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A HIGH PANEL-REACTIVE ANTIBODY RESCUE PROTOCOL FOR CROSS-MATCH-POSITIVE LIVE DONOR KIDNEY TRANSPLANTS¹

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Abstract [TOP](#)

Background. Alloimmunization can present a virtually insurmountable barrier to kidney transplantation. Past protocols to desensitize patients using plasmapheresis and cyclophosphamide have not been broadly applied because of the fear of complications, including high rates of immunologic failure.

Methods. Fifteen patients with a positive donor-recipient cross-match were desensitized with plasmapheresis to permit live donor (LD) transplantation under newer maintenance immunosuppressants. Pretransplant the patients received plasmapheresis three times weekly for a planned maximum of six treatments, plus intravenous hyperimmune globulin, tacrolimus, mycophenolate mofetil, and prednisone. Patients who were successfully desensitized and received transplants were given 10 days of OKT3 postoperatively.

Results. Eleven of the 15 patients became anti-human globulin cross-match-negative after one to five plasmapheresis treatments and underwent LD transplantation. Relatively low initial titers of donor-specific antibody were predictive of successful attainment of a negative cross-match. Few side effects and rejection episodes were observed. All transplant patients remain dialysis-free after 3-26 months of follow-up.

Conclusion. A positive cross-match is not necessarily a contraindication to LD transplantation, especially for patients with low donor-specific alloantibody titers.

Some of the longest waiting times for a kidney transplant are observed in patients who are allosensitized because of a prior transplant, pregnancy, or blood transfusions. For patients placed on the United Network for Organ Sharing waiting list in 1992, those with a peak panel-reactive antibody (PRA) over 20, which represented 40% of that years' kidney waiting list, median waiting times were 2-5 times longer than those for unsensitized patients (1). Some patients with broadly reactive alloantibody will never receive a transplant without treatment to reduce their antibody titer. The problem is especially frustrating for patients with a live kidney donor, as their waiting time could be very short were it not for the positive cross-match.

Desensitization of alloimmunized patients with plasmapheresis or immunoadsorption to prepare for kidney transplantation has been sporadically explored over the years (2, 3). Past studies relied upon cyclophosphamide, cyclosporine, and prednisone as maintenance immunosuppression to prevent early immunologic destruction of the graft. Under these older agents, the rates of immunologic failure and infectious complications were sufficiently high to interdict general acceptance of desensitization by the transplant medical community. However, two new maintenance immunosuppressants tacrolimus (FK) and mycophenolate mofetil (MMF) are now available, which are superior to older agents in reducing rejection of transplanted kidneys (4, 5). It was hypothesized that their use in a desensitization protocol might improve outcomes. The current study explored this approach in a small group of sensitized recipients of live donor (LD) kidney transplants with a positive donor-recipient cross-match.

PATIENTS AND METHODS TOP

Between February 1998 and February 2000, 15 patients with end-stage renal disease completed a high PRA rescue protocol approved by the University of Maryland School of Medicine Institutional Review Board. To qualify for the study, recipients and their live donors had to meet standard criteria for LD transplantation, and have a positive anti-human globulin (AHG) cross-match with their donor. Recipients ranged in age from 27 to 66 years, 53% were men, 67% were Caucasian, 47% had received a prior solid organ transplant, and 73% had first degree relatives as their donors (Table 1). Mean peak PRAs for the group were 69%. The average donor HLA alleles mismatched with the recipient were 2.2 AB and 1.3 DR. Two patients were insulin-dependent diabetics who desired a simultaneous cadaver pancreas transplant with the LD kidney, known as an SPLK transplant (6). All kidneys were procured by the laparoscopic technique (7).

Table 1. Patient characteristics^a Number of prior solid organ transplants the recipient had received.^b Peak panel-reactive antibody (%), CDC).^c End point dilution at which AHG cross-match was positive. Values represent the recipient's serum sample taken at the time of the initial transplant evaluation (eval) and immediately prior to first plasmapheresis treatment (start).^d Number of plasmapheresis treatments needed to convert the recipient's AHG cross-match from positive to negative (patients 12-15 did not convert to negative after the six planned treatments).

Recipients in the protocol started taking a standard dose of MMF 3 days before the first plasmapheresis treatment (Table 2). This was done in an attempt to prevent antibody rebound after plasmapheresis through inhibition of B-cell proliferation. Patients were also started on standard doses of FK and prednisone on the day of the first plasmapheresis treatment. These three immunosuppressive agents were continued through the posttransplant period. Patients were also given

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nizatidine and clotrimazole troche as prophylaxis against gastritis and fungal stomatitis. Intravenous immunoglobulin (IVIG, Sandoglobulin; Novartis, Summit, NJ), 500 mg/kg total dose divided over 7 days, was started immediately after the first plasmapheresis treatment to suppress alloantibody rebound. Transplant patients received 10 days of OKT3, with dosage adjustments to keep the percentage of CD3-positive lymphocytes less than 5%.

Table 2. High PRA rescue protocol

Before transplant, the patients received plasmapheresis three times weekly for a planned maximum of six treatments. Plasmapheresis was performed in the mornings on Monday, Wednesday, and Friday for 2 consecutive weeks, if necessary. A one plasma volume exchange with 5% albumin was used, with a Cobe Spectra continuous flow apheresis device. Venous access was achieved with either a central venous catheter or the patient's permanent hemodialysis access. Plasmapheresis times varied with height and weight, on average ranging from 1.5 to 2 hr. Flow rates ranged from 80 to 100 ml/min.

The removal of donor-specific anti-HLA class I antibodies was monitored with serial anti-human globulin complement-dependent cytotoxicity (AHG-CDC) cross-matching, ELISA antibody identification, and frozen cell trays. The titer of donor-specific antibodies was determined by serum dilutions tested by AHG-CDC against donor cells. The HLA antibody specificity was determined by AHG-CDC PRA using panels of fresh or frozen cells. In addition, historical peak, pre- and postpheresis sera were studied to determine donor-specific HLA antibodies. These were measured with a commercial ELISA kit (QID; GTI, Madison, WI) in which HLA class I antigen preparations from 42 donors are immobilized in 96-well trays. Optical density (OD) values were analyzed to estimate the levels of anti-HLA antibodies, using the values recommended by the manufacturer. Changes in OD values corresponded to changes in the strength and specificity of alloantibodies in the recipient's serum. A drop in observed OD values was an indicator for clearance effectiveness in each pheresis session.

Patients whose serum drawn on the morning after a plasmapheresis treatment had reached a negative AHG-CDC cross-match, and no donor-specific reactivity by ELISA, were transplanted within 24 hr. The two SPLK recipients were given several additional alternate day plasmapheresis treatments after the cross-match became negative, in order to remove any alloantibody resynthesized during the brief time it took to find a suitable cadaver pancreas.

RESULTS TOP

Four of the 15 patients who completed the protocol remained cross-match-positive with their donors after the planned six plasmapheresis treatments. These patients did not receive an LD transplant and remained on dialysis. The remaining 11 patients became AHG cross-match-negative after one to five treatments and underwent LD transplantation, including two patients who received a simultaneous cadaver pancreas. In the immediate postoperative period, 3 of the 11 transplant patients required at least one dialysis treatment, the longest period of delayed graft function being 13 days. All 11 patients are currently dialysis-free after a mean follow-up of 13.3 ± 2.4 months (range: 3-26). Mean serum creatinine is now 1.6 ± 0.2 mg/dl (range: 1.1-2.4, [Fig. 1](#)).

Figure 1. Postoperative serum creatinine levels (mg/dl) versus time after transplant (months) for the first 10 transplant patients. Most causes of creatinine elevation were not rejection.

A total of 19 kidney biopsies in eight patients have been performed for creatinine elevation. Only four biopsies in four different patients showed any signs of acute rejection. Three of these biopsies showed a mild neutrophilic vascular infiltrate in the early postoperative period ([Fig. 2](#)), interpreted as possible antibody-mediated rejection (patients 6, 7, and 8). Two of these patients had a transient, concomitant rise in their donor-specific antibody titer by ELISA. These three patients were successfully treated with three to five additional plasmapheresis treatments, 7-10 days of additional OKT3 or

antithymocyte globulin, and IVIG. The fourth biopsy showing rejection was a case of mild cellular rejection on postoperative day (POD) 41 (patient 2), which resolved with pulse steroids alone.

Figure 2. Kidney biopsies of the only two patients in whom the donor-specific antibody titer was found to be transiently elevated. (A) Glomerulus of the kidney of patient 6 showing occasional polymorphonuclear leukocytes (arrows), suggestive of possible, mild vascular rejection. (B) Three arterioles of the kidney of patient 8 on POD 13 showing endothelitis (arrows), indicative of vascular rejection. Both patients were successfully treated with additional plasmapheresis, IVIG, and antilymphocyte antibody.

No other patients' biopsies showed evidence of acute rejection. Patient 1, whose creatinine is now the highest in the group, has never had acute rejection, but has had both histologic and cytologic evidence of polyoma virus infection of the kidney, associated with chronic allograft nephropathy (8). The remainder of the biopsies have been unremarkable or have demonstrated nonimmunologic problems such as drug toxicity.

Immunologic monitoring tests showed that the protocol caused a reduction in both antibody titer and specificity (Table 3). We found that, in general, the HLA class I specificities as measured by AHG-CDC and ELISA were similar, but the ELISA-based method was more sensitive to identify weak antibodies or tail reactions. Patients successfully desensitized and transplanted had a decline in their donor-specific antibody titer to undetectable by ELISA as a result of the protocol. However, not all alloantibody was eradicated in some cases, as indicated by PRAs that were reduced but not eliminated, and the persistence of non-donor specificities even among some of the desensitized patients. Seven of the 11 patients transplanted under the protocol have had at least one ELISA or flow cytometry assay for donor-specific alloantibody in the postoperative period. The only positive tests were in patients 6 and 7, who had positive ELISA assays on POD 7 and 12, respectively. Both patients have had excellent graft function after 13 and 8 months of follow-up, without other evidence of acute rejection.

Table 3. Histocompatibility testing^a Pre means before the first plasmapheresis treatment; post means after the last treatment.^b This patient's PRA was 0% by CDC, 78% by ELISA.

Both of the simultaneous cadaver pancreas transplants have failed. One pancreas (patient 5) was lost to accelerated chronic rejection, associated with a gradual increase in the need for insulin, 4 months after the transplant. The other pancreas (patient 6) was lost to early thrombosis on POD 1, possibly caused by an alloantibody-mediated process. The recipient's blood sample before the transplant had a negative AHG-CDC cross-match with both the kidney and the pancreas donor. Although the cross-match was negative, an alloantibody against the pancreas donor's B63 antigen was weakly positive in the same pretransplant serum when tested with the more sensitive ELISA assay. This weak antibody (not strong enough to cause a positive AHG-CDC cross-match) may have contributed to the pancreas thrombosis, as pathologic exam of the explant specimen indicated vascular rejection. In support of this diagnosis, the patient had a strong spike in the B63 antibody titer after the pancreas was removed.

Plasmapheresis was well tolerated. None of the procedures had to be terminated prematurely because of adverse reactions. Typical plasmapheresis side effects are often attributable to hypocalcemia from the citrate, which is used as an anticoagulant. These include peripheral and circumoral paresthesias, feelings of weakness, as well as nausea and vomiting. None of the patients reported paresthesias, but 40% (6/15) complained of feeling weak and tired. Gastrointestinal side effects occurred in 60% (9/15) of the patients (diarrhea in 6/15, and nausea in 7/15). Because gastrointestinal symptoms are common in patients taking MMF and FK, these complaints may have been the result of these medications rather than plasmapheresis. Other side effects during plasmapheresis were more clearly attributable to immunosuppressants, including 20% (3/15) with hyperglycemia from prednisone and FK, and 27% (4/15) with either headache or tremulousness from FK. None of the patients had an allergic reaction to either the albumin or fresh-frozen plasma, but two patients had an early postoperative hematoma, which may have been caused by plasmapheresis-induced coagulopathy. For this reason, fresh-frozen plasma, rather than albumin, is now being used to replace the plasma removed during plasmapheresis if transplantation is deemed likely within 48 hours.

DISCUSSION TOP

Utilization of a desensitization protocol allowed 11 patients to receive LD kidney transplants, which would not otherwise have been performed because of positive cross-matches. The protocol was well tolerated, and surprisingly few rejection episodes have been observed in the transplanted kidneys. In the early postoperative period, three patients had a transient rise in creatinine, associated with mild neutrophilic infiltrates in the glomeruli or endothelium. These patients were treated for mild vascular rejection with full clinical resolution. One additional case of mild cellular rejection occurred after 41 days, and resolved completely with pulse steroids. The primary nonfunction rate was zero, and all patients remain off dialysis after 3-26 months of follow-up.

The acute rejection rate of 36% (4/11), with no immunologic failure of a kidney to date, is acceptable by modern standards. Acute rejection rates in recent multicenter trials of kidney transplantation were 20% under MMF (5) and 31% under FK (4), and we have recently reported an overall acute rejection rate of 30% among our MMF-treated kidney recipients (9). The low rate and severity of antibody-mediated rejection in this series of patients previously sensitized to their donors' antigens may be the result of properties of the immunosuppressants that were used. Mycophenolate mofetil inhibits proliferation of B cells and blocks alloantibody production in vitro. Tacrolimus inhibits T-cell function, which could reduce the helper function required for optimal production of mature B cells and plasma cells. OKT3 has been shown to be effective in reducing immunologic failures in AHG cross-match-positive patients (10).

IVIg was used as part of the current protocol because of its immunomodulatory effects. It has been used successfully to reduce the PRA of patients waiting for a transplant (11). The beneficial effect of IVIg may be mediated by anti-idiotypic IgG antibody, and by in vivo induction of blocking IgM antibody. Other proposed mechanisms of action include down-regulation of antibody production or B-cell energy (12). Notwithstanding these theoretical benefits, the actual contribution made by IVIg to the outcomes in this study could not be determined, as all patients received it, and it was given with other immunosuppressive therapies. Furthermore, plasmapheresis can abrogate the potential benefits of IVIg therapy (13).

It is encouraging that the protocol was well tolerated. Past desensitization protocols were associated at times with excessive rates of infectious and immunologic complications. Such protocols often included high-dose corticosteroids and cyclophosphamide, and profound antibody depletion with protracted courses of plasmapheresis or immunoadsorption. We believe the current protocol was tolerable because overimmunosuppression was avoided by use of low-dose steroids and no cyclophosphamide. Although plasmapheresis and IVIg can cause deleterious complications such as infections and immune complex disease, short courses of these therapies are generally considered rather safe. The complications of antibody depletion were reduced in the current protocol by limiting the number of plasmapheresis treatments to a maximum of six, and by replenishing recipient serum IgG with IVIg.

During the time frame of the current series, we gained additional experience with plasmapheresis in LD kidney transplant recipients who did not strictly fulfill the inclusion criteria for the protocol. Two patients were cross-match-positive with their donors at the time of evaluation, but had converted spontaneously negative immediately before the first plasmapheresis treatment. These patients were given one and three plasmapheresis treatments before transplantation. Both of these two patients developed histologic evidence of antibody-mediated rejection on POD 3 and POD 14, and both were treated with plasmapheresis, IVIg, and antilymphocyte antibody. Their creatinine levels are now 1.3 and 1.4 mg/dl, at 9 and 6 months after the transplant, respectively. Another patient was referred from another transplant center, which would not perform his second LD transplant because of a positive flow cytometric, but negative cytotoxic, cross-match. This patient was given four plasmapheresis treatments before transplant and was still rejection-free at 6 months. One repeat transplant patient had a negative T-cell cross-match, with a strongly positive B-cell cross-match, and received two pretransplant plasmapheresis treatments to reduce her titer of class 2 antibody. This patient remained rejection-free 4 months after transplant.

It would be useful to have a predictor of the likelihood of success with pretransplant desensitization. In this study all those who successfully completed the protocol had a donor-specific antibody titer of 1:2 or lower immediately before the first plasmapheresis treatment (Table 1). However, because the number of patients in this series is small, it would be premature to exclude those with a higher titer from eligibility based on these findings.

Although this study raises the hope for transplantation of sensitized recipients with a live kidney donor, patients enrolled in such a protocol should be counseled about the risk of immunologic failure. Although there were no kidney graft losses caused by vascular rejection in this series, there was evidence that alloantibody may have caused transient graft dysfunction in three cases. Furthermore, one of the pancreas transplants performed simultaneous with a LD kidney in this series was probably lost to humoral rejection. Further study is needed to more accurately define the short- and long-term risks of immunologic failure.

We conclude that a positive cross-match is not necessarily a contraindication to LD transplantation. Using a protocol to eliminate alloantibody using plasmapheresis, IVIG, FK, MMF, and OKT3, 11 patients have received successful LD kidney transplants, which would not normally have been done because of positive cross-matches.

FOOTNOTES TOP

¹ Presented in part at the 25th Annual Meeting of the American Society of Transplant Surgeons, May 19-21, 1999, Chicago, IL. [\[Context Link\]](#)

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