Published in final edited form as:
Biol Blood Marrow Transplant. 2011 November ; 17(11): 1704–1708. doi:10.1016/j.bbmt.2011.04.013.

Anti-HLA Antibodies in Double Umbilical Cord Blood Transplantation

Claudio G. Brunstein[1,2], **Harriet Noreen**[3], **Todd E. DeFor**[1], **David Maurer**[3], **Jeffrey S. Miller**[1,2], and **John E. Wagner**[1,4]

[1] Blood and Marrow Transplantation Program, University of Minnesota

[2] Department of Medicine, University of Minnesota

[3] Immunology/Histocompatibility Laboratory, University of Minnesota

[4] Department of Pediatrics, University of Minnesota

Abstract

Recent registry data suggest that host-versus-graft alloreactions mediated by anti-donor human leukocyte antigen (HLA) antibodies in recipients of adult allogeneic hematopoietic stem cells or single-unit umbilical cord blood (UCB) contribute to the risk of graft failure. The present study evaluated the impact of anti-HLA antibodies on engraftment and unit predominance in 126 double UCB (dUCB) recipients. Eighteen dUCB recipients were identified with at least 1 of 2 UCB units recognized by anti-HLA antibodies directed against donor antigens (DSAs). Overall, 9 of 12 patients who had DSAs against one of the two UCB units composing the graft and 5 of 6 who had DSAs against both units engrafted. The cumulative incidence of engraftment was similar in patients with and without DSAs (83% vs. 78%). Thus, our data do not support the negative effect of anti-HLA antibodies on engraftment at least in the setting of cyclosporine and mycophenolate mofetil and the conditioning regimens employed at the University of Minnesota and argue against routine screening for use in graft selection prior to dUCB transplantation. Further studies are required to fully understand the value of anti-HLA antibody testing in dUCB graft selection and its impact on transplantation outcomes.

Address correspondence to: Dr. Claudio G. Brunstein, Department of Medicine, Mayo Mail Code 480, 420 Delaware Street, S.E., Minneapolis, MN, 55455, USA. Ph: 612 625-3918, Fax: 612 625-6919, bruns072@umn.edu.

AUTHORS CONTRIBUTIONS

Claudio G. Brunstein was involved in the study conception, design, data analysis, draft and approval of the version to be published.
Harriet Noreen was involved in the study conception, data collection and analysis, review and final approval of the version to be published.
Todd E. DeFor was involved in the study conception, performed the data analysis, review and final approval of the version to be published.
David Maurer was involved in the data collection, review and final approval of the version to be published.
Jeffrey S. Miller was involved in the study conception, review and final approval of the version to be published.
John E. Wagner was involved in the study conception, design, data analysis, review and final approval of the version to be published.

Disclosure: The authors have no relevant conflict of interest to disclose.

INTRODUCTION

The presence of donor-directed human leukocyte antigen (HLA)-specific antibodies (DSAs) has long been associated with an increased risk of graft failure in solid organ transplantation [1]. Recent reports indicate that graft failure is associated with the presence of DSAs in recipients of related haploidentical [2] and unrelated adult donor hematopoietic cell transplantation (HCT) [3] and single umbilical cord blood (UCB) transplantation [4,5]. These data suggest that that antibody screening is needed for optimal donor and UCB unit selection.

For more than a decade, the use of two partially HLA-matched UCB units, referred to as double UCB (dUCB) transplantation, has helped extend the use of this stem cell source to adults and larger adolescents for whom an adequate single UCB unit is not available. Still, the rate of neutrophil recovery and hematopoietic engraftment is suboptimal [6,7]. The median time to neutrophil recovery is 26 days, with the risk of graft failure ranging from 5 to 15% [6,7]. Reasons for graft failure are likely multifactorial, and include the frequent utilization of grafts that are mismatched at 4 of 6 HLA loci and/or have low but acceptable cell doses. Because the presence of relevant DSAs may also increase the risk of graft failure after UCB transplantation, we evaluated whether DSAs present in the recipient prior to dUCB transplantation could predict risk of overall engraftment or unit predominance following transplantation.

PATIENTS & METHODS

Study design

This retrospective cohort study included dUCB transplant recipients treated at the University of Minnesota Blood and Marrow Transplantation Clinic between 2004 and 2009. Only patients with cryopreserved sera collected prior to dUCB transplantation and available for anti-HLA antibody analysis, were included in the study. The cohort was divided into two categories based on exposure to DSAs directed against one or both of the donor UCB units. Control subjects were defined as patients exposed to “irrelevant” anti-HLA antibodies not directed against either of the UCB units or patients testing negative for all of the anti-HLA antibodies. Demographic and engraftment data were collected prospectively and recorded in the University of Minnesota Blood and Marrow Transplantation Database. For the purpose of this study, donor engraftment was defined as ≥ 3 consecutive days with an absolute neutrophil count (ANC) ≥ 500/mcL in the presence of ≥ 5% chimerism. Graft failure was defined as failure to achieve an ANC ≥ 500/mcL or <5% chimerism by day +42 post-transplantation. Long-term donor predominance was defined as the UCB unit with chimerism ≥ 70% at day +100 or beyond [8]. All patients were treated on transplantation protocols approved by the University of Minnesota Institutional Review Board and provided written informed consent according to the principles of the Declaration of Helsinki.

HLA typing and antibody determination

All patients and donors were molecularly HLA typed for HLA-A, -B, -C, -DRB1/3/4/5 and –DQB1 at high resolution (allele level). Molecular HLA typing was performed by reverse sequence-specific oligonucleotide probes (SSOP) (LABType SSO, One Lambda, Inc) and by sequence-based typing (SBT) (AlleleSEQR HLA SBT, Abbott Molecular, Inc). Stored plasma samples were retrospectively tested for the presence of anti-HLA antibody directed against an HLA-A, B, C, DRB1/3/4/5 or DQB1 antigen on either UCB unit. As the UCB units were not typed at DPB1, we were unable to consider that locus. HLA antibody specificities were determined by solid phase, single-antigen bead (SAB) technology using sequential testing with first LABScreen Mixed Antigen and then Single Antigen assays if

samples were positive with LABScreen (One Lambda, Inc, Canoga Park, CA). A positive test for an anti-HLA antibody was defined as an increase in mean fluorescence intensity (MFI) of ≥ 500 above the negative control. In order to verify specificity, each lot of reagents was validated against positive patient samples and standard sera. In addition, in each analytic run two standard sera of known specificity and antibody strength from highly sensitized patients were included as positive controls.

Statistical considerations—We used descriptive statistics to evaluate patient demographic characteristics and frequency of DSAs. While we tested for antibodies to high- and low-expression loci, we made no distinction in the analysis. The cumulative incidence of engraftment was estimated by treating early death as a competing risk (deaths prior to day 21 post transplant).[9] The proportional hazards model of Fine and Gray was used to assess the independent effect of anti-HLA antibodies on engraftment controlling for the CD3 dose (by quartile) and HLA match (match versus mismatch)[10] All factors were tested for proportional hazards prior to inclusion in the regression models. Analyses were performed using SAS 9.2 (SAS Institute) and R 2.4 statistical software.

RESULTS

Patient characteristics and anti-donor HLA alloreactivity

A total of 297 patients received a dUCB transplant between 2004 and 2009. Of these, 126 patients had stored plasma available for retrospective testing for the presence of anti-HLA antibodies. Demographic characteristics for these patients are summarized in Table 1. Anti-HLA antibodies were present in 50 (41%) patients in our study group. Of the 50 patients with one or more anti-HLA antibody, only 12 (24%) had a DSA that targeted one UCB unit (Table 2) and 6 (12%) had a DSA that targeted both UCB units (Table 3). Among the patients with a DSA (n=18), 12 had an antibody directed against a class I antigen(s) and 8 against a class II antigen(s). Only 3 patients had DSAs against both HLA class I and II antigens, and all 3 had antibodies targeting both UCB units.

Outcomes of UCB units targeted by DSAs

Of the 12 patients with a DSA directed against one of two UCB units, the targeted unit was detectable at day +21 by chimerism assay in 9 patients, contributing 8–100% to chimerism (Table 2). Notably, in 4 patients, the UCB unit targeted by the DSA predominated long-term as the sole chimeric unit in 3 patients and one of two chimeric units in 1 patient (i.e., dual chimerism). In 3 patients, the MFI for the DSA was ≥ 3,000 with the unit targeted by DSA predominating in 1 patient who engrafted and persisting short-term in another who engrafted with the non-targeted DSA unit. Graft failure occurred in 2 other patients (one with MFI ≥ 3,000) in which the unit targeted by the DSA never or minimally contributed to chimerism. Six patients had DSAs directed against both UCB donor units (Table 3). In 4 patients, only 1 of the 2 targeted UCB units was detectable by chimerism assay at day +21, while in the other 2 patients both units coexisted at day +21. Alloreactivity against both UCB units was particularly intense (MFI ≥3,000) for 4 patients, only one of which experienced graft failure.

Effect of anti-donor HLA alloreactivity on hematopoietic recovery

The cumulative incidence of neutrophil recovery and engraftment for those patients with a DSA directed against at least 1 of the 2 UCB units (n=18) was 78% (95%CI, 59–93%), with a median time to recovery of 24.5 days (range, 5–39). This incidence of recovery was similar to those patients who had an irrelevant anti-HLA antibody (n=32, 84% [95%CI, 70–94%], median of 24 days [range, 3–38]) and those with no antibody at all (n=76, 86% [95%CI, 85–94%], median of 19 days [range, 0–42]) (p=0.54). A multivariate analysis was performed after adjusting for CD3+ cell dose and HLA-matching, both of which are factors

previously shown to be associated with unit predominance after dUCB transplantation [8]. As compared to patients who had no DSA (n=107), no effect could be discerned for the presence of DSA on engraftment (RR 0.68, 95%CI, 0.36–1.29, p=0.24).

DISCUSSION

In the present study the cumulative incidence of engraftment seems to be unaffected by the presence of DSAs in patients transplanted with two partially HLA-matched UCB units. Unlike previous reports in the context of adult unrelated donor allogeneic HCT [3] and single-unit UCB transplantation [4], the presence of DSAs targeting one or both UCB donor units failed to impair engraftment or affect which unit predominated over the long-term. In a study by Takanashi et al. [4], the incidence of neutrophil recovery was only 32% among 20 patients with a DSA directed against the UCB donor unit. Similar results were observed by Spellman et al. [3] in the context of adult unrelated donor allogeneic HCT, where in 9 of 10 patients with a DSA the donor graft failed, and by Ciurea et al. [2] in the context of haploidentical transplantation, where in 3 of 4 patients with a DSA the donor graft failed. In contrast, the majority of the 18 patients (78%) in our study achieved long-term engraftment despite testing positive for a DSA against one or both UCB units. While it could be speculated that our patients were protected from graft failure by the presence of two UCB units, this may be the only reason for the differences in outcome because engraftment was observed in 5 of 6 (83%) patients with antibodies directed against both units. However, in the first 12 patients, 7 of 10 evaluable patients (excluding one patient with early death and the one with autologous recovery) engraftment occurred with the unit against which there was no DSA. Thus, it is possible that while immediate rejection of units against which there is a DSA does not occur, that DSA could be associated with failure to engraft long-term favoring engraftment of the unit that did not have DSA against. One limitation of the present study is the absence of analysis for DSA against HLA-DPB1. It remains possible that antibodies directed against DP or other antigens on UCB influence graft failure or unit predominance. Taken together, our data suggest that donor-specific HLA alloresponses, may not increase the risk of graft failure in dUCB transplantation. It remains to be determined in larger number of patients whether or not DSA influences unit predominance after dUCB transplantation. While screening for DSAs is certainly feasible [11], it could add to health care costs, delay donor acquisition, and may lead to the selection of a donor unit with a lower cell dose or greater HLA mismatch, all of which are factors proven to negatively impact survival. While we recognize that limited numbers of patients may have prevented our ability to detect the impact of anti-HLA antibodies and that different conditioning regimens and post-transplant immunosuppressive therapies may have altered our results, currently we cannot recommend routine anti-HLA antibody screening for UCB unit selection in the setting of dUCB transplantation as this practice could result in the selection of less suitable units. Additional data, in larger numbers of patients, are required to establish a clear association between DSA and graft failure or unit loss in the setting of dUCB transplantation.

Acknowledgments

This work was supported in part by grants from the National Cancer Institute CA65493 (C.G.B, J.S.M., T.E.D. and J.E.W.) and CA77598 (T.E.D.). The Children's Cancer Research Fund (J.E.W. and T.E.D.). American Society of Blood and Marrow Transplantation Robert A. Good New Investigator Award (C.G.B.) and Leukemia and Lymphoma Society Scholar in Clinical Research Award (C.G.B.).

We thank Michael J. Franklin (University of Minnesota) for editing the manuscript.

References

1. Gebel HM, Bray RA. Approaches for transplanting the sensitized patient: biology versus pharmacology. Nephrol Dial Transplant. 2008; 23:2454–2457. [PubMed: 18364369]
2. Ciurea SO, de Lima M, Cano P, et al. High risk of graft failure in patients with anti-HLA antibodies undergoing haploidentical stem-cell transplantation. Transplantation. 2009; 88:1019–1024. [PubMed: 19855248]
3. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. Blood. 2010; 115:2704–2708. [PubMed: 20089963]
4. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. Blood. 2010; 116:2839–2846. [PubMed: 20628152]
5. Takanashi M, Fujiwara K, Tanaka H, Satake M, Nakajima K. The impact of HLA antibodies on engraftment of unrelated cord blood transplants. Transfusion. 2008; 48:791–793. [PubMed: 18366463]
6. Brunstein CG, Barker JN, Weisdorf DJ, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood. 2007; 110:3064–3070. [PubMed: 17569820]
7. Brunstein CG, Gutman JA, Weisdorf DJ, et al. Allogeneic Hematopoietic Cell Transplantation for Hematological Malignancy: Relative Risks and Benefits of Double Umbilical Cord Blood. Blood. 2010; 116:4693–4699. [PubMed: 20686119]
8. Ramirez P, Wagner JE, DeFor TE, et al. The Kinetics of Chimerism and Factors that Predict Single Unit Predominance after Double Umbilical Cord Blood Transplantation. Blood. 2010; 116:105.
9. Lin DY. Non-parametric inference for cumulative incidence functions in competing risks studies. Stat Med. 1997; 16:901–910. [PubMed: 9160487]
10. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999; 94:496–509.
11. Gutman JA, McKinney SK, Pereira S, et al. Prospective monitoring for alloimmunization in cord blood transplantation: “virtual crossmatch” can be used to demonstrate donor-directed antibodies. Transplantation. 2009; 87:415–418. [PubMed: 19202448]

Table 1

Patient and transplant characteristics

Factors	# of patients (%)
Total patients	126
Median Age (range)	45 (18–68)
Male Gender	72 (57%)
Prior Autologous Transplant	25 (20%)
Disease	
Acute leukemia	75 (59%)
CML/MDS	16 (13%)
Lymphoid malignancy	27 (21%)
Other	8 (7%)
High Risk Disease	78 (62%)
GvHD Prophylaxis	
Cyclosporine A/MMF	124 (98%)
Cyclosporine A ± MTX/MP	2 (2%)
Myeloablative Conditioning	44 (35%)
HLA Disparity	
4/6, 4/6 UCB	38 (30%)
4/6, 5/6 UCB	25 (20%)
4/6, 6/6 UCB	1(1%)
5/6, 5/6 UCB	44 (35%)
5/6, 6/6 UCB	6 (5%)
6/6, 6/6 UCB	12 (10%)
Infused Cell Doses	
Nucleated cell dose ($\times 10^7$/kg) Median (range)	3.8 (2–5.9)
CD34+ ($\times 10^5$/kg) Median (range)	4.6 (1.1–13.7)
CD3+ ($\times 10^5$/kg) Median (range)	140 (50–310)
Median Survival Years (range)	2.1 (1.0–4.1)

CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; GvHD, graft-vs.-host disease; MMF, mycophenolate mofetil; MTX, methotrexate; MP, methylprednisolone; HLA, human leukocyte antigen.

Table 2

Patients with DSAs positivity against 1 of the 2 UCB units

UPN (N=12)	Unit	CD34 (×10^6/kg)	Anti-HLA Antibody Specificity for UCB Unit	MFI	Chimerism at Day +21 (%)	Long-Term Predominant Unit	Days to neutrophil recovery*	Outcome
4398	1	0.09	DR51 (DRB5*01,02)	685	100	yes	39	Engrafted
	2	0.18	NONE	NA	0	no		
4423	1	0.17	A03 (A*03)	12951	97	yes	18	Engrafted
	2	0.06	NONE	NA	0	no		
4473	1	0.16	DR14 (DRB1*14)	1044	26	NA	31	Early death
	2	0.13	NONE	NA	45	NA		
4522	1	0.23	A2 (A*02)	612	0	no	19	Engrafted
	2	0.30	NONE	NA	96	yes		
4599	1	0.35	B7 (B*07)	550	100	yes	14	Engrafted
	2	0.30	NONE	NA	0	no		
4735	1	0.14	DQ7 (DQB1*03:01)	841	35	no	6	Engrafted;Persistent Mixed Chimerism**
	2	0.13	NONE	NA	20	yes		
4755	1	0.21	B7 (B*07)	669	0	NA	5	Graft failure; Autologous Recovery
	2	0.29	NONE	NA	0	NA		
4921	1	0.32	A2 (A*02); Cw6 (Cw*06)	3637; 707	48	no	6	Engrafted
	2	0.25	NONE	NA	37	yes		
4929	1	1.14	Cw7 (Cw*07)	515	62	no	25	Engrafted
	2	0.23	NONE	NA	33	yes		
4930	1	0.15	DR04 (DRB1*04:04)	1337	0	no	NA	Graft failure
	2	0.11	NONE	NA	100	yes		

UPN (N=12)	Unit	CD34 (×10^6/kg)	Anti-HLA Antibody Specificity for UCB Unit	MFI	Chimerism at Day +21 (%)	Long-Term Predominant Unit	Days to neutrophil recovery*	Outcome
5003	1	0.28	B35 (B*35)	8308	8	no	NA	Graft failure
	2	0.35	NONE	NA	79	yes		
5057	1	0.11	DR3 (DRB1*03:01,03:02)	536	19	no	25	Engrafted
	2	0.50	NONE	NA	77	yes		

* > 500/mcL.

** while both units are detectable by chimerism long-term, the unit with no DSA directed against it became represents ≥ 70% of it.

Abbr: DSAs, donor specific antibodies; UPN, unique patient number; HLA, human leukocyte antigen; UCB, umbilical cord blood; MFI, mean fluorescence intensity; NA, not applicable, available or achieved.

Table 3

Patients with DSAs against both UCB units

UPN (N=6)	Unit	CD34 (×10^6/kg)	Anti-HLA Antibody Specificity for UCB Unit	MFI	Chimerism at Day +21 (%)	Long-Term Predominant Unit	Days to neutrophil recovery*	Outcome
4591	1	0.44	A23 (A*23); Cw16 (Cw*16)	9853; 889	0	NA	25	Engrafted; Early Relapse
	2	0.13	Cw5 (Cw*05); DR1 (DRB1*01); DQ5 (DQB1*05)	3797; 14177; 13618	47	NA		
4626	1	0.28	A1 (A*01)	2644	0	no	24	Engrafted
	2	0.27	B44 (B*44)	5417	99	yes		
4693	1	0.46	B60 (B*40:01); Cw7 (Cw*07)	3176; 12354	95	NA	NA	Graft failure
	2	0.49	B51 (B*51); Cw15 (Cw*15); DR4 (DRB1*04)	2130; 519; 19112	0	NA		
4777	1	0.18	A2 (A*02)	646	100	yes	9	Engrafted
	2	0.19	A2 (A*02)	646	0	no		
4547	1	0.42	DR4 (DRB1*04:01)	660	31	yes	32	Engrafted
	2	0.31	DR4 (DRB1*04:01)	660	69	no		
4479	1	0.27	A3 (A*03); Cw3 (Cw*03)	6377; 1104	49	yes	24	Engrafted
	2	0.11	A3 (A*03); Cw3 (Cw*03)	6377; 1474	51	no		

* > 500/mcL.

Abbr: DSAs, donor specific antibodies; UPN, unique patient number; HLA, human leukocyte antigen; UCB, umbilical cord blood; MFI, mean fluorescence intensity; NA, not applicable or not available or not achieved.