

# Antibody-Mediated Rejection: A Review

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**Background:** Chronic antibody injury is a serious threat to allograft outcomes and is therefore the center of active research. In the continuum of allograft rejection, the development of antibodies plays a critical role. In recent years, an increased recognition of molecular and histologic changes has provided a better understanding of antibody-mediated rejection (AMR), as well as potential therapeutic interventions. However, several pathways are still unknown, which accounts for the lack of efficacy of some of the currently available agents that are used to treat rejection.

**Methods:** We review the current diagnostic criteria for AMR; AMR paradigms; and desensitization, treatment, and prevention strategies.

**Results:** Chronic antibody-mediated endothelial injury results in transplant glomerulopathy, manifested as glomerular basement membrane duplication, double contouring, or splitting. Clinical manifestations of AMR include proteinuria and a rise in serum creatinine. Current strategies for the treatment of AMR include antibody depletion with plasmapheresis (PLEX), immunoadsorption (IA), immunomodulation with intravenous immunoglobulin (IVIG), and T cell- or B cell-depleting agents. Some treatment benefits have been found in using PLEX and IA, and some small nonrandomized trials have identified some benefits in using rituximab and the proteasome inhibitor-based therapy bortezomib. More recent histologic follow-ups of patients treated with bortezomib have not shown significant benefits in terms of allograft outcomes. Furthermore, no specific treatment approaches have been approved by the US Food and Drug Administration. Other agents used for more difficult rejections include bortezomib and eculizumab (an anti-C5 monoclonal antibody).

**Conclusion:** AMR is a fascinating field with ample opportunities for research and progress in the future. Despite the use of advanced techniques for the detection of human leukocyte antigen (HLA) or non-HLA donor-specific antibodies, alloimmune response remains an important barrier for successful long-term allograft function. Treatment of AMR with currently available therapies has produced a variety of results, some of them suboptimal, precluding the development of standardized protocols. New therapies are promising, but randomized controlled trials are needed to find surrogate markers and improve the efficacy of therapy.

**Keywords:** Desensitization-immunologic, graft rejection, HLA antigens, kidney transplantation, transplantation tolerance

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## INTRODUCTION

In the past, antibody-mediated rejection (AMR)—or humoral rejection—after renal transplantation was a devastating event that inevitably led to allograft loss. In recent years, an increased recognition of molecular and histologic changes has provided a better understanding of this process as well as potential therapeutic interventions. In the continuum of allograft rejection, the development of antibodies plays a critical role, and antibodies are considered a major cause of allograft failure.

In a seminal paper published in 2012, Terasaki argued that the first formal step in the understanding of AMR occurred in 1914 with the introduction of the dye exclusion test used to distinguish dead cells from living cells in vitro,

allowing for the detection of cytotoxic antibodies.<sup>1</sup> The first description of acute AMR identified neutrophils in peritubular capillaries and de novo donor-specific antibodies (DSAs). Almost concomitantly, C4d, a degradation product of the complement pathway that binds covalently to the endothelium, was identified as marker of endothelial injury and hence of antibody activity.<sup>2</sup> Mauiyyedi et al described the correlation between DSAs and diffuse C4d deposition (>50%) as diagnostic markers for AMR.<sup>3</sup> Recent research has indicated that B cells and plasma cells produce DSAs that interact with the endothelium, which activates the cellular pathways responsible for the development of microcirculatory changes and tissue injury.<sup>2,4</sup>

Allograft rejection is a complex process that involves the interplay of different cellular and molecular pathways that cause a broad range of allograft injuries (acute tubular injury, glomerulitis, capillaritis, and fibrinoid necrosis). Antibody ligation to human leukocyte antigen (HLA) or blood antigens, including non-HLA antigens expressed on the endothelium, can activate the complement system, leading to recruitment of leukocytes and facilitation of natural killer cell-mediated or monocyte/macrophage-mediated cytotoxicity, leading to endothelial damage, loss of vascular integrity, and increased coagulation.<sup>5</sup>

Allograft rejection can be hyperacute (occurring within minutes after the vascular anastomosis), acute (occurring days to weeks after transplantation), late acute (occurring 3 months after transplantation), or chronic (occurring months to years after transplantation). Rejection can also be classified according to the pathophysiologic event: cellular and/or AMR.<sup>6</sup>

Willicombe et al researched the incidence of AMR.<sup>7</sup> In their study, 469 patients received a negative crossmatch renal transplant with alemtuzumab induction. Forty-eight (10.2%) patients were treated for AMR. Allograft survival was inferior in the AMR group (70.2%) compared with the nonrejection group (97%) ( $P < 0.0016$ ).

With the introduction of T cell-depleting drugs, calcineurin inhibitors (CNIs), and antiproliferative agents, the field of transplantation has experienced exceptional improvement in allograft survival, which was considered impossible in the 1960s and 1970s. The understanding of renal allograft rejection has paralleled new discoveries in human immunology and the development of new drugs and biologic products. It is not uncommon for rates of acute rejection to be  $< 15\%$ .<sup>1</sup>

This progress is exciting for transplant physicians because of the current immunologic barriers that prevent or delay kidney transplantation. Significant progress has been made since the recognition of the role of DSAs, such as new techniques to detect antibodies, the use of desensitization protocols, and the introduction of new agents that interfere with complement-mediated allograft injury. We review the current diagnostic criteria for AMR; AMR paradigms; and desensitization, treatment, and prevention strategies.

## CURRENT DIAGNOSTIC CRITERIA

Single-antigen bead testing, which is used to detect anti-HLA antibodies, is the final step in the current techniques used to identify antibodies that can injure the allograft. It is currently used to monitor transplant recipients and used as a diagnostic tool for AMR. Complement-dependent cytotoxicity is still considered the gold standard for the detection of preformed antibodies. Electron microscopy is routinely used for the biopsies of transplant recipients because early changes cannot always be detected with light microscopy.

The Banff classification for allograft pathology has made considerable progress during the last 2 decades in capturing, standardizing, and incorporating histologic, immunohistochemical, and serologic factors believed to improve sensitivity in the diagnosis of allograft rejection and in providing outcome data in terms of allograft survival.

Since 2003, Banff has differentiated acute cellular rejection from AMR. The biology of these 2 entities is inseparable, but for the purpose of this review, our focus is on injuries caused strictly by the interaction of antibodies with the endothelium.

The 12th Banff Conference on allograft pathology was held in Brazil in August 2013. Different working groups presented their findings to reach consensus on the diagnosis of AMR in the presence and absence of a C4d stain; the role of microcirculatory inflammation, including thresholds for glomerulitis; the role of intimal arteritis; comparisons of different methodologies for evaluating interstitial fibrosis; and the role of implantation biopsies in terms of allograft outcomes.<sup>8</sup> Despite the major advances in molecular biology and gene rearrangement, the diagnosis of AMR is still dependent on histologic findings.<sup>9</sup>

## Acute Antibody-Mediated Rejection

According to the Banff 2013 classification,<sup>8</sup> all of the following 3 features are required for the diagnosis of acute AMR:

1. Histologic evidence of acute tissue injury defined by the presence of one or more of the following:
  - a. Glomerulitis ( $g > 0$ ) or peritubular capillaritis ( $ptc > 0$ )
  - b. Intimal or transmural arteritis ( $v > 0$ )
  - c. Acute thrombotic microangiopathy (TMA) of no other obvious cause
  - d. Acute tubular injury of no other obvious cause
2. Histologic evidence of current/recent antibody interaction with vascular endothelium, defined by at least one of the following:
  - a. Linear C4d staining in the peritubular capillaries
  - b. At least moderate microvascular inflammation ( $g + ptc > 2$ )
  - c. Increased expression of tissue gene transcripts indicative of endothelial injury
3. Detection of DSAs (HLA or non-HLA) in the serum

## Chronic Antibody-Mediated Rejection

The histology of acute and chronic AMR overlaps significantly, and acute AMR has been shown to be a major risk factor for the development of chronic AMR.<sup>10</sup> According to the revised Banff 2013 classification,<sup>8</sup> the diagnosis of chronic, active AMR requires 3 features:

1. Histologic evidence of chronic tissue injury, defined by the presence of at least one of the following:
  - a. Transplant glomerulopathy ( $cg > 0$ ) in the absence of chronic TMA
  - b. Severe peritubular capillary basement membrane multilayering identified by electron microscopy
  - c. New-onset arterial intimal fibrosis with no other known etiology
2. Histologic evidence of antibody interaction with vascular endothelium, defined by the presence of at least one of the following:
  - a. Linear C4d staining in the peritubular capillaries
  - b. At least moderate microvascular inflammation ( $g + ptc > 2$ )
  - c. Increased expression of tissue gene transcripts indicative of endothelial injury
3. Detection of DSAs (HLA or non-HLA) in the serum

Redfield et al conducted a study of 123 consecutive patients with chronic AMR based on the revised Banff 2013 classification.<sup>11</sup> They followed the patients for a median of 9.5 years after the diagnosis of chronic AMR. Of the recipients, 76% lost their graft with a median survival of 1.9 years after diagnosis. Chronicity scores  $>8$ , DSA  $>2,500$  mean fluorescence intensity, serum creatinine  $>3$  mg/dL, and urine protein/creatinine ratio  $>1$  g/g were associated with an increased risk of allograft loss. The authors concluded that chronic AMR was associated with poor graft survival after diagnosis.<sup>11</sup>

The Long-Term Deterioration of Kidney Allograft Function (DeKAF) study is an ongoing multicenter, observational study designed to identify and characterize the causes of late ( $>90$  days after transplantation) kidney allograft dysfunction and failure. In this cohort, the investigators were able to identify a high frequency of antibody-mediated injury (as indicated by C4d staining and circulating DSAs) in patients with new-onset late graft dysfunction that leads to allograft failure.<sup>12</sup>

### Moderate Microcirculatory Changes

Microcirculatory changes were introduced in the revised Banff 2013 classification and are recognized by renal pathologists as highly suspicious for antibody injury. Only the presence of vasculitis and the high rate of inflammatory cells in the microcirculation have been associated with poor outcomes.<sup>9</sup> The Banff renal pathologists involved with the evaluation of kidney biopsies established the presence of glomerulitis and graded this finding based on the complete occlusion of at least one glomerular capillary by leucocyte infiltration and endothelial cell enlargement. The g score was determined based on the percent of involved glomeruli: 1%-25%, 26%-50%, and  $>50\%$  equate to g scores of g1, g2, and g3, respectively.

### Phenotypes of Antibody-Mediated Rejection

In the 2011 Banff report, 2 principal phenotypes of acute AMR were defined.<sup>13</sup> Phenotype 1 occurs in the presensitized patient and occurs in the early posttransplant period. Phenotype 2 develops from the emergence of de novo DSAs in the late posttransplant period and is thought to be related primarily to nonadherence or inadequate immunosuppression.

Given the improvement in short-term allograft outcomes, attention has increasingly turned to improve long-term allograft survival.

### PARADIGMS IN ANTIBODY-MEDIATED REJECTION Subclinical Antibody-Mediated Rejection

Protocol biopsies have identified a subgroup of patients with histologic evidence of antibody-mediated injury despite stable creatinine. However, the lack of long-term follow-up data has prevented the development of strong guidelines for effective therapeutic interventions. Patients with subclinical AMR had the poorest graft survival at 8 years posttransplant (56%) compared with the subclinical T cell-mediated rejection (88%) and no-rejection (90%) groups ( $P<0.001$ ).<sup>14</sup> Orandi and colleagues reported that subclinical AMR, if left untreated, increased the risk of allograft loss.<sup>15</sup>

### C4d-Positive and C4d-Negative Rejection

In 1993, Feucht et al were the first to report the presence of C4d deposition in peritubular capillaries and the correlation with allograft loss.<sup>16</sup> C4d has no known biologic action, and it is a split product of C4 activation. C4d positivity without other evidence of allograft injury has been reported.<sup>17</sup> However, the frequency of C4d positivity varies from center to center because of the methodology used to detect C4d, the prevalence of highly sensitized patients, and the threshold for C4d positivity.

C4d was considered a marker for antibody injury; however, we now know that up to 55% of patients can have a C4d-negative rejection with obvious evidence of microcirculatory inflammation.<sup>18</sup> At least 3 well-designed studies have demonstrated a sensitivity of only 50%-60%.<sup>18-20</sup> In addition to complement-independent pathways, the low sensitivity and poor interinstitutional reproducibility make C4d a poor marker for the diagnosis of AMR.<sup>21</sup>

The existence of C4d-negative AMR was discussed at the Banff 2013 conference. Because of the low sensitivity of C4d, the Banff 2013 classification incorporates increased expression of endothelial activation and injury transcripts or other gene expression markers of endothelial injury in the tissue biopsy.<sup>8</sup> C4d-negative AMR, defined by microvascular injury (glomerulitis, peritubular capillaritis, TMA) in the presence of DSAs has been reported both in biopsies performed because of graft dysfunction and in protocol biopsies of grafts with stable function.<sup>19</sup>

In a case-control study, Orandi et al reviewed biopsies of high immunologic risk patients, including ABO- and HLA-incompatible patients.<sup>17</sup> The aim of the study was to determine the risk of allograft loss in patients with C4d-negative AMR ( $n=51$ ) compared with C4d-positive ( $n=156$ ) AMR and matched control subjects. C4d-negative rejection was not different from C4d-positive rejection in any baseline characteristic. Compared to patients with C4d-negative rejection, patients with C4d-positive AMR were more likely to present earlier posttransplantation (median of 14 days vs 46 days for C4d-negative rejections,  $P<0.001$ ) and were 3 times more common (7.8% vs 2.5%). Graft survival at 1 and 2 years in C4d-negative AMR patients was 93.4% and 90.2% vs 86.8% and 82.6% in C4d-positive AMR patients, respectively ( $P=0.4$ ). C4d-negative AMR was associated with a 2.56-fold (95% confidence interval [CI], 1.08-6.05,  $P=0.033$ ) increased risk of allograft loss compared with AMR-free matched controls. In the study, anti-HLA DSA class was not different between the 2 groups (class I DSAs 33.3% and 28.3, class II DSAs 29.2% and 22.8%, and both class I and class II DSAs 37.5% and 49% for C4d-negative and C4d-positive AMR patients with anti-HLA DSAs, respectively;  $P=0.4$ ). No clinical characteristic could distinguish C4d-negative from C4d-positive rejection, and the allograft outcome was worse in the C4d-positive group.<sup>17</sup>

### Antibody-Mediated Vascular Rejection

Traditionally, endarteritis has been associated with cellular rejection; however, a population-based study demonstrated that vasculitis belongs to both T cell-mediated rejection and AMR.<sup>22</sup> In the analysis, 2,079 patients with ABO-compatible transplants were participants. A total of 302 patients had acute biopsy-proven rejection. Antibody-mediated vascular rejection was observed in 21%

of patients (64 patients). The risk of graft loss was 9.07 times (95% CI, 3.62-19.7) higher in antibody-mediated vascular rejection than in T cell-mediated rejection without vasculitis ( $P < 0.0001$ ).<sup>22</sup>

### Non-HLA Antibody-Mediated Rejection

Non-HLA antibodies comprise an evolving field in transplant immunology. Non-HLA antibodies are classified into 2 primary categories: alloantibodies directed against polymorphic antigens that differ between the recipient and donor and antibodies that recognize self-antigens or autoantibodies.<sup>23,24</sup> Non-HLA antibodies use different pathways to cause endothelial injuries that do not involve the presence of integrins, as with HLA antibodies. Although they are not well characterized by current available tests or included in the revised Banff 2013 classification, angiotensin type I receptor and endothelin type A receptor antibodies have been implicated as markers of potential endothelial injury in case reports and clinical studies.<sup>25-27</sup> New specific assays to identify these antibodies would facilitate the understanding and diagnosis of allograft dysfunction and would likely identify molecular pathways for effective treatment.

In a population-based analysis, Loupy et al investigated whether the complement-binding capacity of anti-HLA antibodies played a role in kidney allograft failure.<sup>28</sup> A total of 1,016 patients were screened for the presence of circulating donor-specific anti-HLA antibodies and their complement-binding capacity. Patients with complement-binding donor-specific anti-HLA antibodies after transplantation had a lower 5-year graft survival (54%) compared with patients with non-complement-binding donor-specific anti-HLA antibodies (93%) and patients without donor-specific anti-HLA antibodies (94%) ( $P < 0.001$  for both comparisons). These antibodies were associated with an increased risk of allograft loss, an increased rate of AMR, and a more severe graft injury phenotype with increased microcirculatory inflammation and more C4d deposition.<sup>28</sup>

### Transplant Glomerulopathy

Porter et al first described transplant glomerulopathy in 1968.<sup>29</sup> Recently, it has drawn more interest as a manifestation of AMR. Transplant glomerulopathy is manifested as glomerular basement membrane duplication, double contouring, or splitting<sup>30</sup> and is considered to be a late stage of antibody-mediated injury that is usually irreversible and an indicator of poor graft survival. The prevalence of transplant glomerulopathy is 5%-20% in most series, increasing to 55% in high-risk cohorts (T cell complement-dependent cytotoxicity crossmatch).<sup>31-33</sup> Transplant glomerulopathy causes progressive allograft failure with a poor prognosis and eventual allograft loss in 40%-70% of patients and is considered a histologic feature associated with chronic AMR that results from recurrent events of endothelial activation injury and repair.<sup>34</sup> Transplant glomerulopathy is a frequent cause of proteinuria. Proteinuria  $>2.5$  g/d is associated with a worse outcome (graft loss of 92% vs 33%,  $P < 0.005$ ) and is strongly associated with preexisting or de novo DSAs.<sup>31</sup>

The majority of attendees at the Banff 2013 meeting agreed that electron microscopy unequivocally provides the best tool for the early diagnosis of transplant glomerulop-

athy and should be incorporated into the definition of chronic glomerulopathy.<sup>8</sup>

Patri et al studied 92 patients with transplant glomerulopathy to develop a prognostic index based on the risk factors for allograft failure within 5 years of diagnosis.<sup>35</sup> The index was then validated using an independent cohort of 47 patients. The factors considered in the score included serum creatinine, level of proteinuria, and chronic inflammation score at biopsy based on the Banff classification. Based on the score, the authors developed a prognostic index and classified patients into risk groups. Compared with the low-risk group (median allograft survival  $>60$  months from diagnosis), the median survival was 19 months for patients in the medium-risk group and 1.6 months for patients in the high-risk group. The authors concluded that this risk stratification may provide guidance for prognosis and treatment.<sup>35</sup>

### ANTIBODY REMOVAL FOR HIGHLY SENSITIZED PATIENTS

Approximately one-third of patients awaiting a deceased-donor kidney transplant have circulating anti-HLA antibodies, and almost 15% have a high degree of sensitization to potential kidneys.<sup>36-38</sup> Desensitization to HLA antibodies involves treatment with immunomodulating therapies designed to reduce levels of anti-HLA antibodies to make kidney transplantation a feasible option.<sup>39</sup>

### High-Dose Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) is considered an immunomodulatory agent. Several mechanisms of IVIG action have been proposed, including the inhibition of T cell proliferation, the inhibition of cytokine synthesis, the inhibition of complement activation, and the antiidiotypic blockade of alloantibodies.<sup>40</sup>

Jordan et al published their research based on a National Institutes of Health-sponsored trial.<sup>41</sup> Ninety-eight sensitized patients were randomized to receive a placebo (50 patients) vs IVIG (48 patients). IVIG was given monthly for 4 months and at months 12 and 24 after inclusion. Rejection episodes occurred in 9 of the 17 IVIG and in 1 of the 10 placebo subjects who received transplants. Seven graft failures occurred (4 IVIG, 3 placebo). With a median follow-up of 2 years after transplant, the viable transplants functioned normally with a mean  $\pm$  SEM serum creatinine of  $1.68 \pm 0.28$  mg/dL for patients who received IVIG vs  $1.28 \pm 0.13$  mg/dL for patients who received placebo. Adverse events rates were similar in both groups. The authors concluded that IVIG was better than placebo in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized patients with end-stage renal disease.<sup>41</sup>

### Intravenous Immunoglobulin and Rituximab

Rituximab, a chimeric monoclonal antibody against the CD20 antigen, induces B cell lysis via complement-dependent cytotoxicity and antibody-mediated cellular cytotoxicity. It blocks B cell activation and eventual maturation to antibody-forming plasma cells but does not affect existing plasma cells as they do not express the CD20 antigen.<sup>42</sup>



Vo et al reported on 20 highly sensitized patients who received 2 g/kg of IVIG on days 0 and 30.<sup>43</sup> Mean panel reactive antibodies (PRAs) were  $77\% \pm 19\%$ . Rituximab was given at days 7 and 22. Sixteen patients were offered a kidney transplant within a mean time of  $5 \pm 6$  months (range, 2-18 months). Graft survival and patient survival were 94% and 100%, respectively, and the rejection rate was 50%. The researchers concluded that the combination of IVIG and rituximab was effective as a desensitization regimen, but they acknowledged the need for larger trials to evaluate the efficacy of this intervention.<sup>43</sup>

### Plasmapheresis, Intravenous Immunoglobulin, and Rituximab

Leflaucheur et al compared the outcome of a plasmapheresis (PLEX)-, IVIG-, and rituximab-based protocol vs IVIG alone.<sup>44</sup> Group A consisted of 12 patients treated with high-dose IVIG, and Group B consisted of 12 patients treated with PLEX, IVIG, and rituximab. Graft survival at 36 months was 91.7% in Group B vs 50% in Group A ( $P=0.02$ ). The researchers concluded that high-dose IVIG is inferior to combination therapy.<sup>44</sup>

### Immunoadsorption for Rapid Crossmatch Conversion

Compared to PLEX, immunoadsorption (IA) allows not only a more specific but also a more effective clearance of circulating immunoglobulins without the side effects associated with the substitution of fresh frozen plasma or albumin.<sup>45,46</sup> Three or more plasma exchanges can be processed during a single session. Some authors have proposed that this technique may provide rapid and selective antibody depletion in a few hours. Bartel et al reported on a cohort of 68 deceased-donor renal allografts with PRAs  $>40\%$ .<sup>47</sup> Treatment consisted of a single session of immediate pretransplant IA (protein A) followed by posttransplant IA. Twenty-one patients had a positive crossmatch, and 30 had a negative crossmatch. At 5 years, overall graft survival, death-censored graft survival, and patient survival were 63%, 76%, and 87%, respectively, without any differences among crossmatch-positive, crossmatch-negative/DSA-positive, and crossmatch-negative/DSA-negative recipients. Bartel et al did not find any differences in rates of AMR, cellular rejection, or allograft function. They concluded that IA is an effective strategy for rapid desensitization in deceased-donor transplantation.<sup>47</sup>

## TREATMENT OF ANTIBODY-MEDIATED REJECTION

Because of current immunosuppressive medications, the rates of acute rejection and 1-year graft survival have substantially improved since the early 1990s. The threat of hyperacute rejection has been completely eliminated and is currently likely to only attract historic interest. Since the introduction of flow crossmatch in 1983, it is easy to detect the presence of antibodies that can harm kidneys after transplantation.<sup>48</sup> This technique has been proven to be useful not only in detecting de novo antibodies but also in following DSAs after treatment for rejection. Some centers follow rigorous protocols for the diagnosis of subclinical rejection and early intervention. Protocol biopsies and DSA monitoring at predetermined intervals are gaining wide

acceptance, primarily at centers that perform HLA- and ABO-incompatible transplants. The treatment of subclinical AMR and its potential benefits are still under investigation.

Loupy and colleagues reported that among patients with preformed DSAs, despite aggressive induction and antibody removal, 31% were found to have subclinical AMR at their 3-month protocol biopsies.<sup>49</sup> In addition, 49% had microcirculatory inflammation with negative C4d. No specific treatment was given to patients with subclinical AMR at 3 months, and their 1-year protocol biopsies showed further progression of transplant glomerulopathy and interstitial fibrosis/tubular atrophy, with a corresponding decline in estimated glomerular filtration rate.<sup>49</sup>

The obvious goal for the treatment of AMR should be to reduce the inflammation in the allograft, eliminate the factors that cause inflammation, and effectively prevent antibody formation without jeopardizing the normal immune responses that protect patients from serious infections.

Only a few studies to date have reported on the effect of treatment in patients with DSA-associated AMR. Wiebe et al reported no impact on DSA levels or histopathology from optimization of baseline immunosuppression, monthly high-dose IVIG, and pulse steroids.<sup>50</sup> Only a handful of randomized and nonrandomized controlled trials with a small number of patients has been conducted addressing the treatment of AMR. In a US Food and Drug Administration (FDA) open workshop, Archdeacon et al described 13 case series and controlled trials using different treatment modalities including rabbit antithymocyte globulin, methylprednisolone, PLEX, IVIG, B cell-depleting agents, muromonab and maintenance therapy with CNIs, antiproliferative agents, and oral steroids.<sup>51</sup> Only 150 patients were identified in these trials. Overall, AMR is less common than acute cellular rejection; however, in highly sensitized patients, as Archdeacon et al reported, the incidence of AMR exceeds 25%, and more important, it causes severe allograft injuries that lead to late allograft failure. The researchers concluded that a randomized controlled trial to demonstrate superiority of a treatment will require a large sample size and a lengthy follow-up.<sup>51</sup>

Orandi et al compared 219 patients with AMR (77 subclinical, 142 clinical) to controls matched on HLA/ABO compatibility, donor type, prior transplant, PRAs, age, and year of transplant.<sup>15</sup> Graft survival in subclinical AMR at 1 and 5 years was 95.9% and 75.7%, respectively, compared to 96.8% and 88.4% in matched controls ( $P=0.0097$ ). Subclinical AMR was independently associated with a 2.15-fold increased risk of allograft loss (95% CI, 1.19-3.91,  $P=0.012$ ) compared to matched controls but was not different from clinical AMR ( $P=0.13$ ). Treated subclinical AMR patients had no difference in graft loss compared to matched controls (hazard ratio [HR] 1.73; 95% CI, 0.73-4.05;  $P=0.21$ ), but untreated subclinical AMR patients had a 3.34-fold (95% CI, 1.37-8.11;  $P=0.008$ ) higher risk of graft loss compared to matched controls.<sup>15</sup>

### Plasmapheresis

PLEX rapidly removes preformed antibodies and is considered a standard part of therapy in most protocols developed for the treatment of AMR.<sup>42</sup> A randomized controlled trial by Bonomini et al found PLEX to be beneficial.<sup>52</sup> Two controlled trials by Blake et al and Allen

et al found no benefit.<sup>53,54</sup> Kirubakaran et al found potential harm with PLEX.<sup>55</sup> However, the validity of the current studies is challenging because of the heterogeneity of the patient populations, the low number of patients involved in the analyses, use of historical controls, different treatment protocols, sensitization status, induction therapy, combination of cellular and humoral rejection, severity of histology, and interobserver variability. Additional studies have provided conflicting results.

Pascual et al evaluated the role of PLEX and tacrolimus (TAC)-mycophenolate rescue therapy for AMR.<sup>56</sup> During a 14-month period, 73 renal transplants were performed. During the first month, 5 patients were diagnosed with AMR. PLEX (4-7 treatments) significantly decreased circulating DSAs to almost pretransplant levels in 4 of 5 patients, and improvement in renal function occurred in all patients.<sup>56</sup> In an observational study, Slatinska et al found that the combination of PLEX and IVIG was superior to PLEX alone.<sup>57</sup> The authors retrospectively reviewed kidney allograft survival after a follow-up period of 12 months. Thirteen patients received treatment with PLEX alone, and 11 patients received treatment with a combination of PLEX and IVIG (0.5 g/kg). One-year graft survival was significantly higher in the PLEX plus IVIG group than in the PLEX-alone group (90.9% vs 46.2%,  $P=0.044$ ). Similarly, patient survival was higher in the PLEX plus IVIG group vs the PLEX-alone group (100% vs 76.9%,  $P=0.056$ ).<sup>57</sup>

Currently, strategies for the treatment of non-HLA antibodies are based on the same principle used for AMR, which mainly involves extracorporeal techniques to remove antibodies, including PLEX or IA.<sup>58</sup>

### Intravenous Immunoglobulin

The efficacy of IVIG as a monotherapy for AMR is likely limited. Better allograft outcomes have been reported in combination therapy with PLEX and rituximab.

### Rituximab

In a pilot study that included 8 AMR patients receiving rituximab 375 mg/m<sup>2</sup>/week for 3-5 doses, Faguer et al reported a 75% graft survival at 10-month follow-up with 50% infectious complications.<sup>59</sup> In a pilot study of 7 AMR patients treated using PLEX and IVIG at 100 mg/kg/d for 3 days, then 3 times per week for 2-4 weeks, then rituximab 500 mg/m<sup>2</sup> for 1 dose if AMR was ongoing at week 4, Mulley et al found 100% graft survival at 21-month follow-up.<sup>60</sup> As mentioned earlier, Lefaucheur et al conducted a retrospective study of 12 AMR patients compared with historic control (IVIG).<sup>44</sup> The treatment group received PLEX and IVIG 100 mg/kg  $\times$  4 doses, then IVIG 2 g/kg every 3 weeks  $\times$  4 doses, and rituximab 375 mg/m<sup>2</sup>/week  $\times$  2 doses. Graft survival was 91.7% in the treatment group vs 50% in the control group. Kaposztas et al performed a retrospective study of 54 AMR patients compared with historic control (PLEX and IVIG).<sup>61</sup> The treatment group received rituximab 500 mg/m<sup>2</sup>, PLEX, and IVIG 500 mg/kg if an immunoglobulin G deficiency was noticed. Graft survival was 90% in the treatment group vs 60% in the control group.

### Proteasome Inhibitor

Removing plasma cells that generate antibodies is the rationale behind using a proteasome inhibitor (PI) as

therapy for AMR. Bortezomib, currently approved for the treatment of multiple myeloma, has been used in combination with PLEX, IVIG, or rituximab as a rescue therapy for AMR with some encouraging results. Woodle and colleagues published their experience in a multicenter collaborative study that by May 2010 had treated 60 AMR patients in 15 centers with bortezomib-based therapy.<sup>62</sup> The PI-based protocol included bortezomib (1.3 mg/m<sup>2</sup>/dose  $\times$  4 doses) preceded by a single rituximab dose and PLEX prior to each bortezomib dose. Data are reported for 56 episodes of AMR  $\pm$  acute cellular rejection occurring in 51 patients. Transplanted organs with AMR included adult kidney (43), adult kidney/pancreas (9), and pediatric heart (4). The majority of patients undergoing repeat biopsy demonstrated histologic improvement. This large experience with PI-based AMR therapy demonstrated that it provides effective AMR reversal, including substantial reductions in DSA levels.<sup>62</sup>

### Complement Inhibition

The FDA has approved 2 agents, eculizumab (an anti-C5 monoclonal antibody) and a C1 esterase inhibitor (C1-INH), for complement inhibition. Eculizumab was approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. C1-INH was approved for use in patients with hereditary angioedema. Only limited data, usually single cases, are available on the efficacy of eculizumab in patients with severe AMR. Wongsaroj et al reported on the efficacy and safety of eculizumab in treating patients with severe AMR episodes unresponsive to standard treatment with IVIG plus rituximab with or without PLEX.<sup>63</sup> Eight of 13 patients had significant pathologic findings of AMR, 86% were C4d positive, and 5 of 13 patients had AMR with TMA. Seven TMA patients recovered fully or partially after eculizumab compared with 100% graft failure in TMA-positive patients treated with IVIG, rituximab, and PLEX.<sup>63</sup>

In a 2014 study, Orandi et al found that eculizumab was not effective in severe oliguric early-onset AMR.<sup>64</sup> The authors review their experience using different rescue therapies in 24 patients with AMR including splenectomy alone ( $n=14$ ), eculizumab alone ( $n=5$ ), or splenectomy plus eculizumab ( $n=5$ ), in addition to PLEX. At a median follow-up of 533 days, 4 of 14 splenectomy-alone patients experienced graft loss, compared to 4 of 5 eculizumab-alone patients. No patients treated with splenectomy plus eculizumab experienced graft loss. The researchers concluded that splenectomy plus eculizumab may provide an effective intervention for rescuing and preserving allograft function for patients with early severe AMR.<sup>64</sup>

Yelken et al presented a study of 8 patients treated with eculizumab for refractory AMR.<sup>65</sup> Three of the 8 patients achieved a creatinine level  $<2.2$  mg/dL. Four patients were on dialytic therapy that typically continued for  $<3$  months after the initiation of eculizumab. Their kidney biopsies showed different degrees of cortical necrosis, TMA-positive C4d stains, and acute tubular injury. The authors proposed that the early use of eculizumab before advanced changes in kidney injury are identified can improve responses and allograft survival.<sup>65</sup>

### Chronic Antibody-Mediated Rejection Treatment

The previously mentioned therapies have been used for the treatment of chronic AMR although data are limited. Because of the slow progression of chronic AMR compared to acute AMR, subjecting patients to rigorous and potent immunosuppressive agents might not be feasible.<sup>42</sup> A case series conducted by Fehr et al of 4 adult patients who received steroid pulse and rituximab (375 mg/m<sup>2</sup>) followed by IVIG (0.4 g/kg/d for 4 days) showed improved kidney allograft function.<sup>66</sup> Of the 4 patients, 1 had recurrent acute AMR 1 year later, and another patient developed severe pulmonary toxicity that could have been a rare reaction to rituximab.

In the Redfield et al retrospective review of 123 consecutive patients with biopsy-proven chronic AMR, treatment with steroids/IVIG was associated with improved graft survival despite the limitations of the review.<sup>11</sup> The authors acknowledge that further studies are needed, and despite the best available therapies, patients experienced poor graft survival, as 76% of grafts still failed in this cohort.

### PREVENTION OF ANTIBODY-MEDIATED REJECTION

Since its development in 1991, the Banff classification for AMR has proposed ways to identify and to refine pathologic features and diagnostic criteria to better understand and define this entity. Unfortunately, few tools are available that can be used to stratify and predict allograft outcomes or patients at risk for allograft failures.

The presence of C1q-fixing DSAs has emerged as an independent predictor of allograft loss. In a study published by Loupy et al, investigators differentiated DSAs by C1q binding (as a measure of the ability to fix complement), supporting the pathogenicity of DSAs as a predictor of graft failure.<sup>28</sup> The 5-year graft survival for patients with no DSAs, non-C1q-binding DSAs, and C1q-binding DSAs was 94%, 93%, and 54%, respectively. However, the C1q binding assay currently is neither widely available nor validated.<sup>67</sup>

Further, it is well recognized that pregnancies, blood transfusions, and previous organ transplantation are major risk factors for recipient sensitization and AMR.

Djamali et al proposed several strategies to prevent AMR, including avoiding transplantation for highly sensitized patients, better stratifying immunologic risk by using sensitive donor-specific anti-HLA antibody screening, enrolling highly sensitized patients in a paired kidney exchange program, participating in special programs such as the Eurotransplant Acceptable Mismatch Program, and combining kidney paired exchange programs with desensitization protocols.<sup>2</sup>

Monitoring de novo DSAs, using class II HLA epitope matching, and performing protocol biopsies are also strategies used to reduce or early diagnose AMR and improve allograft survival.

For instance, Wiebe et al studied 315 consecutive transplant patients without pretransplant DSAs.<sup>68</sup> Protocol (n=215) and for-cause (n=163) biopsies were analyzed. Forty-seven of 315 (15%) patients developed de novo DSAs. The median 10-year graft survival for those with de novo DSAs was lower than for patients in the no-DSA group (57% vs 96%,  $P<0.0001$ ). The authors also reported that in stable patients without dysfunction who had protocol biopsies at

the time of initial DSA detection, microvascular inflammation was found in 8 of 14 patients, and evidence of antibody-mediated injury (intimal arteritis) was present in 10 of 14 patients. These findings suggest that DSAs are present before the detection of allograft dysfunction.<sup>68</sup>

Opelz et al published a large database review of the Collaborative Transplant Study that included 25,045 patients undergoing kidney transplantation during 1996-2005.<sup>69</sup> No patient in this analysis had a rejection episode with a creatinine level between 1.4-2.9 mg/dL, which in the opinion of the transplant centers was considered excellent or good creatinine at the end of the first year posttransplant. The authors of this observational study concluded that in kidney transplant recipients with good allograft function, withdrawing maintenance cyclosporine (CyA), TAC, or mycophenolate mofetil (MMF) or reducing the dose of these agents below certain thresholds (CyA <150 mg/d, TAC <2 mg/d, and MMF <500 mg/d) after the first year posttransplant is associated with a statistically significant risk of graft loss.<sup>69</sup>

Although the incidence of subclinical rejection has decreased with modern immunosuppression in patients with conventional immunologic risk, rejection rates remain high among transplant patients with DSAs.

The Jordan et al NIH-sponsored randomized controlled trial involved 101 adult patients with PRAs >50%.<sup>41</sup> Patients received IVIG monthly for 4 months with additional infusions at 12 and 24 months or the equivalent volume of placebo. IVIG significantly reduced PRA levels, and more patients in the IVIG group were transplanted (35% vs 17%).

In a metaanalysis of 7 uncontrolled trials, even low levels of DSA were predictors of allograft failure.<sup>70</sup> Subtherapeutic CN1 level, including a TAC level <5 ng/mL, is a risk factor for allograft failure.<sup>71</sup>

In summary, a reduction of immunosuppression, whether physician driven or because of patient nonadherence, is a well-recognized risk factor for DSA formation and subclinical rejection and hence should be avoided to prevent allograft failure. Education for patients and community nephrologists is advised, as well as appropriate financial evaluations prior to transplantation. Lack of financial support is a major barrier for adherence to the long-term use of immunosuppressive medications. Community physicians tend to lower the dose of CNIs and/or antiproliferative agents based on the longevity of the allograft without considering potential late rejection.

Late allograft failures impose significant emotional and financial burdens on patients, family members, and the healthcare system. The mortality risk increases, and retransplantation becomes challenging because of the high level of DSAs and sensitization of these patients.

### CONCLUSION

Despite the use of advanced techniques for the detection of HLA or non-HLA DSAs, alloimmune response remains an important barrier for successful long-term allograft function. The invaluable contribution of histologic criteria, driven principally by the Banff classification, has helped standardize the diagnosis of AMR. Treatment of AMR with currently available therapies has produced a variety of results, some of them suboptimal, precluding the development of standardized protocols. Nonetheless PLEX and IVIG are still



considered mainstay therapies for the treatment of AMR. More research is required in the field of AMR using homogenous populations, similar thresholds for the detection of DSAs, appropriate follow-up, and possibly the introduction of surveillance biopsies. Newer therapies are promising, but randomized controlled trials are needed to find surrogate markers and improve the efficacy of therapy.

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