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Brief Articles

Sustained Donor Engraftment in Recipients of Double-Unit Cord Blood Transplantation Is Possible Despite Donor-Specific Human Leukoctye Antigen Antibodies

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A B S T R A C T

The impact of human leukocyte antigen (HLA) donor-specific antibodies (DSA) on cord blood (CB) engraftment is controversial. We evaluated the influence of pre-existing HLA-antibodies (HLA-Abs) on engraftment in 82 double-unit CB recipients (median age, 48 years) who underwent transplantation for hematologic malignancies. Of 28 patients (34%) with HLA-Abs, 12 had DSA (median mean fluorescence intensity 5255; range, 1057 to 9453). DSA patients had acute leukemia (n = 11) or myelodysplasia (n = 1) and all received either high-dose or reduced-intensity (but myeloablative) conditioning. After myeloablative CB transplantation (CBT) (n = 67), sustained donor engraftment was observed in 95% without HLA-Abs (median, 23 days), 100% with nonspecific HLA-Abs (median, 23 days), and 92% with DSA (median, 31 days, $P = .48$). Of 6 patients with HLA-Abs to 1 unit, 3 engrafted with that unit and 3 with the other. Of 6 patients with HLA-Abs against both units, 1 had graft failure despite being 100% donor, and 5 engrafted with 1 unit. Successful donor engraftment is possible in patients with DSA after myeloablative double-unit CBT. Our data suggest potential deleterious effects of DSA can be abrogated in patients with hematologic malignancies.

INTRODUCTION

Hematopoietic stem cell (HSC) allograft recipients are often allo-immunized. This sensitization may include antibodies (Abs) directed against mismatched HLA of potential donors. Animal models suggest that such Abs may be a barrier to allogeneic engraftment [1,2]. Moreover, graft failure is observed in approximately 5% of unrelated allograft recipients [3], and analyses have suggested this may relate, at least in part, to pre-existing donor-specific Abs (DSA) [4-7]. In cord blood (CB) transplantation (CBT), marked donor-recipient HLA disparity and low cell dose are additional risk factors for graft failure. The ≥ 20% graft failure rates after single-unit CBT [8,9] have been reduced by new conditioning and immunosuppression and by the introduction of double-unit CBT [10,11]. Nonetheless, graft failure has not been eliminated and DSA is an accepted additional risk factor for graft failure in single-unit CBT [12,13]. However, double-unit CBT studies have yielded conflicting results [14-16]. Although some investigators have recommended avoiding units against which the recipient has DSA [12-14,16,17], this practice is controversial. We, therefore, analyzed the influence of HLA-Ab on the likelihood of engraftment and unit dominance in 82 double-unit CBT recipients. Our hypothesis was that the combination of immunosuppressive conditioning, lack of antithymocyte globulin (ATG), and double-unit grafts in patients with hematologic malignancies may abrogate the adverse effects of DSA upon engraftment described in CBT recipients in the literature.

METHODS

Patient and Graft Characteristics

Consecutive first allograft recipients who underwent transplantation with double-unit CB grafts for the treatment of hematologic malignancies consenting to pretransplantation HLA-Abs analysis were analyzed. Patients and/or guardians also provided informed consent to transplantation and outcome analysis. Patients underwent transplantation during the period from July 2008 to July 2012. Patients received high-dose conditioning (n = 21), reduced-intensity but functionally myeloablative conditioning (n = 46, predominantly with cyclophosphamide [Cy] 50 mg/kg, fludarabine [Flu] 150 mg/m^2, thiotepa [Thio] 10 mg/kg, and total body irradiation [TBI] 400 cGy[11] [Cy 50/Flu 150/Thio 10/TBI 400]), or nonmyeloablative conditioning (n = 15).

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Table 1
Patient and Graft Characteristics According to Presence of HLA Antibodies

Characteristics	Total	No Antibody	Antibody	*P* Value
No. of patients	82	54 (66%)	28 (34%)	
Age, median (range), yr	48 (2-69)	48 (2-69)	49 (10-64)	.53
Weight, median (range), kg	70 (15-125)	76 (15-125)	67 (37-93)	.09
Gender, female, n (%)	34 (41%)	14 (26%)	20 (71%)	<.01
Diagnosis, n (%)				
AML	33 (40%)	17 (31%)	16 (57%)	.06
ALL	14 (17%)	9 (17%)	5 (18%)	
MDS & MPD	9 (11%)	6 (11%)	3 (11%)	
Lymphoma & CLL	26 (32%)	22 (41%)	4 (14%)	
Recipient CMV+	47 (57%)	26 (48%)	21 (78%)	.03
Conditioning, n (%)				
High-dose myeloablative	21 (26%)	12 (22%)	9 (32%)	.16
Reduced intensity*	46 (56%)	29 (54%)	17 (61%)	
Nonmyeloablative	15 (18%)	13 (24%)	2 (7%)	
Number of units	164	108	56	
Unit-recipient HLA-A, -B antigen, -DRB1 allele match				
6/6	7 (4%)	4 (4%)	3 (5%)	.12
5/6	78 (48%)	45 (42%)	33 (59%)	
4/6	79 (48%)	59 (55%)	20 (36%)	
Inf. TNC x 10^7/kg				
Larger	2.9 (1.5-5.6)	2.9 (1.5-5.6)	2.9 (1.5-5.5)	.73
Smaller	2.0 (1.1-4.5)	2.0 (1.4-4.5)	2.0 (1.1-2.8)	.46
Inf. $CD34^+$ cell x 10^5/kg				
Larger	1.4 (.3-4.5)	1.5 (.3-4.5)	1.3 (.4-3.0)	.22
Smaller	.7 (.2-1.6)	.7 (.2-1.6)	.6 (.2-1.5)	.43

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder; CLL, chronic lymphocytic leukemia; CMV, cytomegalovirus; Inf, infused; TNC, total nucleated cells.

* Reduced-intensity conditioning was predominantly Cy 50/Flu 150/Thio 10/ TBI 400 in 44 of 46 patients. This regimen is functionally myeloablative.

Immunosuppression was with a calcineurin inhibitor and mycophenolate mofetil, and no patient received ATG. All patients received post-transplantation granulocyte colony-stimulating factor.

CB units were selected based on 4/6 to 6/6 HLA-A, -B antigen, -DRB1 allele match to the recipient, cryopreserved total nucleated cell (TNC) dose $\geq$ 1.5 x 10^7/kg/unit (increased to 2.0 in 2011) [18], and CB bank. Above the TNC dose threshold, HLA match was given priority. Patients and CB units were also typed at HLA-A, -B, -C and -DQ alleles, but high-resolution match grade at 10 alleles was not used in unit selection during this period. Patients and units were not typed for HLA-DP. Additionally, HLA-Abs screening results were not available at the time of unit selection and were, therefore, not considered in selecting the graft.

HLA-Abs Screening

HLA-Abs screening was performed using LABScreen Mixed beads (One Lambda Inc, Canoga Park, CA) that detect Class I/II Abs with a panel coated with purified HLA antigens according to manufacturer's instructions. Test serum (20 μL) and controls were incubated with LABScreen beads (5 μl) in the dark at room temperature for 30 minutes. After 3 washes, R-Phycoerythrin–conjugated goat antihuman IgG was added, followed by incubation and wash. Data acquisition and analysis were performed using Luminex 100 (Luminex Corporation, Austin, TX). Sample reactivity was corrected for nonspecific binding (One Lambda's HLA Fusion software). Positive samples were further tested using LABScreen Single Antigen Class I/II beads. Positivity was defined as adjusted mean fluorescence intensity (MFI) $\geq$ 1000 and derived per laboratory validations [19-21] and external proficiency programs. HLA-Abs profiles were compared with mismatched CB HLA-antigens to identify DSA.

Assessment of Donor Engraftment and Statistical Analyses

Donor chimerism was determined serially on marrow and blood after transplantation as previously described [10]. Sustained engraftment was defined as sustained donor-derived neutrophil recovery with total donor chimerism $\geq$ 90%. The dominant unit was the only 1 detected or the unit contributing $>$ 50% total chimerism in serial testing. Neutrophil recovery was the first of 3 consecutive days with a count $\geq$ .5 x 10^9/L.

Patient and graft characteristics were compared according to HLA-Abs presence using a Wilcoxon rank-sum test for continuous variables and Fisher exact test for categorical variables. Cumulative incidence functions estimated engraftment incidence according to HLA-Abs and Gray's test was used to compare engraftment incidence. All tests were considered statistically significant based on a 2-sided test at alpha level .05. All analyses were done using R statistical software, version 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient and Graft Demographics According to HLA-Abs

Twenty-eight patients (34%) had pre-existing HLA-Ab. HLA-Ab patients were predominantly female and more likely to be cytomegalovirus (CMV) sero-positive (Table 1). Otherwise, there were no differences. Of the 28 patients with HLA-Abs, 26 received myeloablative conditioning (9 high-dose and 17 Cy 50/Flu 150/Thio 10/TBI 400 [11]), and 2 received nonmyeloablative conditioning.

HLA-Abs were donor-specific in 12 patients. These patients were a median age of 51 years (range, 24 to 64) and a median weight of 67 kilograms (range, 51 to 93); 10 were female; 11 had acute leukemia and 1 had myelodysplasia; and all received myeloablative conditioning that was of high dose (Cy 120 mg/kg, Flu 75 mg/m^2, and TBI 1375 cGy [n = 2]) or of reduced intensity but functionally myeloablative (Cy 50/Flu 150/Thio 10/TBI 400 [11] [n = 10]). DSA patients received a median infused TNC dose (x 10^7/kg) of 2.4 (larger unit, range 1.5 to 3.4) and 2.0 (smaller unit, range 1.4 to 2.5).

Nature of HLA-Abs

The nature of HLA-Abs are summarized in Table 2. Fifteen patients (54%) had class I Abs, 2 (7%) had class II Abs, and 11 (39%) had both. Eight patients had HLA-Abs to 1 locus only (4 HLA-A, 3 HLA-B, 1 HLA-DRB1), and the remaining 20 patients had Abs to combinations of HLA-A, -B, -C, and/or -DRB1, -DQ. Overall, 22 patients had HLA-A Abs, 18 had HLA-B Abs, 8 had HLA-C Abs, 10 had HLA-DRB1 Abs, and 8 had HLA-DQ Abs. Additionally, 7 patients also had Abs against HLA-DP, but the potential contribution to engraftment outcome of such Abs was not analyzed, as patients and CB units were not typed for DP.

Sixteen patients had Abs without specificity for either unit of the graft, and 12 had DSA ([median, 1.5/patient; range, 1 to 4], 6 against 1 unit and 6 against both). The median MFI