among patients who underwent a related allo-SCT in CR1 were 65%, 32%, and 14%, respectively, which were compatible with those observed in the United Kingdom Medical Research Council UKALL XII/Eastern Cooperative Oncology Group E2993 trial (53%, 24%, and 18%, respectively).[3] Some patients were transplanted from a 1-locus mismatched related donor because it was reported that the outcome of allo-SCT from a 1 locus-mismatched related donor was similar to that of matched unrelated allo-SCT in the Japanese population.[15] On the other hand, the rates of OS, relapse, and NRM among patients who underwent an unrelated allo-SCT were 62%, 22%, and 27%, respectively, which were better than those reported from the Center for International Blood and Marrow Transplant Research (39%, 20%, and 42%, respectively).[4] These differences in NRM could be explained by the lower incidence of acute GVHD in our population, which possibly resulted from the genetic homogeneity in the Japanese population.[16,17] Interestingly, abnormal karyotype was associated with NRM. This could be explained by the possibility that patients with abnormal karyotype received intensive chemotherapy before allo-SCT because of persistent minimal residual disease, which might result in increased NRM rates. Another possibility is that rapid taper of immunosuppressive treatment might also cause GVHD leading to NRM.

In this study, NRM rates were higher in unrelated allo-SCT compared with related allo-SCT, whereas comparable NRM rates were reported in some recent reports,[18] suggesting that NRM rates after unrelated allo-SCT could be reduced with further efforts, such as better HLA matching. Because HLA-C was not routinely typed before 2003, most of the HLA-C data in this study were examined retrospectively, revealing that considerable numbers of patients had received class I allele-mismatched unrelated allo-SCT. Better HLA matching might reduce NRM after unrelated allo-SCT in the future. Although slower hematopoietic recovery after bone marrow transplantation compared with peripheral blood stem cell transplantation might affect the timing of deaths, there was no statistical difference in early mortality between the grafts (data not shown).

There was no statistical difference in the incidence of chronic GVHD between related and unrelated allo-SCTs, although acute GVHD was observed more frequently in unrelated allo-SCT. This was compatible with a past report in which the incidence of chronic GVHD was similar between related and unrelated allo-SCTs, whereas acute GVHD was observed more frequently in related allo-SCT.[14]

It was noteworthy that the interval from diagnosis to allo-SCT revealed a different effect on related and unrelated allo-SCTs. In Japanese clinical practice, the JALSG protocols have been common, where 1.5-month induction chemotherapy was followed by

Table 2. Univariate and multivariate analyses of factors influencing OS among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.19 (0.78-1.82)	.42	—		101	0.83 (0.56-1.25)	.38	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.73 (0.34-1.77)	.52	—		54	0.81 (0.44-1.48)	.35	—	
Other	42	0.94 (0.54-1.64)	.84	—		74	1.08 (0.70-1.67)	.72	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.51 (0.14-1.54)	.19	—		11	1.49 (0.54-4.09)	.44	1.59 (0.58-4.36)	.37
Other (not(9;22))	96	1.03 (0.67-7.14)	.89	—		112	1.49 (1.09-2.17)	.04	1.43 (1.13-2.40)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.36 (0.87-2.12)	.18	—		192	1.06 (0.71-1.59)	.77	—	
High	108	1.77 (0.95-3.31)	.07	—		94	1.02 (0.56-1.88)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	2.04 (1.30-3.13)	.002	2.13 (1.36-3.34)	.0009	50	1.05 (0.63-1.73)	.86		
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.95 (0.46-1.96)	.90	—		139	1.44 (1.01-2.06)	.04	1.45 (1.01-2.07)	.04
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.43 (0.94-2.13)	.09	1.40 (0.93-2.11)	.11					
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.75 (1.16-2.63)	.007	1.80 (1.19-2.71)	.005	308	0.33 (0.10-1.04)	.06	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.56 (0.26-1.20)	.14	—		165	1.54 (1.07-2.21)	.02	1.62 (1.12-2.34)	.01
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.72 (0.38-1.35)	.30	—		319	0.59 (0.27-1.26)	.17	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	2.02 (1.15-3.56)	.01	—		160	1.38 (0.96-1.97)	.08	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

6-month consolidation chemotherapy and 16-month maintenance chemotherapy.[8] Therefore, a shorter interval from diagnosis to allo-SCT, which was more common in related cases, might result in insufficient consolidation chemotherapy and worse survival because of increased relapse rates in related allo-SCT. Alternatively, effects from insufficient consolidation chemotherapy might be more prominent in related allo-SCT because graft-versus-leukemia effects might be weaker after related allo-SCT than unrelated allo-SCT.[19] On the other hand, a longer interval from diagnosis to allo-SCT, which was more common in unrelated cases, might result in the cumulative toxic sequelae of chemotherapy responsible for interstitial pneumonia indicated in the past reports.[20-25] Because the JALSG protocols do not define the timing of allo-SCT, it is possible that chemotherapy before allo-SCT might be prolonged because of persistent minimal residual disease. However, we could not confirm this because there were no data concerning minimal residual disease in the registry database.

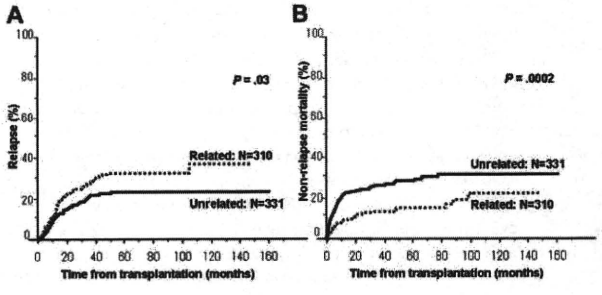

Figure 2. Cumulative incidence of relapse and NRM in patients transplanted in CR1 according to donor type. (A) Cumulative incidence of relapse among patients transplanted in CR1 was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$). (B) Cumulative incidence of NRM among patients transplanted in CR1 was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$).

Table 3. Univariate and multivariate analyses of factors influencing relapse among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	0.88 (0.52-1.47)	.62	—		101	1.11 (0.62-1.98)	.72	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.54 (0.22-1.37)	.09	—		54	1.31 (0.57-3.02)	.62	—	
Other	42	1.21 (0.66-2.21)	.54	—		74	1.06 (0.53-2.11)	.87	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.64 (0.19-2.12)	.36	—		11	1.97 (0.46-8.35)	.91	—	
Other (no t(9;22))	96	1.11 (0.68-1.82)	.67	—		112	2.15 (1.24-3.73)	.01	2.15 (1.24-3.73)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	0.96 (0.59-1.55)	.87	—		192	1.04 (0.57-1.91)	.90	—	
High	108	0.81 (0.35-1.84)	.61	—		94	1.04 (0.43-2.52)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	0.82 (0.41-1.64)	.57	—		50	0.74 (0.42-1.32)	.08	—	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.82 (0.33-2.02)	.66	—		139	0.74 (0.42-1.32)	.31	—	
Stem cell source*										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.07 (0.65-1.76)	.79	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.68 (1.05-2.69)	.03	1.68 (1.05-2.69)	.03	308	0.47 (0.11-1.92)	.29	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.49 (0.18-1.34)	.16	—		165	0.92 (0.54-1.58)	.76	—	
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.62 (0.31-1.25)	.18	—		319	0.47 (0.15-1.52)	.21	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.62 (0.81-3.26)	.18	—		160	1.39 (0.81-2.38)	.24	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.
*Stem cell source (peripheral blood) was not a significant risk factor for relapse in the multivariate analysis.

Although we mainly focused on patients in CR1, our results also indicated that some, but not all, patients with refractory disease could be rescued by allo-SCT. These patients could not have survived long with chemotherapy alone, and complete unresponsiveness, even to allo-SCT, was often assumed. These results were compatible with some reports showing that long-term survival could be achieved for patients receiving allo-SCT, even in refractory disease.[26-28]

Our study has several limitations. First, there might be some selection biases between related and unrelated allo-SCTs. It was possible that eligibility was more stringent in patients who received unrelated allo-SCT, and they might have had better pretransplantation conditions. Second, a time-censoring effect might impact the outcome. The longer interval from diagnosis to unrelated allo-SCT eliminates the effect of patients who die during that period. This bias might improve the outcome of unrelated allo-SCT. Third, we could not make the comparison between chemotherapy and allo-SCT in this study.

The time-censoring effect could be the major bias in this study, which resulted in lower relapse rates, especially in patients transplanted from unrelated donors. We tried to correct this bias by the previously described method.[29] In the JALSG ALL study, it was reported that approximately 80% and 75% of patients were alive 6 months and 10 months after enrollment, respectively.[8] Because 6 months and 10 months were the median interval from diagnosis to related and unrelated allo-SCTs, respectively, a crude way to apply a correction factor for the survival seen in our study is to lower the survival estimate at any given time point by 20% for related allo-SCT and 25% for unrelated allo-SCT, respectively. Thus, the corrected OS rates at 4 years were 52% ± 5% for related allo-SCT and 47% ± 4% for unrelated allo-SCT, which showed no statistical difference between related and unrelated allo-SCTs. Time-censoring effects would not change the results.

The change of transplantation indication for adolescents through the observation period might affect the outcome. In the JALSG protocol ALL202 (from September 2002), we treated patients less than 25 years old with a similar protocol performed for pediatric patients. Because allo-SCT was recommended only for high-risk patients, such as those with t(4;11) or MLL-rearrangement in the pediatric protocol, the outcome of young patients might be affected by the difference in the indication for allo-SCT between pediatric and adult protocols after 2002. However, the effect of this small population would not be so large.

Table 4. Univariate and multivariate analyses of factors influencing NRM among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	n	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.21 (0.63-2.34)	.57	—		101	0.79 (0.48-1.30)	.35	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	1.25 (0.41-3.81)	.53	—		54	0.62 (0.29-1.38)	.17	—	
Other	42	0.87 (0.34-2.26)	.78	—		74	1.08 (0.65-1.81)	.76	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.77 (0.16-3.17)	.73	—		11	1.03 (0.25-4.30)	.63	1.11 (0.27-4.64)	.57
Other (no t(9;22))	96	0.92 (0.47-1.81)	.81	—		112	1.47 (0.94-2.29)	.09	1.67 (1.06-2.64)	.03
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.85 (0.86-3.97)	.12	—		192	1.01 (0.62-1.65)	.96	—	
High	108	2.82 (1.09-7.31)	.03	—		94	1.03 (0.50-2.10)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	3.90 (2.09-7.25)	< .0001	3.90 (2.09-7.25)	< .0001	50	1.26 (0.72-2.20)		.42	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	1.64 (0.64-4.18)	.30	—		139	1.69 (1.10-2.60)	.02	1.69 (1.10-2.61)	.02
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.75 (0.94-3.28)	.08	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.64 (0.87-3.11)	.13	—		308	0.31 (0.08-1.25)	.10	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	1.07 (0.42-2.72)	.89	—		165	1.90 (1.21-2.99)	.01	1.98 (1.26-3.13)	.003
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.63 (0.25-1.61)	.34	—		319	0.67 (0.25-1.85)	.44	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.66 (0.65-3.80)	.29	—		160	1.33 (0.86-2.05)	.52	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

In conclusion, comparable survival rates were observed between adult Ph$^-$ ALL patients who underwent related and unrelated allo-SCTs in CR1, although relapse rates, incidences of NRM, and risk factors for transplantation outcomes were different between them. Better outcomes could be achieved by performing allo-SCT at an appropriate timing and HLA compatibility according to donor type.

Table 5. Causes of death among patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
	n	%	n	%	P
Relapse	44	44	32	26	.01
Infection	12	12	23	19	.20
Organ failure	12	12	17	14	.83
GVHD	9	8.9	16	13	.40
Interstitial pneumonia	5	5.0	15	12	.06
Hemorrhage	3	3.0	6	5.0	.52
Graft failure	2	2.0	3	2.5	1.0
ARDS	1	1.0	3	2.5	.63
Other	8	7.9	6	5.0	.42
Unknown	5	5.0	0	0.0	.02
Total	101	100	121	100	

ARDS indicates acute respiratory distress syndrome.

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Authorship

Contribution: S.N., Y.I., and K.M. designed the research and wrote the manuscript; S.N. and Y.I. performed the statistical analysis and interpreted the data; H.S., M. Kurokawa, H.I., H.O., T.F., Y.O., N.K., M. Kasai., T.M., K.I., T.Y., M.O., and

K.M. provided the patient data; and K.K., Y.M., R.S., and Y.A. collected the patient data.

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ORIGINAL ARTICLE

Reduced-intensity unrelated donor bone marrow transplantation for hematologic malignancies

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Abstract To review a current experience of unrelated bone marrow transplantation (BMT) with reduced-intensity conditioning (RIC) regimens, we conducted a nationwide survey with 77 patients (age, 25–68 years). The backbone RIC regimen was a combination of fludarabine or cladribine, busulfan or melphalan and total body irradiation at 2–4 Gy. Five patients died early, but 71 (92%) achieved initial neutrophil recovery. Thereafter, 36 patients (47%) died of therapy-related complications, 23 (30%) of whom died within day 100. Grades II–IV acute graft-versus-host disease (GVHD) occurred in 34 of the 68 evaluable patients (50%). In a multivariate analysis, a regimen containing antithymocyte globulin (ATG) was significantly associated with a decreased risk of acute GVHD ($P = 0.041$). Thirty-three patients are currently alive with a median follow-up of 439 days (28–2002 days), with an OS of 50% at 1 year. In conclusion, unrelated BMT with RIC regimens can be a curative treatment in a subset of patients.

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Keywords Unrelated transplantation · Reduced-intensity conditioning · Hematologic malignancy

1 Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a possible curative approach for patients with various hematologic malignancies. Recently, the application of reduced-intensity conditioning (RIC) regimens, mostly incorporating fludarabine as a backbone agent, has been explored for patients whose age or concomitant medical conditions contraindicate the use of conventional myeloablative regimens [1–3]. Since only 30–40% of patients have an appropriate family donor available [4], the establishment of an unrelated donor transplantation program with RIC regimens is urgently needed.

Graft rejection, regimen-related toxicities and graft-versus-host disease (GVHD) have been the major problems in unrelated HSCT with RIC [5–13]. In unrelated transplantation, engraftment is influenced by the source of stem cells and superior results have been observed with peripheral blood stem cells (PBSC) compared to bone marrow [9, 14]. Nevertheless, PBSC has not yet been approved as a graft source for unrelated transplantation in Japan [15]. The level of regimen-related toxicities directly depends on the intensity of the regimen, and the incidence of GVHD increases with unrelated donors compared to related donors. Although attempts have been made to overcome these problems, a suitable procedure for unrelated bone marrow transplantation (BMT) with RIC regimens has not yet been established. To accumulate further expertise, we conducted a nationwide survey of Japanese patients with hematologic malignancy who had undergone BMT from an HLA-matched or -mismatched unrelated donor with RIC regimens. Although the present data were obtained from a limited population of patients, these findings may show a current status of unrelated BMT with RIC.

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2 Patients and methods

2.1 Data sources

This survey collected the data of 77 consecutive patients in 17 participating hospitals who received unrelated BMT with RIC for hematologic malignancies between 2000 and 2004. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of GVHD. The minimum data required for inclusion of a patient in this study were age, sex, histological diagnosis, status at transplant, donor information, conditioning regimen, date of transplant, donor chimerism status, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression (PD)/death and cause of death.

This study was approved by institutional review board of each individual center. All patients provided written informed consent according to the Declaration of Helsinki. Unrelated donors provided consent through the Japan Marrow Donor Program as part of its standard procedures. The indications, conditioning regimens, management of GVHD and supportive care for BMT were left to the discretion of each institution. Patients who had previously received allogeneic HSCT and those younger than 20 years were not included. Patients younger than 50 years who had organ dysfunction and/or have previously received high-dose chemotherapy with autologous HSCT were also included.

2.2 Definitions

RIC regimens were defined as previously reported [6, 9, 10], and conditioning regimens that included either beyond 4 Gy of total body irradiation (TBI), 8 mg/kg of busulfan or 140 mg/m^2 of melphalan were excluded from the study. Alleles at the HLA-A, -B, and -DRB1 loci were identified by middle-resolution DNA typing as described previously [16]. Risk status at transplantation was categorized as either standard risk or high risk. Standard-risk diseases included acute leukemia in first complete remission, chronic myeloid leukemia in first chronic phase, and refractory anemia of myelodysplastic syndrome (MDS). Other diseases were categorized as high-risk disease. Graft failure was analyzed in patients who survived more than 28 days posttransplant according to the criteria reported by Petersdorf et al. [17]. Briefly, the definition included failure of the absolute neutrophil count (ANC) to surpass 500/mm^3 before relapse, death or second transplantation, as well as a decrease in the ANC to less than 100/mm^3 on at least three consecutive determinations with a finding of severe hypoplastic marrow. The degree of donor chimerism among peripheral blood T cells was assessed several times

between day 28 and day 100 after HSCT using fluorescence in situ hybridization (FISH) to detect X and Y chromosomes for recipients of grafts from sex-mismatched donors, and polymerase chain reaction-based analyses of polymorphic microsatellite regions for recipients of sex-matched or sex-mismatched transplants. Mixed chimerism was defined as the detection of 5–90% of donor cells in the peripheral blood. Acute and chronic GVHD were graded according to the consensus criteria [18, 19]. Patients who survived 100 days were evaluable for the assessment of chronic GVHD. Overall survival (OS) was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until PD/relapse or death from any cause. Patients who died from transplantation-related causes were classified as non-relapse mortality (NRM) regardless of their disease status.

2.3 Statistical analysis

The primary endpoint of this study was OS and chimerism. The secondary endpoints were PFS, NRM, PD, and the incidence of acute and chronic GVHD. Descriptive statistical analysis was performed to assess patient baseline information. Patients were divided into two groups: age 60 or above and less than 60. OS and PFS were calculated using the Kaplan–Meier method. The cumulative incidence of acute GVHD was calculated using the method described by Gooley et al. [20] to eliminate the effect of competing risks. The competing event for acute GVHD was defined as death without grades II–IV acute GVHD. For each endpoint, a Cox proportional hazard model was used for univariate and multivariate analyses. The factors included in the analysis were HLA disparity (mismatch vs. identical), recipient age (age 60 or above vs. less than 60), use of TBI (yes vs. no), use of ATG (yes vs. no), diagnosis of AML (yes vs. no), risk status (high vs. standard) and acute GVHD (II–IV vs. 0–I). Acute GVHD in the model was treated as a time-varying covariate. We defined statistical significance as a P value less than 0.05. All statistical analyses were performed using STATA version 8 (College Station, TX).

3 Results

3.1 Patients and diagnoses

The patients' characteristics are listed in Table 1. The median age of the patients was 54 years (range, 25–68 years) as a whole. Twenty-one patients (27%) had acute myelogenous leukemia (AML), 2 (3%) had acute lymphoblastic leukemia, 5 (7%) had chronic myeloid leukemia, 20 (26%) had MDS or myeloproliferative disease (refractory anemia, $n = 8$; refractory anemia with excess blasts, $n = 9$; others, $n = 3$), 19 (25%) had non-Hodgkin lymphoma (follicular lymphoma, $n = 12$; diffuse large B-cell lymphoma, $n = 4$; mantle cell lymphoma, $n = 2$; peripheral T-cell lymphoma, unspecified, $n = 1$), 7 (9%) had adult T-cell leukemia/lymphoma, and 3 (4%) had multiple myeloma. Sixty-three patients (82%) had high-risk disease at the time of allogeneic BMT.

3.2 Conditioning regimens

Conditioning regimens are shown in Table 2. None received ex vivo T-cell depleted transplantation.

3.3 HSCT procedure and supportive care

Forty-seven patients (61%) were transplanted from a matched, 24 (31%) were from a 1 allele-mismatched, and 6 (8%) were from a 2 or 3 allele-mismatched unrelated donor. All patients received bone marrow as a source of stem cells. The prophylaxis of GVHD was either cyclosporine- or tacrolimus-based. Thirty-nine patients (51%) received cyclosporine with methotrexate, including five patients who received an ATG-containing preparative regimen. Nine patients (12%) received cyclosporine alone, including five patients who received ATG. Each patient received cyclosporine with mycophenolate mofetil and cyclosporine with prednisolone, respectively. Twenty-five patients (33%) received tacrolimus with methotrexate, including one patient who received ATG. Two patients (3%) received tacrolimus alone, including one who received ATG. Granulocyte colony-stimulating factor was administered intravenously from day +1 or +6 until neutrophil engraftment in all patients.

3.4 Engraftment and chimerism

Five patients died before the engraftment evaluation, with a median survival time of 15 days (range, 2–17 days). Seventy-one patients (92%) achieved initial neutrophil recovery, but three patients (two AMLs and one MDS) later experienced secondary graft failure; one each with AML and MDS after unrelated BMT from an HLA-1 allele-mismatched donor received a second transplantation when they failed to achieve subsequent complete donor-type chimerism, but both died of infectious complications. The other patient with AML after unrelated BMT from an HLA-6 allele-matched donor achieved initial complete chimerism, but later developed secondary graft failure upon the administration of ganciclovir for cytomegalovirus antigenemia. However, this patient achieved the spontaneous recovery of autologous marrow function and is currently surviving beyond 2,000 days.

Table 1 Patient characteristics

Variable	Younger than 60 years ($n = 60$)	60 years or older ($n = 17$)
Patient age (range, median)	25–59, 52	60–68, 63
Disease		
Acute myelogenous leukemia	16 (27%)	5 (29%)
Acute lymphoblastic leukemia	2 (3%)	0
Chronic myeloid leukemia	5 (8%)	0
Myelodysplastic syndrome or myeloproliferative disease	12 (20%)	8 (47%)
Malignant lymphoma	16 (27%)	3 (18%)
Adult T-cell leukemia/lymphoma	7 (12%)	0
Multiple myeloma	2 (3%)	1 (6%)
Risk status		
Standard	13 (22%)	1 (6%)
High	47 (78%)	16 (94%)
HLA disparity		
Matched	37 (62%)	10 (59%)
One-mismatched	19 (32%)	5 (29%)
Two or more mismatched	4 (7%)	2 (12%)
Donor–recipient sex match		
Male–male	20 (33%)	11 (65%)
Male–female	16 (27%)	2 (12%)
Female–male	9 (15%)	4 (24%)
Female–female	15 (25%)	0
GVHD prophylaxis		
Cyclosporine ± methotrexate	38 (63%)	10 (59%)
Tacrolimus ± methotrexate	21 (35%)	6 (35%)
Others	1 (2%)	1 (6%)
Median nucleated cell dose infused ($\times 10^8$/kg, range)	2.80 (0.39–5.52)[a]	2.92 (0.76–4.30)

HLA Human leukocyte antigen, *GVHD* graft-versus-host disease

[a] The data of two patients were excluded because infused nucleated cell dose was unknown

Chimerism was evaluated in 68 patients (88%), with short tandem repeats analysis ($n = 52$), variable number of tandem repeats analysis ($n = 5$) and FISH analysis in the case of sex mismatch ($n = 11$). Complete donor chimerism was confirmed in 58 (85%) within day 100. Mixed chimerism was confirmed in nine patients (13%), but two later reverted to recipient type. One patient failed to achieve donor-type chimerism due to disease relapse on day 20. The incidence of complete donor chimerism was similar in those younger and older than 60 years (85 and 86%), with a similar incidence of mixed chimerism (15 and 14%). No patients received donor lymphocyte infusion.

3.5 GVHD

Acute GVHD occurred in 41 of the 68 evaluable patients (60%), grades II–IV in 34 (50%) and grades III–IV in 14 patients (21%). Chronic GVHD occurred in 26 of the 42 evaluable patients (62%), with extensive type in 23 (55%). The incidence of grades II–IV acute GVHD was the same in patients younger and older than 60 years (50%). The incidence of grades III–IV acute GVHD (22 and 14%) and extensive chronic GVHD (56 and 50%) was similar. In unrelated BMT, from HLA-6 allele-matched ($n = 40$), HLA-1 allele-mismatched ($n = 23$), and HLA-2 or 3 allele-mismatched ($n = 5$) donors, grades II–IV acute GVHD occurred, respectively, in 18 (45%), 10 (43%) and 3 patients (60%), and chronic GVHD occurred in 15 (38%), 9 (39%) and 2 patients (40%). In univariate and multivariate analyses, an ATG-containing regimen was significantly associated with a decreased risk of the onset of grades II–IV acute GVHD (data not shown).

3.6 Survival

Thirty-three patients are currently alive with a median follow-up of 439 days (28–2,002 days), with an OS of 50% at 1 year and 46% at 2 years. The OS of patients younger than 60 years was 49% at 2 years (95% confidence interval [CI], 34–62%), and this could not be defined in older patients (95% CI, 15–45%). Patients younger than 60 years

Table 2 Conditioning regimens

Conditioning regimens	Younger than 60 years ($n = 60$)	60 years or older ($n = 17$)
TBI-containing		
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, TBI 4 Gy	30 (50%)	6 (35%)
Fludarabine 125–180 mg/m^2, melphalan 80–140 mg/m^2, TBI 4 Gy	5 (8%)	3 (18%)
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, TBI 2 Gy	2 (3%)	0 (0%)
Fludarabine 180 mg/m^2, TBI 4 Gy	0 (0%)	1 (6%)
ATG-containing		
Fludarabine 180 mg/m^2 (or cladribine 0.66 mg/kg), oral busulfan 8 mg/kg, ATG	5 (8%)	4 (24%)
Fludarabine 180 mg/m^2, cyclophosphamide 60 mg/kg, ATG	1 (2%)	0 (0%)
Fludarabine 180 mg/m^2, ATG	1 (2%)	0 (0%)
TBI and ATG-containing		
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, TBI 4 Gy, ATG	1 (2%)	1 (6%)
Non-TBI and non-ATG		
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg	6 (10%)	2 (12%)
Fludarabine 125–180 mg/m^2, melphalan 140 mg/m^2	5 (8%)	0 (0%)
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, cyclophosphamide 60 mg/kg	2 (3%)	0 (0%)
Fludarabine 180 mg/m^2, oral busulfan 8 mg/kg, thiotepa 10 mg/kg	1 (2%)	0 (0%)
Fludarabine 180 mg/m^2, cyclophosphamide 60 mg/kg	1 (2%)	0 (0%)

TBI Total body irradiation, *ATG* antithymocyte globulin (ATG-Fresenius 10 mg/kg or thymoglobulin 5 mg/kg)

tended to show better survival than older patients ($P = 0.124$). The HLA disparity (match vs. mismatch), TBI vs. non-TBI, ATG vs. non-ATG-containing regimen, and disease category (AML vs. MDS or myeloproliferative disease vs. lymphoid malignancies) was not significantly associated with OS (data not shown). Patients with standard risk tended to show better survival than those with high risk ($P = 0.129$). In univariate and multivariate analyses, no variables were significantly associated with OS (data not shown).

3.7 NRM and PD

Thirty-six patients (47%) died of therapy-related complications, with a cumulative incidence of NRM at 1 year of 43% (95% CI, 31–56%). Of the patients who died of therapy-related complications, 23 (30%) died within day 100 of transplantation and 13 (17%) died thereafter. The NRM at 1 year in patients younger and older than 60 years was 38% (95% CI, 25–53%) and 61% (95% CI, 36–85%), respectively, as shown in Fig. 1. The causes of NRM were infection (23%), regimen-related toxicity (14%) and GVHD (9%). GVHD-related mortality was found in 26%. Infection was the major cause of death in patients younger than 60 years. Regimen-related toxicity, mainly pulmonary complications, was the major cause of treatment failure for patients older than 60 years. In univariate and multivariate analyses, no variables were significantly associated with

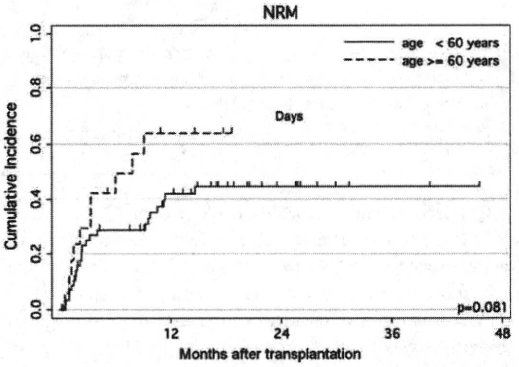

Fig. 1 Non-relapse mortality stratified according to patient age, younger or older than 60 years

NRM (data not shown). Relapse or progression of primary disease after unrelated BMT with RIC regimens was observed in 13 patients (17%; 10 patients younger than 60 years and 3 older than 60 years). There were no relapsed patients after transplantation in standard risk group. The incidence of death due to relapse or progression of primary disease was 14%. In univariate and multivariate analyses, no variables were significantly associated with PD although patients with grades II–IV acute GVHD showed a relatively lower incidence of PD (data not shown).

4 Discussion

This report reviews the current experience of unrelated BMT with RIC regimens in Japan, with particular focus on the risk factors for engraftment, GVHD, NRM, survival and PD. Although the engraftment rate has been reported to be lower when RIC unrelated transplantation was performed with bone marrow compared to peripheral blood cells [9, 10], we observed that sustained engraftment was achieved in 99% of evaluable patients, with complete donor chimerism confirmed in 85%. The incidence of graft failure was not different from that in RIC transplantation from related donors in Japan; 3.7% in recipients with an HLA-matched donor and 5.7% in those with a 1-locus-mismatched donor [21]. Complete donor chimerism in our study was comparable with that reported from the National Marrow Donor Program (85 vs. 84%) [22]. In our study, two-thirds of patients successfully received 2–4 Gy TBI-containing regimens, which were aimed at the enhancement of engraftment, as suggested in a previous report with patients with aplastic anemia [23], while 2 of the 12 patients who received an ATG-containing regimen had late graft failure, similar to a previous report which noted an incidence of 19% [5]. It has been reported that the Japanese population is more homogenous than others in terms of the distribution of HLA. Thus, it would be possible that the impact of minor HLA disparities on engraftment may become prominent after RIC transplantation.

Despite the observed satisfactory engraftment rate, we confirmed a high NRM rate (47%) after unrelated BMT with variable RIC regimens, due mostly to GVHD-related complications, including infections under steroid therapy, as previously designated by Wong et al. [10]. On the other hand, the incidence of death due to relapse or progression of primary disease was low (14%). Hence, successful prophylaxis and treatment of GVHD is particularly important in this procedure, and studies with ATG [5, 24] or alemtuzumab [25–27] have reported encouraging results. Although the number of patients was still small, in our study an ATG-containing regimen resulted in a decreased incidence of acute and chronic GVHD, despite the use of a lower dose (ATG-Fresenius 10 mg/kg or Thymoglobulin 5 mg/kg) than reported elsewhere. This study showed that age older than 60 years tended to be associated with a higher risk of NRM after unrelated HSCT with RIC regimens, though this relation was not statistically significant in a multivariate analysis. This finding, however, is limited by the small sample size. Additional use of ATG may reduce the incidence of GVHD-related NRM even in older patients but ATG should be carefully incorporated since about 20% of patients who received an ATG-containing regimen developed late graft failure in our study.

This study suggested that the onset of grades II–IV acute GVHD was associated with a lower incidence of PD, although this was not statistically significant in a multivariate analysis, possibly due to the small sample size. However, GVHD in turn resulted in a higher incidence of NRM, and a desirable graft-versus-leukemia or lymphoma effect would be offset, particularly in older patients [10, 28]. Hence, our observation echoes the warning that the intentional induction of GVHD should be avoided.

Compared to the long-term follow-up data after unrelated HSCT with RIC from the NMDP reported by Giralt et al. [22], our NRM at 1 year was worse (43 vs. 30%), but OS was likely to be better (50% at 1 year and 46% at 2 years vs. 44% at 1 year, 28% at 3 years and 23% at 5 years). In their report, disease stage, performance status, stem cell source, HLA matching, and timing of transplant were the most important prognostic factors for survival after RIC unrelated donor transplantation. This study suggested that high risk and HLA-mismatched patients were associated with worse OS, although this was not statistically significant in the multivariate analysis. Interpretation of these results, however, should be careful because of relatively short period of follow-up and the small sample size in our study. Although high risk patients was 82%, rate of relapse were unexpectedly low in our study. This might be due to earlier mortality, which precludes estimate of relapse rate. Alternately, more patients (60%) received more intense conditioning composed of 8 mg/kg of busulfan or 80–140 mg/m^2 of melphalan and 4 Gy TBI in our study.

In conclusion, we confirmed that unrelated BMT with RIC regimens can be a curative therapeutic option in a subset of patients with advanced hematologic malignancy, but at the expense of a high risk of severe complications and NRM. The incorporation of low-dose TBI may be advantageous for enhancing engraftment, and a suitable prophylaxis for GVHD still remains a primary target of clinical research. Based on the observed data, a prospective trial is currently underway to determine the value of a lower dose of ATG (ATG-Fresenius 5 mg/kg) to be added to the combination of fludarabine and busulfan.

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Alloantigen expression on non-hematopoietic cells reduces graft-versus-leukemia effects in mice

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Allogeneic hematopoietic stem cell transplantation (HSCT) is used effectively to treat a number of hematological malignancies. Its beneficial effects rely on donor-derived T cell–targeted leukemic cells, the so-called graft-versus-leukemia (GVL) effect. Induction of GVL is usually associated with concomitant development of graft-versus-host disease (GVHD), a major complication of allogeneic HSCT. The T cells that mediate GVL and GVHD are activated by alloantigen presented on host antigen-presenting cells of hematopoietic origin, and it is not well understood how alloantigen expression on non-hematopoietic cells affects GVL activity. Here we show, in mouse models of MHC-matched, minor histocompatibility antigen–mismatched bone marrow transplantation, that alloantigen expression on host epithelium drives donor T cells into apoptosis and dysfunction during GVHD, resulting in a loss of GVL activity. During GVHD, programmed death–1 (PD-1) and PD ligand–1 (PD-L1), molecules implicated in inducing T cell exhaustion, were upregulated on activated T cells and the target tissue, respectively, suggesting that the T cell defects driven by host epithelial alloantigen expression might be mediated by the PD-1/PD-L1 pathway. Consistent with this, blockade of PD-1/PD-L1 interactions partially restored T cell effector functions and improved GVL. These results elucidate a previously unrecognized significance of alloantigen expression on non-hematopoietic cells in GVL and suggest that separation of GVL from GVHD for more effective HSCT may be possible in human patients.

Introduction

Donor immunity in allogeneic hematopoietic stem cell transplantation (HSCT) harnesses beneficial graft-versus-leukemia (GVL) effects; therefore, allogeneic HSCT represents a very potent form of immunotherapy for hematological malignancies (1, 2). Induction of GVL is usually associated with the development of graft-versus-host disease (GVHD), which is a major complication after allogeneic HSCT. T cell depletion of the donor inocula prevents GVHD and leads to a loss of the GVL effect (3–5). Both GVL and GVHD are mediated by donor T cells, which recognize alloantigens presented on host APCs (6, 7). Donor CTLs and inflammatory cytokines are major effectors of GVHD, whereas CTLs are primarily responsible for GVL (8, 9). In patients with advanced-stage leukemia and lymphoma, relapse is still a major cause of mortality after allogeneic HSCT even after the development of severe GVHD. Thus, improvements in our understanding of the pathophysiology of GVHD and GVL are urgently needed to develop more effective therapies for malignant diseases.

Alloantigens are expressed on the three major components in HSCT recipients in the context of GVHD and GVL: hematopoietically derived APCs, GVHD target epithelium, and leukemia cells. Several studies have shown that host APCs are crucial for the induction of both GVHD and GVL (6, 7, 9–11). Alloantigen expression on epithelium is also critical for the induction of GVHD in MHC-matched, minor histocompatibility antigen–mismatched (mHA-mismatched) models of bone marrow transplantation (BMT) (10), but GVHD can occur in the absence of alloantigen expression on epithelium in MHC-mismatched models of BMT (9). However, the effect of alloantigen expression on non-hematopoietic cells such as the epithelium in GVL is not well defined. In this study, we addressed this important issue in mHA-mismatched models of BMT.

Results

Alloantigen expression on host non-hematopoietic cells augments acute GVHD but reduces GVL effects. We generated BM chimeric mice that express alloantigens on APCs, which are essential for the induction of both GVHD and GVL (6, 7, 12). BM chimeras were created by reconstituting lethally irradiated C3H.Sw (C3: H-2^b) mice with 5×10^6 T cell–depleted (TCD) BM cells isolated from C57BL/6 (B6, H-2^b) mice that differ from C3 mice at multiple mHAs ([B6→C3] chimeras). Control chimeras, [B6→B6], were identically created. Four months later, donor repopulation of hematopoiesis was confirmed by flow cytometry as shown previously (6, 9, 12). Thus, [B6→C3] chimeric mice expressed B6-derived mHAs on hematopoietically derived APCs but not on non-hematopoietic target cells. In contrast, [B6→B6] mice expressed B6-derived mHAs on both APCs and target epithelium. These chimeras were used as BMT recipients; they were reirradiated and injected with 5×10^6 TCD BM cells alone or with various doses of $CD8^+$ T cells from C3 donors. After BMT, GVHD mortality was higher in [B6→B6] mice than in [B6→C3] mice (Figure 1A). Clinical GVHD scores (13) in surviving animals were also higher in [B6→B6] mice than in [B6→C3] mice (Figure 1B). Mortality and morbidity from GVHD in [B6→C3] mice were almost equivalent to those in [B6→B6] mice given a 1-log lower T cell dose. This finding confirmed the previous observation of a lack of alloantigen expression on host epithelium significantly reducing GVHD across mHA disparity (10). We

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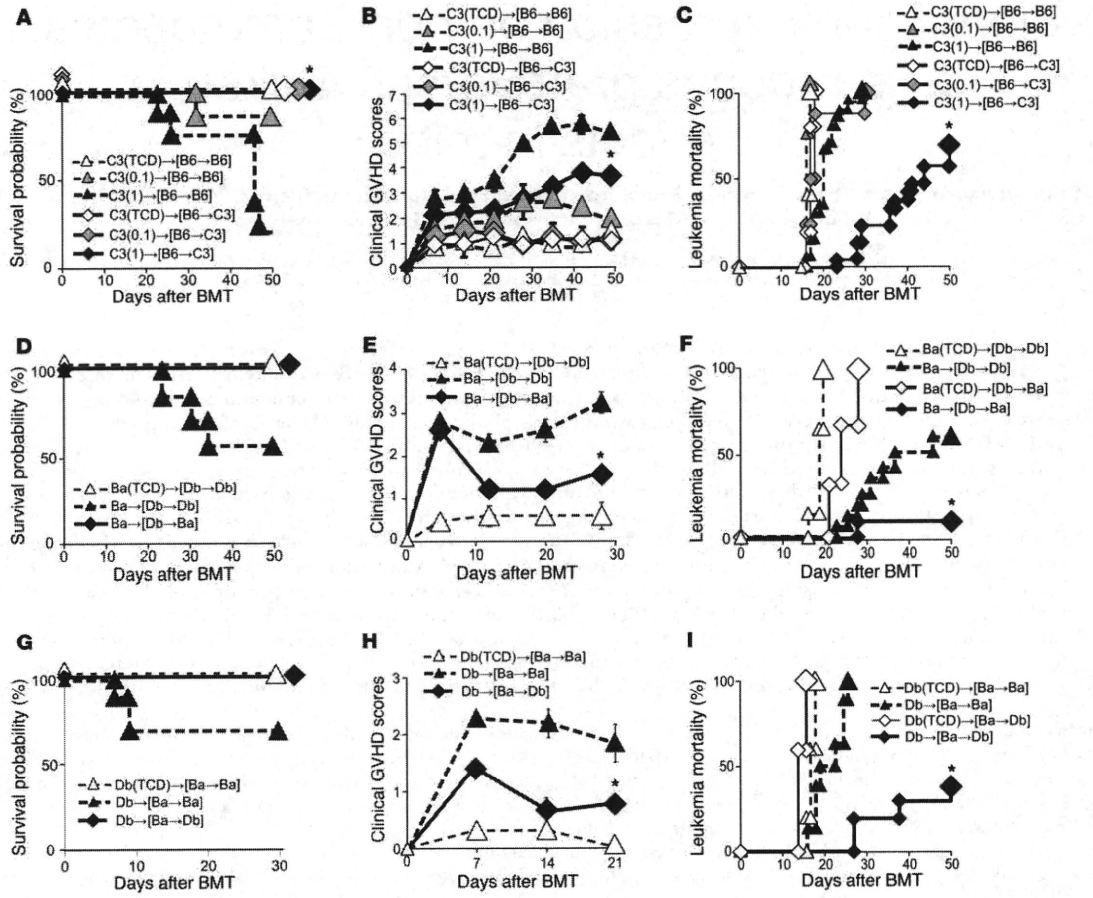

Figure 1

Alloantigen expression on host non-hematopoietic cells augments acute GVHD but reduces GVL effects. (**A–C**) [B6→C3] (diamonds) and [B6→B6] chimeras (triangles) were created by reconstituting lethally irradiated C3 and B6 mice with 5×10^6 TCD BM cells from B6 mice. Four months later, the chimeras were reirradiated and injected with 5×10^5 TCD BM cells alone (open symbols) or with 1×10^6 (black symbols) or 0.1×10^6 (gray symbols) $CD8^+$ T cells from C3 donors (as indicated in parentheses $\times 10^6$). Survival (**A**) and clinical GVHD scores (**B**) after BMT ($n = 3–8$/group). (**C**) Leukemia mortality after BMT in chimeras injected with EL4 cells ($n = 5–21$/group). Data from 3 similar experiments were combined. (**D–F**) [Db→Ba] (diamonds) and [Db→Db] (triangles) chimeras were reirradiated and injected with TCD BM alone (open symbols) or with 2×10^6 T cells from Ba donors (filled symbols). Survival (**D**) and clinical GVHD scores (**E**) after BMT from a representative experiment of 2 similar experiments ($n = 4–7$/group). (**F**) Leukemia mortality after BMT in mice injected with P815 cells. Data from 2 similar experiments were combined ($n = 6–18$/group). (**G–I**) [Ba→Db] (diamonds) and [Ba→Ba] (triangles) chimeras were similarly transplanted with 5×10^6 TCD BM cells alone (open symbols) or with 2×10^6 T cells from Db donors (filled symbols). Survival (**G**) and clinical scores (**H**) after BMT ($n = 3–10$/group). (**I**) Leukemia mortality after BMT in chimeras injected with A20 cells ($n = 5–10$/group). Data from 2 similar experiments were combined. Clinical scores are shown as the mean ± SEM. $^*P < 0.05$ compared with allogeneic controls.

then tested the effect of alloantigen expression on GVHD target epithelium on GVL effects. These chimeric mice were transplanted as described above together with 2,500 B6-derived EL4 cells as a model of residual leukemia after BMT. As expected, 100% of both types of chimeric mice that received TCD BM cells died from leukemia by day +20 after BMT (Figure 1C), whereas leukemia-free survival was significantly prolonged in mice that received donor T cells, demonstrating a significant GVL effect. However, this GVL effect was not potent in [B6→B6] mice, and all mice subsequently died from leukemia. Surprisingly, leukemia mortality was significantly lower in [B6→C3] mice that did not express alloantigens on their non-hematopoietic cells (62% vs. 100%; $P < 0.05$). GVL effects in [B6→B6] mice appeared to be almost equivalent to those in [B6→C3] mice given a 1-log lower T cell dose.

We further confirmed these observations in a different strain combination: BALB/c (Ba, H-2^d) and DBA/2 (Db, H-2^d) mice that

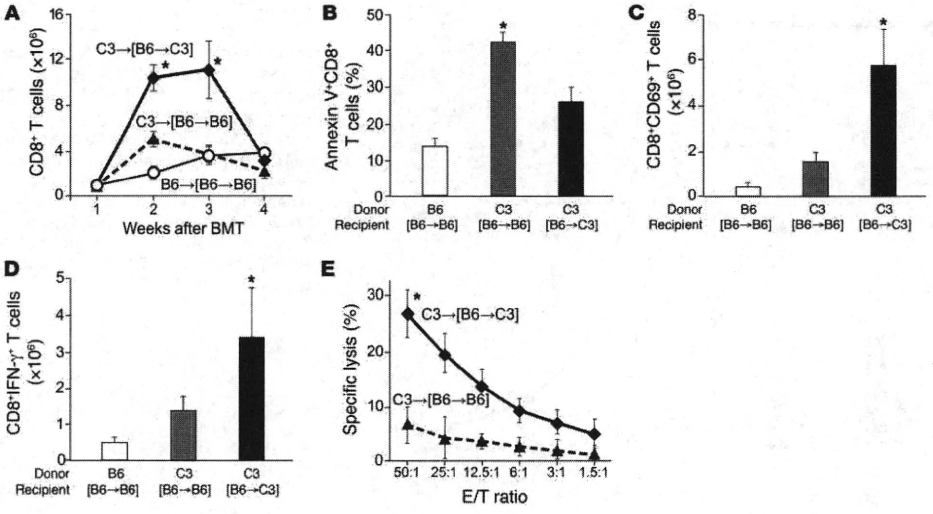

Figure 2

Alloantigen expression on host non-hematopoietic cells enhances the apoptosis and dysfunction of alloreactive T cells. [B6→C3] (diamonds and black bars) and [B6→B6] (triangles and gray bars) chimeras were transplanted as indicated in the legend for Figure 1. Syngeneic controls were [B6→B6] recipients of B6.Ly5.1 (CD45.1+) cells (open circles and white bars). (**A**) Numbers of donor CD8+ T cells in spleens. (**B**) Frequencies of annexin V+ donor CD8+ T cells. (**C**) Numbers of annexin V− donor CD69+CD8+ T cells. (**D**) Numbers of annexin V− IFN-γ–producing donor CD8+ T cells. (**E**) CTL activity against EL4. (**B–E**) Analysis was performed 14 days after BMT (n = 3–8/group). Representative data from 1 of the experiments are shown as the mean ± SD. *P < 0.05 compared with allogeneic controls.

differed at multiple mHAs from each other. [Db→Ba] and control [Db→Db] chimeras were lethally reirradiated and injected with 5 × 10^6 TCD BM cells alone or with 2 × 10^6 Ba T cells. Mortality (Figure 1D, P = 0.08) and morbidity from GVHD (Figure 1E, P < 0.05) were higher in [Db→Db] mice than in [Db→Ba] mice. When cells were transplanted together with 2,000 Db-derived P815 cells, leukemia mortality was significantly lower in [Db→Ba] mice than in [Db→Db] mice (10% vs. 60%; P < 0.05) (Figure 1F).

Similar results were obtained when [Ba→Db] and control [Ba→Ba] chimeras were transplanted with 5 × 10^6 TCD BM cells with or without 2 × 10^6 Db T cells. In [Ba→Db] recipients, in which non-hematopoietic cells do not express alloantigens, mortality (Figure 1G, P = 0.08) and morbidity of GVHD (Figure 1H, P < 0.05) were lower, but GVL effects against Ba-derived A20 lymphoma cells were significantly more potent as compared with [Ba→Ba] controls (leukemia mortality: 30% vs. 100%; P < 0.05) (Figure 1I). Taken together, these results demonstrate that GVHD is decreased but GVL activity is enhanced in the absence of alloantigen expression on non-hematopoietic cells.

Alloantigen expression on non-hematopoietic cells enhances apoptosis and dysfunction of alloreactive T cells. GVHD and GVL in the C3 and B6 strain combination is dependent on donor CD8+ T cells (12, 14). To elucidate the mechanisms responsible for the enhancement of the GVL effect in [B6→C3] chimeric mice, which lack alloantigen expression on non-hematopoietic cells, the kinetics of donor CD8+ T cell expansion and activation were evaluated after BMT. Expansion of donor CD8+ T cells identified as CD5.1+CD8+ cells peaked on day +14 in the spleens of allogeneic [B6→B6] recipients and decreased thereafter (Figure 2A), as previously shown in this model (15). CD8 expansion was significantly greater in [B6→C3] mice than in [B6→B6] mice on days +14 and +21. We next assessed donor T cell apoptosis as a determinant of the kinetics of T cell expansion. Frequencies of annexin V+ apoptotic donor CD8+ T cells were significantly greater in the spleen of [B6→B6] mice as compared with that of [B6→C3] mice on day +14 (Figure 2B). Notably, surviving donor CD8+ T cells were significantly less activated in [B6→B6] mice than in [B6→C3] mice when evaluated based on the expression of CD69 (Figure 2C) and intracellular IFN-γ (Figure 2D) on annexin V− donor CD8+ T cells. We next evaluated CTL activity in donor T cells isolated from the spleen on day +14 after BMT. CTL activity against EL4 targets was significantly reduced in the splenocytes of [B6→B6] mice as compared with [B6→C3] mice (Figure 2E). These results suggest that alloantigen expression on non-hematopoietic cells induces apoptosis and dysfunction of alloreactive T cells.

Absence of alloantigen expression on host non-hematopoietic cells restores GVL effects. Self-recognition in the periphery facilitates the reactivity of mature T cells to foreign antigens (16). Therefore, it is possible that the expression of syngeneic MHC molecules and not the absence of alloantigens on non-hematopoietic cells may be responsible for the enhancement of the GVL effect in [B6→C3] chimeras. This possibility was tested in B6-background $\beta_2m^{-/-}$ mice. [B6→$\beta_2m^{-/-}$] chimeras lacking functional MHC class I molecules on non-hematopoietic cells did not develop GVHD after transplantation with CD8+ T cells from C3 donors, as shown previously (17) (Figure 3A). In these mice, however, leukemia mortality was significantly delayed even in the absence of GVHD as compared with [B6→B6] recipients (Figure 3B, P < 0.05). The expansion and CTL activity of donor CD8+ T cells was significantly greater in [B6→$\beta_2m^{-/-}$] recipients than in [B6→B6] recipients (Figure 3, C and D).

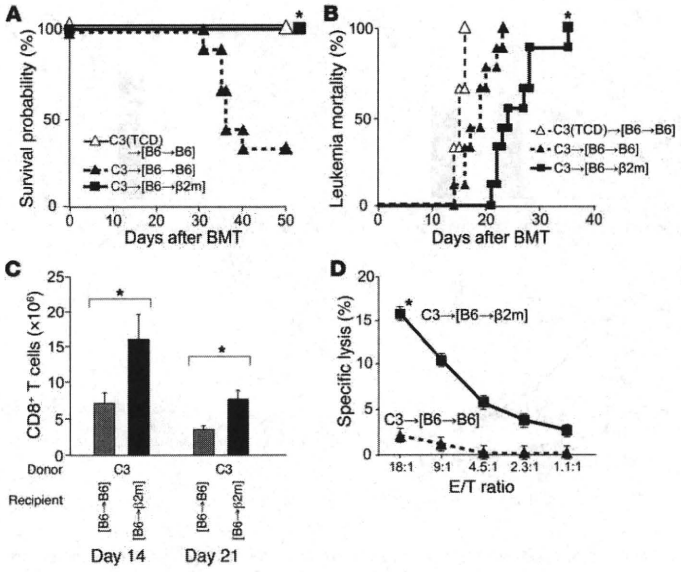

Figure 3
Absence of alloantigen expression on host nonhematopoietic cells restores GVL effects. [B6→B6] (triangles) and [B6→β2m$^{-/-}$] (squares) mice were reirradiated and injected with 5 × 10^6 TCD BM cells alone (open symbols) or with 1 × 10^6 CD8$^+$ T cells from C3 donors (filled symbols). (**A**) Survival after BMT. (**B**) Leukemia mortality in chimeras injected with EL4 cells (n = 6–9/group). Data from a representative experiment of 2 similar experiments are shown. Mean ± SEM numbers of donor CD8$^+$ T cells in spleens (n = 3–6/group) (**C**) and CTL activity against EL4 (**D**). *P < 0.05 compared with allogeneic controls.

These results confirm that alloantigen expression on host epithelium induces apoptosis and dysfunction of alloreactive T cells, which results in impaired GVL effects.

Alloantigen expression on host non-hematopoietic cells stimulates programmed death-1 and its ligand pathway. Programmed death-1 (PD-1) is a negative regulator of activated T cells and regulates T cell exhaustion during chronic infections (18–20). PD-1 interacts with at least 2 ligands: PD ligand-1 (PD-L1) and PD-L2 (21). In particular, the PD-1/PD-L1 pathway has been proposed as one of the most important mechanisms of T cell exhaustion and tolerance induction against infectious agents and tumors (19, 22–25). We therefore hypothesized that the PD-1/PD-L1 pathway plays a role in the loss of GVL effects in [B6→B6] mice. To test this hypothesis, we examined PD-1 expression on donor CD8$^+$ T cells in lymph nodes on day +14 and +21 after BMT. It was significantly upregulated in allogeneic [B6→B6] recipients as compared with syngeneic controls but was low in [B6→C3] mice (Figure 4, A and B). We also investigated the expression of another inhibitory receptor, CTLA-4, on donor CD8$^+$ T cells. Although the expression of cytoplasmic CTLA-4 was slightly upregulated in allogeneic animals as compared with syngeneic animals, its level did not differ between [B6→B6] and [B6→C3] mice (5.5% ± 1.0% vs. 4.5% ± 0.2%, respectively; P = 0.50).

We next examined PD-L1 expression in the liver by real-time PCR after BMT. PD-L1 expression was markedly upregulated in the liver of allogeneic controls as compared with syngeneic controls (Figure 4C). In allogeneic [B6→C3] mice, it was slightly upregulated on day +14 but not on day +21. Immunohistochemical analysis confirmed upregulated expression of PD-L1 in the liver of [B6→B6] mice, as previously reported (Figure 4D) (21, 26). These results showed that alloantigen expression on GVHD target epithelium is associated with upregulation of the PD-1/PD-L1 interactions between donor T cells and GVHD target tissue.

Blockade of the interaction between PD-1 and PD-L1 enhances GVL activity. We next examined whether blocking the PD-1/PD-L1 pathway could enhance GVL activity. [B6→C3] and [B6→B6] chimeras were reirradiated and injected with TCD BM cells and CD8$^+$ T cells from C3 donors. Mice were i.p. injected with 500 μg of anti–PD-L1 mAb on day 0 and then with 200 μg on days +3, +6, +9, +12, +15, and +18 after BMT. In [B6→B6] recipients, injection of anti–PD-L1 mAbs significantly restored T cell functions on day +14, as assessed by CD69 expression (Figure 5A), IFN-γ production (Figure 5B), and CTL activity (Figure 5C). In [B6→C3] mice, it marginally upregulated CD69 expression, IFN-γ production, and CTL activity, although differences were not statistically significant (Figure 5, A, B, and D). As a consequence, anti–PD-L1 mAb administration significantly increased the severity of GVHD in [B6→B6] mice (Figure 5E) but not in [B6→C3] mice (Figure 5F). PD-L1 blockade also significantly augmented GVL activity in [B6→B6] recipients injected with EL4 cells on day 0 (Figure 5G, P < 0.05). It also delayed leukemia death in [B6→C3] mice, although the difference was not statistically significant (Figure 5H, P = 0.38). In controls, PD-L1 blockade did not affect leukemia mortality in TCD-BMT recipients (Figure 5H) or [B6→B6] recipients of syngeneic B6 CD8$^+$ T cells (data not shown).

Discussion

Alloantigens are expressed in three major sites in HSCT recipients: APCs, GVHD target epithelium, and leukemia cells. Alloantigen expression on APCs is essential for the induction of GVHD (6), and an optimal GVL response occurs when alloantigens are expressed on both host APCs and tumor cells (7). Alloantigen expression on the epithelium is also critical for the induction of GVHD across mHA disparities (10), but GVHD can occur in the absence of alloantigen expression on epithelium in MHC-mismatched BMT (9). In this study, we addressed the effect of alloantigen expression on target epithelium in GVL using chimeric mouse models of GVHD and GVL across mHA disparities. Our models mimic clinical BMT in patients not in remission, since most of the mice relapsed after allogeneic BMT. This high tumor burden enabled us to compare the magnitude of GVL activity in our models, and we made sur-

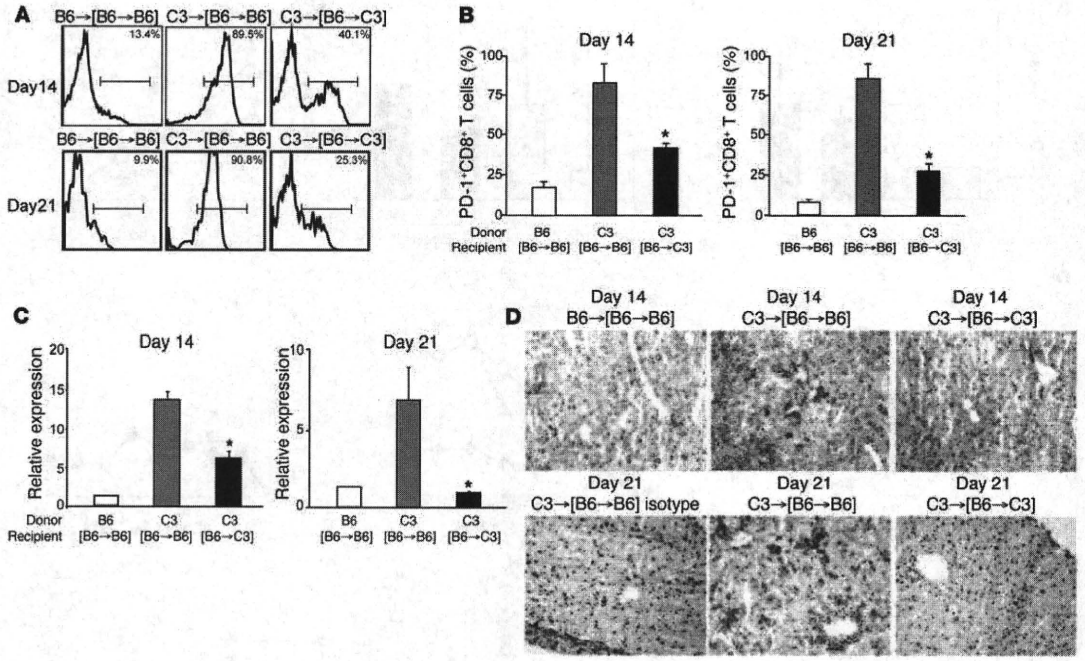

Figure 4

Alloantigen expression on host non-hematopoietic cells stimulates PD-1 and its ligand pathway. [B6→B6] and [B6→C3] chimeras were transplanted as indicated in the legend for Figure 1 ($n = 4–8$). (**A**) Representative histogram of PD-1 expression among donor CD8$^+$ T cells on day +14 and +21 in syngeneic (left), allogeneic [B6→B6] (middle), and [B6→C3] (right) recipients. (**B**) Frequencies of PD-1$^+$ CD8$^+$ T cells (mean ± SD). (**C**) Relative expressions of *Pdl1* mRNA on day +14 and +21 in the livers of allogeneic [B6→B6] (gray bars) and allogeneic [B6→C3] mice (black bars). Data represent the mean (± SD) of *n*-fold difference in the amount of *Pdl1* gene expression relative to that in syngeneic mice. (**D**) PD-L1 expression in the liver on day +14 (top row) and +21 (bottom row) from syngeneic (upper left) and allogeneic [B6→B6] (middle) and [B6→C3] (right) recipients. Isotype control of allogeneic [B6→B6] (lower left) is shown. Original magnification, ×200. *$P < 0.05$ compared with allogeneic controls.

prising observations that alloantigen expression on non-hematopoietic cells inhibited GVL effects but enhanced GVHD. This observation challenges the current paradigm that GVL activity is strongly correlated with the severity of GVHD (1, 2, 27).

We found that alloantigen expression on non-hematopoietic cells induced donor T cell apoptosis and led to a contraction in the size of an alloreactive donor CD8$^+$T cell pool early after BMT. The remainder of the donor T cells were alive, but their ability to produce cytokines and cytotoxicity were impaired. This defect is similar to T cell exhaustion, which is a principal reason for the inability of the host to eliminate the persisting pathogen in chronic infections (18, 28). CD8$^+$ T cell proliferation and differentiation into cytolytic effectors on an encounter with antigens are variable and change as a consequence of the antigen load (29). As the magnitude of the viral load increases, virus-specific T cells become more functionally impaired. During persistent infection, a high antigen load drives a significant number of virus-specific T cells into activation-induced apoptosis, and the remaining virus-specific T cells remain alive but in a dysfunctional state of cytotoxicity (18, 30–33). In tumor models, antigen quantity determines the behavior of the CD8$^+$ effector cells, including their effector function and sensitivity to apoptosis (34–36). In patients with a larger tumor burden, CD8$^+$ T cells were found to undergo apoptosis (37). Thus, a higher alloantigen load in allogeneic controls as compared with chimeras, in which alloantigen expression is limited to hematopoietic cells and tumor cells, may induce apoptosis and the dysfunction of alloreactive T cells, which leads to the inability of the host to eliminate leukemia.

Our results are consistent with seminal observations by Meunier, Fontaine, and colleagues, who showed that the adoptive transfer of immunodominant mHA (B6^{dom1})-specific T cells eradicates B6^{dom1}-expressing leukemia more efficiently in mice lacking B6^{dom1} expression than in mice expressing B6^{dom1} (38). This was because the widespread expression of B6^{dom1} caused activation-induced apoptosis and dysfunction of donor T cells in mice expressing B6^{dom1} (38, 39). These findings along with our results indicate that allogeneic cellular therapy targeting mHAs exclusively expressed on APCs and tumor cells can induce a potent GVL effect while inducing less-severe GVHD than immunotherapy via targeting of ubiquitously expressed mHAs (40).

The PD-1/PD-L1 pathway is critically involved in T cell exhaustion and tolerance induction in infection and tumor immunology (18–20, 23–25, 41). It is also required for protection against chronic rejection of cardiac allograft, and induction of peripheral dele-

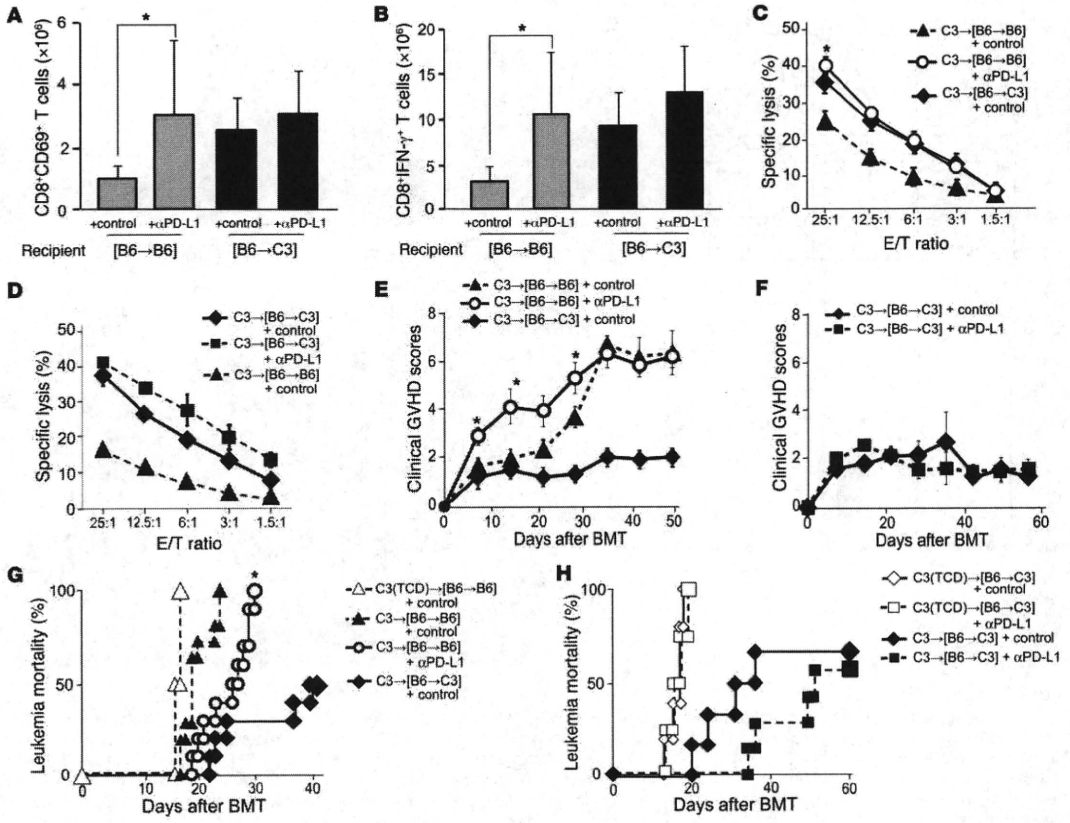

Figure 5

Blockade of the interaction between PD-1 and PD-L1 enhances GVL activity. [B6→C3] and [B6→B6] chimeras were reirradiated and injected with 5 × 10^6 TCD BM cells alone or with 1 × 10^6 $CD8^+$ T cells from C3 donors. Mice were i.p. injected with 500 μg of anti PD-L1 mAbs or controls on day 0 and then 200 μg thereafter on days +3, +6, +9, +12, +15, and +18. Splenocytes were harvested on day +14 to determine the number of $CD8^+CD69^+$ T cells (**A**) and IFN-γ–producing $CD8^+$ T cells (**B**) and CTL activity against EL4 targets (**C** and **D**). Results from a representative experiment of 2 similar experiments (means ± SD, n = 7–8/group). Mean clinical GVHD scores (±SEM) (**E** and **F**) after BMT are shown (n = 5–7/group). (**G** and **H**) Leukemia mortality after BMT in [B6→B6] and [B6→C3] chimeras injected with EL4 cells on day 0 (n = 4–11/group). Data from two similar experiments were combined. αPD-L1, anti–PD-L1 mAbs. *P < 0.05 compared with the corresponding controls.

tional tolerance of alloreactive, anti-donor $CD8^+$ T cells to achieve successful engraftment in BMT (42, 43). In this study, we found that PD-1 expression was upregulated in donor T cells and PD-L1 expression was upregulated in GVHD target organs. The expression of PD-1/PD-L1 was markedly reduced in chimeras lacking alloantigen expression on non-hematopoietic cells. PD-1 and PD-L1 expression is induced upon cell activation and inflammation in GVHD (44); therefore, the absence of alloantigen expression on GVHD target epithelium reduced GVHD in chimeric mice, which resulted in insufficient stimulation of the PD-1/PD-L1 interaction. Target tissue expression of PD-L1 is also critical for the induction of T cell exhaustion or tolerance in chronic viral infection, autoimmune diabetes, and cardiac allografting (19, 42, 45).

Both PD-1 and PD-L1 were markedly upregulated in [B6→B6] mice, but they were also modestly upregulated in [B6→C3] mice. Blockade of PD-1/PD-L1 interactions significantly restored T cell effector functions in [B6→B6] mice but modestly restored them in [B6→C3] mice as well. The relevance of these observations is shown by the PD-1/PD-L1 blockade studies. These data showed that the PD-1/PD-L1 pathway is particularly germane to [B6→B6] mice with widespread expression of alloantigens but also applies, at least in part, to [B6→C3] mice, wherein alloantigen expression is only on APCs. While there is likely to be a role for this pathway in the absence of epithelial alloantigen expression, the full negative impact of this pathway on GVL is only seen when alloantigen expression is present on non-hematopoietic tissues.

Of note, the improvement in GVL by the PD-1/PD-L1 blockade was partial, as has been shown in chronic viral infection (46–48). This may be due to the presence of multiple negative regulatory pathways that contribute to T cell exhaustion, including CTLA-4, IL-10, LAG-3, CD160, and 2B4 (20, 47, 49). In addition, the population of exhausted T cells is heterogeneous, and this interven-