

## NEPHROLOGY

## Rounds®

AS PRESENTED IN THE ROUNDS OF  
THE NEPHROLOGY DIVISION OF  
BRIGHAM AND WOMEN'S HOSPITAL  
BOSTON, MASSACHUSETTS

## The Highly Sensitized Renal Transplant Recipient

By MONICA GRAFALS, MD, and ENVER AKALIN, MD

Kidney transplantation is the treatment of choice for patients with end-stage renal disease (ESRD) because it prolongs survival, decreases morbidity, and improves quality of life. Nevertheless, kidney transplantation is hampered due to decreased organ availability; for example, as of June 2009, >80,000 individuals in the United States (US) were waiting for a cadaveric renal transplant. It is expected that nearly 100,000 individuals will be waiting for a kidney transplant in the US by 2010. Since approximately 9,000 to 10,000 cadaveric renal transplants are performed each year, it is clear that waiting times for a kidney transplant will continue to rise.<sup>1</sup> Furthermore, the number of sensitized patients on transplant waiting lists is also increasing; these are patients who have developed antibodies against human leukocyte antigens (HLAs). The waiting time for sensitized patients is even longer, since they have an additional immunological barrier to transplantation. In some sensitized patients who had living donors, transplantation could not be performed because the recipients had antibodies against their potential donors. Nevertheless, recent advances have made kidney transplantation possible in sensitized patients by decreasing their antibody production. This issue of *Nephrology Rounds* is focused on defining the immunologic features of sensitized patients and describing methods to detect alloantibodies; the issue also outlines current desensitization protocols, and discusses clinical outcomes for the sensitized renal transplant recipient (RTR).

### Characterization of highly sensitized patients

Patients are sensitized to HLA through blood transfusions, pregnancy, and previous organ transplants. Women with ESRD are disproportionately sensitized compared with men; approximately 60%–80% of highly sensitized patients are women. The degree of immunization is much stronger (as determined by the antibody titer) and prolonged when different causes of immunization act together within the same patient.<sup>2</sup> Panel reactive antibody (PRA) titers reflect the patient's degree of sensitization. PRA is defined as the percentage of a panel reacting with a patient's serum or the percentage of donors expected to react based on known antibody activities. Currently, 17% of patients on the waiting list have PRA titers between 10% and 79%, with 8% having PRA titers >80%.<sup>3</sup> In 2000, only 2.8% of all kidney transplants were performed in sensitized patients, even though this population represents approximately 20% of the waiting list.<sup>1</sup> Thus, the highly sensitized patient is destined to wait extended periods of time on dialysis, a factor known to increase morbidity and mortality. In addition, transplant outcomes in highly sensitized patients are inferior to those in nonsensitized patients.<sup>4</sup>

### Crossmatch methods and detection of alloantibodies

Complement dependent cytotoxicity (CDC) methods have formed the basis of anti-HLA antibody detection, since it was first introduced by Patel and Terasaki in 1969.<sup>5</sup> The widespread use of CDC crossmatch has resulted in the almost complete elimination of hyperacute rejection in the modern era. In this test, the serum of the recipient is incubated with lymphocytes from the donor, and a complement is added to determine if the recipient has antibodies that bind to donor cells, activate the complement and the membrane attack complex, and result in cell death. The sensitivity of the cytotoxic assay can be enhanced with the use of an antihuman globulin (AHG) antibody as a secondary reagent (AHG-enhanced crossmatch). A positive cytotoxic T-cell immunoglobulin G (IgG) crossmatch is an absolute contraindication to transplantation.<sup>6</sup>

The flow cytometry (FC) crossmatch was introduced in 1983 as a more sensitive test. This test does not rely on complement fixation, but rather measures the binding of recipient Ig molecules to donor cells. Bound Ig is detected by a second anti-Ig that is conjugated with a fluorescent dye. FC may detect low titers of complement fixing or noncomplement fixing anti-HLA antibodies, as well as non-HLA-related antibodies. Although this test is sensitive, it lacks specificity; therefore, in order to confirm the specificity of a positive FC crossmatch, additional tests should



BRIGHAM AND  
WOMEN'S HOSPITAL



HARVARD  
MEDICAL SCHOOL  
TEACHING AFFILIATE

#### Co-Editors

Joseph V. Bonventre, M.D., Ph.D.,  
(Division Director)

Barry M. Brenner, M.D., F.R.C.P.,  
(Director Emeritus)

#### Nephrology Division Brigham and Women's Hospital

Reza Abdi, M.D.  
Jessamyn Bagley, Ph.D.  
Sangeeta Bhattia, M.D., Ph.D.  
Joseph V. Bonventre, M.D., Ph.D.  
Barry M. Brenner, M.D.  
Steven Brunelli, M.D.  
Anil K. Chandraker, M.B., M.R.C.P.  
David M. Charytan, M.D.  
Mary Choi, M.D.  
Kenneth B. Christopher, M.D.  
Gary C. Curhan, M.D., Sc.D.  
Bradley M. Denker, M.D.  
Jeremy Duffield, M.D., Ph.D.  
John P. Forman, M.D.  
Markus H. Frank, M.D.  
Monica Grafals, M.D.  
Indira Guleria, Ph.D.  
Dirk M. Hentschel, M.D.  
Andreas Herrlich, M.D., Ph.D.  
Li-Li Hsiao, M.D., Ph.D.  
Benjamin D. Humphreys, M.D., Ph.D.  
John J. Iacomini, Ph.D.  
Takaharu Ichimura, Ph.D.  
Vicki Rubin Kelley, Ph.D.  
Julie Lin, M.D., M.P.H.  
Edgar L. Milford, M.D.  
David B. Mount, M.D.  
Nader Najafian, M.D.  
Shona Pendse, M.D.  
Martin R. Pollak, M.D.  
Mohamed H. Sayegh, M.D.  
Julian L. Seifter, M.D.  
Jagesh V. Shah, Ph.D.  
Alice M. Sheridan, M.D.  
Ajay K. Singh, M.B., M.R.C.P. (U.K.)  
Theodore I. Steinman, M.D.  
Eric N. Taylor, M.D.  
John K. Tucker, M.D.  
Takuya Ueno, M.D., Ph.D.  
Vishal Vaidya, Ph.D.  
Sushrut S. Waikar, M.D.  
Wolfgang C. Winkelmayer, M.D., Sc.D.  
Xueli Yuan, M.D., Ph.D.  
Kambiz Zandi-Nejad, M.D.  
Jing Zhou, M.D., Ph.D.

#### Brigham and Women's Hospital

Website: [www.brighamandwomens.org/renal](http://www.brighamandwomens.org/renal)

The editorial content of *Nephrology Rounds* is determined solely by the Nephrology Division of Brigham and Women's Hospital.

**Nephrology Rounds is approved  
by the Harvard Medical School  
Department of Continuing Education  
to offer continuing education credit**

be performed to demonstrate the presence of donor-specific antibodies (DSA) directed against class I and II HLA.<sup>7</sup>

The solid-phase assays (enzyme-linked immunosorbent assay [ELISA], Flow Specific Beads™ and FlowPRA®) are specific tests to detect anti-HLA antibodies. Flow Specific Beads™ and FlowPRA® are membrane-independent flow cytometric techniques utilizing purified HLA antigens coupled to microparticles. The cutoff used for a positive test using HLA antibodies with the Luminex screen assay is a reactivity of >1000 mean fluorescent intensity (MFI), which is the arithmetic average of the fluorescence of a sample number of cells. Mizutani et al<sup>8</sup> demonstrated that titers of alloantibodies were correlated with MFI values obtained by Luminex machines.

Recently, interest in non-HLA antigens as targets of injury in organ transplant recipients has increased. This growth has been spurred by observations that HLA-identical kidney transplants also undergo immunological rejection. Polymorphisms within non-HLA genes associated with eliciting an immune response to alloantigens are currently being studied for their association to transplant outcomes. Non-HLA antigens, such as the polymorphic major histocompatibility complex (MHC) class I-related chain A (MICA), expressed on endothelial cells have been implicated in the pathogenesis of hyperacute, acute, and chronic organ allograft rejection.<sup>9</sup> As with HLA antigens, exposure to allogeneic MICA during transplantation can elicit antibody formation. Soluble MICA can be detected in transplant recipients, and its presence in the serum of heart transplant recipients was associated with an increased incidence of rejection within the first year after transplant.<sup>10</sup> Since MICA antigens are not expressed on lymphocytes, which are the cells commonly used for crossmatching, antibodies directed against MICA are not detected with the methods generally used. However, polymorphic MICA antigens are expressed on endothelial cells and are found to be cytotoxic in the presence of serum complement, so it is likely that such antibodies are harmful to vascularized allografts.<sup>11</sup> Most institutions now test MICA antigens on a routine basis with every potential transplant recipient.

### Definition of antibody-mediated rejection

Patients who are sensitized are at higher risk of developing antibody-mediated rejection (AMR). This entity is different from cellular rejection, which, by definition, is a T cell-mediated process. The term AMR applies to a wide variety of severe vascular and epithelial lesions in the allograft. The presence of DSA directed toward MHC class I and class II antigen in serum indicates, along with the accumulation of complement C4d along peritubular capillaries, a direct interaction of alloantibodies with the allograft.<sup>12</sup> AMR is increasingly recognized as a spectrum of allograft injury ranging from hyperacute rejection, occurring within minutes of exposure to high levels of DSA, to acute AMR, resulting from an increase in DSA activity developing within days to weeks after transplant. Long-term exposure to low levels of DSA may result in chronic AMR, which manifests months to years after transplant. Usually, aggressive therapy for acute AMR is effective in reestablishing renal function, but the long-term prognosis tends to be poor due to chronic

vascular injury, usually manifested as transplant glomerulopathy.<sup>13</sup> The occurrence of slowly progressive antibody-mediated damage, supported by C4d findings and the presence of circulating DSA, is associated with graft loss.

### Mechanisms of injury in antibody-mediated rejection

The endothelium of transplanted organs serves as the primary target of patient immune responses. In the humoral theory of organ transplantation, the endothelium of a donor organ is primarily targeted by alloantibody, either pre-existing or developed *de novo* after transplant.<sup>14-24</sup> The interaction of endothelial cells with high levels of DSA results in complement activation, and causes cell death, loss of vascular integrity, and subsequent ischemic injury.<sup>25</sup> Exposure to DSA may result in complement-mediated allograft injury due to endothelial-cell activation that induces the formation of intercellular “gaps,” the shedding of heparin sulfate molecules with loss of cell-surface electronegativity, and the exposure of subendothelial matrix to plasma coagulation factors and platelets, resulting in vascular thrombosis.<sup>26</sup> In addition to complement-mediated allograft injury, exposure to MHC class I antibodies is capable of inducing apoptosis of vascular endothelium, as described in studies of human allografts experiencing acute AMR.<sup>27,28</sup> Antibody binding and complement activation induce a series of pathological changes in the graft endothelium that promote intravascular thrombosis. Endothelial cells are stimulated to secrete von Willebrand factor that mediates platelet adhesion and aggregation. Complement activation leads to endothelial-cell injury and exposure of subendothelial basement membrane proteins that activate platelets. These processes contribute to thrombosis and vascular occlusion; therefore, the organ suffers irreversible ischemic damage.<sup>29</sup> It is apparent that variable levels of DSA activity may result in allograft injury through a variety of mechanisms, including both complement-dependent and independent pathways, leading to necrosis and endothelial-cell apoptosis.<sup>13</sup>

The speed of any potential pathological change after endothelial injury depends on 3 major factors:

- **Level of alloantibodies:** Patients with <10% PRA have significantly longer allograft half-lives than patients with higher levels of sensitization.<sup>29</sup>
- **The capacity of tissue repair within the transplanted organ:** This is the major mechanism impeding or postponing the development of rejection, but the regeneration capacity is tissue dependent. Some tissue cells have the capacity to regenerate after injury, including renal tubular cells.
- **Immunosuppression and other supportive therapies:** Different immunosuppressants have different effects in inhibiting antibody development.<sup>30-32</sup>

### Current treatment approaches

The financial and emotional costs of maintaining highly sensitized transplant candidates on dialysis for years are enormous. As a result, early transplantation would lead to considerable cost savings, reduced morbidity and mortality, and improvements in quality of life; all of these goals have been difficult to achieve until recently.<sup>33</sup> Various strategies

enable desensitization for renal transplant recipients who are highly sensitized. Therapeutic strategies for desensitization include various combinations of the following:

- intravenous (IV) Ig
- removal of antibodies by plasmapheresis (PP) or immunoadsorption (IA)
- rituximab (anti-CD20)
- splenectomy.

IVIg is prepared by isolating the polyclonal IgG fraction out of plasma that has been pooled from hundreds of healthy blood donors. While the mechanisms of action remain obscure, IVIg has been shown to inhibit complement binding and B- and T-cell activation and proliferation, and to neutralize circulating anti-HLA antibodies through anti-idiotypic activity.<sup>34</sup> Furthermore, studies have suggested that IVIg interferes with alloantigen recognition, resulting in inhibition of the mixed leukocyte reaction (MLR). One of the mechanisms for this action seems to be a reduction in the numbers of intact B cells and monocytes, and a reduction or modulation of CD19/CD20 and CD40 expressions on B cells. Toyoda et al<sup>35</sup> demonstrated that the B-cell modulation is primarily a result of apoptosis. Several other mechanisms of action have been proposed for IVIg, such as: the modification of autoantibody and alloantibody levels through induction of anti-idiotypic circuits; inhibition of cytokine gene activation and anticytokine activity; anti-T-cell receptor activity; Fc receptor-mediated interactions with antigen presenting cells to block T-cell activation; anti-CD4 activity; and inhibition of complement activity.

Recently, other investigators have demonstrated that IVIg inhibits the generation of C5b-C9, the membrane attack complex (MAC), thus preventing antibody-mediated injury.<sup>36</sup> IVIg also inactivates C3b and accelerates C3b catabolism; in fact, it was demonstrated that a critical difference between xenografts surviving through accommodation versus those lost through antibody-mediated rejection was the lack of C5b-C9 MAC in the grafts with accommodation. Furthermore, there are data suggesting that IVIg inhibits the maturation and function of dendritic cells, impairing their antigen presenting-cell activity and inducing interleukin (IL)-10 production.<sup>37</sup> These data are in concert with previous data demonstrating similar effects on B-cells.<sup>35</sup>

IVIg given to highly sensitized patients results in reduced allosensitization and ischemia-reperfusion injury, fewer acute rejection episodes, and more successful long-term allograft outcomes for both cadaveric- and living-donor recipients.<sup>38</sup> The only controlled clinical trial of desensitization therapy is the National Institutes of Health (NIH) IGO2 Study.<sup>39</sup> This controlled, clinical, multicenter, double-blinded trial compared IVIg versus placebo in highly sensitized patients who were awaiting kidney transplantation. The study design for this difficult-to-transplant group was to determine whether IVIg could reduce PRA levels and improve rates of transplantation without simultaneously increasing the risk for graft loss. The results indicated that IVIg was superior to placebo in reducing anti-HLA antibody levels and improving rates of transplantation; in addition, although more acute rejection episodes were seen in the IVIg group, graft survival and allograft function were similar to the placebo group. The study concluded that

transplant rates for highly sensitized patients with ESRD awaiting kidney transplant were improved by IVIg therapy; further, IVIg desensitizes these highly sensitized patients and offers significant transplantation benefits without the patients experiencing excessive allograft loss.

Another approach for desensitization that has been used in living donor kidney transplants is PP plus IVIg. This protocol produces a rapid reduction in anti-HLA titers and allows transplantation after 4–5 PP treatments prior to transplant.<sup>40</sup> It is critical to perform the transplant within a few days of the last PP treatment because rebound of anti-HLA antibodies occurs and can negate the benefits achieved with previous treatments.

While much effort in transplantation has focused on the role of T cells in allograft dysfunction, it is becoming increasingly clear that B-cell-mediated events also affect long-term allograft outcomes; as a result, rituximab is an emerging desensitization approach. Rituximab is a chimeric mouse/human monoclonal anti-CD20 antibody that inhibits B-cell proliferation resulting in rapid B-cell depletion. Rituximab binds specifically to the CD20 molecule, which is present on the surface of pre-B and mature B cells, but not expressed on plasma cells or other tissues.<sup>41</sup> Despite the fact that CD20 is not expressed on plasma cells, rituximab is effective at preventing new alloantibody producing plasma cell formation by eliminating precursor B cells and inhibiting B-cell driven antigen presentation and costimulation of T cells. In combination with strategies including PP and IVIg, rituximab has become an important addition to desensitization protocols. A small Phase 1 study<sup>38</sup> found a decrease in PRA and/or antibody specificity in most of the treated patients; these data were confirmed with the use of single-antigen HLA beads. Currently, rituximab is used as rescue therapy in acute humoral rejection, and as adjunctive therapy to pretransplant PP protocols.

Splenectomy removes a major source of lymphocytes, including antibody-secreting B cells, B-cell precursor cells, and plasma cells. However, the effect of splenectomy on the immune system is permanent and patients are at risk of developing life-threatening sepsis from encapsulated bacteria.<sup>3</sup> Historically, splenectomy has been used for preemptive treatment of antibodies blood group (ABO)-incompatible recipients and in selected circumstances for patients with a positive DSA who are at high risk of AMR.<sup>42</sup> Recently, there have been reports of splenectomy performed as a rescue treatment for severe AMR.<sup>43,44</sup> In the report by Loche et al,<sup>43</sup> 5 patients underwent emergent splenectomy followed by PP + IVIg and had a return of allograft function within 48 hours after the procedure. All patients had functioning grafts and there was a mean follow up of 18 months.

### Early and late transplant outcomes

Since desensitization is a fairly recent procedure, relatively few centers in the United States have attempted to transplant sensitized patients. As a result, outcome data are limited, but the following section reviews the available findings.

The clinical outcome of 13 highly sensitized patients who were desensitized by 3 courses of IVIg pre- and post-transplant was studied by Goeltz et al.<sup>2</sup> Graft survival at 1 year was 84%, but 1-year creatinine was not available and

protocol biopsies were not performed. Similarly, Jordan et al<sup>39</sup> reported findings from a series of 16 patients who underwent transplantation after successful desensitization and received additional IVIg infusions monthly for 4 months. At 2 years, graft survival was 80% and serum creatinine was numerically higher in the IVIg group versus the placebo group (mean  $1.68 \pm 0.28$  vs  $1.28 \pm 0.13$  mg/dL;  $P=0.29$ ); however, no data were available concerning histological progression.

In a recent pilot study,<sup>45</sup> IVIg was given post-transplant to 44 patients who had a previous positive T-cell crossmatch and/or previous or current positive DSA. All patients received a cadaveric renal transplant, had a current negative T-cell crossmatch on the day of transplantation, and were ABO compatible. The patients received IVIg on days 0, 21, 42, and 63. The authors observed that there was a complete disappearance of PRA in the majority of the patients with alloantibodies at transplantation 1 month after the completion of the IVIg administration. This result is not typical of those reported in pretransplant desensitization studies that show only a partial decrease of PRA after IVIg; therefore, these data must be interpreted cautiously because of a lack of long-term follow-up. What makes this study important is the fact that protocol biopsies were performed on every patient at 3 and 12 months post-transplant. The biopsies demonstrated that despite a good glomerular filtration rate (GFR) posttransplant, there was an increasingly high rate of interstitial fibrosis and tubular atrophy. Despite these findings, the patient and graft survival figures were 97% and 95%, respectively, and no graft was lost due to rejection (mean follow-up was 25 months).

AMR is now widely recognized as a major problem in organ transplantation, especially in sensitized renal transplant recipients. Endothelial inflammation is one of the mainstays of AMR. Activation and/or apoptosis of endothelial cells can occur, suggesting that not only HLA-specific antibodies, but also non-HLA antibodies may initiate damage. Recently, a study by Lefaucher et al<sup>46</sup> analyzed the incidence and course of AMR in a cohort of 237 renal transplant patients who were followed for an average of  $30 \pm 20$  months. Factors associated with a negative outcome after an AMR were the presence and/or persistence of DSA posttransplantation, as well as specific histological markers on biopsy, including neutrophilic glomerulitis, peritubular capillary dilatation with neutrophilic infiltrates, and interstitial edema. Interestingly, the persistence of C4d does not predict outcome, since ABO-incompatible transplants can have excellent graft function despite the persistence of C4d. At present, treatment for AMR includes immunological strategies targeting removal or neutralization of alloantibodies, as well as inhibition of B-cell proliferation and new formation of alloantibodies. Combinations of PP, IVIg, and/or rituximab have been investigated. In most studies, short-term graft and patient survival were 80% and 99%, respectively, but long-term data are still lacking.<sup>46</sup>

The Mount Sinai group<sup>47</sup> reported on the use of low-dose IVIg (300 mg/kg) and thymoglobulin induction in 15 patients, who had a positive CDC for B cell and FC for T or B cell. Three patients developed early AMR and, subsequently, 4 more patients developed acute AMR; further, the MFI of all patients with acute AMR had strong DSAs (MFI >6,000). After this experience, the group performed PP on patients with strong DSA. Living-donor kidney transplant candidates received 4-8 sessions of pretransplant PP over 2-3 weeks and underwent transplantation after the MFI values of DSA decreased to <6,000. Deceased-donor kidney transplant recipients with strong DSAs received 3 sessions of PP every other day starting on postoperative day one. This protocol change resulted in a dramatic decrease in the acute AMR rate to 7% in the following 14 patients with strong DSAs.<sup>47</sup>

The study by Vo and colleagues<sup>48</sup> builds on the NIH IGO2 study by applying a new method of desensitization for recipients of kidneys from deceased or living donors. In this method, IVIg and rituximab were administered to 20 highly sensitized patients with a mean PRA level of  $77 \pm 19\%$ . Patients had a mean time on dialysis of 12 years, and were at the top of the deceased donor list, delayed only by their sensitized status, and were likely to receive a transplant offer if desensitization succeeded. Acute rejection occurred in 50% of the patients, 31% were antibody-mediated rejections, mostly in the first months after transplant. Patient and graft survival were 100% and 94% respectively, with all patients having at least 12 months of follow up. Even though there was a high rate of rejection early post transplantation, these were reversible and the 1-year patient and graft survival were excellent, without an unusually high rate of infectious complications.

PRA is strongly associated with long-term graft loss in HLA-identical sibling-kidney transplantations. Opelz et al<sup>49</sup> compared over 4000 HLA-identical sibling transplantations with over 160 000 cadaveric transplants. The presence of PRA in the deceased-donor group was associated with significant reductions in graft survival in the first year, but there was no effect in the HLA-identical transplantations. However, long-term follow-up in the HLA-identical group revealed a major effect of PRA in recipients with high levels of PRA (>50%). In this group, the number of functioning grafts was significantly lower at 10 years compared with patients with a low level of PRA.

Recent data indicate that humoral alloreactivity and slowly progressive antibody-mediated damage can eventually lead to chronic rejection.<sup>50-55</sup> Histological evidence reveals an association between transplant glomerulopathy and C4d glomerular deposits, and peritubular capillary basement membrane multilayering.<sup>50</sup> C4d deposition preceded the development of transplant glomerulopathy in most patients with serial biopsies, suggesting an important role of DSA and local complement activation in the development of transplant glomerulopathy.<sup>56</sup>

Table 1: Desensitization protocols and their outcomes								
Author	Year	N	Protocol	AMR	Mean follow-up	Patient survival	Graft survival	
Schweitzer <sup>57</sup>	2000	11	PP before and after Tx, IVIg and Muromonab-CD3 × 10 doses	27%	3-26 months	100%	100%	
Gloor <sup>58</sup>	2003	14	PP, low dose IVIg, rituximab and splenectomy at the time of Tx. Thymoglobulin as induction.	23%	15 months	86%	78%	
Jordan <sup>59</sup>	2003	14	IVIg and tx when crossmatch is negative. IVIg at time of Tx and one month after. Daclizumab as induction.	43%	24 months	98%	89%	
Akalin <sup>47,60</sup>	2003, 2005	17	Low dose IVIg before Tx and on POD 1 and 2. Thymoglobulin as induction.	18%	15 months	100%	88%	
Stegall <sup>61</sup>	2006	49	Pts received 3 different protocols with thymoglobulin as induction:		1 year actuarial	93%	82%	
			High dose IVIg (n = 5)	80%				
			PP, IVIg, rituximab (n = 30)	37%				
			PP, IVIg (n = 14)	29%				
Magee <sup>62</sup>	2008	28	PP, IVIg, rituximab. Induction with thymoglobulin	39%	22 months	93%	89%	
Akalin <sup>63</sup>	2008	35	Pts received 3 different protocols:					
			High-dose IVIg and thymoglobulin induction (DSA MFI less than 6,000) (n=12)	0%	16 months	100%	100%	
			High-dose IVIg and thymoglobulin induction (DSA MFI >6,000) (n = 9)	44%	22 months	100%	78%	
			High-dose IVIg, PP and thymoglobulin induction (DSA MFI >6,000) (n=14)	7%	12 months	93%	86%	
Vo <sup>48</sup>	2008	16	High-dose IVIg, rituximab and induction with alemtuzumab	31%	12 months	100%	94%	

PP = plasmapheresis; Tx = transplant; IVIg = intravenous immunoglobulin; DSA = donor-specific antibodies; MFI = mean fluorescent intensity; POD = post-operative day; AMR = antibody-mediated rejection

### Future directions

Recently, within Eurotransplant (ET), the Acceptable Mismatch (AM) program was developed. This program consists of a special highly sensitized waiting list consisting of patients with current or historical sensitization of  $\geq 85\%$  PRA. Acceptable mismatches are defined as those against which the recipient has never made antibodies and are considered to be acceptable as mismatch for the potential donor organ. When a blood group compatible deceased donor becomes available, exhibiting HLA-A, -B, and -DR matched to the recipients antigens, it is first offered to a highly-sensitized patient on the waiting list. The standard crossmatch performed in the AM program is the CDC crossmatch. Patients transplanted in the program have a 1-year graft survival similar to that of nonsensitized patients within ET.<sup>64,65</sup> As a result, in a small group of patients in the AM program, donor-directed antibodies detected by single-antigen bead assay may be related to acute rejection episodes, but are not detrimental to long-term graft outcome. However, in this study, the patients enrolled were Dutch, which is a low-risk population for rejection and, furthermore, none of the 34 patients who were studied had received any induction therapy.

In the future, selective inhibition of the complement system could theoretically block the consequences of *in situ* complement activation triggered by alloantibodies binding to the graft endothelial cells. Usefulness of complement inhibitors (soluble CD35+ or eculizumab) to block the proinflammatory consequences

of complement activation will probably open a new era in prevention and treatment of humoral-mediated graft damage. This idea is supported by a recent report demonstrating that eculizumab, a humanized monoclonal antibody against the terminal complement protein C5, can effectively inhibit terminal complement activation in humans.<sup>56</sup>

### Conclusions

The sensitized renal transplant recipient presents a unique challenge to the transplant community; however, several protocols allow for successful transplantation of these patients. Graft outcomes are now acceptable and patient survival and quality of life have improved. The challenge currently resides in the sensitized patients without a living donor. The study by Vo et al<sup>48</sup> is important because it potentially allows for the transplant of patients awaiting a cadaveric allograft by utilizing IVIg and rituximab. This protocol improved the patient's chances of receiving a cadaveric renal transplant by decreasing their antibodies. The results of this study are fascinating and may represent the beginning of a change in the current standard of care for managing sensitized patients with kidney failure for whom there are limited options at present.

*Dr. Grafals is a staff member with the Nephrology Division of Brigham and Women's Hospital. Dr. Akalin is the medical director of kidney transplantation at the Montefiore Medical Center, Albert Einstein College of Medicine, New York.*

## References

1. United Network for Organ Sharing. National data. Available at: <http://optn.transplant.hrsa.gov/latestData/rptData.asp>. Accessed June 15, 2009.
2. Glotz D, et al. Intravenous immunoglobulins and transplantation for patients with anti-HLA antibodies. *Transpl Int*. 2004;17(1):1-8.
3. Akalin E. Posttransplant immunosuppression in highly sensitized patients. *Contrib Nephrol*. 2009;162:27-34.
4. Hariharan S, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. 2000;342(9):605-612.
5. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med*. 1969;280(14):735-739.
6. Gupta A, et al. Pretransplant donor-specific antibodies in cytotoxic negative crossmatch kidney transplants: are they relevant? *Transplantation*. 2008;85(8):1200-1204.
7. Akalin E, Bromberg JS. Intravenous immunoglobulin induction treatment in flow cytometry cross-match-positive kidney transplant recipients. *Hum Immunol*. 2005;66(4):359-363.
8. Mizutani K, et al. The importance of anti-HLA-specific antibody strength in monitoring kidney transplant patients. *Am J Transplant*. 2007;7(4):1027-1031.
9. Sumitran-Holgersson S. Relevance of MICA and other non-HLA antibodies in clinical transplantation. *Curr Opin Immunol*. 2008;20(5):607-613.
10. Suarez-Alvarez B, et al. The predictive value of soluble major histocompatibility complex class I chain-related molecule A (MICA) levels on heart allograft rejection. *Transplantation*. 2006;82(3):354-361.
11. Zou Y, et al. Antibodies against MICA antigens and kidney-transplant rejection. *N Engl J Med*. 2007;357(13):1293-1300.
12. Bohmig GA, et al. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol*. 2002;13(4):1091-1099.
13. Gloor J, et al. The spectrum of antibody-mediated renal allograft injury: implications for treatment. *Am J Transplant*. 2008;8(7):1367-1373.
14. Ogura K, et al. The significance of a positive flow cytometry crossmatch test in primary kidney transplantation. *Transplantation*. 1993;56(2):294-298.
15. Gebel HM, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. *Am J Transplant*. 2003;3(12):1488-1500.
16. Starzl TE, et al. Shwartzman reaction after human renal homotransplantation. *N Engl J Med*. 1968;278(12):642-648.
17. Gollackner B, et al. Acute vascular rejection of xenografts: roles of natural and elicited xenoreactive antibodies in activation of vascular endothelial cells and induction of procoagulant activity. *Transplantation*. 2004;77(11):1735-1741.
18. Feucht HE, et al. Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int*. 1993;43(6):1333-1338.
19. Zhang Q, et al. Development of posttransplant antidonor HLA antibodies is associated with acute humoral rejection and early graft dysfunction. *Transplantation*. 2005;79(5):591-598.
20. Koo DD, et al. C4d deposition in early renal allograft protocol biopsies. *Transplantation*. 2004;78(3):398-403.
21. Podaval RD, et al. Implications of immunohistochemical detection of C4d along peritubular capillaries in late acute renal allograft rejection. *Transplantation*. 2005;79(2):228-235.
22. Girmata AL, et al. HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. *Am J Transplant*. 2005;5(1):131-138.
23. Mitchell RN. Allograft arteriopathy: pathogenesis update. *Cardiovasc Pathol*. 2004;13(1):33-40.
24. Dorling A, et al. In vitro accommodation of immortalized porcine endothelial cells: resistance to complement mediated lysis and down-regulation of VCAM expression induced by low concentrations of polyclonal human IgG antipeptide antibodies. *Transplantation*. 1996;62(8):1127-1136.
25. Cascalho M, Platt JL. Basic mechanisms of humoral rejection. *Pediatr Transplant*. 2005;9(1):9-16.
26. Saadi S, Platt JL. Transient perturbation of endothelial integrity induced by natural antibodies and complement. *J Exp Med*. 1995;181(1):21-31.
27. Caillier JF, Laplante P, Hebert MJ. Endothelial apoptosis and chronic transplant vasculopathy: recent results, novel mechanisms. *Am J Transplant*. 2006;6(2):247-253.
28. Liptak P, et al. Peritubular capillary damage in acute humoral rejection: an ultrastructural study on human renal allografts. *Am J Transplant*. 2005;5(12):2870-2876.
29. Cai J, Terasaki PI. Humoral theory of transplantation: mechanism, prevention, and treatment. *Hum Immunol*. 2005;66(4):334-342.
30. Iwaki Y, et al. Flow cytometry crossmatching in human cadaver kidney transplantation. *Transplant Proc*. 1987;19(1 Pt 1):764-766.
31. Rose ML, et al. Mycophenolate mofetil decreases antibody production after cardiac transplantation. *J Heart Lung Transplant*. 2002;21(2):282-285.
32. Merville P, et al. Lower incidence of chronic allograft nephropathy at 1 year post-transplantation in patients treated with mycophenolate mofetil. *Am J Transplant*. 2004;4(11):1769-1775.
33. Jordan SC, Vo AA, Peng A, Toyoda M, Tyan D. Intravenous gammaglobulin (IVIG): a novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplant*. 2006;6(3):459-466.
34. Beimler JH, Susal C, Zeier M. Desensitization strategies enabling successful renal transplantation in highly sensitized patients. *Clin Transplant*. 2006;20 (Suppl 17):7-12.
35. Toyoda M, et al. Pooled human gammaglobulin modulates surface molecule expression and induces apoptosis in human B cells. *Am J Transplant*. 2003;3(2):156-166.
36. Lutz HU, Stammler P, Bianchi V, et al. Intravenously applied IgG stimulates complement attenuation in a complement-dependent autoimmune disease at the amplifying C3 convertase level. *Blood*. 2004;103(2):465-472.
37. Bayry J, et al. Inhibition of maturation and function of dendritic cells by intravenous immunoglobulin. *Blood*. 2003;101(2):758-765.
38. Jordan SC, Pescovitz MD. Presensitization: the problem and its management. *Clin J Am Soc Nephrol*. 2006;1(3):421-432.
39. Jordan SC, et al. 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. *J Am Soc Nephrol*. 2004;15(12):3256-3262.
40. Montgomery RA, Zachary AA, Racusen LC, et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation*. 2000;70(6):887-895.
41. Becker YT, Samaniego-Picota M, Sollinger HW. The emerging role of rituximab in organ transplantation. *Transpl Int*. 2006;19(8):621-628.
42. Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation*. 2002;74(9):1207-1210.
43. Locke JE, et al. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant*. 2007;7(4):842-846.
44. Kaplan B, et al. Successful rescue of refractory, severe antibody mediated rejection with splenectomy. *Transplantation*. 2007;83(1):99-100.
45. Anglicheau D, et al. Posttransplant prophylactic intravenous immunoglobulin in kidney transplant patients at high immunological risk: a pilot study. *Am J Transplant*. 2007;7(5):1185-1192.
46. Lefaucheur C, et al. Determinants of poor graft outcome in patients with antibody-mediated acute rejection. *Am J Transplant*. 2007;7(4):832-841.
47. Akalin E, Ames S, Sehgal V, et al. Intravenous immunoglobulin and thymoglobulin induction treatment in immunologically high-risk kidney transplant recipients. *Transplantation*. 2005;79(6):742.
48. Vo AA, et al. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med*. 2008;359(3):242-251.
49. Opelz G. Non-HLA transplantation immunity revealed by lymphocytotoxic antibodies. *Lancet*. 2005;365(9470):1570-1576.
50. Mauveydi S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol*. 2001;12(3):574-582.
51. Ivanyi B, et al. Peritubular capillaries in chronic renal allograft rejection: a quantitative ultrastructural study. *Hum Pathol*. 2000;31(9):1129-1138.
52. Vongwiwatana A, et al. Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy. *Am J Transplant*. 2004;4(1):124-129.
53. Theruvath TP, et al. Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. *Transplantation*. 2001;72(1):77-83.
54. Cardarelli F, et al. Prevalence and significance of anti-HLA and donor-specific antibodies long-term after renal transplantation. *Transpl Int*. 2005;18(5):532-540.
55. Lee PC, et al. All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. *Transplantation*. 2002;74(8):1192-1194.
56. Rowshani AT, et al. Humoral immunity in renal transplantation: clinical significance and therapeutic approach. *Clin Transplant*. 2008;22(6):689-699.
57. Schweitzer EJ, et al. A high panel-reactive antibody rescue protocol for cross-match-positive live donor kidney transplants. *Transplantation*. 2000;70(10):1531-1536.
58. Gloor JM, et al. Overcoming a positive crossmatch in living-donor kidney transplantation. *Am J Transplant*. 2003;3(8):1017-1023.
59. Jordan SC, et al. Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation*. 2003;76(4):631-636.
60. Akalin E, et al. Intravenous immunoglobulin and thymoglobulin facilitate kidney transplantation in complement-dependent cytotoxicity B-cell and flow cytometry T- or B-cell crossmatch-positive patients. *Transplantation*. 2003;76(10):1444-1447.
61. Stegall MD, et al. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant*. 2006;6(2):346-351.
62. Magee CC, et al. Renal transplantation in patients with positive lymphocytotoxicity cross-matches: one center's experience. *Transplantation*. 2008;86(1):96-103.
63. Akalin E, et al. Addition of plasmapheresis decreases the incidence of acute antibody-mediated rejection in sensitized patients with strong donor-specific antibodies. *Clin J Am Soc Nephrol*. 2008;3(4):1160-1167.
64. van den Berg-Loonen EM, et al. Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. *Transplantation*. 2008;85(8):1086-1090.
65. De Meester J, et al. Renal transplantation of highly sensitized patients via prioritised renal allocation programs. Shorter waiting time and above-average graft survival. *Nephron*. 2002;92(1):111-119.

**Disclosure:** Dr. Grafals has no disclosures to announce in association with the contents of this issue. Dr. Akalin is on the speaker's bureau for Novartis and the advisory board for Wyeth.

This activity is supported by an educational donation provided by

# Amgen

©2009 Nephrology Division, Brigham and Women's Hospital, Boston, Massachusetts, which is solely responsible for the contents. The opinions expressed in this publication do not necessarily reflect those of the publisher or sponsor, but rather are those of the author based on the available scientific literature. Publisher: **SNELL Medical Communication Inc.** in cooperation with the Nephrology Division, Brigham and Women's Hospital. **Nephrology Rounds** is a registered trademark of **SNELL Medical Communication Inc.** All rights reserved. The administration of any therapies discussed or referred to in **Nephrology Rounds** should always be consistent with the recognized prescribing information as required by the FDA. **SNELL Medical Communication Inc.** is committed to the development of superior Continuing Medical Education.