Recognition and Management of Antibody-Mediated Rejection

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Abstract Previously underdiagnosed and overshadowed by other forms of allograft rejection, antibody-mediated rejection (AMR) is increasingly recognized as a major cause of kidney allograft dysfunction and chronic graft loss. In addition, the problem of sensitization among potential recipients continues to grow, with almost one third of all current waiting-list patients affected. Improved understanding of the pathophysiology of this phenomenon has driven advances in diagnostic and treatment options but also has highlighted the magnitude and complexity of AMR. During the 2013 American Transplant Congress in Seattle, Washington, experts discussed ongoing developments in molecular diagnostics and results of current treatment strategies, with particular emphasis on plasmapheresis and complement inhibition.

Antibody-mediated rejection (AMR) is a major cause of acute and chronic allograft dysfunction.[1,2] Diagnostic difficulty previously led to underestimation of the extent of this problem; its importance continues to become more obvious as new diagnostic tools emerge. Acute AMR occurs in at least 5%–7% of all kidney recipients and as many as 25%–30% of presensitized crossmatch-positive patients.[3] In addition, chronic AMR resulting from sensitization or de novo donor-reactive antigen is a major contributor to long-term allograft loss.[2,4]

OVERVIEW

Diagnosing AMR

AMR may arise early after transplantation from reactivation of antibody responses to preexisting antigens (type I) or de novo to donor-specific antibodies (DSAs) encountered late after transplant (type II), mostly as a result of nonadherence to immunosuppressive therapy.[5] The current Banff classification relies upon three cardinal features essential for diagnosis: positive C4d staining, circulating DSAs, and tissue injury. However, the histologic findings of injury vary—DSA may be absent despite histologic findings of AMR and positive C4d staining. In addition, C4d detection may vary across methods, and findings among patients with chronic AMR often are negative.

The classification of AMR is based upon the clinical setting, underlying pathophysiology, and temporal relationship to transplantation (hyperacute, acute, and chronic). Clinical manifestations range from immediate graft loss to chronic subclinical rejection with gradual loss of function.[1–3]

Outcomes associated with AMR

AMR has a worse outcome than acute cellular rejection (ACR), which likely is the result of diagnostic difficulty and less-effective therapeutic options. Among renal transplant recipients who develop AMR, 15%–20% will lose their grafts within 1 year.[6] In addition, > 40% of patients with AMR eventually develop transplant glomerulopathy, whether or not initial treatment can reverse the acute renal functional impairment. This glomerulopathy is associated with < 50% 5-year graft survival from the time of identification.[6]

Targets and Therapies

As shown in Figure 1, multiple therapeutic options exist for AMR.[1] These treatments include inhibition or depletion of B-cell function with rituximab or corticosteroids; interference with antibody function using plasmapheresis, immunoadsorption, and/or intravenous immunoglobulin (IVIg); interruption of plasma-cell function with bortezomib; or prevention of complement cascade using eculizumab.[1,2,7]

At a session held during the 2013 American Transplant Congress in Seattle, Washington, experts in organ transplantation explored novel molecular scoring approaches to the diagnostic difficulty in AMR. In addition, they discussed emerging therapeutic strategies for type I and type II AMR, including complement inhibitors and multimodal approaches. The symposium was moderated by Elaine F. Reed, PhD, Professor of Pathology and Laboratory Medicine at the University of California, Los Angeles, and Jean I. Tchervenkov, MD, Research Director at the Royal Victoria Hospital of McGill University Health Centre in Montreal, Canada.

THE ROLE OF PLASMAPHERESIS IN TREATING AMR

Based on a presentation by Robert Montgomery, MD, PhD, FACS, Professor of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Over 96,000 patients currently are registered on the waiting list for kidney

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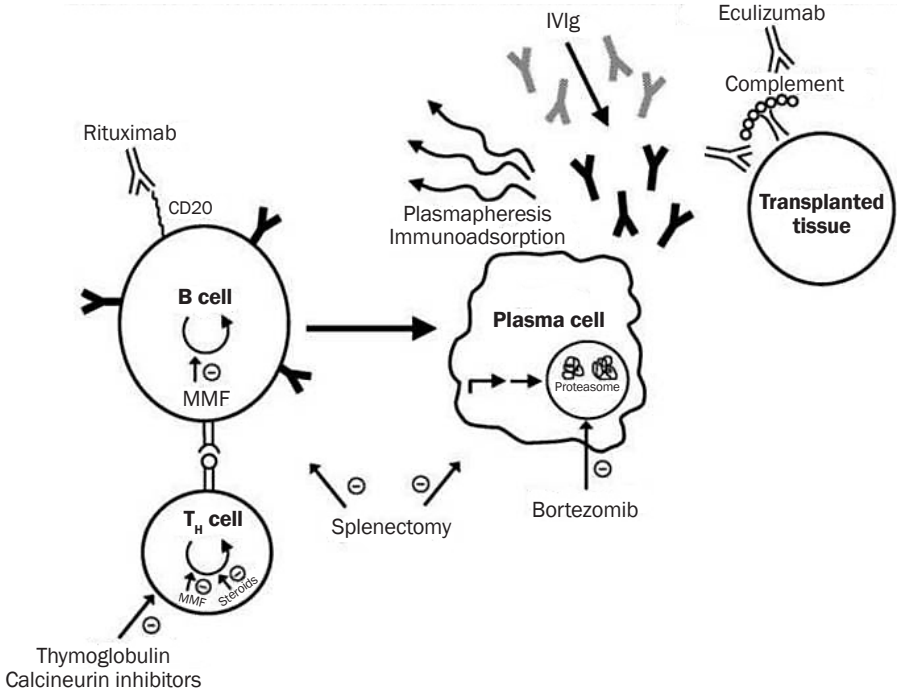

FIGURE 1 Steps in the development of antibody-mediated rejection. MMF = mycophenolate mofetil; T_H cell = T-helper cell; IVIg = immunoglobulin-γ. Adapted, with permission, from Levine and Abt.[1]

transplantation; almost 16% of them are prior organ-transplant recipients, and approximately 30% will be sensitized to human leukocyte antigen (HLA).[8] Highly sensitized patients with > 80% panel reactive antibody (PRA) wait three times longer to undergo transplant surgery than do unsensitized renal transplant recipients and have an average wait time of almost 10 years.[9]

Over the past decade, desensitization protocols have evolved into combination therapies, which include plasmapheresis; immunoadsorption; IVIg; splenectomy; and, more recently, administration of rituximab, bortezomib, or eculizumab. Today, an increasing number of patients are transplanted across previously insurmountable barriers. However, these desensitized patients continue to have an increased incidence of type I AMR and graft loss.[10]

Pathophysiology of AMR in Sensitized Patients

The pathophysiology of type I AMR in desensitized patients apparently involves residual plasma cells and long-lived allospecific memory B cells, which reactivate a recipient response against donor antigen.[11] Approximately one fourth of desensitized recipients experience early AMR, usually within the first week after transplant. Two thirds of these patients will be responsive to plasmapheresis, whereas the remainder may experience severe, oliguric, plasmapheresis-resistant rejection, which often is accompanied by graft loss.[12] The principal effector mechanism of antibody-mediated injury involves activation of the classic complement pathway by the antigen-antibody complex deposition (Figure 2).[13]

Plasmapheresis to treat AMR in Desensitized Patients

Clinicians and surgeons at Johns Hopkins have been at the forefront of desensitization treatments, which enable transplantation across HLA and ABO incompatibility. Montgomery et al[14] used plasmapheresis with IVIg to reduce the strength of DSA prior to transplantation. Induction therapy with antithymoglobulin and corticosteroids together with maintenance immunosuppression (tacrolimus and mycophenolate mofetil) prevented AMR in most desensitized patients. In addition, one third of patients received anti-CD20 immediately prior to transplant due to the presence of high-risk factors, such as antibody titer or combined ABO/HLA incompatibility. Desensitization improved patient survival to 90% and 80% at 1 and 5 years, respectively, as compared with survival of 93% and 65% among patients who waited for compatible donors.

Montgomery and colleagues[12] reported a 22% incidence of early AMR, which is mostly responsive to further treatment with plasmapheresis and IVIg. However, this therapy was insufficient for patients with certain types of high-grade AMR associated with severe dysfunction. This failing may be due to rapid expansion of plasma cells and an inability of plasmapheresis to suppress large-scale antibody production. Splenectomy rapidly reversed oliguria and reduced DSA strength.[12] Examination of splenic tissue demonstrated that donor-specific plasma cells had been removed.

However, approximately 50% of treated patients lose their grafts within 2 years. More recently, the addition of complement inhibitor to splenectomy has increased the rescue rate and significantly reduced the development of tubular glomerulopathy.[12]

Phenotypes and Outcomes

The evaluation of patients treated for AMR suggests that plasmapheresis is better at eliminating type I antibody generated to major histocompatibility complex (MHC) class I than it is at eradicating MHC class II antigen. Additional phenotypes associated with poor outcome include C4d-positive staining with glomerulitis, peritubular capillaritis, and microcirculatory inflammation. These findings differ from results seen in the setting of type II AMR. Further studies must incorporate immunologic and histopathologic parameters into treatment algorithms

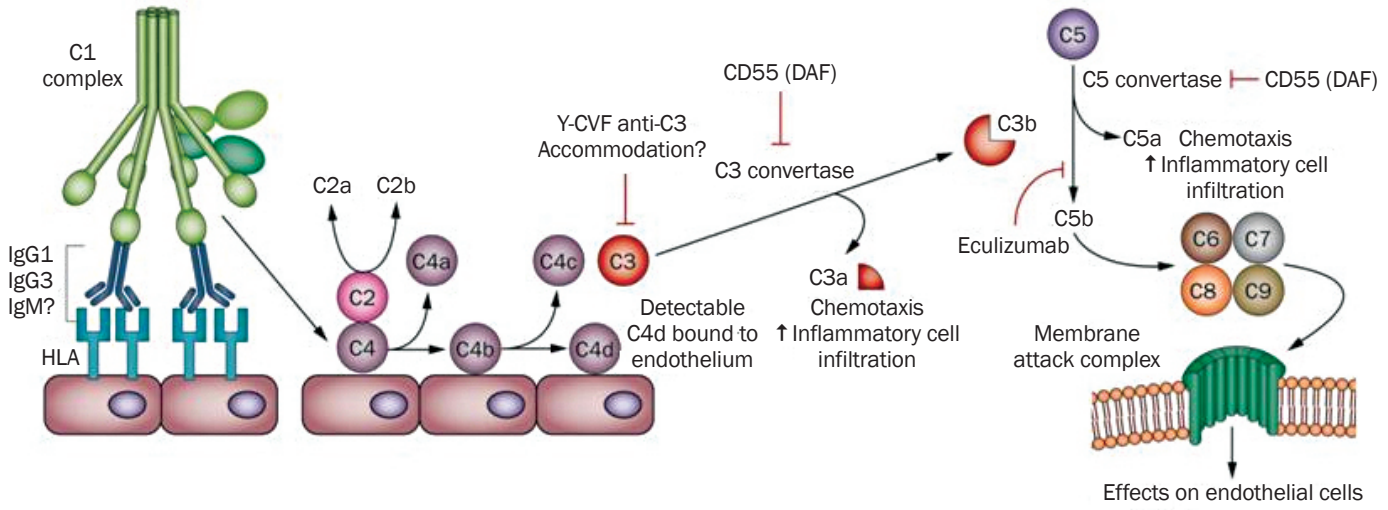

FIGURE 2 The role of complement in antibody-mediated rejection. Ig = immunoglobulin; HLA = human leukocyte antigen; DAF = decay-accelerating factor; Y-CVF = Yunnan-cobra venom factor. Reproduced, with permission, from Stegall et al.[13]

that will balance risk with degree of therapeutic aggression.

THE ROLE OF COMPLEMENT INHIBITION IN TREATING AMR

Based on a presentation by Mark Stegall, MD, Professor of Surgery and Immunology, Mayo Clinic, Rochester, Minnesota.

Whereas the complement-inhibitor treatment strategies used at Johns Hopkins for managing AMR essentially involve a rescue-based approach, the Mayo Clinic approach represents a preemptive strategy that involves the use of eculizumab and anti-CD20.

Stegall and others[15] evaluated the use of eculizumab post transplant to prevent AMR among patients desensitized to levels of low-to-moderate antibody strength. They compared 26 consecutive patients treated with eculizumab with 51 historic matched controls. Within the first 3 months after transplant surgery, AMR occurred in 7.7% of patients given eculizumab and 41% of matched controls. No additional plasmapheresis treatments were planned for the study group post transplant.

The long-term effects and optimal treatment duration associated with the use of complement inhibition remain unknown. However, when protocol-required biopsies were examined up to 1 year after treatment, they showed no evidence of transplant glomerulopathy.

Risk of Rejection Depends on DSA Titer and Type

DSAs are most commonly directed against HLA class I (present on all nucleated cells) or class II (antigen-presenting cells and endothelial cells). DSAs may also develop against non-HLA antigens, including MHC class I–related chain A and B (MICA, MICB), molecules of the renin-angiotensin pathway and platelet-specific antigens.[2] The level of DSA, expressed as mean fluorescent intensity (MFI), is proportional to the risk of rejection. Complement inhibition did not alter the DSA level, but it was particularly effective in reducing AMR in patients with a high DSA concentration (control group, 100%; study group, 15%).[15]

Eculizumab-Resistant AMR

Late acute AMR and chronic AMR often are associated with low DSA titers and low or negative amounts of C4d. The role of complement inhibition and antibody removal in these patients is unclear.[16] Treatment of late AMR with findings suggesting acute cellular rejection and AMR may involve a combination of thymoglobulin, plasma exchange, and eculizumab.

Chronic AMR accompanied by negative C4d staining may be a form of complement-independent rejection and, hence, eculizumab-resistant rejection. The best treatment of chronic AMR appears to be a combination of therapeutic approaches, such as bortezomib therapy plus plasma exchange and IVIg.[17]

THE DOMINANT ROLE OF AMR IN KIDNEY TRANSPLANT FAILURE

Based on a presentation by Phillip Halloran, MD, PhD, Director, Alberta Transplant Applied Genomics Centre, University of Alberta, Edmonton, Alberta, Canada.

Each year, over 5,000 patients develop late renal transplant failure, making it the fourth most common cause of end-stage renal disease. Gaston et al suggested that alloimmunity has a critical role in most chronic lesions previously designated as being chronic allograft nephropathy and, specifically, that AMR plays a key role in many of these late graft losses.[18,19]

Diagnostic Challenge: C4d-Negative AMR and Underestimation of the Role of AMR

The accuracy of AMR diagnosis is questionable due to the heterogeneity of histologic features, the lack of specificity of lesions for rejection pathology, the variability of HLA identification across assays, and the disparate results seen for C4d staining with immunofluorescence

and immunohistochemistry. The detection of C4d has been a requirement for AMR diagnosis. However, many C4d-negative cases have clinical and histologic findings similar to those of rejection and exhibit DSA. A substantial fraction of chronic graft failure previously labeled as calcineurin-inhibitor nephrotoxicity may result from C4d-negative AMR.[4] Sis et al[20] examined 329 biopsy samples from patients with graft dysfunction and found that during the first year after transplantation, peritubular capillaritis and glomerulitis often were not associated with DSA (27%).

Molecular Score

The Banff classification was a significant advance in the diagnosis and treatment of AMR. However, use of this classification tends to underdiagnose the phenomenon, particularly when chronic AMR is considered. Predictive molecular scoring systems based upon levels of gene expression increasingly are being used to augment standard diagnostic histopathology, which fails to improve risk stratification. The Banff Working Group currently is addressing a number of approaches related to deficiencies, including IgG subtyping, MFI levels of DSA, and C1q-fixing DSA.[5]

Sellarés et al[21] used Affymetrix microarray technology to analyze samples from indication biopsies post transplant. Their study biopsies were obtained over 1 year from transplant and represented type II AMR. For many patients, the biopsy results were related to medication nonadherence.[4] Survival was linked to timing of the biopsy and the disease process identified. The greatest risk of graft loss was during the initial 3 years following indication biopsy.

A cohort of 30 genes strongly associated with AMR were identified and validated (Table 1).[21] The genes, which included cadherin 13 *(CDH13),* chemokine (C-X-C motif) ligands 10 and 11 *(CXCL10* and *CXCL11),* and fibroblast growth factor binding protein 2 *(FGFBP2),* all were associated with endothelial injury and cellular trafficking. This molecular fingerprint was applied to all samples and appeared to discriminate between AMR and other causes of acute deterioration in graft function.[21]

TABLE 1
Genes Associated with Antibody-Mediated Rejection

Gene name	Gene symbol	Probable cellular expression
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	*APOBEC3A*	Macrophages
Cadherin 5, type 2	*CDH5*	EC
Cadherin 13	*CDH13*	EC
Caveolin 1	*CAV1*	EC, EP
Chemokine (C-C motif) ligand 4	*CCL4*	NK, T, macrophages
Chemokine (C-X-C motif) ligand 10	*CXCL10*	IFNG-induced
Chemokine (C-X-C motif) ligand 11	*CXCL11*	IFNG-induced
Chemokine (C-X3-C motif) receptor 1	*CX3CR1*	NK
Cholesteryl ester transfer protein, plasma	*CETP*	Cell type unknown
Chromosome 21 open reading frame 63	*C21orf63*	EC
Corticotropin-releasing hormone binding protein	*CRHBP*	Cell type unknown
Duffy blood group, chemokine receptor	*DARC*	EC
Endothelial cell-specific chemotaxis regulator	*ECSCR*	EC
Fibroblast growth factor binding protein 2	*FGFBP2*	NK, T
Granulysin	*GNLY*	NK, T
Guanine nucleotide binding protein (G protein), γ 11	*GNG11*	EC, EP
Kruppel-like factor 4 (gut)	*KLF4*	EC
Mesenchyme homeobox 1	*MEOX1*	EC
Palmdelphin	*PALMD*	EC
Phosphoglucomutase 5	*PGM5*	Cell type unknown
Phospholipase A1 member A	*PLA1A*	EC, EP
Rap guanine nucleotide exchange factor 5	*RAPGEF5*	EC
Receptor (G protein-coupled) activity modifying protein 3	*RAMP3*	Cell type unknown
Roundabout homolog 4	*ROBO4*	EC
Sclerostin	*SOST*	Cell type unknown
Sex-determining region Y–box 7	*SOX7*	EC
T-cell receptor δ locus	*TRD*	NK
Transmembrane 4 L six family member 18	*TM4SF18*	EC, EP
Von Willebrand factor	*VWF*	EC

EC = endothelial cells; EP = epithelial cells; NK = natural killer cells; T = T cells; IFNG = interferon-γ
Source: Sellarés et al[21]

The molecular score may reflect subclinical injury and may predict the emergence of AMR earlier than conventional scoring systems. It remains to be determined whether the molecular score represents a novel method of tracking disease progression, whether it can measure the efficacy of therapeutic interventions, and whether it is altered by successful treatment of AMR.

RECOGNITION AND MANAGEMENT OF SUBCLINICAL AMR

Based on a presentation by Abdolreza Haririan, MD, MPH, Associate Professor of Medicine, University of Maryland Medical Center, Baltimore, Maryland.

The natural progression of AMR is under scrutiny amid growing awareness that within the heterogeneous histologic features seen on protocol-required biopsies, some transplant recipients will develop early chronic AMR with no clinical effects for a period of time.[22]

Subclinical AMR is defined as graft changes that meet established pathologic and serologic criteria for AMR without the presence of associated graft dysfunction or concurrent ACR. The serum creatinine level remains unchanged, and DSAs tend to have low MFI values in the absence of proteinuria. In many instances, patients will repeatedly undergo biopsy without exhibiting clear evidence

of rejection before developing clinical and pathologic changes. Some experts believe that quiescent disease can erupt later as a result of an immunologic trigger.

The risk of graft loss is 77% higher among patients with DSAs. Some DSAs are more likely than others to result in graft rejection. In addition, non-HLA and non-complement fixing antibody may be responsible for subclinical AMR.

Accommodation vs Subclinical Rejection

The presence of C4d without evidence of tissue injury also may suggest the presence of inhibitory mechanisms to the distal complement cascade downstream of C4d cleavage. Known as accommodation, it often is seen in protocol-required biopsies after ABO-incompatible transplantation. However, aside from ABO incompatibility, desensitized patients with C4d-positive staining typically experience tissue injury. At present, there is no way to differentiate C4d-positive biopsies that represent accommodation from subclinical AMR-mediated rejection.[23]

Non–Complement-Activating Alloantibodies

Approximately 10% of biopsies with features of acute AMR are C4d negative, implying that rejection may occur through complement-independent mechanisms. Sis et al[24] used microarray analysis to demonstrate increased endothelial cell gene expression in biopsies having histopathologic findings of AMR and DSA but negative C4d staining; this approach may represent a novel method of AMR detection.

Diagnosis and Treatment of Subclinical AMR

The investigation and diagnosis of subclinical AMR are challenging. Current data suggest that not all DSAs pose the same risk. Molecular phenotyping and electron microscopy have a potential role in detecting and managing subclinical AMR. Gloor et al[25] reported that treatment of desensitized transplant recipients with subclinical AMR using a combination of corticosteroids, plasmapheresis, and IVIg resolved the histologic abnormalities, although the long-term clinical outcome of this strategy remains unclear.

CONCLUSION

Better understanding of the pathogenesis of acute AMR is the foundation for successful treatment of desensitized patients by blocking multiple points of the pathway, from antibody production to complement-dependent tissue injury. Chronic AMR, the more prevalent form of this phenomenon, is a major cause of late graft loss and remains less understood than acute AMR. However, new gene-based molecular approaches to the diagnosis of AMR offer hope of more timely and effective treatment. The challenge remains to assign precise risk to the individual patient and to tailor treatments specific to the characteristics of AMR.

REFERENCES

1. Levine MH, Abt PL. Treatment options and strategies for antibody mediated rejection after renal transplantation. *Semin Immunol.* 2012;24:136–142.
2. Puttarajappa C, Shapiro R, Tan HP. Antibody-mediated rejection in kidney transplantation: a review. *J Transplant.* 2012;2012:193724.
3. Lucas JG, Co JP, Nwaogwugwu UT, Dosani I, Sureshkumar KK. Antibody-mediated rejection in kidney transplantation: an update. *Expert Opin Pharmacother.* 2011;12:579–592.
4. Sellarés J, de Freitas DG, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant.* 2012;12:388–399.
5. Mengel M, Sis B, Haas M, et al. Banff 2011 meeting report: new concepts in antibody-mediated rejection. *Am J Transplant.* 2012;12:563–570.
6. Gloor JM, Cosio FG, Rea DJ, et al. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. *Am J Transplant.* 2006;6:1841–1847.
7. Fehr T, Gaspert A. Antibody-mediated kidney allograft rejection: therapeutic options and their experimental rationale. *Transplant Int.* 2012;25:623–632.
8. Organ Procurement and Transplantation Network (OPTN) Web site. http://optn.transplant.hrsa.gov. Accessed July 5, 2013.
9. Jordan SC, Reinsmoen N, Peng A, et al. Advances in diagnosing and managing antibody-mediated rejection. *Pediatr Nephrol.* 2010;25:2035–2045.
10. Paramesh AS, Zhang R, Baber J, et al. The effect of HLA mismatch on highly sensitized renal allograft recipients. *Clin Transplant.* 2010;24:E247–E252.
11. Stegall MD, Dean PG, Gloor J. Mechanisms of alloantibody production in sensitized renal allograft recipients. *Am J Transplant.* 2009;9:998–1005.
12. Montgomery RA, Warren DS, Segev DL, Zachary AA. HLA incompatible renal transplantation. *Current Opin Organ Transplant.* 2012;17:386–392.
13. Stegall MD, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat Rev Nephrol.* 2012;8:670–678.
14. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med.* 2011;365:318–326.
15. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant.* 2011;11:2405–2413.
16. Stegall MD, Gloor JM. Deciphering antibody-mediated rejection: new insights into mechanisms and treatment. *Curr Opin Organ Transplant.* 2010;15:8–10.
17. Trivedi HL, Terasaki PI, Feroz A, et al. Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation.* 2009;87:1555–1561.
18. Gaston RS, Cecka JM, Kasiske BL, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation.* 2010;90:68–74.
19. Safinia N, Afzali B, Atalar K, Lombardi G, Lechler RI. T-cell alloimmunity and chronic allograft dysfunction. *Kidney Int Suppl.* 2010;S2–S12.
20. Sis B, Jhangri GS, Riopel J, et al. A new diagnostic algorithm for antibody-mediated microcirculation inflammation in kidney transplants. *Am J Transplant.* 2012;12:1168–1179.
21. Sellarés J, Reeve J, Loupy A, et al. Molecular diagnosis of antibody-mediated rejection in human kidney transplants. *Am J Transplant.* 2013;13:971–983.
22. Papadimitriou JC, Drachenberg CB, Ramos E, et al. Antibody-mediated allograft rejection: morphologic spectrum and serologic correlations in surveillance and for cause biopsies. *Transplantation.* 2013;95:128–136.
23. Park WD, Grande JP, Ninova D, et al. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant.* 2003;3:952–960.
24. Sis B, Jhangri GS, Bunnag S, Allanach K, Kaplan B, Halloran PF. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant.* 2009;9:2312–2323.
25. Gloor JM, DeGoey SR, Pineda AA, et al. Overcoming a positive crossmatch in living-donor kidney transplantation. *Am J Transplant.* 2003;3:1017–1023.