Brief report

The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure

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Donor-directed human leukocyte antigen (HLA)–specific allo-antibodies (DSAs) cause graft failure in animal models of hematopoietic stem cell transplantation (HCT). Archived pretransplantation sera from graft failure patients (n = 37) and a matched case-control cohort (n = 78) were tested to evaluate the role of DSAs in unrelated donor HCT. Controls were matched for disease, disease status, graft type, patient age, and transplantation year. Patients had acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome; 98% received myeloablative conditioning regimens 100% received T-replete grafts, 97% received marrow, 95% HLA-mismatched, and 97% received calcineurin-based graft-versus-host disease prophylaxis. Among the 37 failed transplantations, 9 (24%) recipients possessed DSAs against HLA-A, B, and/or DP, compared with only 1 (1%) of 78 controls. Therefore, the presence of DSAs was significantly associated with graft failure (odds ratio = 22.84; 95% confidence interval, 3.57-∞; $P < .001$). These results indicate that the presence of pretransplantation DSAs in recipients of unrelated donor HCT is associated with failed engraftment and should be considered in HCT donor selection. (*Blood*. 2010; 115(13):2704-2708)

Introduction

Hematopoietic stem cell transplantation (HCT) recipients may become alloimmunized to foreign human leukocyte antigens (HLAs) through pregnancy or blood transfusions. The resulting sensitization may include antibodies directed against mismatched HLA antigens of a potential stem cell donor. Recent National Marrow Donor Program (NMDP) analyses suggest that greater than 50% of unrelated donor HCTs are mismatched for at least one classic HLA-A, B, C, or DRB1 locus.[1,2] In addition, mismatching at HLA-DP is observed in approximately 88% of all unrelated donor HSCT.[1,2] Engraftment failure is observed at a rate of approximately 5% in unrelated donor HCT, and donor-directed HLA alloantibodies may increase the risk.[3] In a murine model of allo-sensitization, rapid graft failure was shown to result from alloimmune rejection mediated by antibody-dependent cell-mediated killing.[4] Prescreening of patient serum and the identification of specific HLA antibodies could be used as part of a donor selection strategy designed to avoid a potential deleterious incompatibility.

Only a few studies have demonstrated that recipient sensitization to mismatched donor HLA antigens affects engraftment. In a study of marrow transplantations from HLA-mismatched relatives, graft failure occurred in 13 of 21 patients (62%) with a positive pretransplantation cross-match (patient serum vs donor T or B lymphocytes), compared with 31 of 501 patients (7%) with a negative cross-match ($P < .001$).[5,6] Ottinger et al also found that a positive lymphocyte cross-match was a predictor for graft failure and poor survival after HCT from HLA-mismatched donors.[7]

Although a lymphocyte cross-match is an effective tool to evaluate alloimmunization and potential donor/recipient incompatibility, the procedure is labor intensive, may detect non-HLA antibodies, and is logistically difficult for remotely located unrelated donors because of the requirement for live cells. Non-HLA antibodies may be important in HCT; however, studies have not been done that support this point unequivocally. New solid-phase antibody detection technologies can better identify HLA-specific alloantibodies and are more sensitive than cytotoxicity testing and flow cytometry.[8-11] Using these methods, it may be possible to predict alloreactivity against HLA mismatches for unrelated donor recipient pairs before transplantation. Takanashi et al have reported that virtual cross-match–detected DSAs predict graft failure of unrelated umbilical cord blood transplantation.[12]

Methods

We designed a case-control study to retrospectively evaluate the effect of preexisting DSAs on engraftment in unrelated donor HSCT. Thirty-seven cases with available samples were selected based on the failure to achieve engraftment after transplantation and 78 controls that engrafted were selected for comparison. Cases and controls were matched for disease, disease status, patient age, year of transplantation, conditioning regimen, and graft type. Graft failure was defined as never achieving an absolute neutrophil count more than 500 with survival beyond 28 days. Patients had acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome; 98% received myeloablative conditioning regimens, 100% received T-replete grafts, 97% received

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Table 1. Characteristics of patients included in the study using cases (graft failure) versus controls (engraftment)

	Graft failure		Engraftment	
Variable	**No. evaluated**	**No. (%)**	**No. evaluated**	**No. (%)**
No. of patients		37		78
No. of centers		22		33
Median age, y (range)	37	35 (7-53)	78	36 (5-54)
Age at transplantation, y	37		78	
Less than 10		1 (3)		1 (1)
11-20		4 (11)		9 (12)
21-30		9 (24)		18 (23)
31-40		11 (30)		22 (28)
41-50		10 (27)		24 (31)
More than 50		2 (5)		4 (5)
Male sex	37	17 (46)	78	45 (58)
Karnofsky before treatment $>$ 90%	36	26 (72)	76	51 (67)
Overall HLA matching	37		78	
12/12		2 (5)		4 (5)
11/12		4 (11)		11 (14)
10/12		6 (16)		26 (33)
9/12		11 (30)		18 (23)
8/12		7 (19)		8 (10)
7/12		5 (14)		6 (8)
6/12		2 (5)		5 (7)
HLA mismatch distribution				
HLA-A		13 (35)		25 (32)
HLA-B		13 (35)		25 (32)
HLA-C		22 (59)		38 (49)
HLA-DRB1		3 (8)		8 (10)
HLA-DQB1		10 (27)		13 (17)
HLA-DPB1		33 (89)		63 (81)
Disease at transplantation	37		78	
AML		12 (32)		26 (33)
ALL		2 (5)		5 (6)
CML		20 (54)		41 (53)
MDS		3 (8)		6 (8)
Disease status at transplantation	37		78	
Early		13 (35)		28 (36)
Intermediate		14 (38)		30 (38)
Advanced		9 (24)		19 (24)
Other		1 (3)		1 (1)
Stem cell source	37		78	
Bone marrow		36 (97)		76 (97)
PBSC		1 (3)		2 (3)
Conditioning regimen	37		78	
Myeloablative		36 (97)		76 (97)
Reduced intensity		1 (3)		2 (3)
GVHD prophylaxis	37		78	
CsA or FK506 + MTX $\pm$ other		36 (97)		73 (93)
CsA or FK506 $\pm$ other (no MTX)		0		2 (3)
MTX $\pm$ other		1 (3)		2 (3)
Other		0		1 (1)
Donor/recipient sex match	37		78	
Male/male		5 (14)		31 (40)
Male/female		12 (32)		20 (26)
Female/male		12 (32)		14 (18)
Female/female		8 (22)		13 (17)
Donor/recipient CMV match	37		78	
Negative/negative		4 (11)		29 (37)
Negative/positive		15 (41)		24 (31)
Positive/negative		10 (27)		7 (9)
Positive/positive		5 (14)		17 (22)
Unknown		3 (8)		1 (1)
Donor median age, y (range)	37	36 (21-52)	78	35 (18-54)

Table 1 continues

Table 1. Characteristics of patients included in the study using cases (graft failure) versus controls (engraftment) (continued)

	Graft failure		Engraftment	
Variable	**No. evaluated**	**No. (%)**	**No. evaluated**	**No. (%)**
Donor age, y	37		78	
18-29		11 (30)		21 (27)
30-39		12 (32)		35 (45)
40-49		13 (35)		14 (18)
50 and older		1 (3)		8 (10)
Year of transplantation	37		78	
1990		0		1 (1)
1991		0		0
1992		1 (3)		6 (8)
1993		3 (8)		7 (9)
1994		3 (8)		7 (9)
1995		6 (16)		5 (6)
1996		5 (14)		10 (13)
1997		4 (11)		6 (8)
1998		2 (5)		8 (10)
1999		5 (14)		8 (10)
2000		3 (8)		11 (14)
2001		5 (14)		7 (9)
2002		0		2 (3)
Median follow-up of survivors, mo	3	72 (70-74)	21	65 (38-158)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; and MTX, methotrexate.

marrow, and 97% received calcineurin-based graft-versus-host disease prophylaxis (Table 1). Cases and controls were preferentially selected for the presence of at least one HLA mismatch at HLA-A, -B, -C, DRB1, DQB1, or DPB1 to serve as a potential allogeneic target. All HLA typing was verified using high-resolution DNA-based methods as described previously.[13]

Pretransplantation recipient serum samples were obtained from the NMDP Research Repository. All surviving recipients included in this analysis were retrospectively contacted and provided informed consent for participation in the NMDP research program. Research was approved and conducted under supervision of the NMDP Institutional Review Board. A modeling process was used as previously described to adjust for any bias introduced by exclusion of nonconsenting survivors.[1]

The sera were tested in 2 different laboratories using solid-phase microparticle methods with 10% tested in duplicate for quality control purposes. HLA antibody screening was initially performed on all samples by flow cytometry using FlowPRA (One Lambda, Inc). All samples with a positive panel reactive antibody (PRA) were further evaluated using the Luminex-based LABScreen single antigen assay or single antigen flow beads (One Lambda Inc) to determine HLA specificities. Samples were considered positive for specific HLA antigens based on a background-adjusted mean fluorescence intensity of more than 2000 for Luminex-tested samples or a mean fluorescence channel shift of more than 40 for single antigen flow bead assays. DSA assessments were performed by comparing the HLA antibody profile with the mismatched HLA antigens in the graft-rejection direction.

Conditional logistic regression analysis was performed to compare the PRA and DSA positivity rates between the graft failure cases and engrafted controls. Cox regression models were developed to test the impact of HLA-C mismatches, cytomegalovirus (CMV), and cell dose on graft failure rates, as these were not included in the original matching schema.

Results and discussion

The PRA testing found that 37% of all patients carried preexisting HLA class I or II antibodies before transplantation. Forty-three percent of graft failure cases and 32% of engraftment controls possessed a positive PRA, and the distribution by class I and II is noted in Table 2. There was no significant difference in PRA positivity rates between the 2 groups (χ^2, $P = .221$) or an association found between PRA and graft failure using conditional logistic regression (data not shown).

Table 2. Results of univariate analysis comparing the rates of panel-reactive antibody positivity and donor-specific HLA antibody positivity between graft failure cases and engrafted controls

	Class I positive, no. (%)	Class II positive, no. (%)	Class I and/or II positive, no. (%)	*P*
Panel-reactive antibody results				> .221
Cases (n = 37)	16 (43)	10 (27)	16 (43)	
Controls (n = 78)	21 (27)	13 (17)	25 (32)	
Donor-specific HLA antibodies				< .001
Cases (n = 37)				
	A: 1 (3)	DP: 6 (16)	9 (24)	
	B: 4 (11)	DR: 0 (0)		
	C: 0 (0)	DQ: 0 (0)		
Controls (n = 78)				
	A: 1 (1)	DP: 1 (1)	1 (1)	
	B: 0 (0)	DR: 0 (0)		
	C: 0 (0)	DQ: 0 (0)		

Table 3. Results of conditional logistic regression analysis evaluating the association of DSA directed against HLA class I and/or II and graft failure

	Odds ratio	95% confidence interval	*P*
Class I DSA	11.34	1.49-∞	.017
Class II DSA	12.00	1.46-551.97	.014
Class I and/or II DSA	22.84	3.57-∞	< .001

Screening for the presence of DSAs in the PRA-positive graft failure cases and controls found that 8.7% were positive. Among the cases, 24% (9 of 37) carried DSAs, 3 with class I only, 4 with class II only, and 2 with both class I and II specific antibodies (Table 2). In the control group, only one case was positive for DSA (class I and II). The DSA positivity rates were significantly different between the groups (χ^2, $P < .001$). The conditional logistic regression analysis found that the presence of class I (odds ratio [OR] = 11.34; 95% confidence interval [CI], 1.49-∞; $P = .017$), class II (OR = 12.00; 95% CI, 1.46-551.97; $P = .014$), or either class I or II (OR = 22.84; 95% CI, 3.57-∞; $P < .001$) DSAs in the recipients before transplantation was significantly associated with graft failure (Table 3).

Further analyses were conducted using Cox regression models to evaluate a limited set of covariates not accounted for in the case-control matching, including patient CMV status, cell dose, and HLA-C match, because these variables had been associated with graft failure in a previous study of unrelated donor transplantations.[14] Cell dose ($< 2 \times 10^9$/kg) and CMV status (recipient positive) were independently predictive of engraftment ($P = .01$ and .03, respectively). No effect was observed for HLA-C match ($P = .84$). The presence of antidonor HLA class I or II antibodies was still predictive of engraftment failure when adjustment was made for either cell dose (OR = 15.49; 95% CI, 2.06-697.83; $P = .002$) or CMV status (OR = 7.94; 95% CI, 0.97-367.84; $P = .05$).

Interestingly, antibodies against HLA-DP were quite prominent and were present in 60% of antibody-positive failures (Table 2). HLA-DP mismatching has previously been implicated as affecting graft-versus-host disease and lower relapse rates in the T-cell replete myeloablative bone marrow transplantation setting, suggesting its ability to act as a transplantation antigen.[1,15] This suggests that, even though matching for HLA-DP may not be important for overall survival, if a patient has HLA antibodies directed against the mismatched DP type of the donor, there may be an increased risk for graft failure. Although it is possible that a higher cell dose, such as provided by a peripheral blood stem cell graft, could help overcome the engraftment barrier posed by DSAs, there are no clinical data available to define a cell dose threshold. Thus, HLA antibody screening is indicated for potential HCT recipients. Moreover, if HLA antibody is present, a thorough assessment of antibody specificity and donor mismatches is warranted before transplantation.

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Authorship

Contribution: S.S., R.B., and C.A. participated in the conception and design of this study and wrote the paper; S.S., S.F., M.H., and J.K. selected and defined the study population; R.B. and S.R.-B. performed laboratory testing; M.H. and C.V.-G. prepared the dataset for analysis and performed univariate assessments; J.K. performed the multivariate analysis; and S.F., M.H., S.R.-B., C.V.-G., and J.K. reviewed the manuscript.

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