Table 3 Effect of the *CCR9* genotype on transplant outcome

Events	*Risk factor(s)*	*Multivariate*[a]		*Genotype 926AG*[b]	
		Hazard ratio (CI)	P-*value*	*Hazard ratio (CI)*	P-*value*
Acute GVHD					
Grade II–IV	PBSCT	3.4 (1.7–6.9)	0.001	1.2 (0.30–5.3)	0.76
Skin stage 2–4	PBSCT	2.7 (1.3–5.5)	0.008	3.2 (1.1–9.1)	0.032
Liver stage 2–4	—	—	—	4.2 (0.47–37)	0.20
Gut stage 2–4	PBSCT	5.7 (1.6–20)	0.007	2.4 (0.30–19)	0.41
Chronic GVHD					
Limited/extensive	—	—	—	1.7 (0.52–5.8)	0.37
Eye	—	—	—	6.2 (0.56–68)	0.14
Oral	—	—	—	2.4 (0.71–8.4)	0.16
Skin	—	—	—	4.1 (1.1–15)	0.036
Lung	—	—	—	12 (0.76–196)	0.077
Liver	Female to male	3.5 (1.0–12)	0.05	1.7 (0.21–13)	0.63
Overall survival	Age>40	2.1 (1.3–3.6)	0.004	0.84 (0.30–2.3)	0.73
	High risk	2.1 (1.3–3.6)	0.004		
Non-relapse mortality	Age>40	2.7 (1.2–6.1)	0.015	1.3 (0.40–4.5)	0.64
Relapse	High risk	4.0 (2.0–7.8)	<0.001	0.36 (0.05–2.6)	0.31

Abbreviations: CI = confidence interval; PBSCT = peripheral blood stem cell transplantation.
[a]Covariates used were age, conditioning, disease risk, remission state, donor–recipient sex combination and graft source. For chronic GVHD analysis, history of acute GVHD was included in the covariates. Only significant factors were listed.
[b]Adjusted by significant factors.

chemokines in initiating GVHD. Specifically, we address the association of polymorphism in the tissue-specific chemokine receptor gene with acute and chronic GVHD and the regulation of leukocyte trafficking.

CCL25 and CCR9 (as chemokine and chemokine receptor) are selectively expressed in both the thymus and the small intestine.[22,23] One of their important functions is the selective homing and retention of CCR9-positive T cells and B cells to the small intestine rather than to the colon, which provides a mechanism for regional specialization of the mucosal immune system.[9,24] Another function is regulating intrathymic T-cell development, particularly double-negative to double-positive transition,[25,26] which may be associated with T-cell recovery after allo-HSCT. Therefore, the effect of the *CCR9* genotype on acute GVHD is hypothesized to result from its function in the small intestine because T cells educated in the thymus will appear at least 6 months after transplantation.[27]

Interestingly, donor *CCR9* SNPs affected the incidence of skin GVHD, but did not affect the incidence of intestinal GVHD. This observation may be partially explained by the findings of Beilhack *et al.*,[28] who recently reported the redundancy of secondary lymphoid organs at different anatomical sites in GVHD initiation. They suggested that primed T cells could initiate GVHD at sites other than their original priming sites. As Peyer's patches are important sites of Ag presentation,[29] differences in T cell homing to Peyer's patches between each *CCR9* genotype may produce changes in Ag presentation and result in varying incidences of skin GVHD.

Our results suggest the possibility of CCL25/CCR9-targeting modalities for GVHD. CCL25 and CCR9 have an important role in the adherence of T lymphocytes to the intestinal endothelium under inflammatory and normal conditions, and anti-CCL25 Ab attenuates the TNF-α-induced T-cell adhesion in the small intestine.[30] Although blocking the interactions of CCL25 and CCR9 may delay immunological reconstitution in the thymus, CCR9-deficient mice showed no major effect on intrathymic T-cell development despite a 1-day lag in the appearance of double-positive cells and a diminution of γδ-T cells.[31]

One possible limitation of this study is that genetic associations can be biased by population stratification,[32] and there is also the chance of false-positive associations with the *CCR9* genotype on the basis of multiple statistical tests. Confirmation of the results with another separate cohort is needed for eliminating a possible confounding effect. Another limitation is that this SNP might be in linkage disequilibrium with SNPs in the *CCR9* gene or in the other genes located nearby. Linkage disequilibrium mapping of *CCR9* using the HapMap-JPT database showed one small block in introns of the *CCR9* gene, but G926A was outside the block with no known associations with other SNPs in the *CCR9* gene or with genes located around chromosome 3p21.3. In addition, this SNP alters CCR9 amino acid sequences of the third exoloop, which is an important site for chemokine binding and specificity.[33] Therefore, this SNP can affect biological functions due to altered efficiencies of the receptor or signal transduction from the receptor. Although Transwell assays using SNP-transfected cells showed that biological functions varied according to this SNP, the elucidation of additional mechanisms are matters for future research.

In summary, this study suggests that donor 926AG is associated with an increased incidence of acute and chronic skin GVHD in related HSCT recipients. CCL25 and CCR9

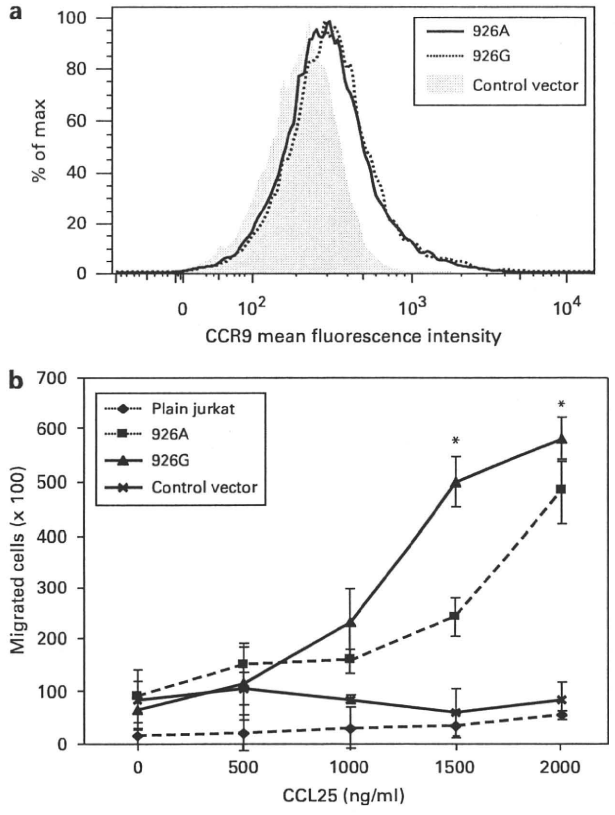

Figure 2 Chemotaxis assays with *CCR9*-polymorphism-transfected Jurkat cells: (**a**) Flow cytometric analysis of CCR9 expression. Transfected Jurkat cells were stained with phycoerythrin (PE)-labeled monoclonal anti-CCR9. Control staining with control-transfected Jurkat cells is also shown (shadow); (**b**) Jurkat cells transfected with cDNAs encoding *CCR9* migrated in response to CCL25. After puromycin selection, 1×10^6 transfected cells and the same number of Jurkat cells were added to porous Transwell tissue culture inserts and placed in wells containing various concentrations of CCL25. After 90-min of incubation, cells migrating through the membranes into the lower wells were counted. Results are expressed as cells migrating per 10^6 input cells. Assays were carried out in triplicate and error bars represent s.d. $*P<0.05$

may be candidates for future therapeutic targets that alter the quality and incidence of GVHD.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References

1 Perfetti P, Carlier P, Strada P, Gualandi F, Occhini D, Van Lint MT *et al.* Extracorporeal photopheresis for the treatment of steroid refractory acute GVHD. *Bone Marrow Transplant* 2008; **42**: 609–617.
2 Kunkel EJ, Butcher EC. Chemokines and the tissue-specific migration of lymphocytes. *Immunity* 2002; **16**: 1–4.
3 Campbell DJ, Kim CH, Butcher EC. Chemokines in the systemic organization of immunity. *Immunol Rev* 2003; **195**: 58–71.
4 Campbell JJ, Butcher EC. Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. *Curr Opin Immunol* 2000; **12**: 336–341.
5 Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001; **2**: 123–128.
6 Pan J, Kunkel EJ, Gosslar U, Lazarus N, Langdon P, Broadwell K *et al.* A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. *J Immunol* 2000; **165**: 2943–2949.
7 Wang W, Soto H, Oldham ER, Buchanan ME, Homey B, Catron D *et al.* Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). *J Biol Chem* 2000; **275**: 22313–22323.
8 Kunkel EJ, Campbell JJ, Haraldsen G, Pan J, Boisvert J, Roberts AI *et al.* Lymphocyte CC chemokine receptor 9 and epithelial thymus-expressed chemokine (TECK) expression distinguish the small intestinal immune compartment: epithelial expression of tissue-specific chemokines as an organizing principle in regional immunity. *J Exp Med* 2000; **192**: 761–768.
9 Papadakis KA, Prehn J, Nelson V, Cheng L, Binder SW, Ponath PD *et al.* The role of thymus-expressed chemokine and its receptor CCR9 on lymphocytes in the regional specialization of the mucosal immune system. *J Immunol* 2000; **165**: 5069–5076.
10 Stenstad H, Svensson M, Cucak H, Kotarsky K, Agace WW. Differential homing mechanisms regulate regionalized effector CD8alphabeta+ T cell accumulation within the small intestine. *Proc Natl Acad Sci U S A* 2007; **104**: 10122–10127.
11 Onishi M, Kinoshita S, Morikawa Y, Shibuya A, Phillips J, Lanier LL *et al.* Applications of retrovirus-mediated expression cloning. *Exp Hematol* 1996; **24**: 324–329.
12 Yu CR, Peden KW, Zaitseva MB, Golding H, Farber JM. CCR9A and CCR9B: two receptors for the chemokine CCL25/TECK/Ck beta-15 that differ in their sensitivities to ligand. *J Immunol* 2000; **164**: 1293–1305.
13 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
14 Sullivan KM, Agura E, Anasetti C, Appelbaum F, Badger C, Bearman S *et al.* Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991; **28**: 250–259.
15 Inamoto Y, Suzuki R, Kuwatsuka Y, Yasuda T, Takahashi T, Tsujimura A *et al.* Long-term outcome after bone marrow transplantation for aplastic anemia using cyclophosphamide and total lymphoid irradiation as conditioning regimen. *Biol Blood Marrow Transplant* 2008; **14**: 43–49.
16 Kallianpur AR. Genomic screening and complications of hematopoietic stem cell transplantation: has the time come? *Bone Marrow Transplant* 2005; **35**: 1–16.
17 Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E. Genetic polymorphisms predicting the outcome of bone marrow transplants. *Br J Haematol* 2004; **127**: 479–490.
18 Lin MT, Storer B, Martin PJ, Tseng LH, Gooley T, Chen PJ *et al.* Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med* 2003; **349**: 2201–2210.

19 Socie G, Loiseau P, Tamouza R, Janin A, Busson M, Gluckman E *et al.* Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Transplantation* 2001; **72**: 699–706.
20 Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH, Middleton PG. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. *Blood* 2001; **98**: 1594–1600.
21 Mehta PA, Eapen M, Klein JP, Gandham S, Elliott J, Zamzow T *et al.* Interleukin-1 alpha genotype and outcome of unrelated donor haematopoietic stem cell transplantation for chronic myeloid leukaemia. *Br J Haematol* 2007; **137**: 152–157.
22 Zaballos A, Gutierrez J, Varona R, Ardavin C, Marquez G. Cutting edge: identification of the orphan chemokine receptor GPR-9-6 as CCR9, the receptor for the chemokine TECK. *J Immunol* 1999; **162**: 5671–5675.
23 Zabel BA, Agace WW, Campbell JJ, Heath HM, Parent D, Roberts AI *et al.* Human G protein-coupled receptor GPR-9-6/CC chemokine receptor 9 is selectively expressed on intestinal homing T lymphocytes, mucosal lymphocytes, and thymocytes and is required for thymus-expressed chemokine-mediated chemotaxis. *J Exp Med* 1999; **190**: 1241–1256.
24 Mora JR, von Andrian UH. Role of retinoic acid in the imprinting of gut-homing IgA-secreting cells. *Semin Immunol* 2009; **21**: 28–35.
25 Wurbel MA, Philippe JM, Nguyen C, Victorero G, Freeman T, Wooding P *et al.* The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur J Immunol* 2000; **30**: 262–271.
26 Norment AM, Bogatzki LY, Gantner BN, Bevan MJ. Murine CCR9, a chemokine receptor for thymus-expressed chemokine that is up-regulated following pre-TCR signaling. *J Immunol* 2000; **164**: 639–648.
27 Heitger A, Neu N, Kern H, Panzer-Grumayer ER, Greinix H, Nachbaur D *et al.* Essential role of the thymus to reconstitute naive (CD45RA+) T-helper cells after human allogeneic bone marrow transplantation. *Blood* 1997; **90**: 850–857.
28 Beilhack A, Schulz S, Baker J, Beilhack GF, Nishimura R, Baker EM *et al.* Prevention of acute graft-versus-host disease by blocking T-cell entry to secondary lymphoid organs. *Blood* 2008; **111**: 2919–2928.
29 Niess JH, Reinecker HC. Lamina propria dendritic cells in the physiology and pathology of the gastrointestinal tract. *Curr Opin Gastroenterol* 2005; **21**: 687–691.
30 Hosoe N, Miura S, Watanabe C, Tsuzuki Y, Hokari R, Oyama T *et al.* Demonstration of functional role of TECK/CCL25 in T lymphocyte-endothelium interaction in inflamed and uninflamed intestinal mucosa. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G458–G466.
31 Wurbel MA, Malissen M, Guy-Grand D, Meffre E, Nussenzweig MC, Richelme M *et al.* Mice lacking the CCR9 CC-chemokine receptor show a mild impairment of early T- and B-cell development and a reduction in T-cell receptor gammadelta(+) gut intraepithelial lymphocytes. *Blood* 2001; **98**: 2626–2632.
32 Wacholder S, Rothman N, Caporaso N. Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 513–520.
33 Youn BS, Yu KY, Oh J, Lee J, Lee TH, Broxmeyer HE. Role of the CC chemokine receptor 9/TECK interaction in apoptosis. *Apoptosis* 2002; **7**: 271–276.

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ORIGINAL ARTICLE

Cytochrome P450 genetic polymorphisms influence the serum concentration of calcineurin inhibitors in allogeneic hematopoietic SCT recipients

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Calcineurin inhibitors are necessary as immunosuppressants during hematopoietic SCT (HSCT) to prevent alloreactivity, but have unfortunate toxicities. So, we investigated the association of gene polymorphisms with the initial calcineurin inhibitor concentration and the subsequent drug dose from day 1 to day 28 among patients who underwent HSCT at a single institution. We analyzed 58 serial cases of Japanese patients receiving GVHD prophylaxis with CsA (21 cases) or tacrolimus (37 cases). We investigated eight single-nucleotide polymorphisms: rs4244285 (*CYP2C19*), rs15524, rs4646450, rs3800959, rs776746 (*CYP3A5*), rs1128503, rs2032582 and rs1045642 (*MDR1*). The CsA concentration was significantly higher when the genotype of *CYP3A5* rs15524 was T/T ($P=0.044$) or rs776746 was G/G ($P=0.027$). The *CYP3A5* rs776746 and rs4646450 genotypes were also associated with tacrolimus concentration ($P=0.013$ and $P=0.0058$, respectively). The dosage of tacrolimus was remarkably reduced from day -1 to day 28 when the patient had the *CYP3A5* rs4646450 C/C and/or rs776746 G/G genotype ($P=0.0010$ and $P=0.0021$, respectively). In this study, we show that genetic variation has a predictable effect on the pharmacological responses to calcineurin inhibitors in HSCT patients.
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Keywords: calcineurin inhibitor; polymorphism; CYP3A5; MDR1

Introduction

The successful outcome of HSCT has been greatly enhanced in the last 40 years not only by improvements in techniques and by progressive understanding of HLA histocompatibility and typing, but also by the development of effective immunosuppressive agents. CsA and tacrolimus have been critical for the improved outcome of both solid organ transplantation and HSCT that has been observed over the last three decades. Although the patients are given CsA and tacrolimus routinely as prophylaxis of GVHD, the agents have a narrow therapeutic window and a spectrum of undesirable side effects that often accompany their administration. These drugs can be both nephrotoxic and neurotoxic (insomnia, tremor and headache), findings consistent with the ubiquitous expression of calcineurin. Other side effects that limit their usefulness for prolonged therapy include hirsutism, hyperglycemia, hypertension, hyperkalemia and glucose intolerance.[1,2]

Clinically calcineurin inhibitors are often administered using the same dose based on the patient's body weight, even though the blood concentration of these agents shows a wide spread in different cases. Although individual differences in drug response can result from the effects of age, sex, disease or drug interactions, genetic factors can also influence both the efficacy of a drug and the likelihood of an adverse reaction.[3] The variability in CsA and tacrolimus disposition has been attributed to interindividual differences in the expression of the metabolizing enzymes cytochrome P450 (CYP) 3A4 and 3A5, and in the expression of the drug transporter P-glycoprotein (encoded by the ABCB1 gene, formerly known as the multidrug resistance 1 gene (*MDR1*)). As these genes have functional polymorphisms affecting drug metabolism, there have been many pharmacogenetics studies.[4–9] Because this type of study helps us predict the patient's biological response to a drug, we can avoid the side effects and provide suitable therapy for each individual.

There are many reports investigating the association of these gene polymorphisms and calcineurin inhibitors in organ transplant recipients, however, there are only a few studies looking at how this association influences the clinical features in HSCT recipients. In order to predict calcineurin inhibitor-related complications, we investigated the association between the initial concentration of these drugs, and cytochrome P450 and P-glycoprotein gene polymorphisms. Because the continuing dose of calcineurin inhibitor is often reduced in HSCT recipients during the first 4 weeks in order to maintain target blood concentrations,

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we also examined the relationship between the drug dose ratio of the day 28 dose compared with the starting dose and gene polymorphisms.

Materials and methods

Study patients, sample collection and drug administration
We analyzed 58 serial cases of adult Japanese patients who underwent HSCT in Tokai University School of Medicine from March 2006 to May 2009. Patient characteristics are shown in Table 1. GVHD prophylaxis was administered, with 21 cases receiving CsA (starting dose of 1.5 mg/kg, twice daily by 3-h i.v. infusion from day −1), and 37 cases receiving tacrolimus (starting dose of 0.02 mg/kg, by continuous i.v. infusion from day −1) and a short course of MTX (15 mg per day i.v. on day 1 and 10 mg per day i.v. on days 3 and 6). Because there were no patients with obesity whose body mass indices were in excess of 30 kg/m^2, we did not adjust the drug dosage according to patient's obesity. All patients were administered omeprazole (20 mg per day), itraconazole (200 mg per day), ciprofloxacin (400 mg per day) and acyclovir (500 mg per day) from 7 days before transplantation.

The initial drug concentration was investigated on day 2 or day 3, and subsequent drug concentrations were monitored three times a week. Blood samples from patients receiving CsA were collected before daily drug administration. The drug dosage was adjusted according to the target serum concentration. The target concentration was 250–300 ng/mL for CsA (at the trough) and 10–15 ng/mL for tacrolimus. If the drug concentration was outside the target range, we increased or decreased the daily drug dose by 20%. All subjects provided informed consent to participate in this study, as approved by the ethical committee of Tokai University School of Medicine.

Table 1 Patient characteristics

	CsA (n = 21)	*Tacrolimus (n = 37)*
Age (years), median (range)	48 (27–58)	42 (18–59)
Gender		
Male/Female	12/9	23/11
Underlying disease		
AML	6	11
ALL	9	8
Lymphoma	3	4
AA	1	5
MDS	1	4
CML	1	4
MM	0	1
Stem cell donor		
Related	14	5
Unrelated	7	32
Conditioning regimen		
Myeloablative	15	28
Reduced intensity	6	9
Acute GVHD[a]		
Grades 0–I	10	23
Grades II–IV	10	13

Abbreviations: AA = aplastic anemia; MDS = myelodysplastic syndrome; MM = multiple myeloma.
[a]One patient from each group died from primary graft failure.

Analysis of gene polymorphisms
Genomic DNA was purified from peripheral blood or BM obtained before HSCT. We studied single-nucleotide polymorphisms (SNPs) in genes encoding CYP2C19, CYP3A5 and MDR1 based on the following criteria: (1) known functional consequences on enzyme activity and drug metabolism and (2) a minor allele frequency higher than 5% in the Japanese population. The selection was based on publicly available databases (dbSNP, International HapMap project) and on Medline publications for selected SNPs. Finally we examined the *CYP2C19* SNP (rs4244285),[10,11] *CYP3A5* SNPs (rs15524, rs4646450, rs3800959 and rs776746)[4,12,13] and *MDR1* SNPs (rs1128503, rs2032582 and rs1045642)[12] by PCR based on suitable methods for each SNP (Table 2 and Supplementary Table 1).

Statistical analysis
Statistical analyses were performed using *t*-tests for polymorphisms and drug concentrations. Renal dysfunction and acute GVHD rates were estimated by the Kaplan–Meier method and *P*-values were calculated using the log-rank test. A significance level of $P<0.05$ was used for all analyses.

Results

Initial drug concentration
Mean initial CsA and tacrolimus concentrations were significantly influenced by the *CYP3A5* genetic polymorphisms. The CsA concentration was significantly higher when the *CYP3A5* rs15524 genotype was T/T (251.1 ± 92.8 and 145.7 ± 36.7 ng/mL for T/T and T/C + C/C, respectively; mean ± 95% confidence interval (CI)]; $P=0.044$; Figure 1a) or the *CYP3A5* rs776746 genotype was G/G (250.8 ± 69.9 and 138.6 ± 30.8 ng/mL for G/G and A/G + A/A, respectively; mean ± 95% CI; $P=0.027$; Figure 1b). There

Table 2 SNP rs number and frequency

Gene	*rs No.*	*Minor allele frequency*	
		CEU	*JPT*
CYP2C19	4244285	0.155	0.284
CYP3A5	15524	0.067	0.273
	4646450	0.175	0.273
	3800959	0	0.216
	776746	0.058	0.25
MDR1	1128503	0.392	0.422
	2032582	0.469	0.448
	1045642	0.429	0.459

Abbreviations: CEU = Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection; JPT = Japanese in Tokyo, Japan; SNP = single-nucleotide polymorphism.

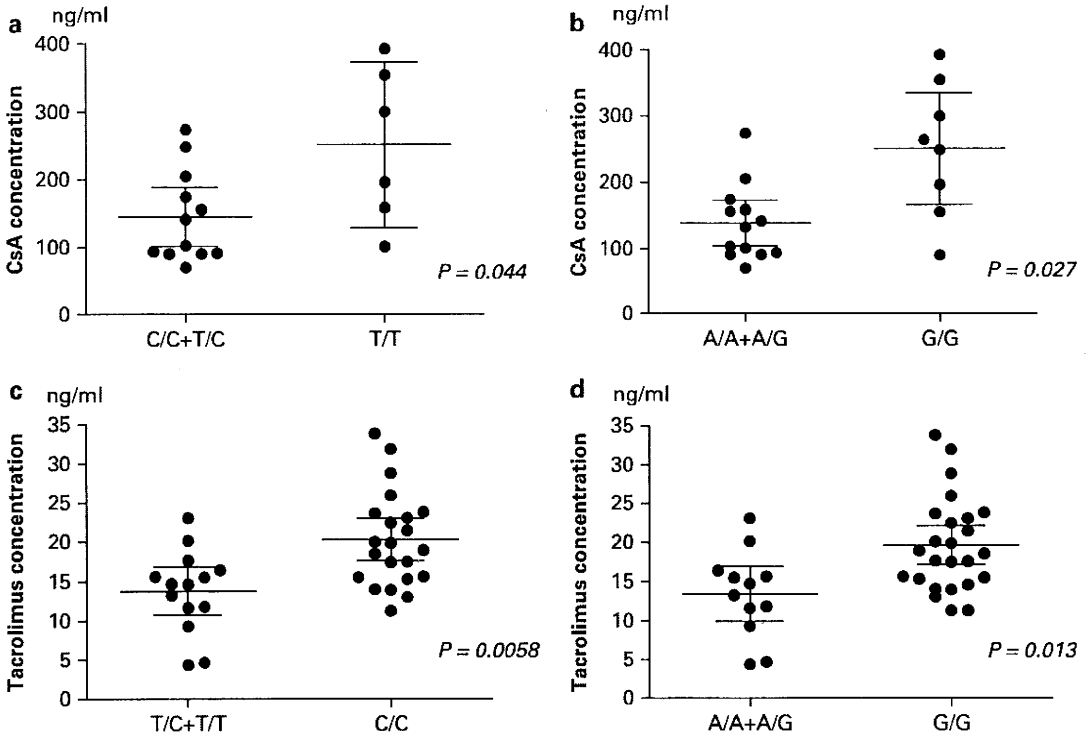

Figure 1 Initial concentration of calcineurin inhibitor and genetic polymorphisms. The initial concentration of CsA was significantly increased in patients with the *CYP3A5* rs15524 T/T genotype (**a**) or the *CYP3A5* rs776746 G/G genotype (**b**) ($P=0.044$, $P=0.027$, respectively). The initial concentration of tacrolimus was also significantly increased in patients with the *CYP3A5* rs4646450 C/C genotype (**c**) or the *CYP3A5* rs776746 G/G genotype (**d**) ($P=0.0058$, $P=0.013$, respectively).

were no significant associations between the initial trough level of CsA and the polymorphisms of *CYP2C19*, *MDR1* and the other investigated *CYP3A5* SNPs.

The tacrolimus concentration was also remarkably influenced by *CYP3A5* SNPs. When the patient had the C/C genotype for *CYP3A5* rs4646450, the tacrolimus concentration was significantly higher than in patients with the T/C or T/T genotype (20.3 ± 2.5 and 13.8 ± 2.7 ng/mL for C/C and T/C+T/T, respectively; mean ± 95% CI; $P=0.0058$; Figure 1c). The *CYP3A5* rs776746 genotype also had a significant association with the serum tacrolimus concentration. When the patient had a G/G genotype, the tacrolimus concentration was higher than in patients who had the A/G or A/A genotypes (19.7 ± 2.4 and 13.4 ± 3.1 ng/mL for G/G and A/G+A/A, respectively; mean ± 95% CI; $P=0.013$; Figure 1d). There were no significant associations between the tacrolimus concentration and the polymorphisms of *CYP2C19*, *MDR1* and other investigated *CYP3A5* SNPs.

Drug dose ratio of day -1 to day 28

To explore the predictive role of the genetic polymorphisms on dose adjustment of the drugs, we compared the initial drug dose with the day 28 drug dose and investigated the drug dose ratio (day 28 dose per day -1 dose) in each patient. The dosage of tacrolimus was remarkably reduced from day -1 to day 28 to maintain the target concentration, when the patient had the *CYP3A5* rs4646450 C/C genotype (39.8 ± 8.4 and $86.1\pm25.5\%$ for C/C and T/C+T/T, respectively; mean ± 95% CI; $P=0.0010$; Figure 2a) and/or the *CYP3A5* rs776746 G/G genotype (41.6 ± 8.2 and $90.5\pm29.4\%$ for G/G and A/G+A/A, respectively; mean ± 95% CI; $P=0.0021$, Figure 2b). There was no association of the drug dose ratio with the *CYP2C19*, *MDR1* or other *CYP3A5* polymorphisms.

Transplantation-related complications and genetic polymorphisms

As renal dysfunction is a common side effect of calcineurin inhibitor treatment, we analyzed the relationship between genetic polymorphisms and renal failure over the 12 months after HSCT. Patients with a creatinine level more than two times over the upper normal limit (men, >2.2 mg per 100 mL; female, >1.6 mg per 100 mL) were diagnosed as having renal failure. Among the CsA and tacrolimus patients, 9/22 (40.9%) and 15/37 (40.5%) developed renal failure, respectively. There were no significant associations between the frequency of renal failure and the genetic polymorphisms. We also investigated these polymorphisms and acute GVHD. There was also no significant association between developing acute GVHD and these genetic polymorphisms.

Discussion

The main aim of genetic association studies involving pharmacogenetics is to avoid treatment toxicity and enhance drug effects.[3,14,15] To date, no studies have been

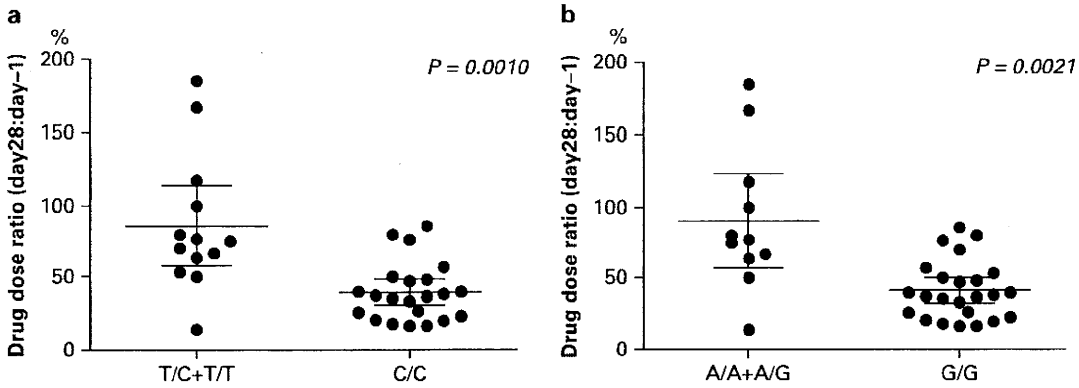

Figure 2 Tacrolimus drug dose ratio (day 28:day -1). The tacrolimus dose ratio comparing day 28 dose to day −1 dose was significantly different according to the *CYP3A5* rs4646450 genotype (**a**) or the *CYP3A5* rs776746 genotype (**b**) ($P=0.001$, $P=0.0021$, respectively).

reported in the field of SCT showing a relationship between complications and genetic polymorphisms of drug metabolism genes. In this study, we have demonstrated that calcineurin inhibitors have a wide range of serum concentrations because of the individual patient's genomic background. On the other hand, we have described a lack of association between the patient's genetic polymorphisms and transplantation-related complications. As we could adjust the drug dosage according to the patient's serum calcineurin inhibitor concentration, the drug monitoring strategy should be effective to reduce the frequency of complications even in the specific genetic backgrounds. In fact, the drug dose ratio results from day -1 to day 28 indicate that a patient who has the *CYP3A5* rs4646450 C/C and/or rs776746 G/G genotype could receive a reduced initial dose of tacrolimus. Thus, patients with the specific genotype tended to receive a reduced dosage, therefore avoiding the drug side effects. Because this consecutive case study included patients with a wide variety of clinical backgrounds, there was no association between clinical outcomes and genetic background. However, strong associations were seen between the drug concentration and genetic background. These results might provide useful information to avoid drug-induced toxicity.

Although calcineurin inhibitors are metabolized mainly by CYP2C and CYP3A, at the same time, these enzymes are also associated with the metabolism of several other drugs.[4,16,17] Because HSCT patients usually need several drugs at the same time to prevent infections and HSCT complications, we must consider all the drug interactions influenced by calcineurin inhibitor metabolism. There are some studies describing a clinically significant drug interaction with calcineurin inhibitors in renal transplants and HSCT recipients.[18–20] In order to reduce the impact of other drug interactions, our study compared the initial concentration of calcineurin inhibitor, because at that time the patients were uniformly administered omeprazole, itraconazole, ciprofloxacin and acyclovir. In our study, the conditioning regimen also did not affect the initial concentration of the calcineurin inhibitor. Therefore, during the early time of HSCT, the concentration of calcineurin inhibitor was influenced by the patient's genetic background rather than drug interactions.

Carriers of a reduced-function *CYP3A5* allele showed significantly higher levels of calcineurin inhibitor in this study. The most well-known functional polymorphism in this study was *CYP3A5* rs776746, which has reduced CYP3A5 enzyme activity when the subject has the G/G genotype.[4] However, our results showed that the SNP rs4646450 within *CYP3A5* also had a significant association, although there have been no reports of the function of the rs4646450 SNP. One genome-wide association study using 550 000 SNPs investigating the maintenance dose of warfarin reported that it was the *CYP3A5* rs4646450 SNP and not rs776746 that had the best association with stabilized warfarin dose.[13] From these results, *CYP3A5* rs4646450 might be associated with common mechanism affecting both warfarin and tacrolimus metabolism.

In conclusion, we have shown that genetic variation has an effect on the pharmacological response to calcineurin inhibitors in HSCT patients. These results are predictable and can therefore help to avoid drug toxicity in the future. Because this study was a serial case study from single institution, the clinical backgrounds of patients were heterogeneous. Moreover, this study was dealing with modest samples and homogeneous genetic background population. In order to define the genetic background influence on clinical outcome of HSCT, it is necessary to investigate further case-controlled study.

Conflict of interest

The authors declare no conflict of interest.

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References

1 U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med* 1994; **331**: 1110–1115.

2 Mayer AD, Dmitrewski J, Squifflet J-P, Besse T, Grabensee B, Klein B *et al.* Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 1997; **64**: 436–443.
3 Weinshilboum R. Inheritance and drug response. *N Engl J Med* 2003; **348**: 529–537.
4 Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J *et al.* Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; **27**: 383–391.
5 Hesselink DA, van Schaik RHN, van der Heiden IP, van der Werf M, Gregoor PJHS, Lindemans J *et al.* Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003; **74**: 245–254.
6 Hesselink DA, van Gelder T, van Schaik RH. The pharmacogenetics of calcineurin inhibitors: one step closer toward individualized immunosuppression? *Pharmacogenomics* 2005; **6**: 323–337.
7 Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; **75**: 13–33.
8 Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour DC *et al.* The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics* 2004; **14**: 147–154.
9 Anglicheau D, Thervet E, Etienne I, Hurault De Ligny B, Le Meur Y, Touchard G *et al.* CYP3A5 and MDR1 genetic polymorphisms and cyclosporine pharmacokinetics after renal transplantation. *Clin Pharmacol Ther* 2004; **75**: 422–433.
10 Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB *et al.* Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 2009; **302**: 849–857.
11 Elmaagacli AH, Koldehoff M, Steckel NK, Trenschel R, Ottinger H, Beelen DW. Cytochrome P450 2C19 loss-of-function polymorphism is associated with an increased treatment-related mortality in patients undergoing allogeneic transplantation. *Bone Marrow Transplant* 2007; **40**: 659–664.
12 Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. *Clin Pharmacokinet* 2010; **49**: 141–175.
13 Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI *et al.* A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood* 2008; **112**: 1022–1027.
14 Tan S-H, Lee S-C, Goh B-C, Wong J. Pharmacogenetics in breast cancer therapy. *Clin Cancer Res* 2008; **14**: 8027–8041.
15 Dai Z, Papp A, Wang D, Hampel H, Sadee W. Genotyping panel for assessing response to cancer chemotherapy. *BMC Med Genomics* 2008; **1**: 24.
16 Stubbins MJ, Harries LW, Smith G, Tarbit MH, Wolf CR. Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics* 1996; **6**: 429–439.
17 de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J Biol Chem* 1994; **269**: 15419–15422.
18 Capone D, Tarantino G, Gentile A, Sabbatini M, Polichetti G, Santangelo M *et al.* Effects of voriconazole on tacrolimus metabolism in a kidney transplant recipient. *J Clin Pharm Ther* 2009; **35**: 121–124.
19 Mori T, Aisa Y, Kato J, Nakamura Y, Ikeda Y, Okamoto S. Drug interaction between voriconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2009; **44**: 371–374.
20 Chang HH, Lee NY, Ko WC, Lee HC, Yang YH, Wu CJ *et al.* Voriconazole inhibition of tacrolimus metabolism in a kidney transplant recipient with fluconazole-resistant cryptococcal meningitis. *Int J Infect Dis* 2010; **14**: e348–e350.

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ORIGINAL ARTICLE

Cytotoxic T-lymphocyte antigen 4 haplotype correlates with relapse and survival after allogeneic hematopoietic SCT

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CTLA-4 is a negative regulator of activated T cells and the association of CTLA-4 polymorphisms with autoimmune diseases and transplant outcome has been reported. We evaluated the effect of donor CTLA-4 polymorphisms on outcome after allogeneic hematopoietic SCT (HSCT). We analyzed 147 Japanese HLA-matched sibling recipients and their donors who had undergone allogeneic HSCT. Genotyping of three single-nucleotide polymorphisms in CTLA-4 (−318, +49, CT60) was performed using TaqMan-PCR. According to the international HapMap database, only these three CTLA-4 haplotypes, classified as C-G-G, C-A-A and T-A-G, are present in the Japanese population. In this study, percentage expression of the C-G-G, C-A-A and T-A-G haplotypes was 59.5, 30.6 and 9.9%, respectively. Recipients of the C-A-A haplotype donor showed a significantly lower risk of relapse (HR: 0.54, 95% CI: 0.30–0.97, $P=0.040$) and a trend toward higher OS (HR: 0.61, 95% CI: 0.36–1.0, $P=0.054$) than did recipients of a donor without the C-A-A haplotype. The presence or absence of the C-A-A haplotype did not affect GVHD or non-relapse mortality. As the presence of the C-A-A haplotype reduced relapse risk and improved survival after allogeneic HSCT, this CTLA-4 haplotype may provide useful information for donor selection.
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Introduction

Allogeneic hematopoietic SCT (HSCT) has been established as an effective treatment for patients with hematological malignancies. GVHD caused by donor-derived T cells is one of the most common causes of morbidity and mortality after allogeneic HSCT.[1] However, donor-derived T cells also mediate a GVL effect, which assists in the eradication of tumor cells.[2] Control of alloimmune reactions and separation of the potent GVL effect from severe GVHD are therefore important for a successful outcome after allogeneic HSCT. Although optimal HLA matching between patients and donors is critical for the prevention of severe GVHD, this can still develop after HSCT from an HLA-identical sibling donor due to non-HLA gene polymorphisms.[3,4] Thus, the association of polymorphisms in genes encoding mHA,[5,6] cytokines,[7,8] chemokines[9] and drug-metabolizing enzymes[10] with transplant outcomes has been reported.

CTLA-4 is a receptor expressed on the surface of activated T cells, and is a homolog of CD28 that is responsible for T-cell activation. Although both CTLA-4 and CD28 bind the two ligands B7.1 (CD80) and B7.2 (CD86) expressed on APCs, CTLA-4 binds B7 molecules with higher affinity and avidity than CD28. CTLA-4 gene polymorphisms correlate with autoimmune diseases such as systemic lupus erythematosus,[11–13] type 1 diabetes mellitus[14,15] and Graves' disease.[16] In addition, recent studies have shown an association of the CTLA-4 polymorphisms (−318 (rs5742909), +49 (rs231775) and CT60 (rs3087243)) with outcome after allogeneic HSCT.[17–19] We therefore focused our study on these three polymorphisms, and analyzed the impact of donor genotypes and haplotypes in the Japanese population on outcome after HLA-identical sibling HSCT.

Patients and methods

Patients

The study population included adult Japanese patients who received hematopoietic stem cells from an HLA-identical sibling donor for the treatment of hematological

Table 1 Patient characteristics

No. of patients	147
Median age in years (range)	39 (15–62)
Sex (M/F)	81/66
Disease	
AML	49
ALL	28
CML	39
Myelodysplastic syndrome	17
Malignant lymphoma	6
Multiple myeloma	5
Others	3
Disease risk	
Standard[a]	81 (55%)
High[b]	66 (45%)
Graft source	
BM	110 (75%)
Peripheral blood	37 (25%)
Preconditioning	
Myeloablative	128 (87%)
Reduced-intensity	19 (13%)
Acute GVHD	
0	82 (56%)
I	37 (25%)
II	19 (13%)
III-IV	9 (6%)
Chronic GVHD	
None	74 (53%)
Limited	11 (8%)
Extensive	54 (39%)
Relapse at 5 years	40%
Non-relapse mortality at 5 years	21%
OS at 5 years	50%

[a]Acute leukemia in first CR, CML in first chronic phase and myeloid displastic syndrome with IPSS score of 1.0 or lower.
[b]More advanced status than standard-risk disease.

malignancies at the Nagoya University Hospital and the Japanese Red Cross Nagoya First Hospital between 1987 and 2006. A total of 147 recipient-donor pairs were selected according to the following criteria: (1) DNA samples and clinical data were available; (2) an unmanipulated graft was transplanted; and (3) short-term MTX and CsA were used as GVHD prophylaxis. MTX was administered i.v. on day +1 (10 mg/m^2) and on days +3 and +6 (7 mg/m^2 each day). CsA was administered by i.v. infusion at a dose of 3.0 mg per kg at beginning on day −1.

Patient characteristics are summarized in Table 1. A total of 81 patients (55%) were classified as having standard-risk disease defined as acute leukemia in first CR, CML in first chronic phase and myelodysplastic syndrome with an international prognostic scoring system (IPSS) score of 1.0 or lower, whereas 66 patients (45%) had high-risk disease defined as disease of more advanced status than standard risk disease. Graft source was BM for 110 patients (75%) and peripheral blood for 37 patients (25%). Conditioning was myeloablative for 128 patients (87%) and reduced-intensity for 19 patients (13%).

Informed consent was obtained from all patients and donors. The study was approved by the ethics committees at the Nagoya University Hospital, the Japanese Red Cross Nagoya First Hospital and the Tokai University School of Medicine.

CTLA-4 genotyping

Genomic DNA was obtained from donor peripheral blood or BM using the QIAamp DNA Blood Mini Kit (QIAGEN sciences, Germantown, MD, USA). The TaqMan PCR method was used to determine the three single-nucleotide polymorphism (SNP) genotypes of CTLA-4: −318 (rs5742909), +49 (rs231775) and CT60 (rs3087243). The respective primers and probes used for the TaqMan PCR were: −318 C/T, forward 5′-AAATGAATTGGACTGG ATGGT-3′ and reverse 5′-TTACGAGAAAGGAAGCC GTG-3′, probe 5′-GTCTCCACTTAGTTATCCAGATCC T[C/T]AAAGTGACATGAAGCTTCAGTTTC-3′; +49 A/G, forward 5′-GCTCTACTTCCTGAAGACCT-3′ and reverse 5′-AGTCTCACTCACCTTTGCAG-3′, probe 5′-G CACAAGGCTCAGCTGAACCTGGCT[A/G]CCAGGA CCTGGCCCTGCACTCTCCT-3′; CT60 A/G, forward 5′-ATCTGTGGTGGTCGTTTTCC-3′ and reverse 5′-CC ATGACAACTGTAATGCCTGT-3′, probe 5′-TCTTCAC CACTATTTGGGATATAAC[A/G]TGGGTTAACACAG ACATAGCAGTCC3′.

PCR reactions were performed in a 10-μL reaction volume containing 1 × TaqMan Universal Master Mix (Applied Biosystems, Tokyo, Japan), 1 μmol of each primer, 1 μL of each probe and 1 μL of genomic DNA. Thermal cycle conditions were 95 °C for 10 min, 40 cycles of 92 °C for 15 s and 60 °C for 1 min. All PCR and endpoint fluorescent readings were analyzed using an ABI7900 sequence detection system (Applied Biosystems).

Statistical analysis

OS was calculated from the date of transplantation to the date of death using the Kaplan–Meier method, and *P*-values were calculated using a log-rank test. EFS was calculated from the date of transplantation to the date of death or relapse, whichever occurred first, and *P*-values were calculated using a log-rank test. Non-relapse mortality (NRM) was defined as mortality due to any cause other than relapse or disease progression. Cumulative incidences of NRM and relapse were estimated using Gray's test, with relapse and NRM, respectively, as a competing risk.

Acute GVHD was diagnosed and graded according to consensus criteria.[20] Chronic GVHD was evaluated in patients who survived beyond day +100, and was categorized as limited or extensive.[21] A multivariate Cox model was created for analysis of grade II-IV acute GVHD, grade III-IV acute GVHD, chronic GVHD, OS, NRM, relapse and EFS using stepwise selection at a significance level of 5%. Age, sex, disease risk, conditioning regimen and graft source were used as covariates, and those variables with a *P*-value of less than 0.2 in the univariate analysis were entered into the stepwise selection method. Hazard ratios of the CTLA-4 haplotype CAA were adjusted using these models. Analysis was carried out using STATA (StataCorp. 2007; Stata Statistical Software: Release 10.0. Special Edition. Stata Corporation, College Station, TX, USA). *P*-values of less than 0.05 were

regarded as statistically significant, and *P*-values between 0.05 and 0.1 as suggesting a trend.

Results

Frequencies of CTLA-4 genotypes and haplotypes

Frequencies at which the three CTLA-4 SNPs were expressed in the 147 donors are listed in Table 2. The SNPs −318 (rs5742909), +49 (rs231775) and CT60 (rs3087243) were included in one haplotype block that was constructed using the international HapMap database. The haplotype analysis revealed only three haplotypes in the Japanese population: −318*C/+49*G/CT60*G (C-G-G), −318*C/+49*A/CT60*A (C-A-A) and −318*T/+49*A/CT60*G (T-A-G). In this cohort, the frequencies of the haplotype C-G-G, C-A-A and T-A-G were 59.5, 30.6 and 9.9%, respectively. All of the donors were distributed among the six haplotype combinations (Table 3).

Effect of the CTLA-4 haplotype C-A-A on transplant outcome

It has been shown that the donor −318 C allele is associated with a lower risk of relapse[19] and that the donor CT60 AA genotype is associated with a lower risk of relapse and a higher OS.[17] We therefore focused our analysis on the C-A-A haplotype, and examined the association between the C-A-A haplotype and the outcome after allogeneic HSCT.

The incidence of grade II-IV acute GVHD was 19% for all patients (Table 1). There was no significant difference between the cumulative incidences of grade II-IV acute GVHD in patients who received stem cells from a donor with the C-A-A haplotype (21%) or from a donor without the C-A-A haplotype (17%) ($P=0.66$) (Figure 1a).

Of 147 patients, 139 could be evaluated for chronic GVHD, and the incidence of chronic GVHD was 47% (Table 1). The incidence of chronic GVHD was not significantly different in the presence or absence of the C-A-A haplotype (51 vs 47%, $P=0.81$) (Figure 1b). Recipients of donors with the C-A-A haplotype showed a significantly lower incidence of relapse (28 vs 45%, $P=0.049$) and a higher OS (58 vs 36%, $P=0.033$) than recipients of donors without the C-A-A haplotype (Figures 2a and b). However, there was no significant difference in NRM between recipients of donors with or without the C-A-A haplotype (17% for both) ($P=0.87$).

Multivariate analyses showed that age >40 years was a risk factor for chronic GVHD, NRM, OS and EFS; that high-risk disease was a risk factor for relapse, OS and EFS; and that reduced intensity conditioning was a risk factor for chronic GVHD and relapse; and that PBSCT was a risk factor for acute and chronic GVHD. The hazard ratios of the C-A-A haplotype, adjusted by these factors, are listed in Table 4. The C-A-A haplotype was significantly associated with a lower relapse rate (HR: 0.54, 95%, CI: 0.30–0.97, $P=0.040$). Additionally, the group with the C-A-A haplotype exhibited trends toward higher OS (HR: 0.61, 95%, CI: 0.36–1.0, $P=0.054$) and EFS (HR: 0.67, 95%CI: 0.41–1.1, $P=0.1$), compared with the group without the C-A-A haplotype. The presence or absence of the C-A-A haplotype did not affect the incidence of acute or chronic GVHD or NRM.

Table 2 Frequency of CTLA-4 genotypes

Polymorphism	n *(%)*
No. of donors	147
−318	
CC	121 (82.4)
CT	23 (15.6)
TT	3 (2.0)
+49	
GG	51 (34.7)
AG	73 (49.7)
AA	23 (15.6)
CT60	
GG	69 (46.9)
AG	66 (44.9)
AA	12 (8.2)

Table 3 Frequencies of the CTLA-4 haplotype

Haplotype	*−318*	*+49*	*CT60*	n *(%)*
C-G-G/C-G-G	CC	GG	GG	51 (34.7)
C-G-G/C-A-A	CC	AG	AG	58 (39.5)
C-G-G/T-A-G	CT	AG	GG	15 (10.2)
C-A-A/C-A-A	CC	AA	AA	12 (8.2)
C-A-A/T-A-G	CT	AA	AG	8 (5.4)
T-A-G/T-A-G	TT	AA	GG	3 (2.0)

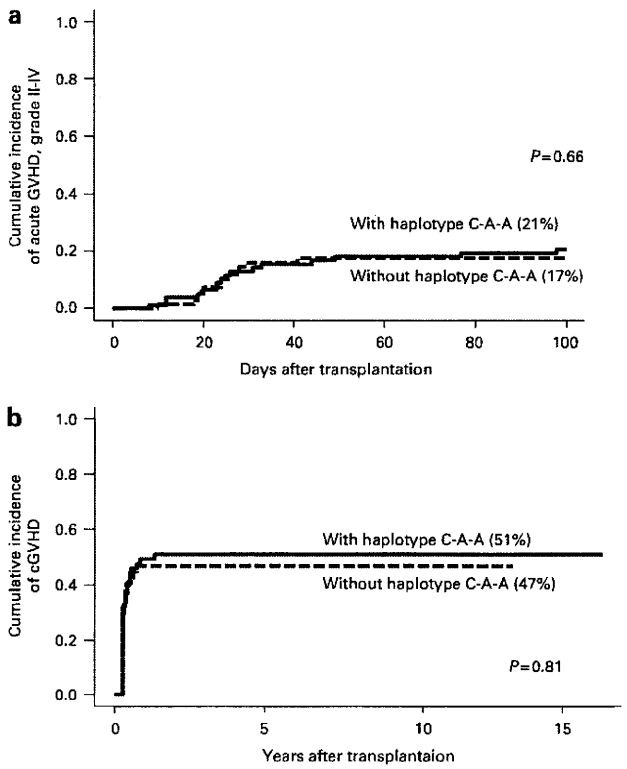

Figure 1 Association of (**a**) the cumulative incidence of grade II-IV acute GVHD and (**b**) chronic GVHD in recipients of donors with (solid line) and without (dotted line) the CTLA-4 C-A-A haplotype.

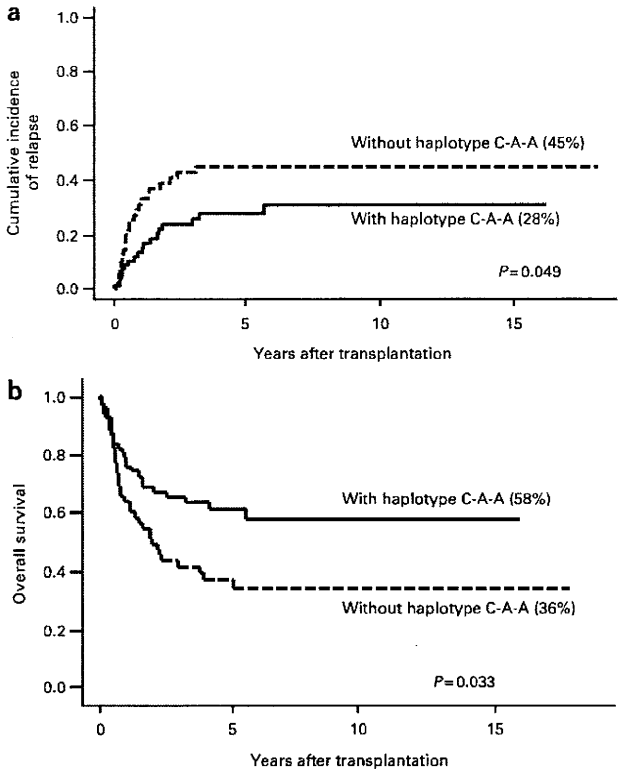

Figure 2 The impact of donors with (solid line) or without (dotted line) the CTLA-4 haplotype C-A-A on the incidence of relapse (**a**) and the OS (**b**) in recipient.

Table 4 Effect of donor CTLA-4 haplotype on transplant outcome

Events	*Factors*	*Multivariate*[a]	
		Hazard ratio (CI)	P-*value*
Acute GVHD Grade II-IV	PBSCT	3.4 (1.6–7.1)	0.001
	Haplotype C-A-A[b]	1.1 (0.53–2.4)	0.77
Acute GVHD Grade III-IV	PBSCT	6.1 (1.5–24)	0.011
	Haplotype C-A-A[b]	1.5 (0.38–6.0)	0.56
Chronic GVHD	Age>40	1.7 (1.0–2.8)	0.05
	PBSCT	2.0 (1.1–3.5)	0.027
	RIC	0.28 (0.11–0.70)	0.0067
	Haplotype C-A-A[b]	1.0 (0.63–1.7)	0.92
Non-relapse mortality	Age>40	2.4 (1.0–5.8)	0.042
	Haplotype C-A-A[b]	0.90 (0.39–2.0)	0.79
Relapse	High risk	2.6 (1.3–4.9)	0.005
	RIC	2.3 (1.2–4.5)	0.014
	Haplotype C-A-A[b]	0.54 (0.30–0.97)	0.04
OS	High risk	1.8 (1.1–3.1)	0.025
	Age>40	1.9 (1.1–3.3)	0.013
	Haplotype C-A-A[b]	0.61 (0.36–1.00)	0.054
EFS	High risk	2.0 (1.3–3.4)	0.004
	Age>40	1.6 (1.0–2.7)	0.046
	Haplotype C-A-A[b]	0.67 (0.41–1.1)	0.1

Abbreviation: RIC = reduced intensity conditioning.
[a]Age, sex, disease risk, conditioning regimen, graft source were used as covariates.
[b]Adjusted by significant factors.

Discussion

Our results highlight the impact of the donor CTLA-4 haplotype−318*C/+49*A/CT60*A (C-A-A) on outcome after allogeneic HSCT from an HLA-identical sibling.

It has been shown that the donor −318 C allele is associated with a lower risk of relapse[19] and that the donor CT60 AA genotype correlates with a lower risk of relapse and a higher OS.[17] Therefore, we focused on the C-A-A haplotype from three different haplotypes in the Japanese population, and examined the association between the C-A-A haplotype and the outcome after allogeneic HSCT. The presence of the CTLA-4 C-A-A haplotype exhibited a significantly lower incidence of relapse and a trend toward of a higher survival rate compared with the absence of the haplotype C-A-A, suggesting that the C-A-A haplotype might suppress the inhibitory function of CTLA-4 on tumor-reactive T cells and enhance the GVL effect.

The mechanism by which the C-A-A haplotype exerts its positive effects is still unclear. Several studies addressing the functional consequences of CTLA-4 SNP-318 and CT60 have been reported. The SNP−318 is located at the CTLA-4 promoter region, and the association of these alleles with promoter activity has been examined. Previous studies showed that the −318 C allele correlates with a lower promoter activity and a lower CTLA-4 expression than those observed with the −318 T allele.[22,23] The *CTLA-4* gene is composed of four exons and has two isoforms: a full-length isoform (flCTLA-4) and a soluble form (sCTLA-4) that lacks exon 3, which encodes the transmembrane domain. Serum levels of sCTLA-4 increase in patients with various autoimmune diseases[24,25] and sCTLA-4 has the potential to bind to CD80/CD86,[26,27] suggesting that sCTLA-4 blocks the interaction of flCTLA-4 with CD80/CD86 and thereby enhances T-cell activation. It has been reported that the CT60 A allele is associated with a higher level of the sCTLA-4 mRNA than the CT60 G allele.[17,28] These results indicate that the −318 C allele and the CT60 A allele contribute to the reduction in CTLA-4 inhibitory function and to T-cell activation. However, association of the +49 A allele with a higher expression of CTLA-4 and augmentation of CTLA-4 inhibitory function has been reported.[16,29] Therefore, further investigation is required to elucidate the effect of the C-A-A haplotype on the anti-tumor activity of donor-derived T cells.

Although the C-A-A haplotype was associated with a low incidence of relapse, in this study it did not affect the incidence of GVHD, suggesting that the C-A-A haplotype may have the potential to separate GVL from GVHD responses. However, it might be because of our small cohort, as Perez-Garcia *et al.*[17] demonstrated that the donor CT60 AA genotype was associated with an increased risk of grade II-IV acute GVHD in a large cohort of 536 donors. All of the patients in our study were Japanese, and many of them (75%) had received BM as a stem-cell graft. It is known that Japanese patients have a lower risk of developing acute GVHD,[30] and that BMT is associated with a decrease in the development of acute GVHD.[31]

Thus, the ethnic population or the stem-cell source might also affect the association between CTLA-4 polymorphisms and the development of acute GVHD.

In summary, the presence of the CTLA-4 C-A-A haplotype reduced the risk of relapse and improved survival after allogeneic HSCT. Therefore, knowledge of the CTLA-4 haplotype may provide useful information for donor selection. The exact effect of the CTLA-4 C-A-A haplotype on transplant outcome should be determined in different cohorts with a substantially larger number of subjects.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med* 1991; **324**: 667–674.
2 Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ *et al.* Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 1990; **75**: 555–562.
3 Dickinson AM. Non-HLA genetics and predicting outcome in HSCT. *Int J Immunogenet* 2008; **35**: 375–380.
4 Hansen JA, Petersdorf EW, Lin MT, Wang S, Chien JW, Storer B *et al.* Genetics of allogeneic hematopoietic cell transplantation. Role of HLA matching, functional variation in immune response genes. *Immunol Res* 2008; **41**: 56–78.
5 Goulmy E, Schipper R, Pool J, Blokland E, Falkenburg JH, Vossen J *et al.* Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. *N Engl J Med* 1996; **334**: 281–285.
6 Nishida T, Akatsuka Y, Morishima Y, Hamajima N, Tsujimura K, Kuzushima K *et al.* Clinical relevance of a newly identified HLA-A24-restricted minor histocompatibility antigen epitope derived from BCL2A1, ACC-1, in patients receiving HLA genotypically matched unrelated bone marrow transplant. *Br J Haematol* 2004; **124**: 629–635.
7 Lin MT, Storer B, Martin PJ, Tseng LH, Gooley T, Chen PJ *et al.* Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med* 2003; **349**: 2201–2210.
8 Keen LJ, DeFor TE, Bidwell JL, Davies SM, Bradley BA, Hows JM. Interleukin-10 and tumor necrosis factor alpha region haplotypes predict transplant-related mortality after unrelated donor stem cell transplantation. *Blood* 2004; **103**: 3599–3602.
9 Inamoto Y, Murata M, Katsumi A, Kuwatsuka Y, Tsujimura A, Ishikawa Y *et al.* Donor single nucleotide polymorphism in the CCR9 gene affects the incidence of skin GVHD. *Bone Marrow Transplant* 2010; **45**: 363–369.
10 Sugimoto K, Murata M, Onizuka M, Inamoto Y, Terakura S, Kuwatsuka Y *et al.* Decreased risk of acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation in patients with the 5,10-methylenetetrahydrofolate reductase 677TT genotype. *Int J Hematol* 2008; **87**: 451–458.
11 Ahmed S, Ihara K, Kanemitsu S, Nakashima H, Otsuka T, Tsuzaka K *et al.* Association of CTLA-4 but not CD28 gene polymorphisms with systemic lupus erythematosus in the Japanese population. *Rheumatology (Oxford)* 2001; **40**: 662–667.
12 Hudson LL, Rocca K, Song YW, Pandey JP. CTLA-4 gene polymorphisms in systemic lupus erythematosus: a highly significant association with a determinant in the promoter region. *Hum Genet* 2002; **111**: 452–455.
13 Lee YH, Harley JB, Nath SK. CTLA-4 polymorphisms and systemic lupus erythematosus (SLE): a meta-analysis. *Hum Genet* 2005; **116**: 361–367.
14 Haller K, Kisand K, Pisarev H, Salur L, Laisk T, Nemvalts V *et al.* Insulin gene VNTR, CTLA-4 +49A/G and HLA-DQB1 alleles distinguish latent autoimmune diabetes in adults from type 1 diabetes and from type 2 diabetes group. *Tissue Antigens* 2007; **69**: 121–127.
15 Balic I, Angel B, Codner E, Carrasco E, Perez-Bravo F. Association of CTLA-4 polymorphisms and clinical-immunologic characteristics at onset of type 1 diabetes mellitus in children. *Hum Immunol* 2009; **70**: 116–120.
16 Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J Immunol* 2000; **165**: 6606–6611.
17 Perez-Garcia A, De la Camara R, Roman-Gomez J, Jimenez-Velasco A, Encuentra M, Nieto JB *et al.* CTLA-4 polymorphisms and clinical outcome after allogeneic stem cell transplantation from HLA-identical sibling donors. *Blood* 2007; **110**: 461–467.
18 Azarian M, Busson M, Lepage V, Charron D, Toubert A, Loiseau P *et al.* Donor CTLA-4 +49 A/G*GG genotype is associated with chronic GVHD after HLA-identical haematopoietic stem-cell transplantations. *Blood* 2007; **110**: 4623–4624.
19 Wu J, Tang JL, Wu SJ, Lio HY, Yang YC. Functional polymorphism of CTLA-4 and ICOS genes in allogeneic hematopoietic stem cell transplantation. *Clin Chim Acta* 2009; **403**: 229–233.
20 Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; **15**: 825–828.
21 Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE *et al.* Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 1980; **69**: 204–217.
22 Ligers A, Teleshova N, Masterman T, Huang WX, Hillert J. CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. *Genes Immun* 2001; **2**: 145–152.
23 Wang XB, Zhao X, Giscombe R, Lefvert AK. A CTLA-4 gene polymorphism at position −318 in the promoter region affects the expression of protein. *Genes Immun* 2002; **3**: 233–234.
24 Sato S, Fujimoto M, Hasegawa M, Komura K, Yanaba K, Hayakawa I *et al.* Serum soluble CTLA-4 levels are increased in diffuse cutaneous systemic sclerosis. *Rheumatology (Oxford)* 2004; **43**: 1261–1266.
25 Wong CK, Lit LC, Tam LS, Li EK, Lam CW. Aberrant production of soluble costimulatory molecules CTLA-4,

CD28, CD80 and CD86 in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2005; **44**: 989–994.
26 Oaks MK, Hallett KM, Penwell RT, Stauber EC, Warren SJ, Tector AJ. A native soluble form of CTLA-4. *Cell Immunol* 2000; **201**: 144–153.
27 Saverino D, Brizzolara R, Simone R, Chiappori A, Milintenda-Floriani F, Pesce G *et al.* Soluble CTLA-4 in autoimmune thyroid diseases: relationship with clinical status and possible role in the immune response dysregulation. *Clin Immunol* 2007; **123**: 190–198.
28 Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G *et al.* Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; **423**: 506–511.
29 Mäurer M, Loserth S, Kolb-Mäurer A, Ponath A, Wiese A, Kruse N *et al.* A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1 +49) alters T-cell activation. *Immunogenetics* 2002; **54**: 1–8.
30 Oh H, Loberiza Jr FR, Zhang MJ, Ringden O, Akiyama H, Asai T *et al.* Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood* 2005; **105**: 1408–1416.
31 Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *J Clin Oncol* 2005; **23**: 5074–5087.

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Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia

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Disease-specific analyses of unrelated cord blood transplantation compared with unrelated bone marrow transplantation in adult patients with acute leukemia

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We made a disease-specific comparison of unrelated cord blood (CB) recipients and human leukocyte antigen allele–matched unrelated bone marrow (BM) recipients among 484 patients with acute myeloid leukemia (AML; 173 CB and 311 BM) and 336 patients with acute lymphoblastic leukemia (ALL; 114 CB and 222 BM) who received myeloablative transplantations. In multivariate analyses, among AML cases, lower overall survival (hazard ratio [HR] = 1.5; 95% confidence interval [CI], 1.0-2.0, $P = .028$) and leukemia-free survival (HR = 1.5; 95% CI, 1.1-2.0, $P = .012$) were observed in CB recipients. The relapse rate did not differ between the 2 groups of AML (HR = 1.2; 95% CI, 0.8-1.9, $P = .38$); however, the treatment-related mortality rate showed higher trend in CB recipients (HR = 1.5; 95% CI, 1.0-2.3, $P = .085$). In ALL, there was no significant difference between the groups for relapse (HR = 1.4, 95% CI, 0.8-2.4, $P = .19$) and treatment-related mortality (HR = 1.0; 95% CI, 0.6-1.7, $P = .98$), which contributed to similar overall survival (HR = 1.1; 95% CI, 0.7-1.6, $P = .78$) and leukemia-free survival (HR = 1.2; 95% CI, 0.9-1.8, $P = .28$). Matched or mismatched single-unit CB is a favorable alternative stem cell source for patients without a human leukocyte antigen–matched related or unrelated donor. For patients with AML, decreasing mortality, especially in the early phase of transplantation, is required to improve the outcome for CB recipients. (Blood. 2009;113:1631-1638)

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) with bone marrow (BM) or peripheral blood, the curative treatment of choice for acute leukemia, is limited by the inadequate supply of human leukocyte antigen (HLA)–identical related donors. Bone marrow from HLA-matched unrelated donors has been a major alternative graft source.[1-3] Umbilical cord blood (CB), an alternative stem cell source to BM or peripheral blood stem cells, has been used primarily in children,[4-10] but its use in adults is increasing.[11,12]

Clinical comparison studies of cord blood transplantation (CBT) and bone marrow transplantation (BMT) for leukemia from unrelated donors in adult recipients showed comparable outcomes.[11-13] Recipients of CBT showed delayed neutrophil recovery and lower incidence of acute graft-versus-host disease (GVHD).[11-13] Overall treatment-related mortality (TRM) was reported to be similar[12] or higher[11] compared with HLA-matched BM. Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are different disease entities that require different chemotherapy regimens for treatment. However, previous comparison studies have included both diseases because of limitation in the number of CBTs given to adults.

In addition, the study periods of previous studies encompass the pioneering period of CBT, when the general practice was to use these grafts in patients in whom there were no other curative options and when the relevance of cell dose and HLA matching had not yet been recognized.[6,7,14]

Accumulation of a larger number of CBT results enabled us to make a controlled comparison with unrelated BMTs. To avoid the inclusion of the pioneering period of CBT, the subjects were limited to those who received transplantations in and after 2000.

Methods

Collection of data and data source

The recipients' clinical data were provided by the Japan Cord Blood Bank Network (JCBBN) and the Japan Marrow Donor Program (JMDP).[15]

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Peripheral blood stem cell donation from unrelated donors is not permitted in Japan. All 11 CB banks in Japan are affiliated to JCBBN. Both JCBBN and JMDP collect recipients' clinical information at 100 days after transplantation. Patients' information on survival, disease status, and long-term complications, including chronic GVHD and second malignancies, are renewed annually by follow-up forms. This study was approved by the data management committees of JMDP and JCBBN.

Patients

Between January 2000 and December 2005, a total of 1690 adult patients at least 16 years of age with acute leukemia (999 AML, 261 CB and 738 BM; and 691 ALL, 178 CB and 513 BM) received first HSCT with myeloablative conditioning either CB or BM from unrelated donors. Of these, patients who received a single CB unit with 0 to 2 HLA mismatches, or HLA-A, -B, -C, and DRB1 allele-matched BM from unrelated donors were analyzed. HLA matching of CB was performed using low-resolution molecular typing methods for HLA-A and -B, and high-resolution molecular typing for HLA-DRB1. Of 1023 BM recipients with complete HLA high-resolution data, the following recipients with HLA HLA-A, -B, -C, and DRB1 allele mismatches were excluded: 306 recipients with 1 of 8 mismatches (39 for HLA-A, 6 for HLA-B, 137 for HLA-C, and 124 for HLA-DRB1), 150 recipients with 2 of 8 mismatches (36 for 2 class I antigens, and 114 for class I and class II antigens), 33 recipients with 3 of 8 mismatches, and 1 recipient with 4 of 8 mismatches. Of 390 recipients of CB with complete HLA data, 95 recipients with 3 mismatches and 8 patients with 4 mismatches were excluded. A total of 484 patients with AML (173 CBTs and 311 BMTs) and 336 patients with ALL (114 CBTs and 222 BMTs) were the subjects for the analyses. Eighty-five centers performed 287 CBTs analyzed in this study, and 114 centers performed 533 BMTs.

Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least 500 cells/mm^3 for 3 consecutive points; platelet recovery was defined by a count of at least 50 000 platelets/mm^3 without transfusion support. Diagnosis and clinical grading of acute GVHD were performed according to the established criteria.[16] Relapse was defined as a recurrence of underlying hematologic malignant diseases. Treatment-related death was defined as death during a continuous remission. Leukemia-free survival (LFS) was defined as survival in a state of continuous remission.

Statistical analysis

Separate analyses were performed for AML and ALL. Descriptive statistical analysis was performed to assess patient baseline characteristics, diagnosis, disease classification, disease status at conditioning, donor-patient ABO mismatches, preparative regimen, and GVHD prophylaxis. The 2-sided χ^2 test was used for categorical variables, and the 2-sided Wilcoxon rank sum test was used for continuous variables. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of neutrophil and platelet recovery, acute and chronic GVHD, relapse, and TRM.[17] For neutrophil and platelet recovery, death before neutrophil or platelet recovery was the competing event; for GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and, for TRM, relapse was the competing event. Gray test was used for group comparison of cumulative incidence.[18] Overall survival (OS) and LFS were calculated using the Kaplan-Meier method. The log-rank test was used for group comparisons. Adjusted comparison of the stem cell source on OS and LFS was performed with the use of the Cox proportional-hazards regression model. For other outcomes, the Fine and Gray proportional-hazards model for subdistribution of a competing risk was used.[19] Adjusted probabilities of OS and DFS were estimated using the Cox proportional-hazards regression model, with consideration of other significant clinical variables in the final multivariate models. The variables considered were the patient's age at transplantation, patient's sex, donor-patient sex mismatch, donor-patient ABO mismatch, disease status at conditioning, and t(9;22) chromosome abnormality or others for ALL, cytogenetic information and French-American-British (FAB) classification of M5/M6/M7 or others for AML, the conditioning regimen, and the type of prophylaxis against GVHD. Factors differing in distribution between CB and BM recipients ($P < .10$) and factors known to influence outcomes (such as patient age at transplantation and chromosome abnormalities and FAB classification of leukemia) were included in the final models. Variables with more than 2 categories were dichotomized for the final multivariate model. The cutoff points of the variables were chosen to make optimal use of the information, with the proviso that smaller groups contain at least 20% of the patients. Variables were dichotomized as follows: patient age greater or younger than 45 years at transplantation, female donor to male recipient donor-recipient sex mismatch versus others for donor-recipient sex matching, donor-recipient ABO major mismatch versus others for ABO matching, M5/M6/M7 FAB classification versus others for classification of AML, chromosome abnormality other than favorable abnormalities for cytogenetics of AML, cyclophosphamide and total body irradiation (TBI) or busulfan and cyclophosphamide or others for conditioning regimen of AML, cyclophospohamide and TBI, or others for conditioning regimen of ALL, and cyclosporine-based versus tacrolimus-based prophylaxis against GVHD. Disease status at transplantation was categorized as first complete remission (1CR), second or later complete remission (2CR), or more advanced disease; which was included in the final model using dichotomized dummy variables. All P values were 2-sided.

The statistical power to detect hazard ratios (HRs) of 2.0 and 1.5 (a regression coefficient equal to 0.6931 and 0.4055, respectively) on Cox regression of the log hazard ratio at a .05 significance level adjusted for event rate were 99% and 78%, respectively, for 484 patients with AML and 97% and 60%, respectively, for 336 patients with ALL. The levels of statistical power for subgroup analyses were as follows: 54% and 22% for 1CR, 51% and 21% for 2CR, 96% and 58% for more advanced in AML patients, 62% and 26% for 1CR, 47% and 20% for 2CR, and 67% and 29% for more advanced in ALL patients.[20]

Results

Patient characteristics

The characteristics of the patients are shown in Table 1. There was no significant difference in recipients' age at transplantation in AML (median age, CB vs BM = 38 vs 38 years, $P = .61$) and in ALL (median age, CB vs BM = 34 vs 32 years, $P = .29$). The female/male ratio was higher (CB vs BM = 54% vs 38% in AML patients, and CB vs BM = 54% vs 38% in ALL patients, $P < .001$ and $P = .005$, respectively) in CB recipients, resulting in the lower donor-patient sex match rate (CB vs BM = 48% vs 69% in AML patients, and CB vs BM = 46% vs 65% in ALL patients, $P < .001$ and $P = .002$, respectively) in CB recipients. The proportion of ALL patients with Philadelphia chromosome abnormality was higher (CB vs BM = 38% vs 23%) in CB recipients. CB recipients were likely to have more advanced disease status at transplantation (relapse or induction failure, CB vs BM = 47% vs 31% in AML patients, and CB vs BM = 26% vs 19% in ALL patients), and the difference was significant in AML ($P = .003$). HLA-A, -B (low-resolution typing), and -DRB1 (high-resolution typing) was mismatched in 93% of both AML and ALL among CB recipients, whereas HLA -A, -B, -C, and -DRB1 were all genotypically matched for BM recipients. The ABO-matched donor-patient pair proportion was consistently lower for CB (CB vs BM = 34% vs 59% in AML patients and CB vs BM = 32% vs 58% in ALL patients).

A preparative regimen with TBI and cyclophosphamide was used in almost all patients, and cytosine arabinoside was supplemented for CB recipients with AML (36%) in addition to TBI and cyclophosphamide. For GVHD prophylaxis, tacrolimus (CB vs BM = 29% vs 56% in AML patients, and CB vs BM = 37% vs 53% in ALL patients) and

Table 1. Characteristics of recipients of cord blood or bone marrow from unrelated donors in 484 patients with acute myeloid leukemia and 336 patients with acute lymphoblastic leukemia

	Acute myeloid leukemia			Acute lymphoblastic leukemia		
Characteristic	**U-CBT**	**U-BMT**	***P***	**U-CBT**	**U-BMT**	***P***
No. of transplantations	173	311		114	222	
Median patient age at transplantation, y (range)	38 (16-69)	38 (16-60)	.61	34 (16-58)	32 (16-59)	.29
Patient sex, n (%)						
Male	80 (46)	194 (62)	< .001	52 (46)	137 (62)	.005
Female	93 (54)	117 (38)		62 (54)	85 (38)	
Sex matching, n (%)			< .001			.002
Matched	83 (48)	216 (69)		52 (46)	145 (65)	
Male to female	44 (25)	57 (18)		35 (31)	42 (19)	
Female to male	46 (27)	37 (12)		27 (24)	35 (16)	
Unknown	0 (0)	1 (0)		0 (0)	0 (0)	
Disease classification						
AML (French-American-British)			.045			
M0	17 (10)	26 (8)				
M1	30 (17)	38 (12)				
M2	52 (30)	88 (28)				
M3	4 (2)	25 (8)				
M4	27 (16)	55 (18)				
M5	23 (13)	41 (13)				
M6	3 (2)	18 (6)				
M7	2 (1)	5 (2)				
Others/unknown	15 (9)	15 (5)				
Cytogenetics			.042			
Favorable*	19 (11)	66 (21)				
Normal	74 (43)	116 (37)				
Other	57 (33)	95 (31)				
Unknown	23 (13)	34 (11)				
ALL cytogenetics						.022
t(9;22)				43 (38)	52 (23)	
t(4;11)				2 (2)	3 (1)	
Others				22 (19)	51 (23)	
Normal				27 (24)	85 (38)	
Unknown				20 (18)	31 (14)	
Disease status			.003			.33
First CR	50 (29)	130 (42)		63 (55)	130 (59)	
Second or after CR	39 (23)	82 (26)		21 (18)	48 (22)	
Relapse/induction failure	81 (47)	95 (31)		30 (26)	42 (19)	
Unknown	3 (2)	4 (1)		0 (0)	2 (1)	
HLA matching†						
0 mismatched loci	12 (7)			8 (7)		
1 mismatched locus	35 (20)			25 (22)		
2 mismatched loci	126 (73)			81 (71)		
ABO matching			< .001			< .001
Matched	59 (34)	185 (59)		37 (32)	128 (58)	
Minor mismatch	48 (28)	57 (18)		30 (26)	48 (22)	
Major mismatch	37 (21)	59 (19)		24 (21)	41 (18)	
Bidirectional	28 (16)	8 (3)		23 (20)	3 (1)	
Unknown	1 (1)	2 (1)		0 (0)	2 (1)	
Nucleated cells infused per 10^7/kg, median (range)	2.44 (1.65-5.49)	26.3 (2.10-58.8)	< .001	2.48 (1.51-4.06)	28.2 (2.30-79.0)	< .001
Preparative regimen			< .001			.38
CY + TBI	43 (25)	142 (46)		42 (37)	92 (41)	
CY + CA + TBI	62 (36)	41 (13)		31 (27)	53 (24)	
CY + BU + TBI	7 (4)	36 (12)		3 (3)	5 (2)	
Other TBI regimen	42 (24)	33 (11)		34 (30)	54 (24)	
BU + CY	18 (10)	55 (18)		4 (4)	12 (5)	
Other non-TBI regimen	1 (1)	4 (1)		0 (0)	6 (3)	
GVHD prophylaxis			< .001			< .001
Cyclosporine A + sMTX	103 (60)	131 (42)		65 (57)	100 (45)	
Cyclosporine A ± other	20 (12)	4 (1)		6 (5)	3 (1)	
Tacrolimus + sMTX	34 (20)	168 (54)		26 (23)	106 (48)	
Tacrolimus ± other	15 (9)	5 (2)		16 (14)	11 (5)	
Others	1 (1)	3 (1)		1 (1)	2 (1)	

U-CBT, indicates unrelated cord blood transplantation; U-BMT, unrelated bone marrow transplantation; CR, complete remission; HLA, human leukocyte antigen; CY, cyclophosphamide; CA, cytarabine; BU, oral busulfan; TBI, total body irradiation; and sMTX, short-term methotrexate.

*Favorable abnormal karyotypes are defined as t(8;21), inv16,or t(15;17).

†Number of mismatches was counted among HLA-A, -B (low-resolution typing), and DRB1 (high-resolution typing).

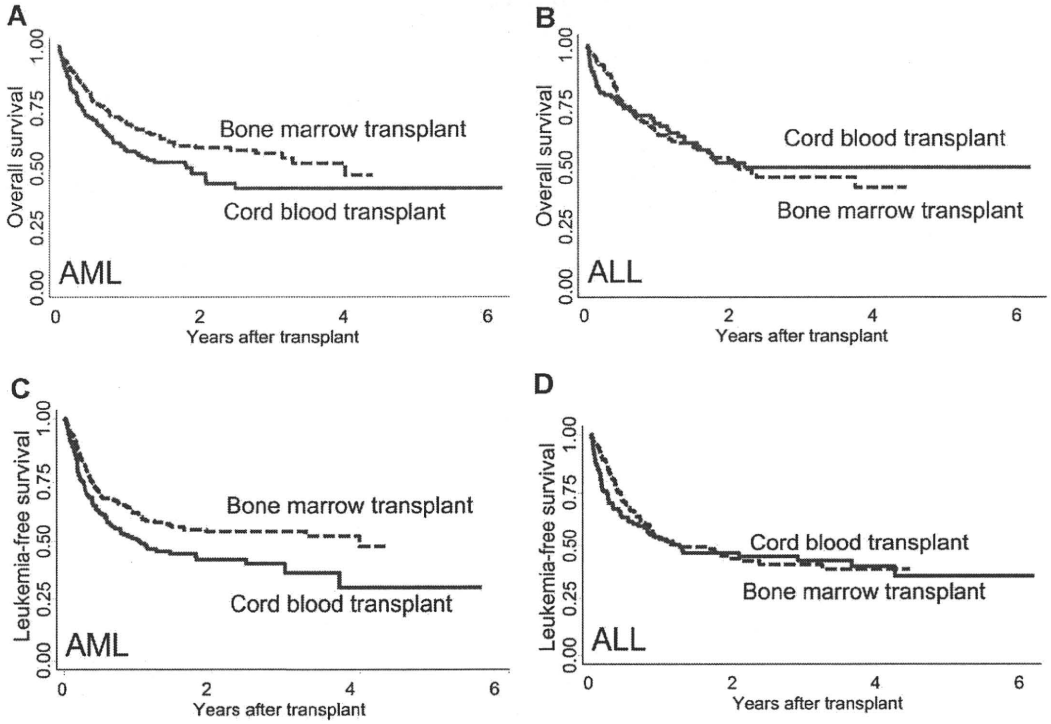

Figure 1. Adjusted OS and LFS of recipients with AML or ALL of CB or BM from unrelated donors. For patients with AML, adjusted probabilities of (A) OS (CB vs BM = 48% vs 59% at 2 years, $P = .010$) and (C) LFS (CB vs BM = 42% vs 54% at 2 years, $P = .004$) were both lower in CB recipients. For patients with ALL, the adjusted probabilities of (B) OS (CB vs BM = 52% vs 53% at 2 years, $P = .99$) and (D) LFS (CB vs BM = 46% vs 44% at 2 years, $P = .41$) were similar between CB recipients and BM recipients.

short-term methotrexate (CB vs BM = 80% vs 96% in AML patients, and CB vs BM = 80% vs 93% in ALL patients) were used preferentially in BM recipients. The median follow-up period for survivors was 1.9 years (range, 0.1-6.2 years) for CB recipients and 1.4 years (range, 0.3-4.5 years) for BM recipients.

Outcome

OS. For patients with AML, the unadjusted probabilities of OS were lower for CB recipients at 1 year (51% vs 69%) and 2 years (43% vs 60%) compared with BM recipients ($P < .001$). For patients with ALL, there were no significant differences between the 2 groups (CB vs BM = 66% vs 66% at 1 year, 49% vs 57% at 2 years, $P = .40$).

Among patients with AML, the use of CB remained a significant risk factor for overall mortality after adjustment for other factors (HR = 1.5; 95% confidence interval [CI], 1.0-2.0; $P = .028$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for overall mortality on multivariate analysis (HR = 1.1; 95% CI, 0.7-1.6; $P = .78$). The adjusted probability of OS was significantly lower for CB recipients (57% vs 69% at 1 year, and 48% vs 59% at 2 years, $P = .010$; Figure 1A) compared with BM recipients for patients with AML, whereas the adjusted probability of OS was similar (69% vs 64% at 1 year, and 52% vs 53% at 2 years, $P = .99$; Figure 1B) between the groups for patients with ALL.

Results of the subgroup analyses showed that the difference in survival among AML patients was prominent in patients demonstrating 1CR at transplantation (RR = 2.9, 95% CI = 1.4-6.2, $P = .005$; Table 3).

LFS. For patients with AML, the unadjusted probabilities of LFS were significantly lower for CB recipients at 1 year (43% vs 62%) and 2 years (36% vs 54%) compared with BM recipients ($P < .001$). For patients with ALL, the unadjusted probabilities of LFS were lower with marginal significance for CB recipients at 1 year (52% vs 58%) and 2 years (45% vs 51%) compared with BM recipients ($P = .06$).

Among patients with AML, the use of CB remained as a significant risk factor for treatment failure (ie, relapse or death) after adjustment for other factors (HR = 1.5; 95% CI, 1.1-2.0; $P = .012$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for treatment failure by multivariate analysis (HR = 1.2; 95% CI, 0.9-1.8; $P = .28$). The adjusted probability of LFS was significantly lower for CB recipients (51% vs 62% at 1 year, and 42% vs 54% at 2 years, $P = .004$; Figure 1C) compared with BM recipients for patients with AML, whereas the adjusted probability of LFS was similar (53% vs 53% at 1 year, and 46% vs 44% at 2 years, $P = .41$; Figure 1D) between the groups for patients with ALL.

Relapse

On univariate analyses, the cumulative incidence of relapse was higher for CB recipients with marginal significance in both AML (27% vs 20% at 1 year, and 31% vs 24% at 2 years) and ALL (27% vs 19% at 1 year, and 31% vs 24% at 2 years) ($P = .067$, and .085, respectively; Figure 2A,B).

On multivariate analyses adjusted by other factors, there was no significantly higher risk of relapse for CB recipients with either AML (RR = 1.2, 95% CI = 0.8-1.9, $P = .38$) or ALL (RR = 1.4, 95% CI = 0.8-2.4, $P = .19$; Table 2).

TRM

For patients with AML, the unadjusted cumulative incidence of TRM was significantly higher for CB recipients at 1 year (30% vs 19%) and 2 years (33% vs 22%) compared with those for BM recipients ($P = .004$; Figure 2C). For patients with ALL, the

Table 2. Results of multivariate analysis of outcomes in 173 recipients of cord blood and 311 recipients of bone marrow with acute myeloid leukemia, and 114 recipients of cord blood and 222 recipients of bone marrow with acute lymphoblastic leukemia

	Acute myeloid leukemia		Acute lymphoblastic leukemia	
Outcome	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
Overall survival*				
BM	1.00		1.00	
CB	1.45 (1.04-2.01)	.028	1.06 (0.71-1.57)	.78
Leukemia-free survival†				
BM	1.00		1.00	
CB	1.48 (1.09-2.01)	.012	1.22 (0.85-1.76)	.28
Relapse‡				
BM	1.00		1.00	
CB	1.21 (0.79-1.87)	.38	1.42 (0.84-2.41)	.19
TRM§				
BM	1.00		1.00	
CB	1.47 (0.95-2.28)	.085	1.01 (0.59-1.73)	.98
Neutrophil recovery‖				
BM	1.00		1.00	
CB	0.41 (0.33-0.51)	< .001	0.37 (0.29-0.48)	< .001
Platelet recovery¶				
BM	1.00		1.00	
CB	0.34 (0.27-0.44)	< .001	0.43 (0.33-0.56)	< .001
Acute GVHD#				
BM	1.00		1.00	
CB	0.80 (0.56-1.15)	.23	0.61 (0.39-0.95)	.028
Chronic GVHD**				
BM	1.00		1.00	
CB	0.94 (0.63-1.42)	.79	1.08 (0.66-1.77)	.77
Chronic GVHD, extensive type††				
BM	1.00		1.00	
CB	0.36 (0.18-0.72)	.004	0.58 (0.28-1.20)	.14

RR indicates relative risk; CI, confidence interval; BM, bone marrow; CB, cord blood; and GVHD, graft-versus-host disease.

*For overall survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

†For leukemia-free survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

‡For relapse, other significant variables for AML were more advanced disease status at conditioning, donor-recipient ABO major mismatch, chromosome abnormality other than favorable abnormalities, and cyclophosphamide and total body irradiation or busulfan and cyclophosphamide conditioning regimen; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and cyclophosphamide and total body irradiation conditioning.

§For TRM, other significant variables for AML were patient age more than 45 years at transplantation, second or after complete remission disease status, more advanced disease status, and chromosome abnormality other than favorable abnormalities; other significant variables for ALL were patient age more than 45 years at transplantation, more advanced disease status at conditioning, and conditioning other than cyclophosphamide and total body irradiation.

‖For neutrophil recovery, other significant variables for AML were second or after complete remission disease status and more advanced disease status; other significant variables for ALL were more advanced disease status at conditioning and cyclosporine-based GVHD prophylaxis.

¶For platelet recovery; other significant variables for AML were second or after complete remission disease status, more advanced disease status, female donor to male recipient donor-recipient sex mismatch, and tacrolimus-based GVHD prophylaxis; other significant variables for ALL were more advanced disease status at conditioning and conditioning other than cyclophosphamide and total body irradiation.

#For acute GVHD, no other significant variables were identified for both AML and ALL.

**For chronic GVHD, other significant variables for AML were more advanced disease status and conditioning other than cyclophosphamide and total body irradiation or busulfan and cyclophosphamide; there were no other significant variables identified for ALL.

††For extensive chronic GVHD, there were no other significant variables identified for AML; another significant variable for ALL was patient male sex.

cumulative incidence of TRM was similar between the 2 groups (CB vs BM = 21% vs 23% at 1 year, 24% vs 25% at 2 years, $P = .83$; Figure 2D).

On multivariate analyses adjusted by other factors, the risk for TRM was higher for CB recipients compared with that for BM recipients among patients with AML (RR = 1.5, 95% CI = 1.0-2.3, $P = .085$; Table 2) with marginal significance. For patients with ALL, the risk for TRM was similar between CB and BM recipients (RR = 1.0, 95% CI = 0.6-1.7, $P = .98$).

Cause of death

Recurrence of the primary disease was the leading cause of death in each group (CB vs BM = 37% vs 33% in patients with AML and 36% vs 41% in patients with ALL). The following causes were infection and organ failure in all groups (Table 4).

Other outcomes of transplantation

Neutrophil and platelet recovery. The unadjusted cumulative incidence of neutrophil recovery or platelet recovery at day 100 was significantly lower in CB recipients for both AML (77% vs 94%) and ALL (80% vs 97%) compared with that among BM recipients ($P < .001$ for both). On multivariate analyses, neutrophil recovery was significantly lower among CB recipients for both AML (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$) and ALL (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$; Table 2).