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Intravenous immunoglobulin, HLA allele typing and HLAMatchmaker facilitate successful transplantation in highly sensitized pediatric renal allograft recipients

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Abstract: The use of intravenous immunoglobulin (IVIG) in sensitized transplant candidates has resulted in reduced HLA antibody levels and shorter transplant wait times. In addition, the HLAMatchmaker program has been used to identify acceptable mismatches to permit transplantation in highly sensitized patients. We used IVIG desensitization in conjunction with high resolution HLA allele typing and HLAMatchmaker grading of donor offers to facilitate successful transplantation in two highly sensitized children who were awaiting second renal transplants. Both patients lost their initial transplant in <10 days to accelerated acute rejection, and were on dialysis for an average of 50 months with high panel reactive antibody (PRA) levels. They were started on monthly IVIG infusions (2 g/kg/dose). Within one wk following their third and fifth IVIG doses, both patients received a crossmatch compatible, deceased donor renal transplant selected by HLAMatchmaker as a suitable donor offer. Both patients remain rejection free with excellent renal function 19 and 15 months post-transplant, respectively. In conclusion, combining IVIG therapy and donor selection by HLA humoral epitope matching permitted successful transplantation of two highly sensitized children. Further studies in larger numbers of patients with longer follow-up are needed to determine the individual role played by, and relative importance of each component of this combined strategy.

Rudolph P. Valentini[1], Sandra L. Nehlsen-Cannarella[2], Scott A. Gruber[3], Tej K. Mattoo[1], Miguel S. West[3], Cheryl Lang[1] and Abubakr A. Imam[1]

[1]Division of Nephrology, The Carman and Ann Adams Department of Pediatrics, Wayne State University School of Medicine, Children's Hospital of Michigan, Detroit, MI, USA, [2]Immunogenetics Laboratory, Detroit Medical Center, University Labs, Wayne State University School of Medicine, Harper University Hospital, Detroit, MI, USA, [3]Section of Transplant Surgery, Department of Surgery, Wayne State University School of Medicine, Harper University Hospital, Detroit, MI, USA

Key words: intravenous immunoglobulin – human leukocyte antigen – HLAMatchmaker – panel reactive antibody – crossmatch – kidney transplantation – pediatrics

Rudolph P. Valentini, MD, Division of Nephrology, The Carman and Ann Adams Department of Pediatrics, Wayne State University School of Medicine, Children's Hospital of Michigan, 3901 Beaubien Blvd., Detroit, MI 48201-2196, USA
Tel.: (313) 745 5604
Fax: (313) 966 0039
E-mail: rvalen@med.wayne.edu

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Renal transplantation is the treatment of choice for ESRD in pediatric patients. Waiting time for an initial renal transplant in children is usually governed by donor availability. On the other hand, wait times for repeat renal transplants may be much more dependent on recipient status. A repeat renal transplant may be unachievable in some children who are highly sensitized to HLA molecules, resulting in an inability to attain a negative cross match.

Children who are exposed to non-self HLA via a prior transplant or blood transfusion may develop IgG to donor HLA, which frequently results in the generation of high PRA levels. These HLA antibodies pose a great challenge to locating a compatible donor organ, and a successful renal transplant can only be attainable in a reasonable time frame by eliminating preformed antibody and downregulating future immunoreactivity.

The immunomodulating properties of IVIG have been exploited in adults seeking deceased donor transplantation with good success rates

Abbreviations: CDC, complement dependent cytotoxicity; CMV, cytomegalovirus; ESRD, end stage renal disease; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; OPO, organ procurement organization; PP, plasmapheresis; PRA, panel reactive antibody.

(1). HLA antibody titers were lowered and wait times on the transplant list were reduced with IVIG treatment. HLAMatchmaker, a matching program that considers the structural basis of HLA molecules, can be a useful aid for selecting donors and/or immunosuppressive protocols to optimize organ transplant survival (2–10). We applied this program, in conjunction with IVIG desensitization, to the transplantation of two highly sensitized pediatric patients in order to select complement dependent cytotoxic (CDC) and flow cytometric crossmatch compatible allograft donors with humoral epitope 'acceptable' HLA mismatches. In this way, we very likely reduced both wait times on the transplant list and risk of antibody mediated rejection, and we report the successful outcomes herein.

Materials and methods

Our inclusion criteria for desensitization candidates included ESRD patients with current and/or peak PRA $> 50\%$, evidence of IVIG responsiveness *in vitro*, and at least one of the following: on the transplant list for > 12 months and/or limited dialysis access necessitating urgent transplantation (11).

HLA typing and antibody testing

Patients are HLA typed to the allele level (molecular, high-resolution sequence-based and sequence-specific typing) to achieve proper antibody identification and donor matching, a practice that has proved necessary in our 65% African American patient population because of the frequency of unusual alleles. Allele typing is used to elucidate the apparent discrepancies of patients seemingly sensitized to a specific donor's HLA while proving to be flow cytometric crossmatch-compatible with that donor. HLA antibody screening is performed using the solid phase ELISA technique with 40-member class I and 30-member class II soluble HLA panels (mixed Caucasian and African American) that have been HLA typed to the allele level (GTI Diagnostics, Inc., Waukesha, WI, USA).

IVIG protocol

An *in vitro* IVIG-inhibition assay was employed to determine whether the patients were candidates for *in vivo* IVIG therapy (11). A serum sample from each patient was tested with and without IVIG in a CDC assay to determine whether the HLA antibody specificities could be eliminated. The PRA levels of both patients were reduced to 0% by this *in vitro* treatment, suggesting that *in vivo* treatment would be successful.

Our desensitization protocol was based on that of Jordan et al. (11). Up to four doses of 10% IVIG [2 g/kg administered over 12 h (maximum dose = 140 g); Gammagard S/D and Polygam S/D; Baxter HealthCare Corp, Westlake Village, CA, USA] were given at one month intervals. Patients were considered eligible for transplantation when their PRA was $\leq 20\%$ on two consecutive months. If a suitable kidney became available within the time period, IVIG was administered on the day of transplant, if not given within the prior seven days. If the patient did not receive a transplant, single annual doses were continued for up to two yr. An additional dose was given one month following transplantation. A 5% vs. 10% IVIG solution was used for the perioperative and one month postoperative IVIG dose because of concerns regarding serious thrombotic events and graft loss from thrombosis occurring with the latter, which may have been related to the higher viscosity or osmolarity of the 10% solution (12–14).

Serum samples were drawn immediately prior to each IVIG dose and assessed for HLA antibody by CDC for PRA. Thirty minutes prior to the IVIG infusion, both patients were premedicated with acetaminophen (15 mg/kg; maximum dose = 650 mg) and diphenhydramine (1 mg/kg; maximum 50 mg).

HLAMatchmaker

The HLAMatchmaker is a structurally based HLA matching computer program that determines donor–recipient compatibility at the molecular level and has been shown to correlate with graft survival, particularly in presensitized recipients (2, 3, 8, 15). HLA mismatch acceptability is determined by intra- and interlocus comparison of donor–recipient HLA polymorphic residues in antibody-accessible positions of donor HLA. The relative difficulty in matching patients in our population with local donors has led us to create a relative match grade for selecting optimal donors for pediatric recipients, with the intent of minimizing the antigen load of mismatched donor HLA (10). To do this, we first reviewed the range of epitope mismatches of our patients' HLA alleles. To this end, we initiated high-resolution HLA typing to define the alleles of all five loci (HLA-A, B, C, DRB1, DQB1) and then assigned individual grades for class I and class II donor HLA depending on the number of mismatched epitopes [0–9 = A (best); 10–19 = B (good); 20–29 = C (fair); 30+ = D (poor)]. This scoring system was further modified with additional information if the mismatches were 'high risk' because they included either: (i) replicate mismatches of apparently highly immunogenic amino acids or (ii) mismatching of amino acids at the 77–83 positions (Bw4,6) (16–17). Although our scoring system exceeds the established HLA triplet mismatches for optimal graft survival in adults, it was designed to improve donor–recipient matches within our local donor population (3, 6, 15). We attempted to avoid strongly mismatched donors while using the epitope match grade to indicate the probable risk of antibody mediated rejection, and therefore preferred a donor match grade of at least B/B and no more than one high risk mismatch.

Immunosuppression protocol

Both patients received a preoperative oral dose of MMF (600 mg/m^2) and an intraoperative dose of rabbit antithymocyte globulin [thymoglobulin (1.5 mg/kg); given over four h, with methylprednisolone (250 mg) as premedication. Postoperatively, thymoglobulin was administered at the same dose for seven to eight days, MMF was continued at 400 mg/m^2 twice daily, and a steroid taper begun. Tacrolimus was introduced on postoperative days two to four with target 12-h whole blood trough levels of 10–12 ng/mL by microparticle enhancement immunoassay (IMx® Tacrolimus II assay; Abbott Laboratories, Abbott Park, IL, USA).

Results

Patient 1 was an 11-yr-old, 32 kg African American, CMV (−) male with ESRD secondary to obstructive uropathy from prune belly syndrome. He had a deceased-donor renal transplant 63 months earlier which was lost to accelerated acute rejection and removed within one wk. His peritoneal dialysis membrane was no longer usable, and he was dialyzed through a left femoral arteriovenous graft. He also had thrombosis/stenosis of his subclavian and internal jugular veins bilaterally and thrombosis of his right femoral vein. Because of severe access limitations and prolonged wait time on the transplant list (>1500 days), he was started on IVIG. Blood drawn immediately prior to his first dose revealed an 88% PRA with antibodies to multiple class I and class II HLA. He required three consecutive monthly infusions of IVIG following hemodialysis to become eligible for transplantation. At this time, his PRA was 0% with all antibody specificities eliminated. Within one wk of his third IVIG dose, a grade B/A (Class I/II, respectively) deceased donor kidney became available. The donor was a 32-yr-old, 79 kg, CMV (+) Caucasian male. There were six class I and one class II HLA mismatches (Table 1) and 17 class I (grade B) and three class II (grade A) epitope mismatches with one high-risk addition (repeated epitope because of a shared mismatch at position 151 on HLA-A3 and A34). A final donor–recipient crossmatch by flow cytometry was HLA antibody-negative. CMV prophylaxis consisted of CMV immunoglobulin and four months of ganciclovir.

The patient experienced delayed graft function and underwent a transplant biopsy on postoperative day eight, revealing acute tubular necrosis without evidence of cellular or antibody-mediated rejection. He was discharged home with a serum creatinine of 0.8 mg/dL. His post-transplant course was complicated by the development of CMV infection at eight months, which was successfully treated with CMV immunoglobulin and ganciclovir without recurrence. He is now 19 months post-transplant on prednisone 5 mg/d, MMF 100 mg twice daily, and tacrolimus (trough levels 5–6 ng/mL) with serum creatinine 0.8 mg/dL (estimated creatinine clearance 100 mL/min/1.73 m^2). He has been free of rejection throughout his post-transplant course.

Patient 2 was a 13-yr-old, 50 kg Caucasian female with ESRD secondary to reflux nephropathy maintained on peritoneal dialysis. She had a living-donor renal transplant 27 months earlier which was lost to accelerated acute rejection and removed within 10 days. As a result of waiting on the transplant list for two yr without any kidney offers, she was started on IVIG. She required four consecutive monthly infusions of IVIG while on nightly peritoneal dialysis to become eligible for transplantation. Five and one half months after the initiation of the IVIG protocol, a grade C/C deceased donor kidney with one/two high risk additions (class I/II, respectively) became available. This kidney, which was a less than optimal match for our high risk patient, was accompanied by a positive final cross match and was not transplanted into our recipient. However, within one wk of her fifth IVIG dose at 12 months, a grade B/A (class I/II, respectively) deceased donor kidney became available. The donor was a 90.8 kg, 34-yr-old African American male. There were five class I and one class II HLA mismatches (Table 1) and 13 class I (grade B) and seven class II (grade A) epitope mismatches with no high-risk additions. A final donor–recipient crossmatch by flow cytometry was negative.

Table 1. Patient demographics and clinical features

	Patient 1	Patient 2
Age at time of second transplant (yr)	11.8	14.3
Race and gender (recipient/donor)	African American male/Caucasian male	Caucasian female/African American male
Time (months) between transplant 1 and 2	63	39
Number of IVIG doses prior to transplant	3	5
Peak PRA	88%	82%
Percent PRA post-IVIG	0%	0%
Recipient HLA	HLA-A*0201,*3001; B*4201,*4901; C*0701,*1701; DRB1*1101; DQB1*0301	HLA-A*0101,*2301; B*0801,*4403; C*0401,*0701; DRB1*0301,*1501; DQB1*0201,*0602
Donor HLA	HLA-A*0301,*3401; B*1402,*3501; C*0401,*0801; DRB1*0101,*1101; DQB1*0301	HLA-A*2402,*3001; B*3901,*4402; C*0501,*1203; DRB1*0301,*0801; DQB1*0201
HLA-A,B,DR (UNOS) Match	1-Antigen (1 of 6)	0-Antigen (0 of 6)
HLA-A,B,C,DR,DQ 5-loci Match	1-Antigen (1 of 10)	1-Antigen (1 of 10)
Epitope mismatch	17 class I + 3 class II (grades B + A + 1 high risk)	13 class I + 7 class II (grades B+A)

The patient's allograft functioned very quickly and led to her discharge home on postoperative day eight with serum creatinine 1.1 mg/dL. She has not experienced any infection or rejection episodes, and is now 15 months post-transplant on prednisone 5 mg, MMF 500 mg twice daily, and tacrolimus (trough levels 6–8 ng/mL) with serum creatinine 1.0 mg/dL (estimated creatinine clearance 90 mL/min/1.73 m^2).

Discussion

Strategies for successful transplantation of the highly sensitized pediatric patient appear to be evolving. Children that are highly sensitized will have a number of HLA antibodies demonstrated on the PRA. Methods to reduce preformed HLA antibodies in prospective renal transplant patients have targeted both the removal of the circulating preformed antibody and impairment of its subsequent regeneration. These therapies have included PP, immunoadsorption, and treatment with alkylating agents (18–20). Glotz et al. (21) first demonstrated that IVIG could suppress the production of panel reactive anti-HLA antibodies for more than three months. The proposed mechanism was the presence of antibodies directed against the idiotypic component of the HLA antibodies, thereby neutralizing their effects and presumably facilitating their clearance.

Combining the advantages of IVIG with PP has also been attempted. Montgomery et al. (22) utilized this combination therapy in a preemptive manner in four adults who required one to six combined treatments of IVIG/PP to abrogate a positive cross match. The graft survival was 100% for the four living donor transplant recipients; all patients, though, had humoral rejection.

Jordan et al. (1) has demonstrated the efficacy of IVIG in a large randomized, placebo controlled trial in adult patients who exhibited a reduction of anti-HLA antibody levels and shorter transplant wait list times when compared with adult patients treated with placebo. A total of 17 patients were treated with IVIG; 16 patients received IVIG prior to transplantation. For the 16 patients pretreated with IVIG, outcomes were very good, with an 80% two yr graft survival. Four of these patients (25%) ultimately lost their allograft. Reasons for graft loss included antibody mediated rejection, recurrent disease, and chronic rejection in two patients (at 14 and 27 months, respectively). However, acute rejection episodes were frequent, with 14 episodes occurring in nine of 17 IVIG-treated patients, many of which occurred in the first six months. Mean serum creatinine was 1.7 mg/dL at follow-up. An earlier study by Jordan et al. (11) of 42 patients [mean age 42 (range 1.5–75 yr)], 21% of whom had a previous transplant, showed two yr graft survival rates of 89%, with an acute rejection rate of 31%. Their immunosuppressive protocol consisted of daclizumab induction, corticosteroids, MMF, and tacrolimus.

Focused reports of IVIG usage in pediatric renal transplantation are limited. Tyan et al. (23) demonstrated successful use of IVIG in a 13-yr-old kidney transplant patient who was successfully re-transplanted after markedly reducing the PRA. Al-Uzri reported its successful use in a sensitized seven-yr-old who received a deceased donor renal transplant 44 months after starting IVIG therapy (24). Despite a mild acute rejection episode approximately 10 days post-transplantation, a good long-term outcome was noted.

Based on the encouraging results with IVIG in highly sensitized patients, we initiated an IVIG desensitization protocol in 2004. As both patients were repeat transplant recipients with early prior graft loss and high PRA, they were deemed at high risk for either antibody mediated or cellular rejection.

The HLA antibodies of our patients were analyzed for identification of the target amino acid epitope(s) and from that, the virtual PRA was calculated through population statistics for African Americans and Caucasians. Our local OPO donor population is approximately 20% African American and 78% Caucasian. In contrast, our pediatric ESRD patient population is about 65% African American, many of whom have been sensitized through transfusion and transplantation from donors of both races, making definition of antibody specificities and donor matching difficult. Identification of the amino acid epitopes and conversion of the PRA to the population-specific virtual PRA through the Duquesnoy HLAMatchmaker program provides more useful information regarding the probability of finding a compatible donor for our patients. This program provides further information when we use it to generate the mismatched humoral epitopes of a potential donor's HLA to determine acceptability by generation of match grades and comparison of the match to the range of donor offers in our area. Takemoto et al. (25) have shown through analysis of the UNOS and Eurotransplant databases that there is a significant drop-off of graft survival when more than two epitopes are mismatched, particularly when calculated in each

of two high-risk groups, non-whites and sensitized. However, it is extremely rare to find a match grade 'A' for both classes of HLA with no high-risk epitopes in donor offers for our patients.

IVIG immunotherapy allowed our highly sensitized patients to become eligible for transplantation. Once eligible, the high resolution HLA allele typing and HLAMatchmaker program permitted the selection of a 'low risk transplant' for our high risk patients. The donors selected were based on their minimal humoral epitope mismatches relative to other available deceased donors. Efforts were made to identify an optimally matched donor kidney to potentially lower the risk of graft loss from rejection. Whether the administration of CMV immunoglobulin in Patient 1 provided an additional immunomodulatory effect is unknown. This overall strategy allowed our two high-risk patients to successfully undergo renal transplantation and continue to enjoy excellent graft function at a mean follow-up of 17 months. Further studies with greater patient numbers and longer follow-up are needed to better generalize this combined approach, determine the relative contribution of each component, and assess its ability to increase the likelihood of successful transplantation in the highly sensitized pediatric recipient.

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