Presensitization: The Problem and Its Management

Stanley C. Jordan* and Mark D. Pescovitz†
*Comprehensive Transplant Center, Transplant Immunology Laboratory, Cedars-Sinai Medical Center, David Geffen School of Medicine, University of California–Los Angeles, Los Angeles, California; and †Departments of Surgery and Microbiology/Immunology, Indiana University School of Medicine, Indianapolis, Indiana

Much attention has been placed recently on transplantation in highly HLA-sensitized patients. In attempts to remove these antibodies and enable successful transplantation, several novel approaches have been developed. These include intravenous Ig (IVIg), mycophenolate mofetil, sirolimus, alemtuzumab, protein A immunoabsorption, and rituximab. IVIg has emerged as a very effective agent when used alone in high dose or when used in low dose and combined with plasmapheresis. Although alemtuzumab has been used to eliminated B cells, it fails to prevent antibody-mediated rejection and therefore probably is not suitable for desensitization. Rituximab, a B cell–specific antibody, seems to be safe and to have some efficacy as a sole agent in elimination of alloantibodies but most likely will require combination therapy with IVIg or other agents. Newer agents, such as humanized anti-CD20, are being developed. Despite the great interest in the problem of allosensitization, with one notable exception, there is a major deficiency in controlled clinical trials, the conduct of which should be a focus for the near future.

intransavenous Ig (IVIg) protocol (Cedars-Sinai Protocol) (5,9–12). The Mayo Clinic (13) also has extensive experience with both protocols. Both protocols have met with significant success and are discussed here. In addition, the use of other techniques, such as B cell elimination with alemtuzumab, newer immunosuppressive agents, protein A absorption, plasma exchange with concomitant Prograf (Ascellas Pharmaceuticals, Deerfield, IL) and Cellcept (Roche Inc., Nutley, NJ) therapy, and rituximab, are being investigated. These approaches also are discussed here.

Mechanism of Action of IVIg

IVIg products are known to have powerful immunomodulatory effects on inflammatory and autoimmune disorders (14). IVIg products are derived from the plasma of thousands of donors, thereby ensuring a wide diversity of antibody repertoire. Numerous proposed mechanisms of action may be relevant to the modification of allosensitization: (1) Modification of autoantibody and alloantibody levels through induction of anti-idiotypic circuits (5,9–12,14), (2) inhibition of cytokine gene activation and anticytokine activity (14), (3) anti–T cell receptor activity (14), (4) Fc receptor-mediated interactions with antigen-presenting cells (APC) to block T cell activation (14–16), (5) anti–CD4 activity (15), (6) stimulation of cytokine receptor antagonists (14), and (7) inhibition of complement activity (14,17,18). Using the mixed lymphocyte culture system, we have shown that IVIg can significantly inhibit T cell activation and reduce the expression of CD40, CD19, intracellular adhesion molecule-1, CD86, and MHC class II on APC in the mixed lymphocyte reaction (15). 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 (15). Samuelsson et al. (19) 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. Therefore, IVIg may have beneficial effects in inflammatory disorders by decreasing B cell activation through interactions with FcγRIIB (19). Another interesting observation that may have relevance, especially for the treatment of AMR, is from Magee et al. (17), who showed that IVIg treatment significantly prolonged the survival of pig-to-baboon xenotransplants (from 30 to 60 min to 10 d). 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 was described recently for both acute rejection and chronic rejection in humans (18,20,21). Other investigators showed recently that IVIg inhibits the generation of C5b-C9 membrane attack complex, thereby preventing AMR. IVIg also inactivates C3b and accelerates C3b catabolism (14,18). IVIg can 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. (21) showed recently that a critical difference between xenografts that survived through accommodation versus those that were lost by AMR was the lack of C5b-C9 membrane attack complex in the grafts with accommodation. Data by Bayry et al. (16) suggest 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 (15). Recently, Abe et al. (22) examined gene expression in patients with Kawasaki disease before and after high-dose IVIg infusion. These investigators demonstrated that in Kawasaki disease, the immunomodulatory effects of IVIg likely were mediated by suppression of an array of immune activation genes in monocytes and macrophages. Gill et al. (23), using an animal model system of ischemia-reperfusion injury, showed that IVIg has direct inhibitory effects on leukocyte recruitment in vitro and in vivo through inhibition of selectin and integrin functions.

Clinical Use of IVIg in Kidney Transplantation

At Cedars-Sinai, we have developed a method to predict which patients would benefit most from IVIg as a desensitization tool. This is summarized in Figures 1 and 2. Figure 1A shows the traditional approach to the highly sensitized patient who has a positive CMX with a living donor. This patient is shifted to the waiting list with monthly antibody screens. The wait times for these patients are exceedingly long (5 to 10 yr) until a suitable CMX(−) kidney becomes available. From our standpoint, this is an unacceptable approach.

In the early 1990s, we developed the IVIg-PRA/CMX test to determine whether IVIg could inhibit PRA or CMX positivity of patients’ sera. Although alternative explanations for the in vitro inhibitory effects of IVIg on cytotoxicity in the PRA and CMX systems have emerged (9,24), we believe that this test is helpful in predicting which patients are likely to benefit from IVIg therapy. This has led to the development of a new paradigm (Figure 1B) in which in vitro reductions of PRA or CMX allow the patients to be treated with high-dose IVIg to enhance their chances for a successful transplant. Transplantation is performed when a negative or acceptable CMX is achieved. In our program, an acceptable CMX is defined as a negative CDC CMX with flow CMX positivity <200 channel shifts for T cells and B cells. Patients who undergo desensitization often have positive flow cytometry CMX at the time of transplantation. Others have described this as well (25).

Figure 2 shows that alternative approaches to desensitization also are necessary. These include plasmapheresis plus IVIg and potential donor exchange programs that have been developed recently at Johns Hopkins. Patients can progress through the various treatment options until successful transplantation is achieved.

IVIg therapy that is 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 (5,9–12,25–29). Pretreatment with IVIg results in reductions of anti-HLA antibodies and is effective in treatment of allograft rejection episodes (10,28,29). The high-dose IVIg protocol that was developed at Cedars-Sinai evolved from re-
ported efficacy with other inflammatory disorders (e.g., Kawasaki disease) (14). Using the high-dose IVIg protocol (2 g/kg) for desensitization requires that antibody specificity be determined. When IVIg shows any reduction of T or B cell cytotoxicity, using the in vitro assay described above, we treat the recipient with 2 g/kg IVIg (maximum dose 140 g) monthly until the CMX is negative or acceptable, typically with no more than four doses. We also have adapted this to use for highly sensitized deceased-donor transplant candidates who have been on the United Network for Organ Sharing list for 5 yr, have a PRA of 50%, and 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 g/kg monthly in hopes of achieving desensitization and receiving a CMX-compatible kidney or other organ (Figure 3).

For patients who do not show reductions in PRA or CMX activity in vitro or those who have very high-titer anti-HLA antibody and are poorly responsive to IVIg, we have developed a modified plasmapheresis/IVIg protocol. This differs from the Johns Hopkins protocol in that only five plasmapheresis treatments are given. This is followed by IVIg 2 g/kg intravenously and Rituxan 375 mg/m²×1. Patients who are entered in this protocol usually are awaiting living-donor transplantation; however, we also would consider this approach for the highly HLA-sensitized patient who is awaiting a deceased-donor transplant if the patient has been on the list for an extensive time (>5 yr) and has frequent offers of deceased-donor organs (dotted line). In addition, patients who are not suitable for IVIg desensitization may be referred to donor exchange programs before or after undergoing plasma exchange therapy. Offering different options will help achieve transplantation for more patients.

Figure 1. (A) The traditional approach to the highly HLA-sensitized patient. Briefly, for living-donor recipients, if the cross-match (CMX) is negative by CDC and flow, then a transplant is performed. However, if the CMX is positive, the patient is referred to the waiting list until a negative CMX kidney becomes available. This may take >5 yr. In addition, the costs of antibody screening (A/S) and quarterly panel-reactive antibodies (PRA; Q/S) as well as repeat CMX tests with deceased donors is required. The same is true for highly HLA-sensitized patients who are awaiting a deceased-donor kidney. Wait times are extensive (>5 yr), and extensive antibody testing is required. (B) How intravenous Ig (IVIg) in vitro testing for both living-donor recipients and those awaiting a deceased-donor transplant with sufficient wait time can enhance transplantability. Briefly, if the IVIg in vitro test shows any inhibition of the CDC CMX test, then the highly HLA-sensitized patients are treated with IVIg monthly ×4 until a negative or acceptable CMX (living donor) or deceased-donor transplant becomes available. Modification of antibody screens and antibody specificity by IVIg treatment can be monitored as usual and used to guide future therapy. If no inhibition is seen, then the work-up would stop or patients would be referred for plasma exchange therapy (Figure 2).

Figure 2. Approach to highly HLA-sensitized patients who fail in vitro IVIg inhibition or who have very high-titer anti-HLA antibody. The protocol included five plasma exchange treatments followed by IVIg 2 g/kg ×1 and Rituxan 375 mg/m²×1. Patients who are entered in this protocol usually are awaiting living-donor transplantation; however, we also would consider this approach for the highly HLA-sensitized patient who is awaiting a deceased-donor transplant if the patient has been on the list for an extensive time (>5 yr) and has frequent offers of deceased-donor organs (dotted line). In addition, patients who are not suitable for IVIg desensitization may be referred to donor exchange programs before or after undergoing plasma exchange therapy. Offering different options will help achieve transplantation for more patients.
From July 2002 to October 2005, we evaluated 89 patients who were highly HLA sensitized and had positive CMX 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 (89%) of eighty-nine received a transplant after IVIg desensitization therapy (46 living donor, 33 deceased donor). Of the 10 patients who did not receive a transplant, 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. Therefore, only two (2.2%) of 89 failed to respond to IVIg sufficiently to allow transplantation to be considered. The mean PRA 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-yr patient and graft survival of 97.5 and 87.1%, respectively. Five grafts were lost to rejection. The mean serum creatinine at 3 yr was 1.4 mg/dl.

National Institutes of Health IGO2 Study

From 1997 to 2000, the National Institutes of Health (NIH) conducted the IGO2 study that was a controlled, clinical, multicenter, double-blinded trial of IVIg versus placebo in highly sensitized patients who were 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 for graft loss in this difficult-to-transplant group. This study represents the only controlled clinical trial of a desensitization therapy (5). IVIg was superior to placebo in reducing anti-HLA antibody levels \((P < 0.004, \text{IVIG versus placebo})\) and improving rates of transplantation. The 3-yr follow-up showed that the predicted mean time to transplantation was 4.8 yr in the IVIg group versus 10.3 yr in the placebo group \((P = 0.02)\). With a median follow-up of 2 yr after transplantation, the viable transplants functioned normally with a mean \((\pm \text{SE})\) serum creatinine of 1.68 \(\pm 0.28\) (IVIg) versus 1.28 \(\pm 0.13\) mg/dl for placebo \((P = 0.29)\). Allograft survival also was superior in the IVIg group at 3 yr. From this multicenter, 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 patients with ESRD. Although more acute rejection episodes were seen in the IVIg treatment group, the 3-yr allo-
graft survival and allograft function were similar to the placebo group. Transplant rates for highly sensitized patients who had ESRD and were awaiting kidney transplants were improved with IVIg therapy. Therefore, 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 (+)CMX barrier. Plasmapheresis + CMV-IgG, (PP/CMV-Ig) and a cocktail of immunosuppressive agents are initiated before renal transplantation (30). After transplantation, additional treatments are delivered during the first 10 d (Table 1). The end point of therapy is the elimination of anti-HLA donor-specific antibody either before or after the transplant. The treatment plan is individualized on the basis of an assessment of the patient’s risk of AMR (30). This group identified recipient features that were associated with increased risk for AMR and graft loss. Patients who were thought to be at low risk (e.g., first transplant with pregnancy as sensitizing event) were treated with PP/CMV-Ig and quadruple sequential immunosuppression, whereas high-risk patients (e.g., third transplant with multiple repeat mismatches) have splenectomy and/or anti-CD20 (rituximab, see below) added to their basic treatment plan.

This protocol produces a rapid reduction in anti-HLA titers that allows for transplantation after four to five plasmapheresis treatments. The JHH group members believe that the addition of CMV-Ig adds an immunomodulatory effector mechanism that benefits in keeping the antibody titers low. It is critical to perform the transplant within a few days of the last plasmapheresis because rebound of anti-HLA does occur and can negate the benefits achieved with previous treatments. The Mayo Clinic has adopted both the PP/low-dose IVIg and high-dose IVIg protocols with similar success (13,25). Table 2 shows the advantages and limitations of the high-dose IVIg versus PP/CMV-Ig protocols. Table 3 compares the results from the two protocols over the last 3 to 5 yr. As can be seen, outcomes for patients at the three institutions (JHH, Cedars-Sinai, and Mayo Clinic) are very similar and comparable to results from nonsensitized patients.

**Complications and Cost of IVIg Therapy**

Unlike the use in immunodeficiency, patients who are highly HLA sensitized require higher doses (1 to 2 g/kg per 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, at first, were not anticipated and were poorly understood. We recently reviewed the complications associated with IVIg infusions in patients who had normal renal function and those who were on dialysis (23). Briefly, the safety of IVIg infusion (2 g/kg) doses given during a 4-h hemodialysis session monthly × 4 versus placebo (0.1% albumin) in equivalent doses was studied in the IGO2 trial (5).

**Table 1. Preconditioning regimen for recipients of a (+)CMX or ABO-incompatible renal allograft (JHH PP + CMV-Ig protocol)a**

<table>
<thead>
<tr>
<th>Pretransplantation</th>
<th>Posttransplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP one volume exchange every other day (replaced with 5% albumin)</td>
<td>3 to 5 protocol every other day PP/CMV-Ig treatments</td>
</tr>
<tr>
<td>low-dose CMV-Ig (100 mg/kg) after each PP treatment</td>
<td>triple-drug immunosuppression (tacrolimus, MMF, and prednisone)</td>
</tr>
<tr>
<td>tacrolimus and MMF begun at the same time as PP/CMV-Ig</td>
<td>dose of anti–IL-2 receptor antibody (1 mg/kg) every 2 wk × 4 doses</td>
</tr>
<tr>
<td>end point of pretransplantation therapy for (+)CMX: (−)AHG CDC CMX</td>
<td>for ABO incompatibility: isoagglutinin titers ≤16</td>
</tr>
<tr>
<td>for ABO incompatibility: isoagglutinin titers ≤16</td>
<td>Day of the transplant</td>
</tr>
<tr>
<td>anti–IL-2 receptor induction antibody (2 mg/kg)</td>
<td>steroid bolus (Solu-Medrol 500 mg)</td>
</tr>
<tr>
<td>splenectomy and/or anti-CD20 for ABO incompatibility or high-risk (+)CMX patients</td>
<td></td>
</tr>
</tbody>
</table>

---

*aCMX, cross-match; AHG, anti-human globulin; CMV, cytomegalovirus; JHH, Johns Hopkins University Hospital; MMF, mycophenolate mofetil; PP, plasmapheresis.

**Table 2. High-dose IVIg versus PP + CMV-Ig: Advantages and limitationsa**

<table>
<thead>
<tr>
<th>High-dose IVIg: Advantages</th>
<th>High-dose IVIg: Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>less expensive/fewer resources required for successful desensitization of living and deceased donors</td>
<td>nonresponders and incomplete responders exist (approximately 10%)</td>
</tr>
<tr>
<td>easy and safe to administer; can be given on dialysis or at home</td>
<td>IVIg may interfere with assays for DSA antibody modulation often less rapid than with PP + CMV-Ig</td>
</tr>
<tr>
<td>desensitization is long lasting in most cases, allowing longer intervals between treatment and transplantation</td>
<td>specific IVIg products have toxicity at high doses (i.e., sucrose and saline excipient products)</td>
</tr>
</tbody>
</table>

**Table 3. Advantages and limitations of high-dose IVIg versus PP/CMV-Ig protocols**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>less expensive/fewer resources required for successful desensitization of living and deceased donors</td>
<td>nonresponders and incomplete responders exist (approximately 10%)</td>
</tr>
<tr>
<td>easy and safe to administer; can be given on dialysis or at home</td>
<td>IVIg may interfere with assays for DSA antibody modulation often less rapid than with PP + CMV-Ig</td>
</tr>
<tr>
<td>desensitization is long lasting in most cases, allowing longer intervals between treatment and transplantation</td>
<td>specific IVIg products have toxicity at high doses (i.e., sucrose and saline excipient products)</td>
</tr>
</tbody>
</table>

*aDSA, donor-specific antibody; IVIg, intravenous Ig.
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 versus 23 placebo). The most common adverse event in the IVIg arm was headache (52 versus 24%; \( P = 0.056 \)). This usually abated with reduction in infusion rate and acetaminophen. Ten serious adverse events were noted; of these, nine were in the placebo group. Therefore, 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 (2,5). Currently, a four-dose course of IVIg for a 70-kg person at 2 g/kg would cost $30,000. However, one must compare this with the cost of maintaining patients on dialysis, which is the only other option. In the IGO2 study (5), the calculated cost savings was approximately $300,000 per patient who received a transplant versus those who remained on dialysis for the 5 yr of the study. Data from the U.S. Renal Data System (2003) also confirm that a considerable cost savings to Medicare is seen in highly sensitized patients who receive a transplant versus those who remain on dialysis (3).

Other Approaches to Desensitization

Mycophenolate mofetil (MMF) has been shown to decrease response to neoantigens in the transplant population (31). Min et al. (32) combined MMF with immunoabsorption and tacrolimus for the treatment of acute AMR. Six of 185 cadaver renal allograft recipients developed C4d-positive AMR approximately 5 d after transplant. After treatment, the PRA fell from...
values as high as 50 to 8% with resolution of the rejection in four of the six patients. Which of the components were responsible for this benefit could not be determined with certainty. In a study of pediatric heart recipients, Jacobs et al. (33) used this modality in the pretransplantation setting. In combination with IVIg and plasmapheresis, they were able to provide transplants to eight children who had an elevated PRA; however, the survival rate was only 50%. They concluded that these sensitized patients remain at high risk despite aggressive immunosuppression. In a study of 388 kidney transplant recipients who had a kidney biopsy within the first 6 mo after transplantation, C4d-positive rejection could be reduced by 50% when calcineurin inhibitor or MMF therapy was started 2 to 4 h before transplantation when compared with initiation after surgery (adjusted odds ratio 0.5; P = 0.03).

Glotz et al. (34) reported on the successful use of high-dose IVIg for desensitization. Removal of donor-specific antibody seems to be effective in allowing transplantation in highly sensitized patients and in treating AMR episodes. This first was reported by Pascual et al. (35), who combined plasma exchange with Cellcept and tacrolimus for treatment of AMR. Lehrich et al. (36) reported that IVIg combined with plasmapheresis is extremely effective in reversing AMR episodes. Schweitzer et al. (37) reported on a protocol using plasma exchange to improve transplant rates in highly sensitized patients. Zand et al. (38) reported in vitro data that suggest that Thymoglobulin has significant effects on B cells and plasma cells in vitro and potentially could be useful as a desensitization agent. These approaches are limited by low numbers and/or no clinical experience but could be developed further if rigorous clinical trials are designed.

Although many variations on the theme of IVIg and plasma exchange have been presented, it is clear that both therapies have a place in the treatment of highly sensitized patients. Many possible combinations of therapy might be considered but should be undertaken with great caution as the risks for this patient population are high and the possibilities for repeat transplantation are few.

Sirolimus has similarly been noted to have anti-B cell activity with reduction of antibody formation in transplant patients (39), an effect that seems to be synergistic to that of IVIg (40). Toyoda et al. (40) suggested that lower concentration in combination with IVIg could represent an effective immunomodulatory drug combination.

Campath-1H (alemtuzumab) is a humanized mAb against CD52, a small glycosylphosphatidylinositol-anchored glycoprotein determinant that is highly expressed on both T and B cells (41). However, despite its reactivity against B cells, it has been unable to prevent and in fact may result in increased rates of C4d-positive, AMR when used without a calcineurin inhibitor (42–44). It also seems that memory T cells are resistant to alemtuzumab therapy (45,46). These findings would question its addition to desensitization protocols.

Protein A column immunoabsorption has been used primarily as part of both ABO incompatible transplant (47) and in the setting of allosensitized patients (48). Lorenz et al. (48) reported
their 10-yr experience with 40 cadaver transplant recipients who had median PRA of 77% (nine of whom had a flow-positive CMX) and were treated with one absorption before and several after transplantation. Three-year graft survival was 71%, with 11% immunologic loss. Although the current dogma is that protein A columns deplete circulating Ig, Goodyear and Silverman (49,50), using murine models, hypothesized that the benefit comes from protein A that leaches off the column into the patient. They found that infusions of small quantities of protein A into mice led to profound and prolonged depletion of circulating and tissue B cells. Silverman and colleagues (49,51) suggested that perhaps direct administration of protein A may be beneficial for certain antibody-mediated diseases, akin to what is currently being tested with anti-CD20 therapy.

Rituximab, a chimeric (Figure 5) anti-CD20 (Figure 6) mAb that is approved for treatment of lymphoma, efficiently eliminates B cells (52). CD20 is expressed early in B cell ontogeny, but expression is absent on plasma cells (Figure 7). Rituximab eliminates B cells by three potential mechanisms: Antibody dependent, cell-mediated cytotoxicity; complement-dependent cytotoxicity; and apoptosis (Figure 8). In a study of patients who had end-stage renal failure, we found that rituximab could be given safely and at doses lower than those recommended for cancer treatment (53). Dosing was associated with release of TNF-α and had minimal effect on the ex vivo T cell immune responses (54). The B cells that recover are substantially depleted of memory CD27+ B cells for as long as 2 yr after a single dose (55). Dosing dramatically impairs response to a neoantigen given at the time of rituximab dosing but ultimately has no effect on preexisting memory responses (56). Last, we reported methods to perform CMX and PRA determinations in patients who had been treated with rituximab either by elimination of the cell surface CD20 by pronase treatment of the cells or by immunomagnetic bead absorption of the serum-containing rituximab (57,58).

In our small phase 1 study, we found that there was a decrease in PRA and or antibody specificity in most of the patients, data that were confirmed recently using single-antigen HLA beads (59). Most other reports also are small trials and focus on rituximab in ABO-incompatible transplants of either kidney (60) or liver (61,62) and as a method of sparing splenectomy (63,64). The largest experience is of 11 patients in whom rituximab was combined with immunoabsorption and IVIg without splenectomy and resulted in normal renal function in all 11 (64). However, a more recent report questioned the need for rituximab in such transplants (65). This highlights the major problem with rituximab: The absence of controlled clinical trials. Future mechanistic studies will be facilitated by the recent generation of a mouse strain that expresses human CD20 in a normally regulated manner on B cells (66). These animals, when treated with rituximab, undergo B cell elimination in a manner analogous to what is seen with rituximab treatment in humans. Future clinical trials might be facilitated by a humanized anti-CD20, 2H7 (Figure 5), now in early clinical trials for treatment of rheumatoid arthritis.

**Figure 7.** CD20 expression first is weakly detectable at the immature B cell stage. Expression fades again at the plasma cell stage. It is expressed to the greatest amount on germinal center (GC) B cells.
Alternative, Nonpharmacologic Approaches to Improve Transplantation Rates in 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. Claas et al. (67) reported on The Acceptable Mismatch Program, which has been developed for allocating kidneys to highly sensitized patients. These investigators reported a schema that was developed for Eurotransplant using a computer program, HLA Matchmaker, that allocates kidneys to patients on the basis of avoidance of antigen sensitization. The authors reported that 112 transplants had been performed with a 2-yr graft survival of 87% but gave no data on the incidence and the severity of rejection episodes or current serum creatinine values. They also suggested that this could be implemented in conjunction with desensitization protocols in an effort to provide transplants to the most highly sensitized patients. Other potential protocols include donor exchange programs that may improve access of highly sensitized patients to transplantation (59). If these approaches are successful in the United States, then they should be tested before initiation of desensitization therapy because of the reduced expense.

Conclusion

On the basis of multiple observations of the in vitro and in vivo effectiveness of IVIg in modulation of anti-HLA antibodies, the experience 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 (5,9–12,15), we believe that IVIg alone or in combination with PP has an important role in the treatment of highly sensitized patients who are awaiting transplantation. However, newer agents and combinations of agents are being tried with early evidence of anecdotal success. Clearly, large multicenter, well-controlled trials are needed in this field to provide optimal benefit to these patients who are otherwise unlikely to receive a transplant unless therapeutic interventions are undertaken to reduce anti-HLA antibodies.

References


Figure 8. Rituximab is postulated to eliminate B cells by one of three mechanisms: Antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity, or activation of the apoptotic pathways.
58. Kaplan I, Houp JA, Lefell MS, Hart JM, Zachary AA: A...


