Effects of HLA-Matched Blood Transfusion for Patients Awaiting Renal Transplantation

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Background. HLA sensitization in potential renal transplant recipients hinders opportunities of receiving suitable organs. To alleviate this, we sought to determine if supplying closely HLA Class I matched leukodepleted blood would minimize sensitization.

Methods. Patients received HLA selected or random units of packed red cells. Selected units were sourced from blood donors included in the British Bone Marrow Registry and had no HLA-A and HLA-B mismatches where available, or alternatively, no HLA antigens with more than five immunogenic triplet mismatches as determined by the HLA-Matchmaker algorithm. Posttransfusion antibody screening confirmed development of de novo Class I and Class II HLA-specific IgG antibody(s) or increases in preexisting antibody levels of at least 20%.

Results. Thirty-seven and 31 patients received HLA selected (mean, 2.5 units) and random (mean, 3.4 units) blood, respectively. A total of 20 of 37 (54.1%) patients receiving selected units and 10 of 31 (32.3%) patients receiving random units were previously sensitized. No patient receiving HLA selected units demonstrated any change in antibody levels. In patients who received random units, 7 of 31 demonstrated changes in antibody levels with three developing de novo HLA-specific antibodies and four an increase in panel reactive antibody (PRA) of at least 20% (P=0.002).

Conclusions. The risk of developing HLA-specific antibody is significantly reduced in renal patients awaiting transplantation when transfused with HLA selected units of blood compared with random units. With planning, access to HLA typed blood is achievable as many blood transfusion centers recruit donors for stem cell donor registries.

Keywords: HLA matched blood, HLAmatchmaker, Renal transplantation, Antibody sensitization.

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The presence of preformed donor-specific HLA antibody (DSA) is associated with graft loss after renal transplantation (*1*). Antibody formation can occur after exposure to foreign HLA antigen as a consequence of pregnancy, solid organ transplantation, or blood transfusion. This can limit the opportunities of patients with end-stage renal disease from receiving a kidney transplant. Minimization of HLA-specific antibody formation is therefore good practice particularly in view of increasingly long waiting times for suitable deceased donor organs. Modern immunosuppressive therapy and desensitization regimens now facilitate transplantation across a peak positive crossmatch if DSA levels are absent or at very low levels in current serum samples (*2, 3*). Prevention of de novo HLA-specific antibody formation and maintenance of the lowest possible antibody levels in renal patients will maximize opportunities for successful transplantation.

It is not possible to influence HLA-specific antibody development occurring through pregnancy or previous transplantation; however, through selection of suitably HLA matched blood, it should be possible to reduce sensitization occurring through red cell transfusion. From November 1999, all allogeneic blood components produced in the United Kingdom have been subjected to a leukodepletion process to produce blood units containing less than 1×10^6 total leukocytes. This concentration of leukocytes, however, in association with the presence of soluble HLA class I antigen and low level expression of HLA Class I molecules on erythrocytes is still capable of stimulating an immune response resulting in antibody development (*4*). In many instances, this leads to the formation of transitory IgM antibodies, which are considered clinically irrelevant because they do not undergo isotype switching to IgG but preformed IgG-specific

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antibodies can be restimulated, and de novo antibody formation can also occur as a direct consequence of transfusion.

We previously investigated the beneficial effect of blood transfusion on renal allograft survival with a cohort of patients awaiting transplantation who were transfused with units selected to have zero or one HLA-DR mismatch. We observed however that sensitization was predominately influenced by the degree of HLA Class I mismatching (*5, 6*). In the present study, we attempted to minimize immunologic risk to HLA Class I by selecting units from a local cohort of British Bone Marrow Registry (BBMR) blood donors who were HLA typed for HLA-A,-B and -DR loci (*7*). We assessed the efficacy of this approach to reduce antibody sensitization by comparing posttransfusion antibody development between patients who received HLA selected blood and a group of patients who received random units. When zero HLA-A and HLA-B mismatched blood was not available, the HLAMatchmaker program was used to identify blood units from donors considered least likely to stimulate an immune response (*8*).

RESULTS

Demographics

Thirty-one patients (22 male) received only HLA selected transfusions. Twenty-five patients (12 male) received only random units. Six patients (4 male) received both selected and random units at differing periods throughout the course of the study and were therefore included in analysis in both groups. The 37 patients who received selected units had a total of 47 transfusion episodes, and the 31 patients receiving random units had 41 transfusion episodes. The number of units of blood issued ranged from 1 to 8 (mean, 2.5 units) for patients receiving HLA selected blood and 1 to 11 (mean, 3.4 units) for patients receiving random units (Table 1). This was not statistically significant (P=0.487). The degree of mismatching at HLA-A and B for recipients receiving selected HLA units is outlined in Table 2. Random units were sourced from donors in the general blood transfusion population whose HLA types were unknown.

TABLE 1. HLA selected and random blood units transfused per 12-week time episode

No. units transfused per episode	HLA Selected (%) n=47 episodes	Random (%) n=41 episodes
1	11 (23.4)	9 (22.5)
2	22 (46.8)	17 (42.5)
3	3 (6.4)	3 (7.5)
4	4 (8.6)	3 (7.5)
5	5 (10.6)	1 (2.5)
6	0	1 (2.5)
7	1 (2.1)	0
8	1 (2.1)	4 (10)
9	0	0
10	0	2 (5.0)
11	0	1 (2.5)
Total no. units transfused	120	138
Average no. units per 12-week episode	2.5	3.4

TABLE 2. Degree of mismatching at HLA-A and-B loci for patients receiving HLA selected units of blood

No. mismatches at HLA-A and -B loci[a] (mm)	Number of units (% of total 120 units)
0	33 (27.5)
1	41 (34.1)
2	36 (30.0)
3	8 (6.7)
4	2 (1.7)

[a] All mismatched HLA antigens contained ≤5 immunogenic triplets mismatches as determined by the HLAMatchmaker.

HLA Sensitization Pretransfusion

Patient histories and sources of sensitization are listed in Table 3. For patients who received HLA selected blood 20 of 37 (54.1%) had HLA IgG specific antibody detectable before transfusion (13 Class I, 3 Class II, and 4 Class I+II) compared with 10 of 31 (32.3%) of those receiving random blood (6 Class I, 2 Class II, and 2 Class I+II). This was not statistically significant (P=0.1).

HLA Sensitization Posttransfusion

None of the 37 patients (0%) who received HLA selected blood demonstrated any change in antibody profile. In significant contrast, 7 of the 31 patients (22.6%) who received random units had a change in antibody status, (P=0.003; Table 4). When the six patients who received both random and selected units at differing periods in the study were eliminated from the analysis, the result still remained statistically significant: none of the 31 patients receiving selected units had an alteration in HLA antibody status compared with 5 of the 25 (20%) who had random units of blood (P=0.014).

The sensitizing history and pretransfusion HLA profile of those patients demonstrating a change in posttransfusion HLA-specific antibody levels is detailed in Table 5. Patients 1 to 4 demonstrated increases in PRA levels of 20% or more after transfusion. These four female patients produced either de novo antibody, restimulated antibody or a combination of both. Patient 2 exhibited a rise of 40% in her HLA Class I PRA level. She was Class I negative immediately pretransfusion but had demonstrated HLA-B51–specific antibody more than 10 years previously, which could be accounted for by HLA antigen exposure through pregnancy.

Patients 5 to 7 developed de novo antibody. Patient 5 demonstrated a significant increase in Class I specific antibody after multiple transfusions; however, because she had been deemed unsuitable for transplant during the clinical workup process, additional antibody identification could not be performed. Patient 6 was a pediatric patient who required transfusion because of a clinical emergency and subsequently developed Class I specific antibody with a PRA value of 85%. Both of his parents expressed interest in becoming living related donors; however, after transfusion, he developed anti-HLA-A11 and HLA-B7, which were mismatched antigens with his mother and father, respectively. Patient 7 was negative for HLA-specific antibodies, had been previously transplanted, and returned to the transplant wait list. His tissue

TABLE 3. Pre transfusion HLA-specific antibody sensitization history of patients receiving HLA selected and random units of blood

			Potential source(s) of pre transfusion sensitization				
	HLA antibody status	No. patients (%)	Transplant alone	Transfusion alone	Pregnancy alone	Combination	None or unknown
HLA selected units	Positive	20/37 (54.1)	5	3	2	3	7
	Negative	17/37 (45.9)	2	1	1	1	12
Random units	Positive	10/31 (32.3)	0	1	6	3	0
	Negative	21/31 (67.7)	1	4	4	1	11

type contained HLA-B8 and B14 (both Bw6 associated), and he received no B locus mismatches through transplantation; however, after transfusion, newly formed Bw4-associated HLA-specific antibodies, which could not be attributed to any other sensitization source, were identified.

DISCUSSION

The immunologic impact of blood transfusion is complex. In some instances, there is down-regulation of the immune process and pretransplant transfusion can potentially improve renal transplantation outcome (*9*). This was widely accepted in the 1970s and 1980s when survival rates were poorer than those now achieved with modern immunosuppressive regimens. However blood transfusion can also result in increased levels of HLA-specific antibody sensitization as observed with anamnesic (memory) responses of patients previously exposed to HLA alloantigens from pregnancy, transplantation, or blood transfusion (*10*). The number of transfusions also influences the immunologic response. Results from a randomized trial reported similar posttransfusion antibody levels when individuals were transfused with a single unit that either had the buffy coat removed or a unit that had undergone additional leukocyte reduction through filtration (*11*), but the incidence of sensitization was greater in those with multiple transfusions (*12, 13*).

There is increasing awareness of the potential benefit in minimizing sensitization in those requiring transplantation (*14*). Suggested strategies include identification of those at risk of increased antibody production, minimization of blood transfusions, immunosuppression cover in a peritransfusion setting, and HLA-matched transfusions. Although biologically plausible, there is limited data to support the hypothesis that blood that is closely matched is beneficial in reducing the risk of sensitization. In the late 1980s when the practice in some centers was for patients to receive blood from their prospective donors, the lowest observed levels of sensitization occurred in those that were more closely matched (*15*). In patients with low level alloantibodies resulting from pregnancy, none receiving zero mismatched units developed any new HLA-specific antibodies (*16*). There is an absence of evidence, rather than evidence of absence, of a beneficial effect of HLA-matched blood products, primarily because of logistical requirements in securing such units.

In our center since 2004, we have, when practically possible based solely upon clinical urgency, provided selected blood units that are least likely to stimulate deleterious antibody production for individuals on the renal transplant waiting list requiring blood transfusion. Our results are consistent with a reduced risk of sensitization when blood products are closely matched at HLA Class I loci. No patients who received selected products had an increase in alloantibody production compared with 23% of those that had received random units.

There was a difference in the degree of HLA sensitization before transfusion in the selected and random groups (although this was not statistically significant). More than half (54%) of the patients who received HLA selected blood had been already sensitized compared with a third (33%) of those who received random blood. It could be hypothesized that although both groups should react similarly in terms of de novo antibody production because of immunologic priming, the patients receiving selected blood might be more subject to restimulation of HLA-specific antibody than the randomized group. However, although study numbers are relatively small, the converse was true. None of the patients receiving selected blood showed any de novo antibody production or an increase in their antibody levels, although of those receiving random units, 10% showed de novo antibody production and 13% an increase in PRA of 20% or more. More blood was also transfused in the cohort which received the random units; however, the difference was less than one unit on average (3.4 vs 2.5) and was neither statistically nor clinically significant.

It is difficult to predict how individual patients will respond to antigenic stimuli as antibody production is affected by many factors including the immunologic status of patients and their immunosuppressive regimens. Of particular interest therefore were the six patients who received both random and selected units during differing periods of the study. Four of these (three male) produced no HLA-specific antibody after transfusion with either the random or selected units. One had no history of known sensitization, two had been previously transfused before the beginning of the study, and the

TABLE 4. Posttransfusion HLA-specific antibody levels of patients receiving HLA selected and random units of blood

PRA[a] levels	No change	Change from negative to positive	>20% increase in peak levels
HLA selected units	37/37 (100%)	0/37 (0%)	0/37 (0%)
Random units	24/31 (77.4%)	3/31 (9.7%)	4/31 (12.9%)

[a] Panel reactive antibody.

TABLE 5. Pretransfusion and posttransfusion HLA-specific antibody profiles of patients demonstrating a change in PRA levels

					Pretransfusion			Post transfusion		
Patient	Gender	Age (yr)	No. units received	Sensitization source and exposure to HLA	Class I % PRA	Class II % PRA	HLA specificities detected	Class I % PRA	Class II % PRA	Additional HLA specificities detected
1	Female	62	2	Pregnancy A11	25	0	A1 A2 A9 B8 B12	65	0	A68 A34 A10 A11 A19 B62 B27 B49 B57 B13
2	Female	54	1	Pregnancy A23 A30 B51 B57 Cw18 DR12 DR16 Transfusion	0	30	DR15 DR16 DR7	40	60	A3 A25 A32 B8 B13 B18 B45 B49 B51 B52 B57 Cw6 DQ9
3	Female	44	2	Pregnancy A2 A28 B44 B14 Cw5 Cw8	16	0	A2 B44 B45 B57	60	0	A3 A11 A25 A26 A29 A30 A31 A32 A33 A34 A68 B13 B49 60 Cw5 Cw6 Cw18
4	Female	52	4	Pregnancy A2 B51 B44	26	0	A2 A68 A31 B13 B27 B44 B52 B57	86	0	A3 A11 A30 A34 B7
5	Female	34	10	None	0	0		70	0	antibody specificity not identified, patient removed from transplant list
6	Male	2	2	None	0	0		85	0	A11, B7, A2, A3, B60
7	Male	52	6	Transplantation	0	0		20	0	Bw4 associated

fourth who received random blood (he was transfused with 11 units during one transfusion episode) had been previously transfused and had also received two kidney transplants. The remaining two patients did however, show differing responses to HLA selected and random units. One (Patient 4, Table 5) who had preexisting HLA-specific antibody was transfused with two HLA selected units in 2008 but demonstrated no change in antibody levels until the issue of four random units in 2009 when her Class I PRA increased by 60%. The second patient (Patient 6, Table 5) who was negative initially was transfused in 2006 with two random units, which immediately resulted in development of 85% Class I PRA. Three HLA selected units were also issued for this patient in 2007; however, these had no additional deleterious effects on his antibody levels.

Both study groups comprised a subgroup of previous transplant patients (10 in the selected group and 5 in the random group) who had returned to the transplant wait list. Some of these continued with low levels of immunosuppression (but this did not include Patient 7 in Table 5). This may have impacted upon their ability to produce HLA-specific antibody after transfusion; however, no conclusions can be made, given the variability in immunosuppressive regimens and the small numbers involved in this subgroup.

When assigning unacceptable antigens and selecting serum samples for donor crossmatching, it is essential to have comprehensive knowledge of a patient's immunologic history and HLA-specific antibody profile including any HLA mismatching through previous transplantation and also the HLA types of the partner(s) of parous women. As demonstrated here in addition to de novo antibody production, transfusion can elicit restimulation of HLA-specific antibodies, which may have developed historically. It has been shown here that HLA-specific antibody development can occur up to 3 months after transfusion, and so, this should be considered a higher risk period if a patient is crossmatched for a donor organ.

Although the introduction of erythropoietin-stimulating agents to clinical practice in the 1990s has greatly reduced the number of transfusions required by renal failure patients, it is inevitable that there will always be a subgroup who will require red cell transfusion because of blood loss or erythropoietin resistance. Based upon our observations, we currently consider it best practice to search for HLA selected units if patients are awaiting transplantation, are not on immunosuppression, have current PRA levels less than 85%, have fewer than 10 multi-locus specificities, and the transfusion can be delayed for two to three working days if determination of patient HLA type or identification and obtaining a unit from a potential donor(s) is required.

The logistical complexity in sourcing HLA selected units for patients awaiting transplantation may vary from center to center. HLA-matched platelets are required for patients who are highly sensitized and in whom random donor platelets are therefore ineffective. The same mechanism for identifying and sourcing such blood donors is also in current usage in our center.

CONCLUSION

This small scale study provides clinical evidence that HLA-specific antibody sensitization can be minimized or

prevented by administration of HLA selected units. Additional work is required, however, ideally on a multi-center basis to determine the minimal degree of matching required to achieve a beneficial effect for patients and the relative merits of matching for HLA Class I and Class II loci. Transfusing patients with blood selected to minimize HLA-specific antibody production is a beneficial process and should be considered when clinically practical and especially for pediatric patients who are more likely to require retransplantation.

MATERIALS AND METHODS

Patients

This study comprised all patients awaiting renal transplantation between June 2004 and December 2009 who had pretransfusion and posttransfusion clotted blood samples available for HLA-specific antibody screening and identification. The patients who had been HLA typed by complement-dependent cytotoxicity, sequence-specific oligonucleotide (SSO), and/or Luminex molecular methodologies were allocated to the selected or random groups. This was based solely upon clinical urgency as proceeding with transfusion of random units was more appropriate for patients where the potential delay in sourcing selected units would have compromised their safety.

British Transplantation Society (BTS) guidelines recommend the optimum period to detect new HLA-specific antibody development is 14 and 28 days after transfusion (*17*); however, because some patients received multiple transfusions over weeks to several months, it was impossible to assess the impact of each individual unit of blood transfused. Consequently, we compared antibody development in the two groups by investigating transfusion episodes where one episode encompassed any units given over the course of a 12-week period. An antibody response was considered significant if pretransfusion HLA antibody levels changed from negative to positive, or in the case of those individuals who already had preexisting HLA-specific antibody, where the panel reactive antibody (PRA) value increased by at least 20%.

HLA-Specific Antibody Screening and Identification

Screening of pre- and post- transfusion serum samples was performed using EPICS XL and FC500 flow cytometers (Beckman Coulter UK Ltd, High Wycombe, UK) using Flow PRA Screening (*18*) and ID Beads (*19*) for HLA Class IA, B, and C and Class II DR and DQ antibody according to the manufacturer's instructions (One Lambda Inc., Miami, FL).

HLAMatchmaker

The HLAMatchmaker is a computer algorithm designed to identify compatible Class I HLA antigens at the level of polymorphic amino acid triplets or eplets for highly sensitized patients awaiting transplantation (*20*). The HLAMatchmaker is based on the principle that every HLA molecule possesses a distinct array of polymorphic triplets and those that are present on antibody accessible portions of the molecule are immunogenic. Patients will not produce HLA-specific antibodies against self-antigenic epitopes but will form alloantibodies to HLA epitopes, which are foreign. By comparing the similarity between the HLA molecules of a potential donor and recipient, it is possible to determine how many immunogenic triplets that patient will encounter. We used the Class I Serological SER1.2 version of the HLAMatchmaker as this was in local use at the beginning of the study period. Any mismatched HLA antigens possessing five or fewer immunogenic triplets were considered acceptable for selection primarily because this degree of mismatching was deemed achievable in terms of the numbers of blood donors available.

British Bone Marrow Registry Donor Selection

The BBMR comprises blood donors who have volunteered to be bone marrow donors and have been HLA typed for HLA-A, B, and DR loci. We selected suitable donors based upon their HLA-A and HLA-B type from a Northern Ireland cohort (n=11400) HLA typed since 1988 by a succession of methodologies: complement-dependent cytotoxicity, restriction length fragment polymorphism, in house SSO and Luminex technologies.

A search of the local BBMR database was performed based upon the patient's blood group and HLA-A and HLA-B locus type. The search was restricted to HLA Class I antigens based upon previous work demonstrating that sensitization is predominately influenced by the degree of HLA Class I mismatching. Any previously designated unacceptable specificities and any HLA antigens possessing more than five immunogenic triplet mismatches as identified by the HLAMatchmaker were excluded. All remaining potential blood donors were then ranked according to the degree of HLA-A and HLA-B matching with the renal recipient.

Blood Products

Leukodepletion was performed on all HLA selected and random blood units to produce a minimum of 90% containing less than 1×10^6 total leukocytes per pack. Selected blood units were sourced where possible from stock already within the blood bank if less than 14 days old; however, if none were readily available then suitable potential donors were contacted and requested to donate a unit of blood. All selected units were gamma irradiated to reduce the possibility of graft versus host transfusion effects.

Statistical Analysis

Statistical analysis was performed using Fisher exact test and the independent samples *t* test.

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