Evaluation of Intravenous Immunoglobulin as an Agent to Lower Allosensitization and Improve Transplantation in Highly Sensitized Adult Patients with End-Stage Renal Disease: Report of the NIH IG02 Trial

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Abstract. Reported are the reduction of anti-HLA antibody levels and improvement of transplant rates by intravenous immunoglobulin (IVIG) in a randomized, double-blind, placebo-controlled clinical trial. Between 1997 and 2000, a total of 101 adult patients with ESRD who were highly sensitized to HLA antigens (panel reactive antibody [PRA] ≥50% monthly for 3 mo) enrolled onto an NIH-sponsored trial (IG02). Patients received IVIG or placebo. Subjects received IVIG 2 g/kg monthly for 4 mo or an equivalent volume of placebo with additional infusions at 12 and 24 mo after entry if not transplanted. If transplanted, additional infusions were given monthly for 4 mo. Baseline PRA levels were similar in both groups. However, IVIG significantly reduced PRA levels in study subjects compared with placebo. Sixteen IVIG patients (35%) and eight placebo patients (17%) were transplanted. Rejection episodes occurred in 9 of 17 IVIG and 1 of 10 placebo subjects. Seven graft failures occurred (four IVIG, three placebo) among adherent patients with similar 2-yr graft survival rates (80% IVIG, 75% placebo). With a median follow-up of 2 yr after transplant, the viable transplants functioned normally with a mean ± SEM serum creatinine of 1.68 ± 0.28 for IVIG versus 1.28 ± 0.13 mg/dl for placebo. Adverse events rates were similar in both groups. We conclude that IVIG is better than placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized patients with ESRD. Transplant rates for highly sensitized patients with ESRD awaiting kidney transplants are improved with IVIG therapy.

Kidney transplantation is the preferred treatment for patients with ESRD. The benefits are evidenced by prolonged survival and improved quality of life for both children and adults. Despite these well documented benefits, transplant frequency remains lower than desirable as a result of limited organ availability (1,2). In patients with high levels of preformed anti-HLA antibodies (high panel reactive antibody [PRA]; highly sensitized), transplant rates are very low because of the additional immunologic barrier. Approximately 30% of patients on the waiting list are classified as sensitized, meaning they have peak PRA levels >20%, with about half of these having peak PRA levels >80%. These antibodies result from exposure to nonself HLA antigens; usually from previous transplants, blood transfusions, or pregnancy. Accordingly, women with ESRD are disproportionately sensitized compared with men.

Patel and Terasaki (3) established in 1969 that the presence of anti-donor IgG antibody (positive crossmatch) was a contraindication for kidney transplantation. Accordingly, the higher the PRA, the more difficult it becomes to find an immunologically compatible match. Transplant rates are lower...
for sensitized patients, and the waiting times for a compatible crossmatch are longer. Furthermore, although many of these patients may have living donors, transplantation cannot proceed because of the presence of a positive crossmatch.

In 2000, only 2.8% of all kidney transplants were performed in patients with PRA >80% at the time of transplantation (1,2), despite representing approximately 20% of the waiting list. In the last decade, the transplant rates for these patients has actually decreased over 50%, in part because of increased sensitivity of antibody detection techniques, but also because the pool of wait-listed patients competing for the scarce donor kidney resource is much greater. When finally transplanted, these patients experience an increased number of rejection episodes and have poorer graft survivals (2). The highly sensitized patient is destined to remain wait-listed for extended periods of time on dialysis, an added risk factor for patient and graft survival (4,5). The financial and emotional costs of maintaining highly sensitized transplant candidates on dialysis for years are enormous. Thus early transplantation would result in considerable cost savings, reduced morbidity and mortality, and improvement in quality of life, all desirable results.

Intravenous immunoglobulin (IVIG) are known to have powerful immunomodulatory effects (6). There are compelling clinical and laboratory data from our group and others that suggest that IVIG therapy administered to highly sensitized patients reduces allosensitization, ischemia-reperfusion injuries, and acute rejection episodes, and results in better long-term outcomes for cardiac and renal allograft recipients (7–13). Other investigators have confirmed these findings (14–17).

Here, we examined the utility of IVIG as an immunomodulatory agent in highly sensitized patients with ESRD awaiting transplantation. We hypothesized that IVIG would decrease anti-HLA antibodies in sensitized patients awaiting kidney transplantation. Our objective was to determine whether dialysis time could be minimized by improving successful transplant rates.

Materials and Methods
Patients and Treatments

Between October 1997 and July 2000, 12 U.S. transplant centers entered 101 adult patients with ESRD who were highly sensitized to HLA antigens (locally determined PRA ≥50% monthly for 3 mo) into the IG02 trial. Three randomized patients (two placebo, one IVIG) were discontinued before enrollment. Ninety-eight patients were analyzed. Patients were randomized 1:1 to receive IVIG (Gamimmune N 10% SD, Bayer Corp., or placebo 0.1% albumin, Bayer Corp.) (n = 48 IVIG; n = 50 placebo). Subjects received IVIG 2 g/kg (maximum dose 180 g) monthly for 4 mo or equivalent volume of placebo with additional infusions at 12 and 24 mo after entry and were followed to 30 mo. The statistical center prepared a center-blocked randomization plan, and registration was performed by calling the central registration desk, who then instructed the central pharmacy to prepare appropriate blinded study material, which was shipped for individual patient use. If transplanted, additional blinded infusions were given monthly for 4 mo. The dosing-adherent subgroup excluded six patients (two IVIG, four placebo) who failed to initiate therapy (n = 4) or who received crossover therapy (n = 2).

Posttransplantation immunosuppression was per center protocol. In addition, each center decided independently whether individual patients would receive offered organs after IVIG or placebo therapy.

PRA and PRA IVIG Tests

To ascertain panel-reactive antibody in study patients, sera were tested retrospectively in a microlymphocytotoxicity assay on an HLA-typed lymphocyte panel of 50 individuals at the central laboratory. Cells were stained with C-FDA (1:150 dilution of 10 mg/ml stock in 1× PBS, 15 min at 37°C), washed, and dispensed (1 µl/well for peripheral blood lymphocyte (PBL) or 1 µl/well at 200 to 300 cells/ml on beads) into oiled Terasaki trays containing 1 µl of patient test serum per well. PBL or T cells were incubated with serum for 30 min at 21°C and then with complement for 3 h (PBL) or (2 h for T cells isolated on Dynabeads) (HLA Cell Prep 1; Dynal, Great Neck, NY). The incubation times used to determine PRA were to mimic the crossmatch conditions so that the PRA would always be a reflection of the likelihood of obtaining a negative crossmatch. Crossmatching requires the use of a technique with enhanced sensitivity in comparison with the basic microlymphocytotoxicity test (e.g., extended NIH technique (11)).

Statistical Analyses

Wilcoxon tests and t tests were used in testing for group differences in continuous variables. Fisher’s exact tests were used in testing for group differences in categorical variables and the Mantel-Haenszel test was used to examine the treatment effect adjusting for prior transplant status. The log-rank test was used to test for treatment or group differences in time to event data and covariate adjustment used proportional hazard models. Kaplan-Meier estimates and Greenwood’s formula for the variance were used to provide estimates and confidence intervals for the cumulative incidence of time to event data. Mean time to transplantation was estimated by an exponential distribution under assumptions of a constant hazard and the cumulative hazard plots were inspected for deviations from linearity (18). To estimate the average treatment or group difference in PRA values over time, we used a generalized linear regression model, with a linear link function. We related the PRA value to time points and strata group while controlling for baseline measurements. The generalized estimating equations method of Zeger and Liang, which takes into account the correlation of repeated measurements for the same individual, was used to fit the model.

Results

Study Characteristics

The patients were mostly young adults, with women (58%) and minorities well represented. Common primary causes of renal failure included chronic glomerulonephritis, systemic immunologic disease), diabetes, and hypertension. Over half had previously received a renal transplant. Pretransplant PRA as measured by the participating clinics averaged over 80% at each of the prestudy time points; the medians were 86%, 84%, and 87% at months −3, −2, and −1, respectively (Table 1).

Among the adherent cohort, 87% of planned first-year infusions were administered to surviving nontransplanted participants. The administration rate was lower at the 12-mo time point (54 of 75, 72%) than during months 0 to 3 (331 of 367, 90%).
Adverse Experiences

Infusion symptoms, including headache, were monitored during, at the end, and 1 h after infusion. Overall, there was a significant increase in headache in the treatment group versus placebo (52% versus 30%) (\(P = 0.056\)). The rates of moderate/severe headache were also higher in the IVIG group (24% versus 13%). There was no difference in headache during the infusion period, but over the course of the five first-year infusions, significantly more IVIG patients experienced at least one headache at the end (50% versus 24%) and 1 h after (45% versus 20%) an infusion. For any particular infusion, the headache rate was small; for example, headache was reported at any time for 14% of the first infusions (20% IVIG, 9% placebo).

Both the number of patients reporting adverse events and their frequency were similarly few in the two treatment arms. Before transplantation, two IVIG patients experienced infusion reactions judged to be serious and potentially related to treatment. Each patient received subsequent IVIG infusions without serious reactions. Consequently, IVIG is considered safe to administer on dialysis.

Transplantation

A total of 27 patients—17 IVIG recipients (35%) and 10 placebo recipients (20%)—received a transplant during the study period (one-tailed \(P = 0.069\)). Of these six, two IVIG and four placebo were from living donors, so the overall cadaver transplant rates were 31% versus 12% (one-tailed \(P = 0.0137\)). Three transplants occurred among the six patients in the nonadherent group (one patient assigned to IVIG was transplanted before therapy was initiated; two patients assigned to placebo received IVIG therapy). Thus, in the adherent group, the IVIG transplantation rate for IVIG recipients was twice as great as that for placebo (16 of 46 versus 8 of 46; 35% versus 17%; one-tailed \(P = 0.048\)). For patients with a previous transplant, the transplantation rate for the adherent group of patients was three times higher after IVIG than after placebo (10 of 34 versus 3 of 28; 22% versus 7%; Table 2). Time to transplantation is significantly shortened with IVIG pretreatment (log-rank one-tailed \(P = 0.049\)) (Figure 1). This improvement is also seen after adjusting for receipt of a previous transplant (\(P = 0.034\)). Assuming a constant hazard rate, the estimated projected mean time to transplantation is 4.8 yr for the IVIG arm versus 10.3 yr for the placebo arm.

Graft Survival

Of 24 adherent patients who received kidney transplants during the 30-mo study period, graft failure occurred in 4 (25%) of 16 IVIG versus 3 (38%) of 8 placebo subjects. For adherent patients, the 2-yr graft survival rates were 80% for IVIG and 75% for placebo (\(P = 0.57\)). There were three graft failures in the first posttransplant year (two IVIG, one placebo). The IVIG recipients had failures attributed to antibody-mediated rejection at 2 mo and recurrent glomerulonephritis at 9 mo, and the two other IVIG graft failures were attributed to chronic rejection at 14 and 27 mo after transplantation. One placebo patient died with a functioning graft from diabetic hyperglycemia 17 d after transplantation. Two other placebo-
treated patients died with functioning grafts, one of malignancy at month 20 and the other of hyperkalemia at month 26 after transplantation (and after the 30-mo study period concluded).

**Allograft Rejection**

Fourteen acute rejection episodes occurred in 9 of 17 IVIG recipients, 6 within the first month after transplantation. Only 1 of 10 placebo transplants experienced rejection \( (P = 0.042) \). With a median follow-up after transplantation of 2 yr, the mean ± SEM serum creatinine for the patients with viable grafts was 1.68 ± 0.28 mg/dl (IVIG) versus 1.28 ± 0.13 mg/dl (placebo) \( (P = 0.29) \).

**Patient Survival**

During the 30-mo study period, 12 patients died, 8 in the placebo arm and 4 in the IVIG arm \( (P = 0.22) \). Ten deaths occurred from typical causes for dialysis patients (four IVIG) while waiting for transplants. No deaths were related to either IVIG or placebo infusions. In the 30 mo after randomization, two deaths occurred in the posttransplantation phase, both in placebo recipients.

**PRA Changes**

PRA levels in participant sera were analyzed for IgG + IgM and for IgG only (dithioerythritol [DTT] reduction) at specified intervals during the study period before transplantation. Figure 2 shows the results over time. Significant reductions for the IVIG group relative to placebo were seen via repeated-measures analysis for both the IgM + IgG end point \( (P = 0.033) \), and the IgG end point \( (P = 0.007) \). Despite the decrease associated with IVIG administration, the mean PRA at each time point remained >40%. Even over this restricted range, baseline PRA level is predictive of time to transplantation \( (P = 0.009) \). Of interest is the return of PRA levels to near baseline at 6 mo after IVIG infusion. This does not correlate with the improvement in transplantation in the IVIG group (Figure 1) beginning at 6 mo.
Discussion

Our previous work in inflammatory and autoimmune disorders and experience with treating refractory rejection (7,11,14,19,20) in transplant recipients formed the basis for this controlled clinical trial in highly sensitized patients at centers around the United States. On the basis of the observations made in this clinical trial, we think that IVIG has an important role to play in the management of highly sensitized patients before transplantation. The average wait times for cadaveric renal transplantation are long for nonsensitized patients with ESRD (4 to 5 yr) but are nearly exclusionary for very highly sensitized patients (2). Because these patients are unlikely to receive a transplant without an intervention, we tested the hypothesis that repetitive IVIG administration would reduce anti-HLA antibody and thereby improve the chances for a successful kidney transplant. The data presented here show that IVIG decreases, but does not eliminate, sensitization. Transplant rates improve and waiting time is decreased, and graft survival is acceptable.

IVIG is an expensive therapy. Is it cost-effective to use as an agent to desensitize patients with ESRD and improve transplantability? Data do exist in this regard (32). The cost of IVIG is about $35/g. For a 70-kg person, a 2- g/kg dose (140 g) costs $4900. A four-dose course costs $19,600. The costs of maintaining a patient on dialysis is about $80,000/yr, and the costs of a functioning transplant is about $20,000/yr (1). The Johns Hopkins group determined that the costs savings to Medicare (the sole provider for ESRD services in the United States) to remove half the highly sensitized patients from dialysis by performing a successful transplantation would be $1.4 billion over a 3-yr period. Although the numbers of patients transplanted were small in this study, the cost savings (including the cost of the drug) would be more than $300,000 per patient over a 5-yr observation period. Thus we can conclude that IVIG is a cost-effective therapy when one considers the cost of the only alternative therapy, dialysis. Medicare has approved this therapy in many regions on the basis of these data (32).

The effectiveness of IVIG in treating inflammatory and autoimmune disorders has prompted many investigations into potential mechanisms of action, which appear to be relevant to reduction of allosensitization. These include: (1) modification of autoantibody and alloantibody levels through induction of antiidiotypic circuits (6,11,14,19), (2) inhibition of cytokine gene activation and anti-cytokine activity (6,21,22,24), (3) anti-T cell receptor activity (25), (4) Fc receptor–mediated interactions with antigen presenting cells to block T cell activation (23,24), (5) anti-CD4 activity (28), (6) stimulation of cytokine receptor antagonists (24), and (7) inhibition of complement activity (29).

We have shown that IVIG significantly inhibits T cell activation and reduces the expression of CD40, ICAM-1, CD86, and MHC class 2 on antigen presenting cell (APC) in the mixed lymphocyte reaction (MLR) (23). The primary effect is on B cells, and indeed, we have demonstrated that IVIG induces significant B cell apoptosis in vitro through Fc receptor–dependent mechanisms. The expression of an important B cell costimulatory molecule (CD19) (23) is also reduced. Samuelsson et al. (27) have recently described another unique immunoregulatory effector function for IVIG in a mouse model of antibody-induced thrombocytopenia. These investigators demonstrated that IVIG induces the expression of the inhibitory receptor FcγRIIB. This suggests that IVIG may exert many of its beneficial immunomodulatory effects through induction of inhibitory receptors on immune cells with subsequent inhibition of cell proliferation and/or induction of apoptosis (26,27). Another interesting observation that may have relevance, especially for the treatment of antibody-mediated rejection is the high affinity of the Fc receptor portion of IVIG for activated complement components C3b and C4b. The evidence comes from a study that showed that IVIG treatment significantly prolonged the survival of pig-to-baboon xenotransplants (from 30 to 60 min to 10 d) (29). This beneficial effect of IVIG was through inhibition of complement-mediated endothelial cell injury. This could have relevance for the inhibition of complement mediated injury to allografts that has been recently described for both acute rejection and chronic rejection in humans (30,31).

From this multicenter, double-blinded, placebo-controlled trial, we can conclude that IVIG is superior to placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized patients with ESRD. The relevance of this approach to increase transplantation rates in both living donor and cadaver donor recipients is noted. Although more acute rejection episodes were seen in the IVIG treatment group, the allograft survival and mean serum creatinines (SCrs) were similar after a mean of 2 yr of follow-up. It was interesting to note that transplantation rates began to increase in the IVIG group at approximately 1 yr after IVIG therapy. We initially thought that this was because of an issue of organ availability, but recently, we demonstrated that the levels of HLA class I and class II antibodies are suppressed for more prolonged periods of time compared with the PRA. The PRA may not truly represent the level of anti-HLA antibodies in these patients and thus the improved transplantation rates despite return of PRA to higher levels (35).

Finally, our group (20,34) and Glotz et al. (33) have shown that more dramatic rates of PRA and crossmatch reductions and transplantation can be achieved by use of IVIG than were shown in this study. It is important to realize that the I戈2 study was not specifically designed to increase rates of transplantation. Subjects entered did not have readily available donors, and there was no requirement for being on the transplant list for a time sufficient to insure cadaver donor offers. This likely accounts for most of the differences between this study and subsequent trials. When IVIG is provided to patients with living donors or to those who are likely to receive a cadaver donor kidney because of wait time, the transplantability of these patients increases dramatically. We have recently shown that IVIG treatment allows us to transplant >80% of patients referred with positive crossmatches at our center (Cedars-Sinai Medical Center) (34). Efficacy of IVIG relies on both its ability to inhibit anti-HLA activity and the ability to have an available donor when crossmatches or PRA are re-
duced. IVIG pretreatment should improve the transplant potential for highly sensitized patients with ESRD awaiting kidney transplantation, something that was previously not available for them.

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References

2. USRDS Renal Data Systems Report, 2001


