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Diagnosis, Treatment, and Outcomes of Antibody-Mediated Rejection in Kidney Transplantation

Vasishta S. Tatapudi and Bonnie E. Lonze

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Abstract

Antibody mediated rejection remains an important barrier to optimal long-term outcomes after kidney transplantation. Donor specific antibody, while not the formidable barrier to transplantation it once was, remains a major risk factor for antibody mediated rejection and its consequences of premature graft failure. Recent advances in understanding of the cellular and molecular mechanisms of antibody production and antibody-mediated injury have led to refinements in diagnostic techniques, and have paved the way for the development of novel therapies to treat rejection and prolong allograft function. The purpose of this chapter is to review the current level at which we understand the pathophysiology of antibody mediated rejection, describe the current diagnostic criteria for antibody mediated rejection, and discuss available and emerging treatments as well as their outcomes.

Keywords: kidney transplantation, donor specific antibody, antibody mediated rejection

1. Introduction

The first successful kidney transplant was performed between identical twins by Joseph E. Murray and his colleagues at the Peter Bent Brigham Hospital in 1954 [1]. Since then, the field of kidney transplantation has progressed immensely owing to greater understanding of the immune mechanisms underlying allograft rejection at a cellular and molecular level and development of increasingly potent immunosuppressive drug therapies [2]. Today, kidney transplantation is considered the treatment of choice for patients with end stage renal disease (ESRD) since it is associated with lower mortality and cardiovascular morbidity while offering
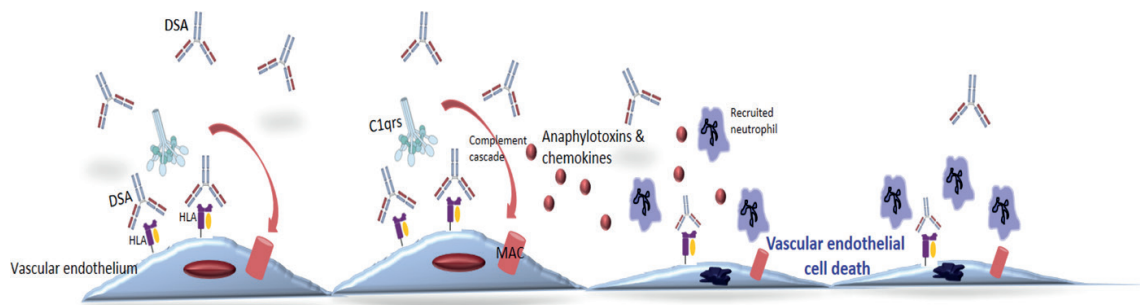
Figure 1. In AMR, DSAs bind to human leukocyte antigens on graft vascular endothelium. This is followed by activation of complement, membrane attack complex-mediated cellular injury and infiltration of mononuclear cells. Reproduced with permission from Montgomery et al. [4].

improved quality of life [3]. However, allograft rejection remains a major impediment to the longevity of renal allografts. Recognition of donor antigens as "non-self" by the host immune system elicits humoral and cell mediated immune responses that if left unchecked result in the destruction of the allograft (**Figure 1**).

2. Human leukocyte antigen (HLA) system and allograft rejection

Human leukocyte antigens (HLA) play a vital role in host defense against foreign pathogens and in immune surveillance of tumors. These antigens are encoded by the major histocompatibility complex (MHC) which is a family of genes that encompasses a 3.6 million base pair genomic region, 6p21, on the short arm of chromosome 6 [5]. The MHC complex is divided into three regions representing three classes of genes—classes I, II, and III. The HLA genes that are involved in the immune response belong to classes, I and II [6]. Class I antigens are expressed on all nucleated cells whereas class II antigens are expressed only on professional antigen presenting cells (APCs)—dendritic cells, B-cells, and macrophages [7]. In the setting of infection, pathogen derived foreign antigens are presented to T-cells as peptides on the surface of major histocompatibility complex (MHC) molecules expressed on the surface of APCs. The ensuing signaling cascade results in the activation, proliferation, and differentiation of naïve T lymphocytes into subtypes with distinct cytokine profiles. Type 1 helper T (Th1) cells drive the cellular immune response mediated by CD8+ cytotoxic T lymphocytes, and type 2 helper T (Th2) cells drive B-cell mediated humoral immune response [2].

The heterogeneity of human MHC molecules enables the immune system to protect us from a variety of foreign pathogens. However, in the context of transplantation between genetically distinct individuals, these MHC polymorphisms elicit immune responses that can result in rejection of the allograft [7]. Two pathophysiologically distinct flavors of renal allograft rejection are recognized—cell mediated, and antibody mediated rejection. Both these types of rejection can manifest as acute or chronic clinicopathologic variants. Acute cell mediated rejection is characterized by infiltration of the allograft by effector T cells resulting in the typical features such as tubulitis, interstitial inflammation and in more advanced cases, endothelial arteritis [2, 8].

3. Clinical relevance of antibody mediated rejection

AMR is estimated to occur in 3–10% of transplant recipients and it represents 20–30% of episodes of acute rejection [7]. Although less common than cell mediated rejection, AMR is generally recognized to have a worse prognosis and requires different forms of therapy [9]. In the 1960s anti-HLA antibodies were recognized as a cause for allograft rejection following reports of hyperacute antibody mediated rejection in patients with antibodies reactive to donor lymphocytes [10, 11]. Patel and Terasaki's landmark study documented immediate graft failure in 24 of 30 (80%) of the patients with circulating donor reactive antibodies identified by a positive cytotoxicity crossmatch [12]. This led to the universal practice of antibody screening by complement dependent cytotoxicity (CDC) crossmatch prior to renal transplantation and the avoidance of transplantation in patients with a positive crossmatch. Therefore, until the mid-1980s, acute cellular rejection, as opposed to antibody mediated rejection (AMR), was considered the major barrier to successful [13]. The advent of calcineurin inhibitors (CNIs) in the 1980s led to a significant decline in incidence of acute rejection and a consequent improvement in short term graft survival rates [14]. Today, cellular rejection seldom causes graft loss [15]. However, contemporary data suggests that these gains have not led to sustained improvement in long-term graft survival [16]. Reasons for the lack of improvement in long-term graft survival have been a topic of much debate and most late graft losses were attributed to either chronic allograft nephropathy (CAN) or death with a functioning graft [17]. Although, the multifactorial nature of late renal allograft loss makes therapeutic intervention challenging [18] prevention and treatment of AMR holds the key to optimizing long term graft survival.

Exposure to non-self HLA by way of pregnancy, blood transfusion or transplantation may lead to the development of circulating anti-HLA antibodies. ESRD patients who are sensitized to HLA by prior exposure have a prolonged wait-time for transplantation and reduced transplant rates. Removal of pre-formed circulating donor specific antibodies (DSA) by various desensitization techniques allows transplantation of many of these biologically disadvantaged patients [19–21] However, such HLA incompatible kidney transplants recipients are at increased risk for developing AMR. A high percentage of episodes of AMR are difficult to treat and may cause immediate graft loss or delayed transplant glomerulopathy [22]. Therefore, AMR remains a significant impediment to the success of transplantation in this subset of patients.

4. Diagnosis of antibody mediated rejection

The Banff classification schema has been used internationally for scoring and classifying kidney transplant pathology findings since its first iteration was published in 1993. However, earlier versions dealt with AMR in an imprecise manner. The development of more sophisticated methods of detection of DSAs by means of solid-phase assays together with the sensitivity and specificity of C4d staining in peritubular capillaries in identifying AMR paved the way for rigorous morphological classification of AMR [23]. The cornerstones for the diagnosis for AMR are (1) Histologic evidence of acute tissue injury; (2) Evidence of current/recent antibody interaction with vascular endothelium; (3) Serologic evidence of DSAs. The updated 2015 Banff classification system recognizes acute active AMR and chronic active AMR and outlines detailed criteria for the diagnosis of each (**Table 1**) [24].

Acute/active AbMR	All three features must be present for diagnosis. Biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious for acute/active ABMR. Lesions may be clinically acute or smoldering or may be subclinical; it should be noted if the lesion is C4d-positive or C4d-negative, based on the following criteria: 1. Histologic evidence of acute tissue injury, including one or more of the following: • Microvascular inflammation ($g^a > 0$ in the absence of recurrent or *de novo* glomerulonephritis, and/or $ptc^b > 0$) • Intimal or transmural arteritis ($v^c > 0$) • Acute thrombotic microangiopathy in the absence of any other cause • Acute tubular injury in the absence of any other apparent cause 2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections or C4d >0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute T-cell mediated rejection (TCMR), borderline infiltrate, or infection; ptc ≥2 alone is not sufficient, and g must be ≥1 • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated 3. Serologic evidence of DSAs (HLA or other antigens) Biopsies suspicious for AMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing
Chronic active ABMR	All three features must be present for diagnosis. As with acute/active ABMR, biopsies showing histological features plus evidence of current/recent antibody interaction with vascular endothelium or DSA, but not both, may be designated as suspicious, and it should be noted if the lesion is C4d-positive or C4d-negative, based on the criteria listed: 1. Histologic evidence of chronic tissue injury, including one or more of the following: • TG ($cg^d > 0$), if no evidence of chronic thrombotic microangiopathy; includes changes evident by EM only • Severe peritubular capillary basement membrane multilayering (requires EM)c • Arterial intimal fibrosis of new onset, excluding other causes; leukocytes within the sclerotic intima favor chronic ABMR if there is no prior history of biopsy-proven TCMR with arterial involvement but are not required 2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2), although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥2 alone is not sufficient and g must be ≥1 3. Serologic evidence of DSAs (HLA or other antigens): • Biopsies suspicious for AMR on the basis of meeting criteria 1 and 2 should prompt expedited DSA testing

C4d staining without evidence of rejection	All three features must be present for diagnosis:
	1. Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d >0 by IHC on paraffin sections
	2. g = 0, ptc = 0, cg = 0 (by light microscopy and by EM if available), v = 0; no TMA, no peritubular capillary basement membrane multilayering, no acute tubular injury (in the absence of another apparent cause for this)
	3. No acute cell-mediated rejection (Banff 1997 type 1A or greater) or borderline changes

g-glomerulitis, ptc-peritubular capillaritis, v-vasculitis, cg-chronic glomerulopathy (transplant glomerulopathy)

Table 1. Criteria for antibody mediated rejection as outlined by the Banff 2015 Meeting Work Group [24].

Glomerular and/or peritubular capillary infiltration with polymorphonuclear leukocytes and/ or macrophages represents microvascular inflammation and is the classic histologic feature of acute tissue injury in acute AMR (**Figure 2**). However, intimal or transmural arteritis, acute TMA and ATN can also denote acute AMR (**Figure 3**). Splitting or double contouring of the GBM (transplant glomerulopathy) as well as severe multi-lamination of peritubular capillary basement membrane are histologic features of chronic AMR. Detection of inert complement split product, C4d in peritubular capillaries by IF or IHC indicates antibody interaction with vascular endothelium (**Figure 4**). However, recognizing that complement independent pathways may be involved in the etiopathogenesis of AMR, the Banff classification also sets forth certain criteria (listed in **Table 1**) that allow for the diagnosis of acute or chronic AMR in patients without detectable C4d staining. Demonstration of circulating DSAs is a pre-requisite for diagnosis of AMR. Noting that non-HLA DSAs may result in clinical and histopathologic findings indistinguishable from AMR, Banff criteria for serologic evidence of DSAs require detection of either DSAs directed against donor HLA or "other" antigens [24].
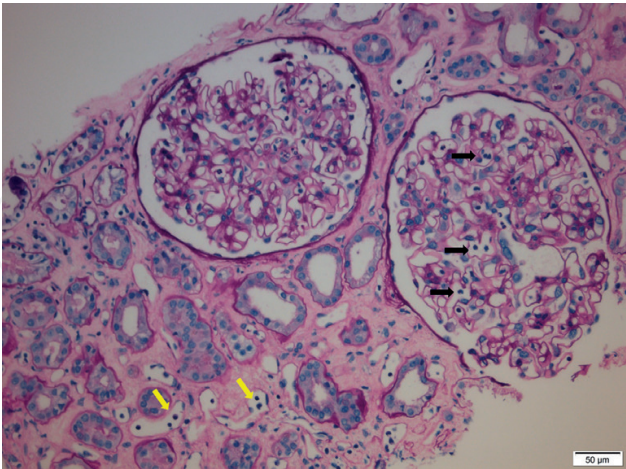

Figure 2. Black arrows show infiltrating polymorphonuclear leukocytes in glomerular capillary loops (glomerulitis) in a patient undergoing acute oliguric antibody mediated rejection. Yellow arrows point to demarginated polymorphonuclear leukocytes in peritubular capillaries (peritubular capillaritis). Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.
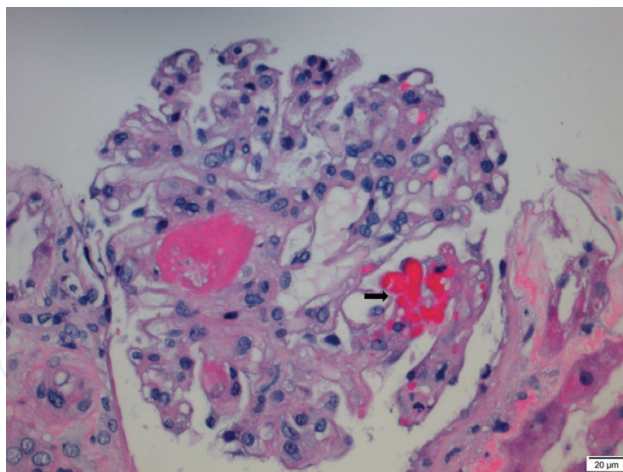
Figure 3. Platelet fibrin thrombi in glomerular capillary loops (black arrow) due to acute thrombotic microangiopathy in a patient with acute antibody mediated rejection. Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.
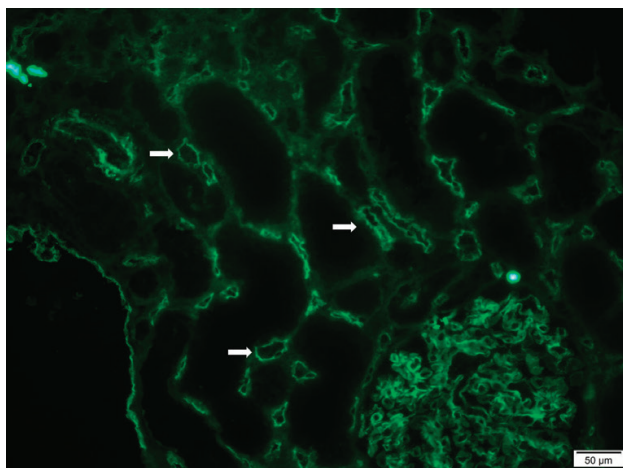

Figure 4. Immunofluorescence staining showing diffuse linear C4d positivity in peritubular capillaries (white arrows). Pathology slides courtesy: Dr. Ming Wu, Department of Pathology, NYU Langone Medical Center.

5. HLA antibody detection assays

5.1. Calculated panel reactive antibody (cPRA)

In the complement-dependent cytotoxicity (CDC) assay, recipient serum is mixed with donor lymphocytes and complement is supplemented. Presence of DSAs is indicated by the appearance of cytotoxicity. The CDC crossmatch was principal technique for detecting DSAs in kidney transplant candidates till the mid-1980s [12, 15]. The panel reactive antibody (PRA) assay, a simple test that predicts the likelihood of a transplant candidate finding a HLA compatible donor (with a negative CDC crossmatch). This test involves treating a panel of cells derived from a pool of individuals representative of the local donor population with recipient serum and noting the percentage of cells that develop cytotoxicity. Therefore, a patient with a high PRA percentage can be predicted to be HLA incompatible with a majority of the potential donors. Consequently, such patients have prolonged wait times for transplantation

and may die waiting for a compatible organ [25]. The emergence of more sensitive solid phase assays that employ HLA antigen-coated beads have revealed a greater prevalence of pre-sensitization to HLA in potential transplant recipients than was previously appreciated. Using these sensitive techniques of HLA antibody detection (in recipient serum) in conjunction with modern HLA typing methods (to determine donor HLA typing), we can now estimate a transplant candidate's calculated panel reactive antibody (cPRA). This is an alternative to standard PRA testing. Transplant centers can designate HLA antigens to which the patient has been sensitized as "unacceptable." The cPRA is computed from HLA antigen frequencies among kidney donors in the United States and represents the percentage of actual organ donors that express any "unacceptable" HLA antigens [25]. One of the key elements of the Organ Procurement and Transplantation Network's (OPTN) new Kidney Allocation System (KAS) introduced in December 2014 is to allocate additional points to waitlisted kidney transplant candidates based on a CPRA sliding scale [26]. The aim of this policy is to increase access to transplantation for sensitized candidates [27]. Soon after the new KAS came into effect, the proportion of transplants being performed in patients with CPRA >98% rose from 2.4 to 13.4% [28]. Therefore, the new KAS appears to be accomplishing the goal of equitable allocation of scarce deceased donor organs to highly sensitized patients who are biologically disadvantaged.

5.2. HLA antibody detection assays

Techniques for detection of HLA antibodies in transplant candidates have evolved considerably since the 1960s when the traditional cell-based complement dependent cytotoxicity (CDC) assay was first developed. Modern flow-cytometry and solid phase assays are far more sensitive than the CDC crossmatch. In the CDC crossmatch, recipient serum is incubated with donor lymphocytes, along with complement. A positive crossmatch is said to occur when DSAs present in the recipient serum bind the corresponding antigens expressed on the surface of donor T or B lymphocytes and activate complement leading to cell lysis [29]. The flow cytometry crossmatch (FCXM) developed in the 1980s was shown to be more sensitive than the CDC crossmatch and can detect lower strength, but clinically significant antibodies that are imperceptible to the CDC crossmatch [30]. In the FCXM, like the CDC crossmatch, donor-specific anti-HLA antibodies, if present in the recipient serum, bind HLA molecules on the surface of donor T or B lymphocytes. The flow cytometry technique detects these HLA bound anti-HLA antibodies by means of secondary fluorescein-labelled antihuman IgG [29]. The advent of solid phase assays for HLA antibody screening in the 1990s has redefined what it means to be sensitized to HLA. There are two varieties of solid phase assays – enzyme linked immunosorbent assay (ELISA) based methods and single antigen bead based methods. Single antigen bead based assays may employ either a flow cytometry platform or a Luminex platform to detect HLA [31]. The Luminex platform employs fluorochrome impregnated microbeads that are coated with specific HLA molecules. Donor-specific anti-HLA antibodies, if present in the recipient serum, bind HLA molecules coated on the surface of the beads. The microbeads are then incubated with phycoerythrin (PE)-labeled anti-human IgG antibodies. The Luminex dual laser system identifies the specificity of the bound anti-HLA antibodies[Ref]. The Luminex based assay has now become the most popular method of HLA antibody detection, both due to its superior sensitivity, as well as its ability to identify the antigenic specificity of the detected HLA antibody [29].

6. Treatments for antibody mediated rejection

The success of desensitization techniques, which enabled transplantation in the setting of pre-existing DSA, represented a breakthrough in the ability to offer transplantation to highly-sensitized patients, who previously had little hope of receiving a transplant [32–34]. But early success was tempered by observations of antibody rebound, early AMR, and suboptimal long-term graft survival [35, 36]. Thus the ability to successfully perform incompatible transplants and optimize long-term outcomes is contingent upon the ability to successfully treat AMR. The first reported efforts at allograft rescue in the setting AMR employed similar techniques as were used for desensitization, namely, techniques that remove or reduce circulating antibody [37]. While removal of antibody remains the cornerstone of AMR therapy, improved understanding of the pathophysiological mechanisms of antibody production and antibody mediated injury have yielded several adjunctive treatment options which are now in various stages of application or new development. For treatment of AMR, no standard protocols exist. Published reports are generally small patient series, and reported techniques vary based on center-specific experience and expertise, as well as center-specific access to emerging therapies [38–40]. Thus, randomized-controlled trial data do not exist for most of these treatments. Meta-analyses are limited by patient heterogeneity, treatment regimen heterogeneity and sample size [39, 41]. Below are brief descriptions of currently existing treatment modalities, though it is important to understand that these are rarely, if ever, used as monotherapies. Most AMR treatment strategies employ a technique for antibody removal in combination with adjunctive agents to minimize antibody production and/or act at the level of the graft to minimize antibody-mediated injury.

6.1. Therapeutic plasma exchange

Though often referred to simply as "plasmapheresis," the procedure utilized in the treatment of AMR is more accurately described as therapeutic plasma exchange (TPE). While plasmapheresis [42] technically describes plasma removal without replacement, TPE entails plasma removal with replacement of a substitute colloid component. A 1–1.5 L plasma volume exchange generally removes approximately 70% of plasma components, including anti-HLA antibodies [43]. For immunoglobulins, the durability this treatment differs dependent upon the tissue compartments in which each immunoglobulin subclass resides. IgM, which resides solely in the intravascular space, and does not significantly repopulate by re-equilibration following TPE, much unlike IgG and IgA. Re-equilibration into the intravascular space generally means that for IgG present in high concentration initially in the serum, multiple TPE treatments are required to make measurable impact on the circulating concentration [44–46]. Rates of antibody removal with TPE, as well as characteristics of rebound following treatment vary with antibody subclass and specificity, and mechanistically this remains poorly understood [47]. TPE was one of the first reported successful strategies for treatment AMR and remains a cornerstone of most current treatment protocols [37, 38, 48, 49].

6.2. Immunoadsorption

Immunoadsorption (IA) is a therapy not available worldwide, but where applied has been used successfully in both desensitization and treatment of AMR. IA has the benefit of specifically removing circulating IgG, while sparing desired plasma protein components such as clotting factors [50]. IA can rapidly and efficiently deplete IgG after a small number of treatments [51–53]. A single randomized controlled trial reporting IA plus pulse steroid compared to pulse steroid alone as treatment for AMR was stopped early after an excessive number of graft failures in the control group [54].

6.3. Intravenous immune globulin (IVIG)

The precise mechanisms of IVIG action both in desensitization and in treatment of AMR remain unclear, but there is evidence to support that it is multimodal [55, 56]. IVIG has been shown to have inhibitory effects on B-cells [57–59], antigen presenting cells [60], and on complement [61, 62]. IVIG in the treatment of AMR has been reported as high-dose therapy (1–2 gm/kg) used without plasma exchange treatments [63–65], and more commonly, as low-dose therapy (100 mg/kg/dose) used in combination with TPE [39, 40, 66].

6.4. Splenectomy

Removal of the spleen, the largest lymphoid organ in the body, is postulated to deplete the plasma cell reservoir, and thereby yield a rapid decrease in circulating HLA antibody in patients with severe acute rejection [67, 68]. Due to its associated morbidity, splenectomy is generally reserved as a rescue therapy [67, 69–71] when all other less invasive interventions are failing and a graft is at risk for imminent loss. Potentially less morbid alternatives to operative splenectomy, including angioembolization or splenic irradiation, may prove to be beneficial in select patient situations [72].

6.5. B-cell and plasma cell targeted medical therapies

Rituximab (Rituxan®, Genentech) is a monoclonal antibody directed against the B-cell CD20 antigen [73]. This recombinant antibody is constructed as a chimeric protein with human IgG_1 constant regions linked to murine anti-human CD20 variable regions [74]. Binding of rituximab to CD20 leads to antibody-dependent complement-mediated cytotoxicity and apoptosis of the bound cell. Rituximab thereby depletes the memory B-cell population and this is hypothesized to, in turn, reduce the plasma cell population and decrease HLA-antibody production [75–77]. Rituximab has been used as an adjunctive therapy in combination with various treatment modalities including: IVIG [78], TPE plus steroid pulse [79, 80], and TPE plus IVIG [66, 81]. While rituximab was perhaps the earliest used adjunctive agent in the treatment of AMR, to date only a single randomized controlled trial of its use has been performed, in which it was compared to placebo in addition to standard therapy (TPE with low dose IVIG). No difference was observed in this underpowered study though there was a trend toward improved outcomes with the addition of rituximab [82].

Whereas the postulated effect of rituximab on antibody-production is indirect, bortezomib (Velcade®, Takeda Oncology) acts directly at the level of the antibody-producing plasma cell. Bortezomib is a proteasomal inhibitor that depletes circulating plasma cells by inducing apoptosis [83–85]. The first reported use bortezomib in transplant recipients was in a small series where graft salvage was attempted in the cases of AMR refractory to therapies including TPE, IVIG, and rituximab [86]. Following bortezomib therapy, circulating DSA strength has been reported to decrease substantially [87], though interestingly, class I and class II DSAs may be not be reduced with equal efficacy [88]. Bortezomib, like rituximab, has been used in combination with TPE, with and without steroids and rituximab [89–92] and is thoroughly reviewed elsewhere [85].

6.6. Complement inhibition

The realization that the tissue injury associated with AMR was, at least in part, mediated by the complement cascade led to the hypothesis that complement inhibition may afford tissue-level protection while TPE or other antibody removing techniques were implemented. The first reported use of the terminal complement inhibitor eculizumab (Soliris®, Alexion Pharmaceuticals) was in a patient with severe accelerated oliguric AMR who was deemed an inappropriate candidate for a rescue splenectomy [93]. With TPE, IVIG, rituximab, and eculizumab, recovery of renal function was achieved. Several reports describe the use of eculizumab as a salvage therapy, either in lieu of or in combination with splenectomy [94–96], but reports of successful salvage are not universal [97]. Eculizumab's mechanism of action of action led to studies of its pre-emptive use in incompatible kidney recipients at high risk for AMR, and while eculizumab may decrease the incidence of AMR [98], it does not prevent it [99].

Additional complement inhibitors have since become available and are being evaluated for their relative efficacy in AMR. C1-esterase inhibitor (C1-INH) is an endogenous protein that is a more proximal inhibitor of the complement cascade and is commercially available as a purified plasma preparation (Berinert®, CSL Behring, and Cinryze®, Shire). A recently reported double-blinded randomized controlled trial of C1-INH as an add-on to standard TPE therapy for AMR suggested a benefit in terms of improved long term renal allograft function in those who received C1-INH [100]. Like eculizumab, C1-INH may have promise in the prevention of AMR in high-risk patients [101], or as a graft protective agent in the setting of severe or treatment refractory AMR [102].

6.7. IL-6 inhibition

IL-6 is a pro-inflammatory cytokine with properties that activate numerous cell lines including B-T- and plasma cells. Tocilizumab (Actemra®, Genentech)is a humanized monoclonal antibody which blocks IL-6 signal transduction by binding and inhibiting the IL-6 receptor [103]. In animal models, IL-6/IL-6R signaling has been found to promote renal injury [104] and may be associated with the injury of acute rejection [105]. In human studies, it may affect a decrease in HLA antibody production [106]. A recent trial of tocilizumab in patients with refractory chronic AMR reported improved long-term graft survival rates in those who received tocilizumab [107].

7. Outcomes and unanswered questions

Generally reported estimates of the incidence of AMR are around 7% for all recipients [108], and may be as high as 50% among recipients of HLA-incompatible grafts [109, 110]. Despite improved abilities to diagnose and treat AMR, it remains an important cause of premature graft loss [111, 112]. Clinically silent AMR identified on biopsy in the setting of normal renal function, if left untreated, is associated with a two-fold increased risk of graft loss [109, 113]. If the AMR is clinically apparent and associated with graft dysfunction, the risk of graft loss can increase to six-fold [109]. Even when recognized and treated promptly, AMR portends recurrent AMR, and ultimately, chronic AMR and transplant glomerulopathy [114, 115].

The pathophysiology of AMR and the molecular mechanisms of antibody-mediated injury have never been better understood, however the fact that such heterogeneity is observed clinically from case to case suggests that much remains yet to be clarified. The spectrum of AMR severity, acuity, and treatability is broad and not easily predictable even when clinical parameters appear relatively constant. While some lines of evidence suggest that any DSA [116, 117] even historical DSA not present at transplant [111], has the potential to be harmful, others have reported clinically silent DSA that, although detectable, has no apparent impact the incidence of rejection or on long-term outcomes [118]. Whether sensitization alone, not just DSA, is an independent risk factor for AMR, is unclear [115]. Whether this variability lies in the DSA specificity, in differential expression of the target HLA molecules on the allograft, or on other factors, remains to be determined. Multiple lines of evidence suggest that complement activating, C1q-binding DSA are associated with greater risks for rejection and for worse outcomes [119–122], compared to non-C1q binding DSAs. The ability to identify and test for the more virulent DSAs may prove to be of benefit in terms of surveillance and directing treatment. There is evidence that class II DSA is associated with worse long-term outcomes [123–125], and poorer responses to treatments [126] compared to class I DSAs. What underlies this difference, remains uncertain. Whether, and how, antigens vary in terms of their immunogenicity and risks for inciting AMR, remains to be determined. Whether any of the available therapies is optimally suited for different DSA patterns or specificities, or AMR phenotypes, also remains to be determined.

Perhaps the most effective means of minimizing the risks of AMR may be in maximizing efforts to prevent it. Experience with HLA-incompatible transplant recipients have demonstrated, both in single-center and multi-center series, that long-term outcomes are inversely correlated with the starting crossmatch strength [19, 20]. Thus careful attention paid to donor selection, and making any effort possible to minimize incompatibility, can pay great dividends in the long-term post-transplant [115]. And while prevention will not always be feasible, the ability to more readily and accurately detect AMR will enable more rapid treatments and improve the chances of their success. Just as new agents are being developed to remove antibody [127], and interrupt the pathways that impart antibody-mediated injury, so too are innovative, increasingly specific, and less invasive procedures for the diagnosis of AMR. The ability to identify AMR, and perhaps even characterize AMR phenotypes based on gene expression profiles in biopsy tissue [128, 129] should allow a clearer determination of AMR severity and ultimately help guide therapy. The identification of serum and/or urinary biomarkers [130–133] should enable better surveillance, earlier diagnosis of AMR, and

prompt treatment to prevent irreversible tissue injury. Ultimately, optimizing the diagnosis and treatment of AMR will lead to greater graft longevity and thus, better utilization of this vastly limited resource.

Conflict of interest

The authors have no conflicts of interest relevant to this publication.

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