DESENSITIZATION THERAPY WITH INTRAVENOUS GAMMAGLOBULIN (IVIG): APPLICATIONS IN SOLID ORGAN TRANSPLANTATION

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ABSTRACT

Intravenous immunoglobulin products (IVIG) are derived from pooled human plasma and have been used for the treatment of primary immunodeficiency disorders for more than 24 years. Shortly after their introduction, IVIG products were found to be effective in the treatment of autoimmune and inflammatory disorders. Over the past 2 decades, the list of diseases where IVIG has a demonstrable beneficial effect has grown rapidly. These include inflammatory diseases such as Kawasaki disease, Guillain-Barre syndrome, myasthenia gravis, dermatomyositis and demyelinating polyneuropathy. Recently, we have described a beneficial effect on the reduction of anti-HLA antibodies with subsequent improvement in rates of transplantation for highly human leukocyte antigen (HLA) sensitized patients as well as a potent anti-inflammatory effect that is beneficial in the treatment of antibody-mediated rejection (AMR). These advancements have enabled transplantation of patients previously considered untransplantable and in concert with new diagnostic techniques has resulted in new approaches to management of AMR.

Introduction

Kidney transplantation results in improved survival rates and quality of life for both children and adults with end-stage kidney disease. However, rates of transplantation are low, due to organ availability (1–4). In patients with high levels of pre-formed anti-HLA antibodies (high Panel Reactive Antibody [PRA]; highly-sensitized), transplant rates are extremely low because of the additional immunologic barrier with increased risk of AMR. From 1994–2003, the numbers of highly-

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sensitized patients on the transplant list have continued to increase (12,808 in 1994 vs 17,814 in 2003) (1). In 2003, 32% of the transplant list was considered sensitized to HLA antigens with 13.7% having PRAs > 80% (1). Due to the many variations in tests used to determine PRA, this number is likely under reported. These antibodies result from exposure to non-self HLA antigens; usually from previous transplants, blood transfusions, and/or pregnancies (5). Thus, female patients are more likely to be sensitized than males.

If transplanted, these patients experience an increased number of rejection episodes and have poorer graft survival (6). 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 (1–4,12). The financial and emotional costs of maintaining highly-sensitized patients on dialysis for years are considerable and contrast greatly with the benefits provided by a successful transplant. Thus, early transplantation results in considerable cost savings, reduced morbidity and mortality and improvement in quality of life. However, until recently no therapeutic approaches were available to deal with this difficult patient group.

Patel and Terasaki demonstrated that kidneys transplanted across a positive crossmatch (CMX) barrier had very poor graft survival. These observations established the basis for modern CMX testing as a means of allocating kidneys (6). Sensitization is a significant barrier to obtaining a successful transplant.

The presence of IgG complement fixing antibody specific for donor HLA antigen (class I or class II) represents an unequivocal contraindication to transplantation. Patients transplanted across this barrier are at a *very high* risk for AMR and allograft loss. Other factors such as history of sensitizing events, titer and duration of anti-HLA antibody are also important risk factors for AMR.

Until recently, no therapeutic approaches were available to deal with this problem. Currently, there are two protocols which have been successfully employed. These include the plasmapheresis/CMVIg protocol (Johns Hopkins Protocol) (7) and the high-dose IVIG protocol (Cedars-Sinai Protocol) (8–12). The Mayo Clinic (13) also has extensive experience with both protocols.

Clinical Use of IVIG in Kidney Transplantation

Intravenous immune globulin products (IVIG) are known to have powerful immunomodulatory effects on inflammatory and autoimmune disorders (14). Data from our group and others suggests that

IVIG therapy given to highly sensitized patients results in reduced allosensitization, reduced ischemia-reperfusion injuries, fewer acute rejection episodes, and higher successful long-term allograft outcomes for cardiac and renal allograft recipients (8–12,15–18). We and others have confirmed that pre-treatment with IVIG results in reductions of anti-HLA antibodies, and is effective in treatment of allograft rejection episodes (10,16,17). We have also shown that IVIG is effective in reducing anti-HLA antibody levels and significantly improving transplant rates in highly-HLA sensitized patients in a controlled clinical trial (12).

The high-dose IVIG protocol developed at Cedars-Sinai evolved from reported efficacy with other inflammatory disorders (i.e., Kawasaki Disease) (14). Using the high dose IVIG protocol (2 gm/kg) for desensitization requires that antibody specificity be determined. To predict which patients will benefit from IVIG therapy prior to its administration, we developed an in vitro test using IVIG in the PRA assay (8,9,11). IVIG is added 1:1 and we then determine the extent of inhibition of T & B-cell cytotoxicity. In our experience, this in vitro assay provides an idea of the expected efficacy of IVIG when given in vivo.

It is important to mention that alternative explanations for the in vitro reduction of anti-HLA antibody-mediated cytotoxicity have emerged. These include inhibition of complement activation by the Fc fragment of IgG molecules in the IVIG preparations (23,27), or possible contamination of IVIG products with soluble HLA molecules (9). Wassmuth et al (18) showed that significant inhibition of the in vitro CDC assay was accomplished with IgM/IgA containing products only and this was likely due to inhibition of complement. These authors also showed that significantly lower inhibitory effects were seen when ELISA techniques for measurement of anti-HLA antibodies were performed.

Our data (9–11) contrast with these observations since no nonspecific inhibition (i.e. complement inhibition by IVIG (IgG) was seen and no soluble HLA was detected in products used. In addition, patterns of inhibition vary from patient to patient.

Despite the limitations of the in vitro assay, we have adapted it to determine the efficacy of IVIG in single donor/recipient pairs who have a positive CMX. If IVIG shows any reduction of T or B-cell cytotoxicity, we then treat the recipient with 2 gm/kg IVIG (maximum dose 140 gm) monthly $\times 4$ doses until the CMX is negative or acceptable. An acceptable CMX is defined in our program as a negative T & B-cell CMX by CDC but still flow cytometry positive at <200 shifts.

We usually give only 4 doses. We have also adapted this to use for

highly-sensitized deceased donor transplant candidates who have been on the UNOS waitlist for >5 years, have a PRA of >50% and who receive frequent offers for kidneys from donors with whom they have a positive CMX. These patients have an in vitro IVIG PRA, and if suppression or inhibition of the PRA is seen with IVIG, the patients are offered IVIG 2 gm/kg monthly ×4 in hopes of achieving desensitization and receiving a CMX compatible kidney or other organ.

From July 2002–October 2005, we evaluated 89 patients who were highly-HLA sensitized and had positive CMXs with potential donors in the in vitro IVIG-PRA test system. Eighty five percent showed inhibition to some degree in the in vitro PRA or CMX system. Seventy nine of eighty nine (89%) were transplanted after IVIG desensitization therapy (46 LD, 33 CAD). Of the 10 patients who were not transplanted, 6 are awaiting a cadaver transplant offer and two did not respond to IVIG. Two others were successfully desensitized for living donors, but medical conditions prevented transplantation. Thus, only 2/89 (2.2%) failed to respond to IVIG sufficiently to allow transplantation to be considered. The mean PRAs for the cadaver recipients were 83% and nearly all patients had antibodies specific to their donors that were eliminated or reduced by IVIG therapy. The incidence of allograft rejection is 28% with a 3 year patient and graft survival of 97.5% and 87.1%, respectively. Five grafts were lost to rejection. The mean serum creatinines at 3 years were 1.4 mg/dl.

The NIH IGO2 Study

From 1997–2000, the NIH conducted the IGO2 study. This study was a multi-center, controlled clinical, double blinded trial of IVIG vs placebo in highly sensitized patients awaiting kidney transplantation. The study was designed to determine whether IVIG could reduce PRA levels and improve rates of transplantation without concomitantly increasing the risk of graft loss in this difficult to transplant group. This study represents the only controlled clinical trial of a desensitization therapy.

Data from this trial were recently published (12). Briefly, IVIG was superior to placebo in reducing anti-HLA antibody levels ($p = 0.004$, IVIG vs placebo) and improving rates of transplantation. The 3 year follow up shows the predicted mean time to transplantation was 4.8 years in the IVIG group vs. 10.3 years in the placebo group ($p = 0.02$). With a median follow-up of 3 years post-transplant, the viable transplants functioned normally with a mean (±SE) serum creatinine of $1.68 \pm .28$ (IVIG) vs 1.28 ± 0.13 mg/dl for placebo ($p = .29$).

Allograft survival was also superior in the IVIG group at 3 years. From this multi-center, double-blinded, placebo controlled trial we concluded that IVIG is superior to placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized ESRD patients. Although more AR episodes were seen in the IVIG treatment group, the 3-year allograft survival and mean serum creatinines were similar to the placebo group. Transplant rates for highly-sensitized ESRD patients awaiting kidney transplants were improved with IVIG therapy.

Thus it appears that IVIG, alone, offers significant benefits in desensitizing highly-HLA sensitized patients and improves the rates of transplantation in this difficult to transplant group without patients experiencing excessive allograft loss.

Plasmapheresis/CMV-Ig Protocol

In 1998 Johns Hopkins University Hospital (JHH) began using an intensive preconditioning protocol to allow transplantation across a (+)CXM barrier. The protocol developed at JHH (plasmapheresis + CMV-IgG, {PP/CMV-Ig}) and a cocktail of immunosuppressive agents are initiated prior to renal transplantation (32). Post-transplant, additional treatments are delivered during the first 10 days (Table 1).

TABLE 1
Preconditioning Regimen for Recipients of a (+) XM or ABOi Renal Allograft (JHH PP + CMV-Ig Protocol)

Pre-transplant:
➢ Plasmapheresis (PP) 1 volume exchange QOD (replaced with 5% albumin)
➢ Low dose CMVIg (100 mg/kg) following each PP treatment
➢ Tacrolimus and mycophenolate mofetil begun at the same time as PP/CMVIg
➢ Endpoint of Pre-transplant Therapy_For (+) XM: (−) AHG CDC XM
➢ For ABOi: Isoagglutinin titers ≤16
Day of the transplant:
➢ Anti-IL-2 receptor induction antibody (2 mg/kg)
➢ steroid bolus (solumedrol 500 mg)
➢ splenectomy and/or anti-CD20 for ABOi or high risk (+) XM patients
Post-transplantation:
➢ 3 to 5 protocol QOD PP/CMVIg treatments
➢ triple drug immunosuppression (tacrolimus, mycophenolate mofetil, prednisone)
➢ dose of anti-IL-2 receptor antibody (1 mg/kg) every 2 weeks × 4 doses

(Abbreviations: CMVIg: cytomegalovirus-specific immune globulin. XM: lymphocytotoxicity crossmatch. ABOi: Blood group ABO incompatible. AHG-CDC XM: Anti-human globulin augmented complement-dependent cytotoxicity crossmatch. Anti-CD20: anti-B-cell Rituxan therapy.)

The endpoint of therapy is the elimination of anti-HLA donor specific antibody (DSA) either before or after the transplant.

The treatment plan is individualized based on an assessment of the patient's risk of antibody-mediated rejection (AMR) (32). This group identified recipient features that were associated with increased risk of AMR and graft loss. Patients thought to be at low risk (e.g. first transplant with pregnancy as sensitizing event) were treated with PP/CMVIg and quadruple sequential immunosuppression whereas high risk patients (e.g. third transplant with multiple repeat mismatches) have splenectomy and/or anti-CD20 added to their basic treatment plan.

This protocol produces a rapid reduction in anti-HLA titers that allows for transplantation after 4–5 plasmapheresis treatments. The JHH group feel that the addition of CMV-Ig adds an immunomodulatory effector mechanism that helps to keep the antibody titers low. It is critical to perform the transplant within a few days of the last plasmapheresis since rebound of anti-HLA does occur and can negate the benefits achieved with prior treatments. The Mayo Clinic has adopted both the PP/low-dose IVIG and high-dose IVIG protocols with similar success (13).

Table 2 shows the advantages and limitations of the high-dose IVIG vs PP/CMV-Ig protocols. Table 3 compares the results from the two protocols over the last 3–5 years. The outcomes for patients at the three institutions (JHH, Cedars-Sinai and Mayo Clinic) are very similar and comparable to results from non-sensitized patients.

We have also adopted a protocol that combines IVIG and plasmapheresis for those who do not respond adequately to IVIG alone. Briefly, we use a total of 5 plasmapheresis treatments that are followed by a single dose of IVIG (2 gm/kg) after the last plasmapheresis treatment, then by a single dose of the B-cell depleting agent Rituxan® at 375 mg/m2. A crossmatch is performed after the last plasmapheresis treatment before the addition of IVIG and Rituxan. If acceptable, we go ahead with the transplant. To date we have transplanted 4 patients with this approach and none has exhibited a rejection episode.

Alternative Approaches to Improve Transplantation for Highly-HLA Sensitized Patients

As more transplant centers in the U.S. and around the world develop protocols to improve transplantation for the highly-HLA sensitized patients, other approaches have emerged. Claas et al (20) reported on The Acceptable Mismatch Program which has been developed for al-

TABLE 2
High-Dose IVIG vs PP + CMV-IgG: Advantages & Limitations

High Dose IVIG: Advantages
➢ Less expensive/Less resources required
➢ Successful desensitization of living and deceased donors
➢ Easy and safe to administer. Can be given on dialysis or at home
➢ Desensitization is long-lasting in most cases allowing longer intervals between treatment and transplantation
High-Dose IVIG: Limitations
➢ Non-responders and incomplete responders exist (~10%)
➢ IVIG may interfere with assays for DSA
➢ Antibody modulation often less rapid than with PP + CMV-Ig
➢ Specific IVIG products have toxicity at high doses (i.e., sucrose and saline excipient products)
PP + CMV-Ig: Advantages
➢ Highly effective, few non-responders
➢ DSA easy to follow
➢ Kinetic modeling can predict number of treatments necessary to achieve desensitization
➢ DSA rapidly removed
➢ Effective in ABO incompatible transplants
PP + CMV-Ig: Limitations
➢ Effective for living donor transplant only
➢ More expensive and resource intensive than IVIG
➢ DSA can rebound post-transplant
➢ Transplant must be done after last PP + CMV-Ig treatment or rebound of antibody is seen
➢ More immunosuppressive

locating kidneys to highly-sensitized patients. These investigators reported that a schema developed for Eurotransplant using a computer program, HLA Matchmaker, which allocates kidneys to patients based on avoidance of HLA antigen sensitization allowed 112 transplants to be performed with a 2 year graft survival of 87%. The authors give no data on the incidence and severity of rejection episodes and current serum creatinine values. They also suggest this be implemented in conjunction with desensitization protocols in an effort to transplant most highly-sensitized patients. Other potential protocols include donor exchange programs that may improve access of highly-sensitized patients to transplantation (21). If these approaches are successful in the U.S. they should be adopted prior to initiation of desensitization therapy.

Complications and Cost of IVIG Therapy

Unlike the use of IVIG in immunodeficiency, patients who are highly-HLA sensitized require higher doses (1–2 gm/kg/dose) to achieve a

TABLE 3
Long-Term Outcomes of Desensitization Protocols for H-S ESRD Patients

Transp. Program Patient #:	Protocol Used	Patient Survival (3-5 Yrs)	Graft Survival (3-5 Years)	AR Rates	Mean SCr (3-5 Yrs.)
Mayo Clinic #94	HD IVIG PE/IVIG LD	97% at 5 yrs.	80% at 5 yrs.	35%	1.6 ± 0.6 mg/dl
JHU #90	PE+ CMV-Ig	95% at 3 yrs.	80.9% at 3 yrs.	62%	1.2 ± 0.3 mg/dl
CSMC #96	HD IVIG	97% at 5 yrs.	87% at 5 yrs.	36%	1.5 + 0.4 mg/dl

beneficial outcome. The use of higher doses and concentrations of IVIG products results in higher rates of infusion-related complications that were, at first, not anticipated and were poorly understood. We have recently reviewed the complications associated with IVIG infusions in patients with normal renal function and those on dialysis (22).

Briefly, the safety of IVIG infusions (2 gm/kg) doses given over a 4 hour hemodialysis session, monthly ×4 vs placebo (0.1% albumin) in equivalent doses was studied in the IG02 trial (12). The results are shown in Table 1. There were more than 300 infusions in each arm of the study using Gamimune N 10% vs. placebo. Adverse events were similar in both arms of the study (24 IVIG vs. 23 placebo). The most common adverse event in the IVIG arm was headache (52% vs 24%, $p = 0.056$). This usually abated with reduction in infusion rate and Tylenol®. Ten serious adverse events were noted, nine were in the placebo group. Thus we concluded from this double-blind placebo-controlled trial that high-dose IVIG infusions during hemodialysis are safe.

IVIG is an expensive therapy and ultimately, insurers and hospitals question the use of this drug for desensitization. The ultimate question relates to the cost effectiveness of IVIG for desensitization. Data do exist in this regard (5,12). Currently, a 4 dose course of IVIG for a 70 kg person at 2 gm/kg would cost ~$25,000–26,000. However, one must relate this to the cost of maintaining patients on dialysis, which is the only other option. In the IG02 study (12), the calculated cost savings was ~$300,000/patient transplanted vs those who remained on dialysis for the 5 years of the study. Data from USRDS (2003) also confirms that a considerable cost savings to Medicare is seen in highly-sensitized patients transplanted vs those who remain on dialysis (2).

Discussion:

IVIG products are derived from the plasma of thousands of donors. This insures that a wide diversity of antibody repertoire can be administered to patients. We also know that the Fc portion of IgG is critical for many of the beneficial effects seen in inflammatory and autoimmune disorders (13,23,24,28). The Fc region interacts with Fcγ receptors on immune cells that can either up regulate or dissipate an immune response. The Fc portion of IgG also has the ability to interact with complement components and regulate inflammation by absorption of active complement components and inhibition of C3 convertase activity (14,28).

Based on our observations on the effectiveness of IVIG in modulation of anti-HLA antibodies, our experiences in the use of IVIG in inflammatory and autoimmune disorders and the use in the treatment of severe AMR episodes in cardiac and renal allograft recipients (8–12,25), we feel IVIG has an important role in the management of highly-HLA sensitized patients awaiting transplantation. These patients are unlikely to receive a transplant unless a therapeutic intervention is used to reduce anti-HLA antibodies.

The data briefly reviewed here suggest that IVIG offers significant benefits in reducing PRA levels and improving the chances for transplantation. The reason(s) for the beneficial effects observed in acute rejection and specific mechanism(s) of action of IVIG have remained unclear until recently. The effectiveness of IVIG in treatment of inflammatory and autoimmune disorders has prompted many studies into potential mechanisms of action. There are numerous proposed mechanisms of action that may be relevant to the efficacy of IVIG in desensitization. These include: a) modification of autoantibody and alloantibody levels through induction of anti-idiotypic circuits (8–12,14), b) inhibition of cytokine gene activation and anti-cytokine activity (14), c) anti-T-cell receptor activity (14), d) Fc receptor-mediated interactions with antigen presenting cells to block T-cell activation (14,25,27), e) anti-CD4 activity (25), f) stimulation of cytokine receptor antagonists (14) and g) inhibition of complement activity (14,24,28).

Using the mixed lymphocyte culture system, we have shown that IVIG can significantly inhibit T-cell activation and reduce the expression of CD40, CD19, ICAM-1, CD86, and MHC-class II on APCs in the MLR (25). 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 (25).

Samuelsson et al have recently described another unique immunoregulatory effector function for IVIG. These investigators demon-

strated that IVIG induces the expression of FcγRIIB, an inhibitory receptor on B-cells. This suggests that IVIG may exert many of its beneficial effects on the rejection process through induction of inhibitory receptors on immune cells with subsequent inhibition of cell proliferation and/or induction of apoptosis (23).

Another interesting observation that may have relevance, especially for the treatment of antibody mediated rejection, is from Magee et al (24) who showed that IVIG treatment significantly prolonged the survival of pig-to-baboon xenotransplants (from 30–60 minutes to 10 days). This beneficial effect was through inhibition of complement-mediated endothelial cell injury by IVIG. The Fc portion of IVIG has high affinity for activated complement components (C3b and C4b) and could represent a novel mechanism for inhibition of complement-mediated injury to allografts that has been recently described for both acute rejection and chronic rejection in humans (26,28–29).

Other investigators have recently shown that IVIG inhibits the generation of C5b-C9 MAC, thus preventing antibody-mediated injury. IVIG also inactivates C3b and accelerate C3b catabolism (14,28). IVIG can also inhibit the activation of endothelial cells in in vitro models of inflammation. These observations may have relevance to acceptance of human solid organ transplants since Williams et al (29) recently showed that a critical difference between xenografts that survived through accommodation versus those lost by AMR was the lack of C5b-C9 MAC in the grafts with accommodation.

Data by Bayry et al (31), suggests that IVIG inhibits the maturation and function of dendritic cells, impairing their APC activity and inducing IL-10 production. These data are in concert with data from our laboratory demonstrating similar effects on B-cells (24).

Recently, Abe et al (30) examined gene expression in patients with Kawasaki disease (KD) before and after high-dose IVIG infusion. These investigators demonstrated that in KD, the immunomodulatory effects of IVIG were likely mediated by suppression of an array of immune activation genes in monocytes and macrophages. Another paper by Gill et al (31) using an animal model system of ischemia-reperfusion injury, demonstrated that IVIG has direct inhibitory effects on leukocyte recruitment in vitro and in vivo through inhibition of selectin and integrin functions.

Regardless of the mechanism(s) involved, current data suggest that IVIG represents a novel and effective approach to the reduction of anti-HLA antibodies pre-transplant and treatment of allograft rejection episodes post-transplant, especially those resistant to other therapies or where antibody-mediated mechanisms are present.

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DISCUSSION

Luke, Cincinnati: There have been renal side effects in patients with immunological diseases, and I just wondered if you would discuss this, particularly after transplantation.

Jordan, Los Angeles: Thank you, Robin. It's hard to cover all these issues in the time allotted, but as Robin has indicated, some IVIG products can be nephrotoxic, and this is a frequent question posed to me regarding the safety of IVIG use overall. Is it safe to use IVIG in transplant patients? In our NIH study, we found that there were equal numbers of side effects or AE's (adverse events) in the IVIG and placebo groups. There were actually more significant adverse events (SAEs) in the placebo group. Thus, IVIG is very safe to give on dialysis. However, getting back to Robin's question . . . the IVIG used in this study was from Bayer (Gamimune N 10%) and was isosmolar. All of the renal problems (acute renal failure, osmotic nephropathy) are caused by osmotically active ingredients added to the IVIG products by some manufacturers. This is usually sucrose and can add considerable osmolality to the products, and when given in high doses can cause osmotic nephropathy due to sucrose. There are some reports, going back to 1950's, showing that if you give more than 50 grams of sucrose IV to some patients, renal failure will ensue. Thus, our objective is to avoid very high osmolar products, especially in patients at risk for acute renal failure. This would include patients with cardiovascular disease, diabetes, and pre-existing renal impairment.

Gotto, New York: I wanted to ask about another potential application. Norm Relkin at our institution is involved in a multi-institutional center clinical trial of IV gamma globulin in patients with Alzheimer's diseases. Would you speculate on what the mechanism might be there?

Jordan: I think the idea is that there're antibodies to beta actin in the IVIG products that accumulate in Alzheimer's patients, and the IVIG-treated patients seem to have reduced CSF content of beta actin. This has been associated with some evidence of clinical improvement as well. The data are early and provocative, but it's going to certainly increase the demand for IVIG.

Abboud, Iowa City: I just hope this is not too naïve a question. As I understand it, you give the IVIG for three or four months, and then it takes sometimes months or years before the HLA antibodies can be normal. What's going on in this situation?

Jordan: That's not a naïve question at all. It's an excellent question. Initially we thought that the *in vitro* mechanisms that I showed happened *in vivo* and were the result of pre-existing blocking antibodies present in the IVIG. This seems to be part of it, but long-term suppression goes well beyond the half-life of IVIG, and we think there is Fc fragment-mediated deletion of B-cells that may give long-term deletion of specific antibody-producing clones. This may result from IVIG-induced B cell apoptosis. It's hard to measure *in vivo*, but we do see what appears to be deletion of antibody-producing cells by the IVIG. This does not happen in everyone, but we often see that we can reduce the panel reactive antibody from 90% to 50% or 40% and dramatically improved the patient's chances of getting a kidney.