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High Risk of Graft Failure in Patients with Anti-HLA Antibodies Undergoing Haploidentical Stem Cell Transplantation

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Abstract

BACKGROUND—While donor-specific anti-HLA antibodies (DSA) have been implicated in graft rejection in solid organ transplantation, their role in hematopoietic stem cell transplantation (HSCT) remains unclear.

METHODS—To address the hypothesis that the presence of DSA contributes to the development graft failure, we tested 24 consecutive patients for the presence of anti-HLA antibodies determined by a highly sensitive and specific solid-phase/single-antigen assay. The study included a total of 28 haploidentical transplants, each with 2–5 HLA allele mismatches, at a single institution, from 9/2005 to 8/2008.

RESULTS—DSA were detected in five patients (21%). Three out of 4 (75%) patients with DSA prior to the first transplant failed to engraft, compared with 1 out of 20 (5%) without DSA (p=0.008). All 4 patients who experienced primary graft failure had second haploidentical transplants. One patient developed a second graft failure with persistent high DSA levels, while 3 engrafted, 2 of them in the absence of DSA. No other known factors that could negatively influence engraftment were associated with the development of graft failure in these patients.

CONCLUSIONS—These results suggest that donor-specific anti-HLA antibodies are associated with a high rate of graft rejection in patients undergoing haploidentical stem cell transplantation. Anti-HLA sensitization should be evaluated routinely in hematopoietic stem cell transplantation with HLA mismatched donors.

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S.O.C. collected and analyzed the data, contributed to study design and wrote the paper. M.L., M.K., S.G., E.J.S. contributed with patient accrual, reviewed and approved the manuscript. P.C. and M.F.V. performed the antibody testing and contributed to study design and manuscript writing. X.W. and P.F.T. performed the statistical analysis. R.E.C. contributed to data analysis, manuscript writing, reviewed and approved the manuscript.

Keywords

Donor-specific anti-HLA antibodies; primary graft failure; haploidentical stem cell transplantation

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from mismatched relatives represents an alternative treatment for patients with hematologic malignancies when no matched sibling or unrelated donor exists (1–3). Historically, its use has been limited due to the high rates of graft rejection and acute graft-versus-host disease (aGVHD), the latter primarily due to the presence donor T-cells in the graft (4,5). Decreased rates of GVHD have been accomplished with T-cell depletion, but these transplants have been complicated by a high rate of graft rejection. Increased rates of engraftment have occurred with the use of “megadoses” of hematopoietic progenitor cells (2,6). However, approximately 10–20% of patients still develop graft failure (7–9).

The degree of human leukocyte antigen (HLA) matching and the presence of anti-HLA antibodies have been associated with solid organ rejection and survival (10–16). Anasetti and colleagues reported almost 2 decades ago that, primary graft failure (PGF) in patients with unmodified marrow from a mismatched donor was higher (12.3%) compared with patients with a fully HLA matched related donor (2%), and that the incidence of graft failure correlated with the degree of donor HLA incompatibility (17). A positive cross-match for anti-donor lymphocytotoxic antibody associated strongly with graft failure, and the risk of graft failure in alloimmunized patients with a positive donor cross-match was significantly greater then among non alloimmunized patients (39% versus 10%) (17). The association between a positive cross-match and PGF in HSCT has been confirmed by other investigators (18). The use of novel humanized monoclonal antibodies in transplantation may interfere with the classical cross-match method by reacting with donor lymphocytes, and may affect the sensitivity and specificity of the test for the presence of anti-HLA antibodies (19). Preformed antibodies present at the time of marrow infusion in multiply transfused mice, rather than primed T-cells, have been shown to be a major barrier against marrow engraftment (20). The antibody-mediated rejection was extremely rapid in primed mice, and a high bone marrow cell dose could partly overcome the predisposition to graft failure in sensitized mice (20).

In recent years novel solid phase immunoassays (SPI) that utilize purified preparations of molecules corresponding to a single HLA antigen allow accurate identification of HLA-antibody specificities have been developed (21). The information gained from the application of these sensitive technologies can be used to predict cross-match results in solid organ transplantation (16); however, the accuracy of these predictions may be sub-optimal when HLA typing of the donor is incomplete (low resolution, loci not tested).

We proposed that with the advent of accurate HLA typing, the use of SPI with fluorescent-beads coated with single HLA antigens can be applied with high precision for the detection and characterization of donor specific anti-HLA antibodies (DSA) in HSCT, and hypothesized that prospective testing for the presence of DSA detected in this manner would

be associated with a higher risk of primary graft failure after T-cell depleted haploidentical HSCT.

METHODS

PATIENTS

We evaluated the incidence of graft failure in 24 consecutive patients with hematologic malignancies treated at the University of Texas M. D. Anderson Cancer Center (UTMDACC) with a T-cell depleted HSCT from a haploidentical donor tested for the presence of DSA from September 2005 to August 2008. All patients provided informed consent approved by UTMDACC Institutional Review Board according the Declaration of Helsinki. Six patients developed graft failure and five were identified to have DSA. Characteristics of these patients are presented in Table 1.

TRANSPLANT CONDITIONING REGIMEN

All patients received a haploidentical graft from a mismatched related donor (at least 2-antigen-mismatch at HLA-A, -B, -C and DR loci), and “megadoses” of CD34+ stem cells. Peripheral blood progenitor cells were mobilized from donors using filgrastim (Neupogen™, Amgen, Thousand Oaks, CA) and collected by apheresis. CD34+ progenitor cells were positively selected using a CliniMACS system as a means of T-cell depletion of the donor graft (22). A minimum acceptable post-selection CD34+ cell dose was 5.0×10^6 CD34+ cells/kg. The conditioning regimen included melphalan 140 mg/m^2 I.V. administered on day -8, thiotepa 1mg/m^2 I.V. on day -7, fludarabine 160mg/m^2 in divided doses on days -6, -5, -4 and -3 and 1.5 mg/kg/day of rabbit anti thymocyte globulin (ATG) on days -6, -5, -4, and -3 (23). No post transplant immunosupression therapy and no growth factors were administered. All patients received anti-microbial prophylaxis with trimethoprim-sulfamethoxazole, gancyclovir or valacyclovir and voriconazole or caspofungin (23).

Four patients who experienced graft failure received second T-cell replete haploidentical transplants, 3 from the same donor and one with a different donor. Conditioning for the second transplants included alemtuzumab (Campath™, Genzyme, Cambridge, MA) for 3 patients, and fludarabine and ATG the remaining patient. Tacrolimus was used for GVHD prophylaxis.

MEASUREMENT OF ANTI-HLA ANTIBODY LEVELS

The presence of DSA was determined by testing the patients’ sera with a panel of fluorescent-beads coated with single HLA antigen preparations (LABScreen® Single-Antigen, One Lambda, Canoga Park, CA). The anti HLA antibody reactivity was detected in a Luminex platform (Luminex™, Austin, TX) [24]. The reactivity of HLA antibodies was defined by testing two panels of HLA molecules, which included a set of single-antigen preparations for assignment of specificity, and confirmed by comparison with the reactivity against a panel of HLA class I or class II antigen preparations extracted from lymphocytes of single subjects. The final DSA was assessed by comparing the high-resolution types of the donor and antigen panels. Antibody levels were interpreted as normalized fluorescence

intensity (FI) as defined by the kit's manufacturer against DSA mismatch. FI < 500 was considered negative, while positive FI ranging 500–1500, 1500–3000, 3000–7000, and > 7000 were classified as weak, intermediate, strong and very strong, respectively. HLA A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 high-resolution typing was accomplished by PCR amplification combined with nucleotide sequencing. Retrospective MICA typing of one patient and the corresponding donor was kindly performed by oligonucleotide hybridization of PCR products by Dr. Jar How Lee (One Lambda, Canoga Park, CA).

TREATMENT WITH RITUXIMAB AND PLASMA EXCHANGE

After the first patient was identified to have PGF in the presence of DSA, the subsequent four patients were treated with a combination of rituximab (Rituxan™, Genentech, San Francisco, CA) and plasma exchange in an attempt to decrease DSA levels and prevent rejection. Two weekly doses of 375mg/m^2 of rituximab and two sessions of plasma exchange (1 × total plasma volume each) starting two weeks prior to transplant were administered in such patients.

STATISTICAL CONSIDERATIONS

Proportions were compared between groups using Fisher's Exact test (25). The ability of patient covariates (DSA prior to transplant, CD34+ cell count, number of HLA mismatch sites, disease status at transplant) to predict the probability of engraftment was evaluated by exact logistic regression (26). Covariates also were compared between the 20 patients without and 4 patients with graft failure using a Wilcoxon-Mann-Whitney test for quantitative variables or exact test for discrete variables (27). Exact computations were carried out using StatXact and LogXact (Cytel corp.). All the reported p-values are 2-sided.

RESULTS

Twenty four patients were treated with this regimen. Five patients (21%), all females, were identified to have intermediate or strong DSA levels (Table 2). We identified DSA against antigens of HLA class I, class II or both. The most common reactivity was detected against anti-HLA DR (Table 3).

Four (17%) of the 24 patients experienced primary graft failure. Overall, 3 out of 4 (75%) patients with donor-specific anti-HLA antibodies treated with haploidentical transplants developed graft failure, as compared with 1 out of 20 (5%) patients who did not have DSA (p=0.008, Fisher's Exact test). Similarly, initial DSA (prior to the treatment with rituximab and plasma exchange) and first graft failure were also associated (p=0.02, Fisher's Exact test). After the first patient was identified to have graft failure in the presence of DSA, a combination of plasma exchange and rituximab was used to decrease the DSA levels and prevent graft failure. Four patients were treated in this fashion, two patients (#3 and #5) had a significant decrease in the antibody levels and achieved engraftment; one of these patients became negative for DSA while the other had persistence of only low levels of DSA (Table 3). The other two patients (#2 and #4) maintained high levels of DSA and experienced PGF (Table 3).

All 4 patients with graft failure underwent second non-T-cell depleted haploidentical transplants, 3 of them from the same donor; 3 engrafted, 2 without DSA and 1 in the presence of DSA. The remaining patient failed to engraft in the presence of persistent DSA. In one of the patients with graft failure, DSA FI decreased significantly within a few days after transplantation; this patient successfully engrafted after the second transplant (Table 3).

In order to exclude other potential causes for PGF we analyzed the degree of HLA matching, the number of KIR-ligand mismatches in the rejection direction (host-versus-graft, HVG), number of progenitor cells infused, degree of ABO mismatching and the disease status at transplant between the study and the control group (Table 1). No significant differences were identified for any of these parameters (Table 1). Since the number of CD34+ cells were actually higher in patients with primary graft failure (mean=12.47, SD=4.64) than in patients without graft failure (mean=9.62, SD=4.07), the significantly higher graft failure rate in patients with DSA versus without DSA could not be explained by number of CD34+ cells. To assess the possibility that other characteristics may have been associated with graft failure, the probability of graft failure was modeled, in turn, as a function of degree of HLA mismatch (< 3 versus ≥3 antigen mismatch) and the disease status at the time of transplant using exact logistic regression. Neither the number of HLA mismatches (p=1.00), nor remission status at transplant (p=1.00) were associated with the development of PGF.

The fact that all patients with DSA were females prompted a review of the pregnancy history in the 11 female patients in the analysis. The median number of pregnancies was 3 in the PGF group (N=5) as compared with 0 in the control group (N=6) (p=0.10). A review of the transfusion history to assess possible alloimmunization, identified a median of 37 units of packed red blood cells transfused in the DSA group (N=5) compared with 17 units the control group (N=17) (p=0.10).

DISCUSSION

We have analyzed prospectively the relationship between the development of primary graft failure and the presence of donor-specific anti-HLA antibodies in 24 consecutive patients receiving T-cell depleted haploidentical HSCT at a single institution. A high rate of graft failure was identified in patients with DSA present at the time of transplant. Three out of 4 patients with DSA experienced PGF. Interestingly, DSA levels became undetectable in 2 patients shortly after transplant, indicating that they may have been absorbed during this procedure.

Serum/cell cross-matches were not consistently performed during this time. Notably, in the HSCT setting, some patients with hematologic malignancies undergo treatment with various agents like ATG or humanized monoclonal antibodies (like rituximab or alemtuzumab), that may affect the viability of lymphocytes, and interfere with the cross-match testing. Therefore, the use of flow-cytometric or complement dependent cytotoxicity assays in this setting may produce results that are difficult to interpret, as recently reviewed by Zachary and Leffell (19).

The most common DSA identified in this study involved reactivity against the DR molecules carrying a subunit encoded by the DRB1 in the patients who rejected their grafts. The deleterious effect of anti-DR (DRB1) antibodies on engraftment appeared to be as important as the effect of the anti HLA-class I antibodies. The DQ, DRB4 and DP loci encode for proteins that are found at lower density on the cell membrane (low expression). It is possible that the anti-HLA antibodies directed against antigens encoded by DQ, DRB4 and DP are less deleterious, as seen in case #5, in which persistence of high levels against DP was not associated with graft failure (Table 3), probably as the result of a lower target density.

The mechanism by which DSA may cause PGF is debatable. Both humoral and cellular immunity have been invoked as playing a role in graft rejection; however, humoral rejection, through preformed antibodies, appeared to be the primary mechanism responsible for graft failure in models of xenotrasplantation (28). Taylor et al. showed that preformed antibodies in mice can mediate rapid rejection in allogeneic bone marrow transplantation (20). They also demonstrated that antibody neutralization by immunoglobulin administration may facilitate engraftment in allosensitized hosts (20). Removal of preformed antibodies through plasmapheresis and intravenous gammaglobulin has been used to prevent rejection in solid organ transplantation (29), while rituximab, in addition to the elimination of B-cells, can decrease de novo antibody production and facilitate graft tolerance in newly transplanted organs (30). Four of our patients were treated with a combination of rituximab and plasma exchange when high DSA levels were identified. Overall, this intervention decreased the DSA levels substantially in two patients, who both achieved engraftment. Conversely, DSA levels remained at higher levels in 2 patients who both failed to engraft.

No differences were identified between the number of mismatches in the high expression or low expression HLA loci, and the number of KIR-ligand mismatches in the HVG direction, suggesting that graft failure was not mediated by mismatches in the rejection direction. Moreover, patients in the graft failure group received a greater median numbers of CD34+ cells as compared with patients that engrafted, and rejection could not be explained by any other parameters that could have negatively influence engraftment in these patients. Antibodies against Major-Histocompatibility-Complex class I-related chain A (MICA) recently have been recently identified as a cause for graft failure in kidney transplantation (31). Our patients were not tested for MICA antibodies as this study was initiated before such testing was available. In the single case where there was a graft loss with no detectable DSA the patient and the donor had identical MICA types (008,012); therefore, MICA allorecognition cannot be invoked as causing primary graft failure in this case. The cause for graft failure in this case is unknown. The role for allo-reactive NK cells can be ruled out since the donor carried all known inhibitory KIR-ligand mismatches (data not shown) while the effect of allo-reactive T-cells could not be excluded in this case. Future studies that investigate the role of antibodies against MICA are warranted not only in solid organs but also in HSCT.

All 5 patients with DSA were young women, 4 with at least 2 prior pregnancies and all heavily transfused prior to transplant, suggesting that alloimmunization occurred due to at least one of these two events.

We find that the assay utilizing fluorescent-beads coated with the single-antigen mismatch may be a more practical approach for evaluating risk of humoral causes of graft rejection in allogeneic HSCT. Ideally, the mismatched HLA alleles of the donor defined by high-resolution typing methods should be represented in the panel of fluorescent-beads coated with single HLA antigen molecules based on the characterization of the specificity of the antibodies in terms of epitopes born by different HLA alleles.

DSA could also be important in other categories of HLA mismatched HSCT including umbilical cord blood transplantation where the use of units presenting several HLA mismatches is a common practice. Because the levels of HLA expression may differ substantially in hematopoietic stem cells from adults and UCB, studies of HLA antibody effects should be performed separately according to the cell source.

In conclusion, a high rate of graft rejection was observed among patients receiving a T-cell depleted haploidentical hematopoietic stem cell transplant performed in the presence of donor-specific anti-HLA antibodies detected by modern techniques. This suggests that donors should be selected if possible based upon a negative DSA screen. For cases with donor-specific antibodies, an attempt to decrease the antibody levels prior to transplant may be required for successful engraftment. Therapeutic strategies for such cases need to be assessed in prospective clinical trials.

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Table 1

Characteristics and factors known to influence engraftment in 24 consecutive HLA haploidentical stem cell recipients receiving their first transplant.

	Engrafted	Graft failure	p-value
Number	20	4	
Median age (yrs)	33.5	38	0.85
Diagnosis			1.0
AML/MDS	16	4	
ALL	2	0	
CML	1	0	
Lymphoma	1	0	
Disease status at transplant			
Not in remission	12	3	0.59
Alloreactive KIR-ligand mismatch in HVG direction			0.62
0	14	4	
1	5	0	
2	1	0	
Sex mismatch			0.89
Male recipient/female donor	4	0	
Female recipient/male donor	6	2	
Female recipient/female donor	4	1	
Male recipient/male donor	6	1	
CMV mismatch			1.0
Recipient+/Donor+	11	2	
Recipient+/Donor−	8	2	
Recipient−/Donor+	1	0	
Recipient−/Donor−	–	–	
Median # HLA mismatches			
High expression loci (A, B, C, DRB1)	4	4	0.63
Low expression loci (DBR3, 4, 5, DQB1)	1	1	0.47
ABO mismatch			0.27
Compatible	9	0	
Minor	5	2	
Major	4	1	
Minor and major	2	1	
Median # CD34+ cells infused ($\times 10^6$/kg) (range)	8.7 (6–20.8)	12.8 (6.72–17.5)	0.22

AML – acute myeloid leukemia; ALL – acute lymphoblastic leukemia; CML – chronic myeloid leukemia; KIR – killer immunoglobulin-like receptors; HVG – host-versus-graft; CMV – cytomegalovirus; ABO – Blood group mismatches; major mismatch occurs when donor's RBC-ABO blood group is incompatible with the recipient's plasma, minor mismatch occurs when recipients RBC-ABO blood group is incompatible with the donor's plasma.

Table 2

Characteristics of five patients who received a haploidentical stem cell transplant at UTMDACC and had donor-specific anti-HLA antibodies.

Patient #	**1**	**2**	**3**	**4**	**5**
Age	43	37	26	39	50
Gender	F	F	F	F	F
Diagnosis	AML	AML	AML	AML	AML
% BM blasts at transplant	0	13	1	23	4
Donor	Sib	Sib	Sib	Son	Daughter
# HLA mismatches*	3	4	2	5	2
Cell type	PB	PB	PB	PB	PB
# CD34+ cells infused (×10e6/kg)	14.8	6.6	17.5	17.5	9.4
# Pregnancies	0	3	3	2	3
# PRBCs transfused prior to transplant	37	41	17	65	32

F- female; AML – acute myeloid leukemia; BM – bone marrow; Sib – sibling; PB – peripheral blood; PRBCs – packed red blood cells;

* Number of allele mismatches in HLA A, B, C, DRB1, DQB1 loci

Table 3

Donor-specific HLA antibody (DSA) levels and engraftment in 5 patients treated with haploidentical transplantation. Changes in DSA levels after rituximab/plasma exchange and post-transplantation. Patients with moderate to high DSA levels against HLA A, B or DRB1 in the pre-transplant serum rejected their graft.

PT #	AB type[a]	Initial titer[b]	R/PE[c]	After R/PE / Pre 1st SCT[d]	Engr Y/N[e]	After 1st SCT /Pre 2nd SCT[f]	Engr Y/N[g]	After 2nd SCT[h]
1	A*3201	NT*	N/A	++++	N	++++	Y	−
2	A*0211	+ + +	Y	+ +	N	+ +	N	NT
	B*3913	+ + +		+ +		+ + +		
	Cw*0702	+ + +		NT		NT		
	DRB1*0404	+		+		+		
3	DRB1*0701	+ +	Y	−	Y	N/A	N/A	N/A
4	DRB1*0701	+ +	Y	+ + +	N	−	Y	N/A
	DRB4*0101	+ + +		+ + +		−		
	DQB1*0202	+ + +		+ + +		−		
5	DRB1*0401	+ +	Y	+/− (borderline)	Y	N/A	N/A	N/A
	DRB4*0103	+ +		+				
	DPB1*0401	+ + +		+ + +				

[a] HLA alleles mismatched in the donor for which the patient had DSA at the initial work-up

[b] Intensity of donor specific anti-HLA antibodies at the initial work-up

[c] Treatment with rituximab and plasma exchange prior to the first transplant

[d] Strength of donor specific anti-HLA antibodies before the first transplant (after treatment with rituximab and plasma exchange)

[e] Engraftment after the first transplant

[f] Strength of donor specific anti-HLA antibodies after the first transplant and/or before the seconds transplant

[g] Engraftment after the second transplant

[h] Strength of donor specific anti-HLA antibodies after the second transplant

Symbols and abbreviations: - < 500; + 500–1,500; + + 1,500–3,000; + + + 3,000–7,000; + + + + >7,000 fluorescence intensity; PT – patient; AB – antibody ; R – rituximab, PE – plasma exchange, SCT – stem cell transplant; Y – yes; N – no; N/A – not applicable; NT – not tested; NT* - not tested, considered positive for the analysis; Engr – engrafted;