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Shared Cadaver Donor-Husband HLA Class I Mismatches as a Risk Factor for Renal Graft Rejection in Previously Pregnant Women

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ABSTRACT: During the last few years, we have observed four cases in which accelerated rejection of a cadaver donor kidney in a previously pregnant woman could be clearly attributed to the rapid emergence of anti-human leukocyte antigen (HLA) antibodies that had been stimulated by mismatched paternal antigens but were completely undetectable at the time of transplantation. In addition to reviewing those cases, we also reviewed data on 19 other women with a history of at least one pregnancy who underwent transplantation with a first cadaveric kidney since 1991 and were followed for at least six months. The HLA antigens of the husbands had to have been determined and all accelerated rejection or early graft losses due to confirmed or presumed immunological causes were considered. Of the 19 additional women meeting these inclusion criteria, three suffered early immunological graft loss. As in our index cases, two of these women had also received kidneys from donors who shared at least one major immunogenic mismatched antigen with the respective husband for a total of six of seven women with early immunological graft loss. Only one of the 16 women without accelerated rejection or early immunological graft loss had a donor who shared a mismatched antigen with her husband. The difference between the two groups is statistically significant ($p = 0.0005$). These findings, considered with individual cases reported by other groups, indicate that transplantation from a cadaver donor with immunogenic mismatched class I HLA antigen(s) shared with the husband should be avoided in women with a previous history of pregnancy even when anti-HLA antibodies are not currently detected. *Human Immunology 60, 1150–1155 (1999).* © American Society for Histocompatibility and Immunogenetics, 1999. Published by Elsevier Science Inc.

KEYWORDS: kidney transplantation; cadaver donor; pregnancy

ABBREVIATIONS

HLA human leukocyte antigen

PRA panel reactive antibody

INTRODUCTION

The outcome of cadaver donor kidney transplantation has been related to several factors [1, 2]. Among these, the risk associated with human leukocyte antigen (HLA) mismatching has been extensively documented [3–5]. Pretransplant sensitization to HLA antigens through previously rejected allografts, pregnancy or blood transfusion, as measured by tests for panel reactive antibodies (PRA), is also a major risk factor [6].

In current practice, it is widely believed that the use of very sensitive crossmatch tests will avoid transplants in which these kinds of antibodies can cause acute or accelerated rejection. In fact, many husband-to-wife renal transplants are highly successful despite the possibility of prior immunization through pregnancy. However, there are major differences between living and cadaver donor transplants that can impact the immunogenic state of the graft [7]. Moreover, in some cases with antibody medi-

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ated accelerated rejection of cadaver donor kidneys, there is no evidence of current anti-HLA antibodies at all in the PRA, or in serology or flow-cytometry crossmatch studies. We believe that our clear documentation in this study of an association in previously pregnant women of cadaver donor/husband sharing of mismatched class I HLA antigens and accelerated rejection attributable to HLA antibodies suggests that this type of latent sensitization may explain other cases.

The literature regarding this issue is sparse. To our knowledge, no explanation exists as to why these cases occur and why they occur only occasionally. However, recognition of this possibility by avoiding these transplants whenever possible may not only help to decrease even more the prevalence of accelerated rejection and early graft loss, but may also help to improve long-term graft survival.

MATERIALS AND METHODS

Patients and Test Methods

The clinical histories of the four women who were specifically determined to have anti-HLA antibody mediated accelerated rejection were reviewed along with the records of all 51 previously pregnant cadaver donor recipients who underwent transplantation between 1991 and 1997 at Baylor College of Medicine, Houston. Previously pregnant recipients whose husbands had not been available for HLA typing were excluded. Recipients of living donor grafts, with previous graft loss or with primary graft loss due to technical causes or noncompliance, were also excluded to ensure that all patients included in the analysis were at similar immunological risk. An additional 19 previously pregnant primary cadaver donor renal transplant recipients met the inclusion criteria. These patients, and their husbands and donors had been typed for HLA-A, -B, and -C antigens, and for DR and DQ antigens using standard microcytotoxicity techniques with positively selected T and B cells, respectively, and a large number of typing sera collectively defining all well characterized HLA types.

Pretransplant crossmatches were performed using both Amos-Wash and anti-globulin techniques for T cells, using an Amos-Wash technique for B cells, and with DTT or DTE to identify positive results due to IgM antibodies, as indicated. Crossmatches for sensitized (high PRA) patients were also performed prospectively using standard flow-cytometry techniques with both T and B cells as targets, and flow crossmatches were performed retrospectively in the four index cases. All pretransplant crossmatches were negative for IgG antibodies.

Data were gathered regarding the number of pregnancies, shared husband-donor mismatched class I HLA antigen(s), and the time and type of rejection, if any. Analysis of shared DR or DQ mismatches was not considered because numerous studies indicate that antibodies to class II antigens are not clinically significant in relation to primary kidney transplants unless they are very high titered and DR specific [8, 9]. We chose patients transplanted since 1991 to ensure consistency in crossmatch techniques and criteria for transplant. Other data collected were age, race, cause of end stage renal disease and husband-patient crossmatch test results. The minimum follow-up time post-transplantation was 6 months.

Data Analysis

The data collected were tabulated in a 2×2 table according to accelerated rejection/early immunological graft loss vs. graft success, and shared versus nonshared donor-husband HLA antigens. The statistical analysis was performed using the chi-square test (Fisher's exact test).

RESULTS

A summary of the HLA typing and immunological test results for the four patients who were documented to have antibody mediated acute/accelerated vascular rejection despite negative pre-transplant crossmatch tests is shown in Table 1. In all four patients pre-transplant and post-transplant sera were tested for the presence of IgG or IgM antiphospholipid antibodies, and all were found to be negative. Neither patient TC nor DH had any detectable HLA antibodies during the two years prior to transplant. Both these patients were treated prophylactically and continuously with OKT3 at the time of rejection but rejection was irreversible.

Although patient PP did have detectable HLA antibodies at the time of transplant, the specificities identified at that time did not correspond to the donor's antigens or to either of the husband's mismatched antigens that were highly crossreactive with the donor's mismatched antigens. Retrospective analysis indicated that relevant specificities had been briefly detectable two years earlier in that patient (Table 1). This patient refused treatment for accelerated acute rejection and the transplanted kidney was removed on post-transplant day 3. The fourth index case, patient GC, was a primary transplant recipient whose previous sensitization apparently resulted from 2 miscarriages rather than term pregnancies. This patient recovered graft function after she underwent plasma exchange. During the previous year monthly PRA had fluctuated between 0 and 6% in 9 of 11 tests, and it was 2% at the time of transplantation. Although two PRA tests had somewhat higher values (12% and 16%) and the specificity A2 could be deduced to be present retrospectively in the highest PRA sample,

TABLE 1 Four patients with accelerated rejection due to previously latent anti-HLA antibodies

A. HLA class I phenotypes

Patient ID	Paient HLA type	Husband HLA type[a]	Donor HLA type	Shared HLA mismatches
TC	A2; B44,62; Bw4,w6	A2,3; B8,44; Bw4,w6	A1,3; B8,38; Bw4,w6	A3; B8
DH	A11,26; B35,55; Bw6	A3,29; B13,44; Bw4	A2,26; B44; Bw4	B44
PP	A3,24; B7,38; Bw4,w6	A29,31; B45,48; Bw6	A2,30; B38,61; Bw4,w6	A30/31; B61/48[b]
GC	A24,25; B57,62; Bw4,w6	A2,31; B35; Bw6	A1.2; B44.57; Bw4	A2

B. Patient antibody specificity analysis

Patient ID	% Pre-transplant PRA[c]	%PRA at repeat analysis	Specificities identified-post-transplantation
TC	0	54, 5 months post-transplant	A1, A3, A11; B8
DH	0	90, 1 week post-transplant	Multispecific, including B44
PP	25 (A29; B44,45,49,50)	58, 27 months pre-transplant	A29.30; B44.45,49.50,60,61 (not tested for B48)
GC	2 (none)	16, 2 months pre-transplant	A2± (8/50 cells; 6/24 A2 cells)

[a] The patient husbands were not HLA typed until after the accelerated rejection occurred.

[b] A30 and 31, and B61 and 48 would be considered to be highly crossreactive HLA antigens. For many years these were difficult to distinguish by serological testing [26].

[c] Pre-transplant T cell (class I) PRA with identified specificities indicated. The anti-A30/31 and -B 60/61(48) activities of patient PP pre-transplant serum and the anti A2 activity of patient. GC serum were undetectable at the time of transplant, even by flow cytometry crossmatch with the donor or panel cells. Since those antibody activities had disappeared two years before the transplant or had not been identified, those antigens were not considered to be unacceptable. The husbands had not yet been HLA typed.

the reactions were weak and no specificity had been clearly identified prior to the transplant (Table 1).

Three of the 19 additional patients meeting inclusion criteria suffered accelerated rejection or presumptive immunological graft loss within six months of the transplant. Table 2 depicts the demographic and immunological data in this combined group of seven patients. Two of the three additional patients for a total of six of seven patients had donors who shared one or more immunogenic mismatched antigens with the husband of the patient. Although several of these women had demonstrated a positive crossmatch against their respective husbands one or more months prior to the transplant, none had detectable antibodies against cells of the donors at the time of transplant. Only one of the 16 nonrejecting patients had a donor who shared a single immunogenic mismatched class I antigen with her husband. Since this woman had had only one previous pregnancy there was in any case only a 50% chance that the fetus even carried this mismatched type.

TABLE 2 Characteristics of patients suffering early immunological renal allograft rejection

Patient	Race	Age	ESRD etiology	Number of pregnancies	%Pre-transplant PRA	Crossmatch husband	Shared HLA mismatches[a]	Time to accelerated rejection or graft loss	Diagnosis
TC	White	28	HTN	1	0	Neg.	A3, B8	4 days	Accelerated rejection
DH	White	62	Pyelonephritis	3	0	Pos.	B44	12 days	Acute and accelerated rejection
PP	Hispanic	52	IDDM	2	25	Pos.	A30/31 B48/61	2 days	Accelerated rejection
GC	Hispanic	52	IDDM and pyelonephritis	2 (miscarriages)	2	Neg.	A2	3 days	Accelerated rejection
CY	White	40	Reflux nephropathy	2	0	Neg.	Bw6	6 months	Acute rejection
SD	White	36	Alport's syndrome	3	25	Pos.	Bw4	33 days	Humoral rejection
LL	Hispanic	35	HTN	2	0	Pos.	None	18 days	Accelerated rejection

ESRD: end stage renal disease; IDDM: insulin dependent diabetes; HTN: hypertension

[a] Immunogenic class I HLA antigens mismatched to the patient that the cadaver donors shared with the patient husbands.

TABLE 3 Analysis of relationship between shared class I mismatched antigen(s) and graft outcome

Early graft loss	Shared mismatched antigen(s) Yes	No	Total
Yes	6	1	7
No	1	15	16
Totals	7	16	23

Odds ratio: 90.0 (95% confidence interval: 4.81–1683.86 [Woolf]).
p-Value (Fisher's exact test): 0.0005.

The data distribution with respect to shared class I HLA antigen mismatches and accelerated rejection and/or early graft loss is shown in Table 3. A statistically significant difference was found in the outcome of the graft in previously pregnant patients whose donors shared immunogenic mismatched class I antigens with their respective husbands in comparison with those whose donors did not share mismatched antigens.

DISCUSSION

Previous pregnancy is one of the major causes of transplant candidate pre-sensitization, as manifested by a high PRA and frequent positive crossmatch test results [6, 10–12]. Pregnancy also apparently acts as a co-factor in sensitization by transfusion since the probability of becoming highly sensitized because of blood transfusions alone is low in men (approximately 15%) in comparison with that in multiparous women who subsequently are transfused (approximately 40%) [13–15]. High PRA, in turn, results in lower one and two-year allograft survival, despite the presence of a negative pretransplant crossmatch. One-year allograft survival is 85% and 76% respectively, in patients with 0% to 49% and 90 to 100% PRA [11]. Of course, kidneys transplanted in the face of a positive crossmatch were demonstrated many years ago to be at risk for hyperacute rejection and very early loss of the graft [16], and transplants are never performed now unless there is a negative donor-recipient T-cell (class I HLA antigen) crossmatch. However, accelerated rejection or early immunological graft loss is still occasionally seen. Some cases of accelerated rejection with negative crossmatch reported in the literature have been attributed to laboratory error or insufficient crossmatch test sensitivity [17], or they were documented to be caused by antiphospholipid antibodies not involving rejection at all [18]. When these considerations are not relevant, the occurrence of accelerated rejection suggests the presence of occult pre-sensitization against donor antigens that is not detected by sensitive crossmatch techniques.

That occult or latent sensitization to HLA antigens through pregnancy that is not detectable by analysis of current serum antibodies must occur is documented not only by the higher response rate to transfusions of multiparous women referred to above, but also by the fact that the frequency with which HLA antibodies are detected after pregnancy is directly proportional to the number of pregnancies experienced [19]. Since these data include results in more than four pregnancies, repeat exposure to the same antigens obviously increases the chances for detection of sensitization which must have already occurred. In the cases we were able to study, repeat crossmatches with the same donor became positive after the rejection episode and the specificities of the post-transplant antibodies were clearly demonstrated to involve the specific shared antigen(s) of their husbands (Table 1).

Other transplant programs have also reported similar, presumably isolated, cases of accelerated graft rejection due to occult sensitization through pregnancy. Two such cases [20, 21] involved accelerated rejection demonstrated by biopsy to be due to antibodies and by specificity analysis to be due to shared mismatching for one of the so-called broad or public antigens, Bw4 or Bw6. Two of our seven patients with early graft loss also had shared mismatches for one of these broad antigen specificities (Table 2). This should not be surprising since antibodies with these specificities are frequently identified in highly sensitized patients [22] and antibodies of any specificity can be presumed to become latent several years after pregnancy.

Our results suggest a correlation between an unfavorable graft outcome and sharing of immunogenic mismatched HLA-A or B antigens between cadaveric donors and husbands of previously pregnant recipients. This observation suggests that avoiding cadaveric transplants in which the donor carries an antigen to which the recipient previously had been exposed through multiple pregnancies, including miscarriages, could contribute to overall cadaveric graft survival even if it only affects a few cases. This caution may not be germane to living-related transplantation because, despite the greater likelihood of presensitization of a previously pregnant woman to the donor (offspring) antigens, the transplant outcome is superior nevertheless. Other factors, such as handling of the organ and the time of cold ischemia, appear to be major determinants [7]. Recent evidence also indicates that recipients of living donor kidneys are not exposed to inflammatory immunological changes in the graft due to accompanying brain death itself [23]. However, it appears that even in this situation the effects of previous pregnancy can be demonstrated. Although the difference was not statistically significant, the first analysis of data from the UNOS registry [24] indicated that the three-

year survival rate for husband-to-wife grafts was only the same as for wife-to-husband grafts (87%) if the wife had never been pregnant but decreased to 76% if the wife had been pregnant. A study from Norway [25] reported earlier graft rejection episodes and more frequent use of antibody therapy for husband-to-wife transplants. Although the differences were not significant, the incidence of previous pregnancy was unknown. In a single case of acute rejection involving a husband-to-wife transplant in a woman with 2 previous pregnancies recently referred by R. Allen, Tulsa, to our own laboratory (MSP) for flow studies, the flow crossmatch became positive after transplant but had been completely negative prior to transplant. Her PRA had been 0% in all previous tests. Rejection was successfully reversed with OKT3, perhaps thanks to the superior quality of living donor kidneys. These reports, nevertheless, confirm that latent sensitization can be clinically relevant and should be considered in cadaver donor transplantation when multiple donor choices are available.

Although the additional factors that cause latent sensitization to become clinically relevant in only a small proportion of cases remain to be determined, avoiding these situations for cadaver donor transplantation whenever possible is easy. Although the possible role of latent sensitization to class II antigens was not addressed in these studies, the results reported here suggest that avoiding this problem would require only the HLA-A,B typing of the husbands of women with multiple previous pregnancies, and the cost-benefit ratio is clearly favorable.

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