Intravenous Gammaglobulin (IVIG): A Novel Approach to Improve Transplant Rates and Outcomes in Highly HLA-Sensitized Patients


Intravenous immunoglobulin (IVIG) products 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 also 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 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 transplantation of highly 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. These studies and relevant mechanism(s) of action will be discussed here.

Key words: Antibody-mediated rejection, desensitization, HLA antibody, immune modulation, IVIG, kidney transplantation

Introduction

The benefits of kidney transplantation are evidenced by prolonged survival and improved quality of life for both children and adults. Despite these well-documented benefits, transplant frequency remains low due to limited organ availability (1–4). In patients with high levels of pre-formed anti-HLA antibodies (high panel reactive antibodies (PRA); highly sensitized), transplant rates are extremely low because of the additional immunologic barrier with increased rejection risk. From 1994 to 2003, the number of highly sensitized patients on the transplant list has 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). These antibodies result from exposure to nonself HLA antigens; usually from previous transplants, blood transfusions and/or pregnancies (5).

In 2003, only 6.5% of all kidney transplants performed in the United States were in-patients with PRAs >80%, despite representing ~14% of the waiting list (1,2). If transplanted, these patients experience an increased number of rejection episodes and have poorer graft survival (6–8). The highly sensitized patient is destined to remain waitlisted for extended periods of time on dialysis, an added risk factor for patient and graft survival (1–4,14). 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; a goal that has been difficult to achieve until recently.

Patel and Terasaki demonstrated the poor outcomes for kidneys transplanted across a positive crossmatch (CMX) barrier, and established the basis for modern CMX testing as a means of allocating kidneys (6). Sensitization is a significant barrier to both access and success in organ transplantation. The risks for transplantation can be assessed using standard assays currently available. Today, the technique(s) used to detect anti-HLA antibody include cytotoxicity (CDC) with/without anti-human globulin (AHG), ELISA and flow cytometry (using cells and antigen coated beads). The development of newer, more sensitive assays has led to an increased ability to define highly sensitized patients and identify donor-specific antibody in patients with antibody-mediated rejection (AMR). Sensitization can be defined further in terms of risk for allograft loss and AMR.

The presence of IgG complement fixing antibody specific for donor HLA antigen (class I or class II) without the addition of AHG represents an unequivocal contraindication to transplantation. Patients transplanted across this barrier are at a very high risk for AMR. The risk is considered moderate to high if antibody detection requires the use of an anti-globulin reagent in the cytotoxicity assay or the use of
a binding assay (e.g. ELISA, flow beads). The patient’s history of sensitizing events (pregnancies, transplants, transfusions), the duration and thoroughness of the antibody screening history of the patient, the sera used in the CMX (number, timing), antibody titer and potential repeat mismatches are also considered important (7,8).

Until recently, no therapeutic approaches existed to deal with this problem. Currently, two protocols have emerged. These include the plasmapheresis/CMV/IgG protocol (Johns Hopkins Protocol) (9) and the high-dose IVIG protocol (Cedars-Sinai Protocol) (10–14). Our center has extensive experience with the high-dose IVIG protocol, which will be discussed here.

Clinical Use of IVIG in Kidney Transplantation

Intravenous immunoglobulin (IVIG) products are known to have powerful immunomodulatory effects on inflammatory and autoimmune diseases (15). Data from our group and others suggest 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 (10–14,16–19). 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 (12,18,19).

End stage renal disease (ESRD) patients awaiting a living-donor or deceased donor transplant who exhibit positive donor-specific CMXs or have high PRAs are candidates for desensitization with the IVIG protocol.

A summary of the Cedars-Sinai high-dose IVIG protocol was recently presented (13). Briefly, we utilize the in vitro IVIG inhibition CMX test (Figure 1A, B) to determine if highly sensitized patients are candidates for the IVIG protocol. Patients first undergo a standard T-cell and B-cell cytotoxicity assay against a random panel of 50 donors to determine PRA. If positive, we then assess the utility of IVIG by incubating IVIG with the positive PRA sera. IVIG is added 1:1 and we then determine the extent of inhibition of T-cell and B-cell cytotoxicity. In our hands, this in vitro assay provides an idea of the expected efficacy of IVIG when given in vivo. Post-treatment, the patients can continue to be monitored using the CDC assay. Nonspecific binding of IgG (i.e. IVIG) can interfere with other assays for anti-HLA antibody, especially binding assays (flow cytometer, ELISA and flow beads). All assays used to measure anti-HLA antibody may be used after ~3 weeks (the half-life of IVIG) since the IVIG will have dissipated from the circulation and will no longer interfere with binding assays.

It is important to mention that alternative explanations for the 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 (20,21), or possible contamination of IVIG products with soluble HLA molecules (11). Wassmuth et al. (20) 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 much lower inhibitory effects were seen when ELISA techniques for measurement of anti-HLA antibodies were performed. Watanabe et al. (21) recently showed similar results, discounting the possibility that the IVIG-induced inhibition seen in the complement-dependent PRA system was due to anti-idiotypic antibody.

Our data (11–13) contrast greatly with these observations since no nonspecific inhibition (i.e. complement inhibition by IVIG (IgG)) was seen and no soluble HLA was detected in the products used. In addition, patterns of inhibition vary from patient to patient. Cytotoxicity can be completely inhibited against T and B cells, or inhibited partially. We also see inhibition of T-cell cytotoxicity with no inhibition of B-cell cytotoxicity. In addition, a role for blocking antibody can be postulated based on concomitant in vitro inhibition of flow cytometry CMXs, and our clinical observations that immediate post-IVIG infusion anti-HLA antibody titers are markedly decreased compared with pre-infusion.

Despite the limitations of the in vitro assay, we have also 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 g/kg IVIG (maximum dose 140 g) monthly until the CMX is negative or acceptable. An acceptable CMX in our program is defined as a negative CDC, but a flow cytometry CMX (B, T or B and T cell) that remains positive at a flow channel shift of <200 CS (normal: <50 CS T cell and <100 CS B cell). This is based on our personal experience and that of others (22). We usually give four doses of IVIG, and have adapted this for use in highly sensitized deceased donor transplant candidates who have been on the UNOS list 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 IVIG PRA assay, and if suppression or inhibition of the PRA is seen with IVIG, the patients are offered IVIG 2 g/kg monthly 4 times in hopes of achieving desensitization and receiving a CMX compatible kidney or other organ. These protocols are summarized in Figure 2A, B.

From July 2002 to July 2005, we evaluated 77 highly HLA-sensitized patients who had positive CMXs with potential donors in the IVIG-PRA test system. Eighty-one percent showed inhibition to some degree in the PRA or CMX system. Sixty-seven of seventy-seven (87%) were transplanted after IVIG desensitization therapy (42 LD,
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Figure 1: (A) The panel reactive antibody status of a patient who is highly sensitized. The patient’s sera contains antibodies that kill 34/50 (68% PRA) lymphocytes on the panel. The cytotoxicity is dependent upon complement as shown in the figure. (B) Shows that IVIG can inhibit the cytotoxicity completely in vitro and this is due to blocking antibodies present in the IVIG preparations. Other explanations (i.e. IVIG’s complement inhibitory capacity) also exist for this observation. If IVIG shows inhibition of PRA or CMX tests in vitro, it is very predictive of in vivo responses.

25 CAD. Of the 10 patients who were not transplanted, six 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 2/77 (2.6%) 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 to 2000, the NIH conducted the IGO2 study that was a controlled clinical, multi-center, double blinded trial of IVIG versus 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 (14). 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 versus 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 ($\pm$SE) serum creatinine of 1.68 $\pm$ 0.28 (IVIG) versus 1.28 $\pm$ 0.13 mg/dL for placebo ($p = 0.29$). 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.

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Although more AR episodes were seen in the IVIG treatment group, the 2-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.

**Alternative Approaches to Improve Transplantation for Highly HLA-Sensitized Patients**

As more transplant centers in the United States and around the world develop protocols to improve transplantation for the highly HLA-sensitized patients, other approaches have emerged. These include the use of plasmapheresis and IVIG currently used at Johns Hopkins and the Mayo Clinic (9,22). Claas et al. (23) reported on The Acceptable Mismatch Program, which has been developed for allocating kidneys to highly sensitized patients. This protocol involves the use of a computer program, HLA Matchmaker, which allocates kidneys to patients based on avoidance of antigen sensitization. The authors report that 112 transplants have been 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 (24). If these approaches are successful in the United States, they should be adopted prior to initiation of desensitization therapy.
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Figure 2: (A) It is a summary of the IVIG desensitization protocol for patients with living donors. If IVIG shows in vitro inhibition or reduction of the donor-specific CMX, IVIG is given at 2 g/kg (maximum dose: 140 g) until a negative CMX is achieved (usually maximum of four doses given). When a negative or acceptable CMX is achieved, the patient is scheduled for transplant. The patient receives an additional 2 g/kg at 1 M post-transplant. (B) Shows the protocol for patients awaiting deceased donor transplantation. Briefly, a similar protocol for living donors is used. If IVIG shows in vitro reduction of PRA, patients receive 2 g/kg IVIG monthly 4 times. Sera are sent to the Organ Procurement Organization after each infusion and CMX with potential deceased donors.

Complications and Cost of IVIG Therapy

Unlike the use in immunodeficiency, patients who are highly HLA-sensitized require higher doses (1–2 g/kg/dose) to achieve a 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 (25). Briefly, the safety of IVIG infusion (2 g/kg) doses given over a 4-h hemodialysis session, monthly 4 times versus placebo (0.1% albumin) in equivalent doses was studied in the IGO2 trial (14). The results are shown in Table 1. There were more than 300 infusions in each arm of the study using Gamimune N 10% versus 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. Is it cost-effective? Data do exist in this regard (5,14) Currently, a four dose course of IVIG for a 70 kg person at 2 g/kg would cost $25,000–$26,000. However, one must
tion. There are numerous proposed mechanisms of action that may be relevant to the modification of alloantisensitization. These include (a) modification of autoantibody and alloantibody levels through induction of anti-idiotypic circuits (10–15), (b) inhibition of cytokine gene activation and anti-cytokine activity (15), (c) anti-T-cell receptor activity (15), (d) Fc receptor-mediated interactions with antigen presenting cells to block T-cell activation (15,28,30), (e) anti-CD4 activity (15), (f) stimulation of cytokine receptor antagonists (15) and (g) inhibition of complement activity (15,27,31). 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 (28). The primary effect is on B cells and we have demonstrated that IVIG induces significant B-cell apoptosis in vitro through Fc receptor-dependent mechanisms (28). Samuelsson et al. have recently described another unique immunoregulatory effector function for IVIG. These investigators demonstrated that IVIG induces the expression of FcγRIIB, an inhibitory receptor on B cells. This suggests that IVIG may regulate B-cell function through induction of inhibitory receptors on immune cells with subsequent inhibition of cell proliferation and/or induction of apoptosis (26). Another interesting observation that may have relevance, especially for the treatment of AMR is from Magee et al. (27) who showed that IVIG treatment significantly prolonged the survival of pig-to-baboon xenotransplants (from 30–60 min 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 (29,31–32).

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 (15,31). 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. (32) 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. (30) suggest that IVIG inhibits the maturation and function of dendritic cells, impairing their APC activity and inducing IL-10 production. This data are in concert with data from our laboratory demonstrating similar effects on B cells (28).

Recently, Abe et al. (33) 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. (34) using an animal model system of
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Figure 3: This figure summarizes the relevant mechanisms of action known for IVIG in modification of autoimmunity, alloimmunity and inflammation. These are divided into Fab- and Fc-mediated events. Many beneficial regulatory mechanisms have been described for IVIG that have relevance to transplantation.

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.

References


