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Successful renal transplant outcome after intravenous gamma-globulin treatment of a highly sensitized pediatric recipient

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Abstract: Approximately 10% of patients on the renal transplant (Tx) cadaver waiting list have high (>20%) panel-reactive antibody (PRA) levels to human leukocyte antigens (HLA). Intravenous gamma-globulin (IVIG) has been shown to reduce anti-HLA cytotoxic antibody levels through an anti-idiotypic antibody-blocking effect. We report a successful renal Tx outcome in a 7-yr-old-girl with high PRA levels owing to a failed renal Tx who experienced a significant reduction in PRA levels (from 96% to 0%) concomitant with IVIG therapy. IVIG was infused weekly (500 mg/kg/week) for 3 consecutive weeks every 12 weeks. Thirty-four months after starting IVIG therapy, the PRA activity dropped to zero and IVIG was stopped. Then IVIG therapy was resumed after 8 months due to a rebound in PRA activity to 52%. Forty-four months after starting IVIG therapy, the patient was cross-matched with a cadaver donor who shared three antigens with the first living donor. The cross-match was positive with the recipient's sera obtained prior to IVIG therapy and negative with the recipient's sera obtained post-IVIG therapy. A successful cadaver renal Tx was performed using anti-thymocyte globulin (ATGAM) induction therapy and a tacrolimus-based immunosuppression protocol. IVIG was given (1 g/kg) prior to Tx and at day 4 post-operatively. A single mild acute rejection episode occurred 10 days post-transplantation that responded to pulse methylprednisolone therapy and an increase in the tacrolimus oral dose. We conclude that a prolonged course of IVIG infusions, without immunosuppressive medications or plasmapheresis, is likely to have been beneficial in modulating the immune response in this highly sensitized recipient. Randomized multicenter trials are required to define the role of IVIG in this specific population.

Amira Y. Al-Uzri[1], Barry Seltz[2], Peter D. Yorgin[4], Catherine M. Spier[3] and Kenneth Andreoni[5]

[1]Department of Pediatrics, Oregon Health & Science University, Portland, Oregon, USA, Departments of [2]Pediatrics and [3]Pathology, University of Arizona, Tucson, Arizona, USA, [4]Department of Pediatrics, Stanford University, Stanford, California, USA, [5]Department of Surgery, University of North Carolina, Chapel Hill, North Carolina, USA

Key words: kidney transplantation – panel-reactive antibodies – child – tacrolimus – lymphocyte cross-match – HLA antigens

Amira Al-Uzri, MD, Oregon Health & Science University, Department of Pediatrics, Division of Pediatric Nephrology, 707 SW Gaines Road-CDRCP, Portland, OR 97201, USA
Tel.: 503-494-7327
Fax: 503-418-6718
E-mail: aluzria@ohsu.edu

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Approximately 10% of patients with ESRD on the Tx cadaver waiting list have high (>20%) PRA levels to HLAs. A high PRA limits the number of negative cross-matches with potential donors; thus, the waiting time for this group of patients is expected to be longer than that for patients with lower PRA levels. The waiting time can often exceed 5 yr (1). In addition, highly sensitized patients are at a greater risk of developing acute rejection (2) post-Tx due to the persistence of memory cells in their presensitized immune system.

Modulation of the immune system, and consequently PRA levels, by immunosuppression therapy in the pre-Tx period had been previously reported, most commonly in con-

Abbreviations: ATGAM, anti-thymocyte globulin; CAD, cadaver donor; ESRD, end-stage renal disease; HLA, human leukocyte antigen; IVIG, intravenous gamma-globulin; PRA, panel-reactive antibodies; Tx, transplantation.

junction with plasmapheresis. Prednisone (3–7), methotrexate (6), cyclosphosphamide (3–7), and azathioprine (6) are commonly used concurrently with plasmapheresis (4, 5,7–10) or immunoadsorption therapy (3, 7, 9, 11) to lower antibody levels. Several reports (12–17) have described the use of combined plasmapheresis/immunoadsorption and immunosuppressive medications in the post-Tx period to reverse an antibody-mediated rejection. Nevertheless, these therapies are associated with a high risk of infection and antibody rebound after cessation of therapy (18, 19).

IVIG has been shown, both in the pre- and post-Tx periods, to reduce anti-HLA cytotoxic antibody levels by exerting an anti-idiotypic antibody-blocking effect (18–24). We report a successful renal Tx in a 7-yr-old girl with a high PRA level due to a previously failed renal Tx that responded to repeated infusions of IVIG over 4 yr.

Case report

A 2995-g female term infant was born with a history of bilateral atrophic kidneys and oligohydramnios on prenatal ultrasound. A repeat post-natal renal ultrasound was consistent with bilateral renal dysplasia. The infant was discharged from the hospital at 3 weeks of age with a serum creatinine of 3.3 mg/dL. At 23 months of age she was referred to the Tx program at the University of Arizona. A living related renal Tx from her aunt was performed concurrently with bilateral native nephrectomies. The pre-Tx final cross-match between the donor and recipient was negative. The HLA type of the patient and her first donor are presented in Table 1. The graft failed within 24 h owing to vascular thrombosis of the renal artery. The patient was placed on home peritoneal dialysis. Immunosuppression therapy was tapered within a month after Tx. Serial measurements of PRA levels demonstrated a dramatic conversion from 0% pre-transplant, to 96% positivity within 2 months after the failed Tx. The patient was placed on the kidney cadaver waiting list.

The high level of sensitization in this child meant the commitment to extended years of dialysis therapy. Long-term dialysis therapy in young children is associated with known medical comorbidities, repeated hospitalizations and interference with normal daily activities and schooling. This prompted us to aggressively seek other venues for early retransplantation in this patient despite her high PRA levels. The few preliminary reports of the positive effect of IVIG in reducing cytotoxic antibody levels *in vitro* and *in vivo* in highly sensitized individuals (21, 22, 25), encouraged us to attempt IVIG therapy. Other modalities of lowering PRA levels such as plasmapheresis, immunoadsoprtion or the use of immunosuppression therapy were excluded due to the high rate of side-effects associated with such modalities (5, 26). Infusion of IVIG was started 8 months post-Tx according to the following protocol: 500 mg/kg/week of IVIG for 3 consecutive weeks followed by a 9-week period without IVIG infusions. The protocol was based on abbreviated reports published previously showing that high doses of IVIG at 1.6–2 g/kg reduced anti-HLA antibody *in vivo* and *in vitro* (19, 20, 27). The derived modified dose of 1.5 g/kg was divided equally over a 3-week period in order to reduce side-effects and to limit the large volume associated with giving a single high dose of IVIG in a child on peritoneal dialysis. IVIG was concentrated to a 12% solution. The positive response after the first cycle as demonstrated by the decline in PRA levels prompted us to repeat the infusions regularly every 3 months. The patient was premedicated with oral acetaminophen and intravenous diphenhydramine prior to each infusion. No adverse side-effects were noted with IVIG therapy including fever, volume overload or allergic reactions. PRA levels decreased in response to the therapy (Fig. 1). Thirty-four months (42 months post-initial Tx) after initiating IVIG therapy, the patient's PRA level decreased to zero, after which IVIG infusions were discontinued. Eight months later there was a noticeable rebound in the PRA level to 52%. The high PRA values at that time were attributed to high anti-HLA (A2) antibodies. IVIG infusions were resumed but as a result of multiple medical problems associated with her dialysis, a desperate search for a potential living donor was attempted. Unfortunately, two potential living related donors were screened and tested positive with the patient's 8-month post-Tx serum (prior to initiation of IVIG therapy) and also with her most recent serum after the IVIG therapy. Both donors typed for the HLA-A2 antigen.

Forty-four months after initiating IVIG therapy (52 months post-initial Tx) the patient was cross-matched with a potential cadaver donor. Three serum samples were tested at three phases with unseparated T and B lymphocytes from cadaveric lymph nodes. The three phases were: (1): room temperature with AMOS

Table 1. Human leukocyte antigens (HLA) for the patient and her two donors

HLA	Patient	First donor	Second donor
A	1, 33	2, 24	3, 24
B	57, 65	35, –	35, –
C	6, –	3, 4	Not tested
DR	3, 7	8, –	1, 8
DQ	2, 3	4, 7	4, 5
DW	52, 53	52	–

Note that the second donor shares similar A, B and DR antigens with the first donor.

wash; (2) 37°C. (warm) cross-match, and (3) antihuman globulin augmented. The three serum dates were 5 months post-failed Tx (pre IVIG therapy), 51 months post, and 52 months post-failed Tx (post-IVIG therapy). The 5 months post-failed Tx serum was strongly positive at all three phases, whereas the two post-IVIG therapy sera were negative at all three phases. A CAD renal Tx was performed based on the negative cross-match of her most recent sera. Her PRA level at the time of Tx – which was 1 month after resuming her IVIG infusion – was 56%. The HLA type of the CAD donor are shown in Table 1.

Prior to the Tx the patient received a single dose of 1 g/kg of IVIG, that was repeated 4 days post-Tx. A 10-day induction with antithymocyte globulin (ATGAM®) at a dose of 20 mg/kg/day was coupled with early initiation of oral tacrolimus therapy aiming at target therapeutic trough levels of 10–15 ng/mL. Immediate graft function with a rapid decline in serum creatinine to 0.8 mg/dL was observed by post-operative day 3.

Ten days post-Tx, the patient developed fever, rigors and an increase in serum creatinine to 1.1 mg/dL. Blood and urine cultures were obtained and she was empirically started on vancomycin and ampicillin/sulbactam (Unasyn). The early onset of high fever post-Tx in a highly sensitized recipient together with marginal tacrolimus trough levels (6–8 ng/mL) prompted the initiation of anti-rejection therapy without obtaining a renal biopsy for a presumed diagnosis of acute rejection. Three pulses of methylprednisolone (10 mg/kg/day) were administered for 3 consecutive days together with an increase in the oral tacrolimus dose to achieve target trough levels of 20–25 ng/mL. The patient responded favorably to this therapy with disappearance of systemic symptoms and lowering of her serum creatinine to 0.7 mg/dL. In addition, an extra dose of IVIG of 1 g/kg was administered during the acute rejection episode. Twenty days post-Tx, her serum creatinine increased again to 1.1 mg/dL, a percutaneous

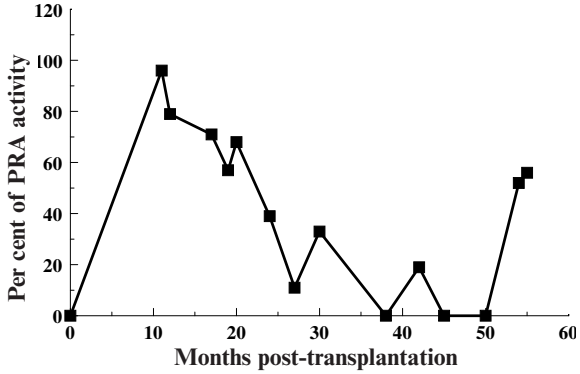

Fig. 1. Changes in panel-reactive antibody (PRA) activity level during prolonged infusion of IVIG over 52 months post a failed primary Tx. IVIG infusion was stopped for 8 months at 42 months post-Tx (34 months after starting IVIG infusion). A rebound in PRA activity to 52% was noted 51 months post-Tx concomitant with withholding the IVIG infusion for 8 months.

Tx renal biopsy was performed which showed no signs of acute rejection. The elevated serum creatinine was attributed to high serum tacrolimus levels that ranged from 17.6 to 25.5 ng/mL. The remainder of hospital course was uneventful and the patient was discharged home with a serum creatinine of 0.6 mg/dL. Immunosuppressive medications upon discharge were prednisone 25 mg/day (1.4 mg/kg/day), mycophenolate mofetil 250 mg TID (1070 mg/m^2/day) and tacrolimus 5 mg BID (0.55 mg/kg/day) with target trough levels ranging from 8 to 15 ng/mL. On her last follow-up visit 10 months post-transplant her serum creatinine was 0.5 mg/dL with no further acute rejection episodes.

Discussion

Plasmapheresis and plasma/immunoadsorption are reported to lower PRA levels and prevent acute rejection in highly sensitized individuals pre- and post-Tx with various success rates (8, 11, 13, 14, 16, 26). Most of these reports combine plasmapheresis with the use of cyclophosphamide therapy. We elected not to use plasmapheresis in our patient because of the higher risk of side-effects and infections associated with this aggressive therapy (13). Other important practical aspects that inhibited us from considering plasmapheresis or immunoadsorption in our patient relate to the lack of a hemodialysis access in a child on peritoneal dialysis. In the study reported by Montgomery *et al.* plasmapheresis was performed in highly sensitized patients combined with a relatively smaller dose of IVIG (100 mg/kg) in preparation for live donor Tx (28). Their proposed

protocol is more difficult to follow for long periods of time in children on the cadaver Tx waiting list.

IVIG has been shown *in vitro* and *in vivo* to modulate the immune system by modification of autoantibody and alloantibody levels through anti-idiotypic circuits (21, 25), anti-CD4 activity, inhibition of γ–IFN, and complement activation (29–31). IVIG can also suppress memory cells that develop from previous exposure to HLA antigens. Pre-Tx IVIG therapy, without additional immunosuppression, had been shown previously to effectively lower PRA levels by decreasing pre-formed anti-HLA idiotypic antibodies, thereby precluding preformed antibody-mediated acute allograft rejection (18, 19, 25, 32). In our patient, IVIG induced the reduction of PRA levels by 26% in 1 yr and from 96% to 0% over 34 months. Furthermore, IVIG was observed to profoundly decrease or eliminate anti-HLA idiotypes (HLA- A24, B35 and DR8) which would have precluded a negative final cross-match. Our findings of IVIG-mediated decreases in PRA levels, in our patient, are similar to those previously reported in adult series. Tyan *et al.* (22) reported a reduction in absolute PRA by a mean of 35% in 18 patients awaiting Tx. Interestingly, as in our patient, the residual antibody specificity often was due to HLA-A2. Tyan *et al.* also reported the successful re-transplantation of a 13-yr-old recipient after a PRA drop from 95% to 15% (22). Jordan *et al.* (20) reported the results of using IVIG in 25 patients with high PRA>50% at a dose of 500 mg/kg twice weekly for 3–4 weeks or as a single 2 g/kg dose. Approximately 70% of patients showed a dramatic and sustained reduction in PRA with IVIG infusion. Six patients successfully received kidney Tx, two received hearts, and two received lung Tx. Although most of the patients showed sustained reduction in PRA levels lasting more than 6 months, a transient increase in PRA activity was noted in most patients 2–4 weeks after initiation of IVIG therapy.

Given the dramatic initial response to IVIG in our patient, we elected to continue with the prolonged cyclic administration of IVIG until PRA activity was down to zero. The gradual decline of PRA observed in our patient may have been partially attributed to the natural decline in HLA antibodies that occurs over time; however, no published data are available on the natural rate of decline of PRA levels in children. The consistent and almost predictable decline in PRA levels observed over time in this patient point strongly towards a positive response to our medical intervention rather than just the effect of time on PRA levels. Furthermore, the discontinuation of IVIG therapy in our patient caused a significant rebound in PRA values. The rebound in PRA activity in response to discontinuation of IVIG had been previously reported by Jordan *et al.* (19). In order to prevent the rebound in PRA activity we elected to continue with our cyclic IVIG administration until the time of Tx.

In our patient, the pre-IVIG treatment sera reacted positively with CAD donor lymphocytes; however, the sera obtained prior to Tx (post-IVIG therapy) did not. This is of particular interest as the second donor shared three of the same HLAs with the first donor, including HLA- A24, B35 and DR 8. The absence of antibody-mediated hyperacute or accelerated acute rejection in this patient's 20-day post-Tx biopsy supports the observation that like naturally declining PRA levels, a negative cross-match post-IVIG treatment is more predictive of outcome then the historic sera prior to IVIG therapy. Patients with high PRA have an increased incidence of humoral mediated acute rejections post-Tx (2). The presumed steroid-responsive acute rejection episode experienced by our patient is thought to be caused by low tacrolimus blood levels that responded dramatically to methylprednisolone therapy and IVIG. Shapiro *et al.* had reported a low incidence (5%) of steroid-resistant acute rejections in tacrolimus-based immunosuppression protocols for pediatric kidney Tx (33). This might have been the case; however, there is ample evidence in the literature regarding the use of IVIG to ameliorate refractory acute rejection (20, 23, 27, 34). We believe that the combined therapy of pulse steroids, higher tacrolimus dose, and IVIG in this high-risk patient was critical in reversing the acute rejection without the need for additional anti-lymphocyte globulin therapy.

In summary, we report the successful use of IVIG in reducing PRA levels by the reduction of preformed anti-HLA idiotypic antibodies. The reduction of PRA permitted a favorable cadaveric Tx outcome in a highly HLA-sensitized child despite the fact that the transplanted graft had similar HLA antigens to the recipient's preformed antibodies. We postulate that IVIG therapy may be beneficial in modulating the humoral immune response in highly sensitized recipients. A multicenter trial supported by the NIH is underway at the present time to address these issues.

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