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Prospective monitoring for alloimmunization in cord blood transplantation: “virtual crossmatch” can be used to demonstrate donor-directed antibodies

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Abstract

Preformed host antibodies may contribute to graft rejection following hematopoietic stem cell transplantation. In cord blood transplantation (CBT), donor-directed host antibodies may be particularly relevant because patients are often markedly mismatched to donors, and limited donor cells preclude cross matching. The recent development of single HLA antigen microbead array assays allows characterization of host alloreactivity to individual HLA antigens with sufficient sensitivity and specificity to allow consideration of “virtual crossmatch” testing as a surrogate for conventional crossmatch testing in the CBT setting. We report results of prospectively monitoring for alloimmunization in our recent CBT experience. Among 46 consecutive patients, four patients (9%) (5 out of 88 units (6%)) had evidence of at least moderate antibodies to HLA antigens on cord units originally selected for transplantation. Virtual crossmatch can be used to screen for donor-directed antibodies in CBT. As possible, units should be changed to avoid sensitized mismatches.

Keywords

Cord blood transplantation; graft rejection; antibodies

Brief report

Graft failure is a significant issue in cord blood transplantation (CBT). Cord blood patients are at increased risk for graft failure by virtue of the low cell dose in the graft as well as often marked HLA mismatching to the donor. Unlike the traditional donor setting, where donor lymphocyte infusion (DLI) may be used to salvage a failing graft, CBT patients have no supply of reserve cells and likely require second transplantation following graft failure. Though recent innovations, including the introduction of double unit transplantation to increase cell dose and augmentation of reduced intensity conditioning (RIC) regimen with additional immunosuppressive therapy, have reduced the incidence of graft failure following CBT, the problem persists. Recent series continue to report graft failures following CBT occurring in at least ten percent of patients.^{1,2,3}

Graft failure in hematopoietic cell transplantation (HCT) may be caused by host anti-donor T and natural killer (NK) cells or host reactive alloantibodies. Among HCT patients, an association of positive anti-donor crossmatch tests with graft rejection was described two decades ago, and recent retrospective analyses demonstrate that donor-directed, HLA-specific alloantibodies in recipients of unrelated and cord blood HCT are predictive of graft failure.⁴⁻⁷ Recent laboratory investigations have further characterized this process and demonstrated that preformed antibody is a significant barrier to bone marrow engraftment in allosensitized mice.⁸

Increasingly refined matching among unrelated HCT donors as well as cross matching of donors with recipients deemed at risk for alloimmunization have abrogated concerns about antibody mediated reactions in the traditional HCT population. However, among CBT patients, HLA antibodies are a potential significant concern. CBT donors are often markedly mismatched with the recipient, creating the possibility of donor-directed HLA antibodies, and the limited donor cells available in cord blood units precludes cross matching.

The recent development of single HLA antigen Luminex and flow beads, as well as the solid phase immunoassays employing single HLA phenotypes assayed on ELISA trays, allows the characterization of host alloreactivity to individual HLA antigens with sufficient sensitivity and specificity to allow consideration of “virtual crossmatch” testing as a surrogate for conventional crossmatch testing in the CBT setting. At our center, all potential CBT patients are screened for the presence of alloantibodies. If antibodies are identified, further testing is performed to characterize the specific antigens against which the patient is sensitized. Donor unit selection is evaluated and altered as possible to avoid use of donors with antigens to which a patient is sensitized. To demonstrate the potential significance of the virtual crossmatch, we report the results of monitoring for donor-specific HLA antibodies in our recent CBT experience.

Between February 2006 and May 2008, 46 patients were scheduled for CBT on protocols for the treatment of high risk malignancies (Table 1). One patient's donor was changed to mismatched unrelated donor because of donor-directed antibodies to cord units.

Patients were first screened for the presence of antibodies against HLA antigens using a panel reactive antibody (PRA) ELISA-based assay in which patient serum was tested against pools of purified class I and class II HLA antigens bound in wells of a plastic microtiter plate (Lambda Antigen Tray for Elisa, One Lambda, Inc. Canoga Park, CA). Serum from patients noted to have evidence of anti-HLA antibodies (PRA positive) prompted further testing to identify the specificity of the antibodies generated using panels of color coded plastic microspheres each coated with a single purified class I or class II HLA antigen (LABScreen Single Antigen HLA Class I and II Antibody Detection Kit, One Lambda, Inc. Canoga Park, CA). Patients' individual antibody profiles were reviewed in order to identify antibodies directed against HLA antigens expressed on selected cord units. The strength of reactivity of each pertinent antibody was evaluated as a function of the mean fluorescence intensity (MFI) of the individual bead carrying the relevant antigen. If moderate levels of donor-directed antibodies (MFIs greater than 4000) were identified, alternative donors were sought. The establishment of MFI thresholds was based on preliminary validation studies using external proficiency testing samples which suggested that MFIs above 4,000 correlated with positive cytotoxic crossmatches for Class I antigens. Among 24 samples tested, six samples had no reactivity against any of the single antigen Luminex beads tested and all six had negative clinical cytotoxic crossmatches with randomly selected external proficiency cells. Thirteen samples generated MFIs in the 5,700-15,000 range with select Luminex single antigen beads; all 13 had positive clinical cytotoxic crossmatches with external proficiency cells bearing the corresponding antigens. The remaining five samples had MFIs between 1,000 to 3,000 with select Luminex single antigen beads; two of these

samples had negative and three had borderline positive clinical cytotoxic crossmatches with cells bearing the relevant antigen. In sera containing antibodies directed against Class II antigens, obtaining a positive cytotoxic crossmatch required higher MFI thresholds with the relevant single antigen beads, but insufficient data were available to establish correlation.

The alternative CB units that were selected were required to meet established cell dose and matching thresholds. If no alternative donors were available, surrogate crossmatching was performed with blood from a donor with the antigen against which the patient was sensitized to help assess the risk of antibody-mediated rejection. Final decisions regarding the use of a unit against which a patient was sensitized were based on the clinical situation (Figure 1).

Engraftment was defined as the first of three consecutive days with absolute neutrophil count (ANC) greater than 500 thousand/uL. Primary graft failure was defined as absence of 3 consecutive days with absolute neutrophil count (ANC) greater than 500 thousand/uL by day 42 post-transplantation without signs of imminent engraftment. Chimerism studies were performed beginning day 21 post transplantation.

Among 46 patients screened, 11 patients (24%) had a positive PRA. Upon subsequent investigation, four patients (9%) had evidence of at least moderate antibodies to HLA antigens expressed on 5 cord units of the 88 (6%) originally selected for transplantation. Forty-two of these patients received double unit CBT; four patients received single unit CBT. (Table 1) Treatment courses for the four patients affected by antibody monitoring are summarized below. Within this cohort, no primary graft failure has occurred and one patient (1/39 (3%) evaluable patients) experienced secondary graft failure. Seven patients are not evaluable, six due to death before day 42 without engraftment and one due to donor source change to mismatched unrelated donor because of donor-directed antibodies to cord units.

Patient 1

The patient was planned for double CBT with two 5/6 matched units. Both units expressed HLA-B49, to which the patient had strong antibody (Figure 2). No alternative adequate units were identified. A 9/10 single allele B mismatched unrelated donor was identified with negative crossmatch, and the donor source was switched. The patient engrafted neutrophils day 26 following peripheral blood stem cell transplantation.

Patient 2

One of the original units selected for double CBT expressed HLA-DRB1*0101 to which the patient had moderate antibody. An alternative safe unit not expressing HLA-DRB1*0101 was selected. The patient engrafted neutrophils day 16 following CBT.

Patient 3

The patient expressed HLA-DRB1*12 and had moderate DQ7 antibodies. The patient's DQ typing was DQB1*06ABYZ, DQB1*0501. The patient was African American, and in African Americans HLA-DRB1*12 is commonly linked to DQB1*0501. In Caucasians, however, HLA-DRB1*12 is commonly linked with HLA-DQB1*0301 (serologic DQ7). The patient's cord units were of mixed ethnicity, including Caucasian, and both units expressed DRB1*12. High resolution DQ typing was therefore performed on both units. One unit was found to express HLA-DQB1*0301. Surrogate crossmatching with a DQB1*0301 donor was performed by cytotoxicity and flow cytometry. The cytotoxic crossmatch was negative and flow cytometric crossmatch, though slightly elevated (median channel shift 66), was below the positive threshold of 94. No alternative donors were rapidly available, and transplantation was performed because the patient had refractory leukemia and active infection. The patient died

day 12 following transplantation with no evidence of engraftment. No chimerism tests were performed.

Patient 4

One of two units for double CBT expressed HLA-Bw6 to which the patient had moderate antibody. No alternative units were available, and the unit was used in transplantation. The patient engrafted neutrophils day 24 following RIT. No evidence of the unit expressing HLA-Bw6 was ever present on chimerism studies, which began day 21 post transplantation.

To our knowledge, we are the first to report on using virtual crossmatch prospectively to monitor for alloimmunization among CBT patients. Using the single HLA antigen bead technology, we have demonstrated the presence of at least moderate antibodies to antigens on cord units originally planned for transplantation in four out of 46 (9%) patients (5 out of 88 units (6%)).

Because we are committed at our center to avoiding units with donor-directed antibodies, establishing a direct link between donor-directed antibodies and graft failure will be challenging. However, we believe that the association between alloimmunization and graft failure is sufficiently well-established in HCT to warrant our policy. Importantly, this association has been confirmed in a recent retrospective study examining the relationship between donor-directed antibodies and graft failure in CBT. Takanashi et al found that in CBT patients without evidence of donor-directed antibodies engraftment rates were 93%, while in patients with evidence of donor-directed antibodies engraftment rates were 58%.⁴

Our graft failure rate (no primary graft failures and 1/37 secondary graft failure among evaluable patients) is lower than that reported at other centers,^{1,2,3} and while numerous confounding factors may contribute to this observation, it may be in part due to screening for alloantibodies. Additionally, our data suggest that it is possible that one of the reasons that double unit CBT has been reported to significantly increase engraftment rates⁹ is that engraftment can still occur if at least one of the two units is crossmatch compatible with the patient. In three of the four cases in which we have identified relevant alloantibodies in patients, the antibodies have been to only one of two transplanted units.

Although our report does not directly demonstrate the clinical relevance of these donor-specific antibodies, our preliminary analysis suggests that Luminex single antigen bead MFIs can be correlated with the likelihood of a positive cytotoxic crossmatch. Larger validation studies will be necessary to optimize the sensitivity and specificity of MFI thresholds for specific antigens, and different centers may prefer different receiver operating characteristics. The significance, moreover, of low level donor-directed antibodies below the correlative threshold for a positive cytotoxic crossmatch is uncertain and remains to be explored.

Because our series is small, larger numbers will be required to better define the true incidence of donor-directed antibodies. In their recent retrospective study, Takanashi et al. reported donor-directed antibodies in 3% of 285 units tested though no threshold for defining positive testing was reported.⁴

Alloimmunization and host reactive HLA antibody may be an important factor influencing graft failure in CBT. Donor-directed antibodies occur in only a minority of patients, but among these patients, ample evidence suggests that graft failure is significantly more likely. We believe CBT patients with evidence of alloreactivity should be screened for donor-directed antibodies by a high sensitivity and high specificity assay as described here. In the presence of a positive virtual crossmatch, we suggest that an appropriate alternative donor be sought.

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Figure 1. Algorithm for anti-HLA antibody monitoring and interpreting the virtual crossmatch

Figure 2. Analysis of the serum of patient 1 for Class I anti-HLA antibodies by flow analysis of HLA conjugated micro beads

Values in the Y-axis (mean fluorescence intensity (MFI)) are an indication of the strength of the antibody. Patient 1 demonstrates strong HLA-B49 antibodies (MFI>9000). HLA-B49 was present on both units originally planned for transplantation.

Table 1

Summary of results of antibody screening

Number of patients screened	46
Ages, median (range)	28 (0.8-68)
Diseases	
AML	23
ALL	12
CML	3
AML/MDS	5
MDS	2
Mycosis Fungoides	1
Myeloablative transplants ^a	31 (70%)
RIT transplants ^b	14 (30%)
Number of patients with positive PRAs	11 (24%)
Ages, median (range)	39 (11-64)
Diseases	
AML	6
ALL	1
CML	1
MDS	1
AML/MDS	1
Number of patients with PRAs affecting unit selection	4/46 (9%)
Number of units with donor-directed antibodies	5/88 (6%)
Primary graft failures in cohort	0 *
Secondary graft failures in cohort	1 (3%) *

* Six patients undergoing CBT not evaluable due to death prior to day 42 without engraftment

^aConditioning: fludarabine 75 mg/m², cyclophosphamide 120 mg/kg, TBI 1320 cGy

^bConditioning: fludarabine 200 mg/m², cyclophosphamide 50 mg/kg, TBI 200 cGy +/- ATG