Antibody-Mediated Rejection

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Summary: The introduction of both complement 4d (C4d) staining in renal allograft biopsies and sensitive methods to detect anti–human leukocyte antigen antibodies, such as single antigen bead flow assays, into tissue-typing techniques have shown the importance of antibody-mediated alloimmune response in kidney transplantation. The use of these sensitive methods, combined with the increased number of transplants in highly sensitized patients with donor-specific antibodies, or patients receiving desensitization protocols, have increased the awareness and thus the incidence of acute antibody-mediated rejection. Chronic rejection also can be mediated through alloantibodies, and the term *chronic antibody-mediated rejection* recently was proposed. In this review article we summarize the current knowledge of the role of alloantibodies in transplantation, the diagnosis and treatment of acute and chronic antibody-mediated rejection, and their effect on graft function and outcome.
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The detrimental role of alloantibodies in transplantation first was shown by Patel and Terasaki[1] in 1969 when they introduced the complement-dependent cytotoxicity (CDC) cross-match as a simple test, which for the first time allowed the detection of patients presensitized to alloantigen before transplantation. With this technique, embraced by the transplant community and rapidly established as routine practice, hyperacute rejection was almost eliminated because patients with a positive cross-match were excluded from transplantation. Having solved this barrier to successful transplantation, research focused on cellular rejection processes and their mechanisms and treatment during the decades to follow. The tremendous progress in diagnosing cellular mechanisms and in treating their effects led to a further reduction in rejection episodes and resulted in impressive improvement in graft survival rates, for which the acute rejection rate decreased to less than 10% to 15%, and the 1-year graft survival rate increased to more than 90% in most transplant centers. The introduction of complement 4d (C4d) staining in renal allograft biopsies, which allows the visualization of a complement split product bound within the allograft endothelium, marked the belated revival of interest in the humoral mechanisms of graft destruction.[2] This interest has expanded dramatically through the recent addition of sensitive tests to detect anti–human leukocyte antigen (HLA) antibodies, such as anti-human globulin (AHG)-CDC and flow-cytometry (FC) cross-match, along with solid-phase assays (enzyme-linked immunosorbent assay, single antigen bead flow assays).[3] Alloantibodies have now re-emerged as a topic of significant interest in transplantation research. These new technologies have since provided information that has led to a better understanding of the role of antibodies, not only in the early phase after transplantation but also during later stages. An increasing number of transplant centers have started desensitization protocols to abrogate cross-match positivity in patients with donor-specific anti-HLA antibodies (DSAs), or to decrease anti-A or anti-B titers in ABO-incompatible transplant recipients. Despite desensitization protocols, these patients still show higher rates of early acute antibody-mediated

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Table 1. Target Antigens in Antibody-Mediated Rejection

HLA or HLA-related antigens
Major MHC antigens
MHC class I (HLA-A, B, and C)
MHC class II (HLA-DR, DP, and DQ)
Minor MHC antigens
MICA and MICB
Non–HLA-related antigens
Angiotensin II type 1 receptor
Endothelium/monocyte antigens
Perlecan
Collagen types IV and VI
Agrin
Vimentin
Myosin
ABO blood group antigens

rejection (AMR). Not only acute rejection but also chronic rejection can be mediated through alloantibodies, and the term *chronic antibody-mediated rejection* recently was proposed. In this overview, we summarize the current knowledge of the role of alloantibodies in transplantation, the diagnosis and treatment of acute and chronic AMR, and the influence of those antibodies on graft outcomes.

TARGET ANTIGENS

The major histocompatibility complex (MHC) class I or II antigens are the principal targets for alloantibodies in transplantation and are the main focus of our discussion. The minor histocompatibility antigens, a variety of non-HLA antigens, and ABO blood group antigens also may trigger the development of antibodies and are summarized in Table 1. Alloantibodies are now believed to play a crucial role in the pathogenesis of both acute and chronic rejection processes. It has been suggested as "humoral theory" by Terasaki[4] that the engagement of alloantibodies with antigen mediates acute and chronic rejection and leads to a series of graft-destructing events.

HLA- or HLA-Linked Antigens

MHC class I or II antigens

MHC molecules are required to present foreign antigens to the host immune system. Foreign MHC molecules (also known as *HLA molecules*) are the most relevant structures against which DSAs can be formed. In the first step of the allorecognition process, MHC molecules are identified by alloreactive T cells either as intact molecules (direct pathway), or as processed peptides when presented within the groove of host HLA molecules (indirect pathway). This recognition process leads to the activation of B cells and the production of DSAs. MHC molecules are predisposed as targets for sensitization because potential graft recipients can be exposed to them during pregnancy, through blood transfusions, or by previous transplants, and because of the extreme polymorphic structure of these molecules.

Anti-HLA antibodies can be directed against MHC class I (known as A, B, and C antigens) or MHC class II molecules (known as DR, DQ, and DP antigens). Although MHC class I molecules can be found on the surface of all nucleated cells of the body, MHC class II molecules are more limited in distribution and are expressed mainly on the surface of B cells, antigen-presenting cells, and endothelial cells.

MHC class I polypeptide–related sequences A and B

MHC class I polypeptide related sequences A (MICA) and B (MICB) are classified as human minor histocompatibility antigens. Both are members of the highly polymorphic HLA class I genes, which are located in close proximity to the HLA-B locus and encode for cell-surface glycoproteins, expressed on endothelial cells, monocytes, gut epithelium, and fibroblasts, but not lymphocytes.[5] MICA and MICB expression was shown in kidney and pancreas allografts with acute and chronic rejection, and the incidence of immunoglobulin (Ig)M or IgG antibodies to MICA and MICB was higher in the serum of rejected kidney transplant recipients, compared with patients with stable grafts.[6-8] Soluble MICA can be detected in transplant recipients, and its presence in the serum of heart trans-

plant recipients was associated with a reduced incidence of rejection within the first year after transplantation.[9] Soluble MICA was able to downmodulate the NKG2d receptor (a common activating natural killer cell receptor) in vitro and to inhibit its cytotoxic activity.

Non–HLA-Linked Antigens

Findings by Opelz[10] suggested that antigen targets that differ from HLA may trigger the formation of clinically unfavorable antibodies. The long-term outcome in kidney transplants in HLA-identical siblings depended widely on the degree of sensitization before transplantation. Although nonsensitized HLA-identical siblings had a 72% 10-year graft survival rate, recipients with 1% to 50% and more than 50% panel reactive antibodies (PRA) had a 63% and 55% 10-year graft survival rate, respectively.[10] Whether PRA reflects a higher responsiveness or a reaction against non-HLA antigens remains to be elucidated.

Activating IgG autoantibodies directed against the angiotensin II type 1 (AT1) receptor were identified in selected patients presenting with marked hypertension, renal allograft dysfunction, and histologic findings of fibrinoid necrosis.[11] These results suggest that a non-HLA pathway, taking advantage of the AT1 receptor, may contribute to refractory vascular rejection. Whether affected patients will benefit from the removal of AT1-receptor antibodies or from medical treatment with AT1-receptor antagonists remains to be determined. Antibodies to endothelium/monocyte antigens were found to be associated with hyperacute rejection, although this is probably rare.[12] Two previous studies showed higher titers of antiendothelial cell antibodies in failed renal transplant patients owing to chronic rejection compared with patients with stable allograft function.[13,14] In rat models of allograft rejection, antibodies against perlecan and collagen types IV and VI were associated with chronic renal allograft rejection.[15] Agrin, a basement membrane protein, can become an antibody target. Anti-agrin antibodies can be found in about 40% of patients with transplant glomerulopathy.[16] Anticardiolipin antibodies present at the time of transplantation were not associated with an altered transplant outcome. Antibodies against vimentin have been shown to play a role in cardiac transplantation. Their presence is associated with transplant coronary artery disease and reduced long-term allograft survival.[17] Myosin, analogous to vimentin, can serve as a target for the production of autoantibodies. These particular antibodies are associated with inferior cardiac transplant outcome.[18]

ABO Blood Group Antigens

The ABO blood group antigens are carbohydrate moieties on glycolipids that are present on the surface of endothelium and erythrocytes. These antigens are the targets of AMR in patients receiving kidney transplantation from ABO-incompatible donors after receiving desensitization protocols.

METHODS TO DETECT ANTI-HLA ANTIBODIES

CDC methods have formed the basis of anti-HLA antibody detection. Lymphocytes from a single donor or a panel of donors are mixed with sera of the recipient, and complement is added to determine if the recipient has antibodies that bind to donor cells, activate complement and the membrane attack complex, and lead to cell death. The CDC method is a nonspecific test and determines the existence of antidonor antibodies, and, depending on the nature of the cells used in the panel, it may be possible to determine the anti-HLA antibody specificity. The addition of anti-human globulin (AHG) to the cytotoxicity assay increases the sensitivity of the test by binding to antidonor antibody that already is bound to lymphocytes. AHG-CDC is now the standard lymphocytotoxicity assay in most tissue-typing centers. This refinement is important for detecting low-titer antibodies or antibodies directed against antigens present at a low surface density.

The FC cross-match was introduced in 1983 as a more sensitive test. The FC cross-match does not rely on complement fixation, but rather measures the binding of recipient immunoglobulin molecules to donor cells. This bound immunoglobulin is detected by a second anti-immunoglobulin conjugated with a fluorescent dye. FC may detect low titers of either complement-fixing anti-HLA antibodies or non–

complement-fixing anti-HLA antibodies, and also non–HLA-related antibodies. It is a sensitive test but lacks specificity with regard to HLA specificity. The solid-phase assays (enzyme-linked immunosorbent assay, Flow Specific Beads, and FlowPRA) are specific tests to detect anti-HLA antibodies. Flow Specific Beads and FlowPRA are membrane-independent flow cytometric techniques using purified HLA antigens coupled to microparticles.[3,19] These methods can identify anti-HLA antibodies that are missed by CDC methods, and are the most specific and sensitive tests currently available but lack the ability to detect non–HLA-antibodies or define if antibodies do or do not activate complement. A new test based on FlowPRA beads, which allows the selective detection of complement-activating anti-HLA antibodies, was tested recently in presensitized patients and awaits broad validation in the clinical setting.[20,21]

C4d

Feucht et al[22] introduced C4d staining in renal transplantation pathology more than a decade ago and reported that the presence of C4d is an adverse prognostic marker for renal allograft survival. Surprisingly, C4d received little attention in the years to come. The "Rediscovery of C4d" a few years ago led to many studies that now have confirmed the role of C4d as an indicator of AMR. The ability to visualize C4d in the tissues of rejecting renal allografts has refined the diagnostic capabilities for transplant pathology considerably. Previously the detection of antibody-mediated damage in target tissues was uncertain because immunoglobulins are subject to high turnover at the level of graft endothelium, and thus it is difficult to show their presence. Other components of the classic complement cascade also undergo rapid degradation. Complement C4d, the α-2 portion of complement C4, results from the cleavage from C4b during the activation of the classic complement pathway. C4d is bound covalently to the graft tissue and serves as a durable and visible marker of complement activation.

C4d can be shown by 2 different methods. A monoclonal anti-C4d antibody detects C4d on frozen sections by means of immunofluorescence. In contrast, the polyvalent anti-C4d antibody can be used for immunohistochemistry studies, which is of great value for transplant centers that exclusively use paraffin sections for histopathologic analysis of biopsies. Both techniques have proven extremely useful in the clinical settings. The "widespread linear circumferential peritubular capillary staining in cortex or medulla, excluding scar or necrotic areas" is the criteria for positive C4d (C4d+) staining of paraffin sections.[23] Focal staining is defined as less than 50% C4d+ in peritubular capillaries, and the clinical importance of focal C4d+ is not clear.[24] Further studies are required to correlate focal C4d staining with the existence of circulating alloantibodies and graft outcome. Glomerular staining alone, however, is not considered relevant for the diagnosis because frozen sections of normal kidneys show C4d in the glomerular mesangium. However, normal glomeruli do not stain with C4d in paraffin sections and positive staining might be important, especially in chronic rejection. The results of a number of studies investigating C4d have shed light on humoral mechanisms and have led to a more accurate definition of renal allograft rejection. AMR is now accepted in the BANFF classification as an independent entity.[25] In addition, it is now suggested that C4d staining be performed in all renal allograft biopsies.

Although the early studies by Feucht[22] suggested an association between C4d deposits in biopsies, the presensitization status of the recipient, and humoral immunity, Collins et al[26] were able to show a strong correlation between de novo DSAs and C4d in peritubular capillaries. These findings were supported by larger investigations in different patient populations, and more than 90% of patients with C4d+ biopsies had circulating DSAs.[27-29] The negative impact of the presence of C4d on long-term allograft function also has been shown by independent investigators.[26,28,30-32] In a large European multicenter trial using 551 protocol and 377 indication biopsies, diffuse and focal C4d staining was found in 2.0% and 2.4% of protocol and 12.2% and 8.5% of indication biopsies, respectively, and correlated with the morphology of humoral rejection.[33]

The clinical significance of C4d+ biopsies in ABO-incompatible kidney transplantation is not

certain because more than 70% to 80% of protocol biopsies showed positive staining without histopathologic findings of acute rejection and tissue injury.[34-37] These findings suggest the possibility of accommodation in those patients. The existence of C4d without tissue injury raises the possibility of potential inhibitory mechanisms involved at the distal end of the complement cascade after C4d cleavage, such as at the level of C3 or C5 activation. However, this finding is rare in ABO-compatible crossmatch–positive kidney transplant recipients receiving desensitization protocols, so that the majority of C4d+ is associated with findings of tissue injury, suggesting acute AMR.[35,36]

CLINICAL AND PATHOLOGIC CLASSIFICATIONS OF ANTIBODY-MEDIATED REJECTION

Hyperacute Antibody-Mediated Rejection

Clinically hyperacute AMR is a severe syndrome of patients with nonfunctioning kidneys that become cyanotic within minutes or hours after revascularization. The antibody-mediated damage to the graft endothelium leads to intravascular thrombosis and accelerated necrosis of graft tissue, which usually necessitates graft nephrectomy. Histologic findings include margination of platelets and neutrophils; deposition of IgG, but not IgM, in glomerular and peritubular capillaries; thrombosis of the microvasculature; acute tubular injury; and cortical necrosis. Hyperacute rejection has been observed in transplant recipients with pre-existing antibodies against donor ABO and HLA antigens in the early years of renal transplantation.[38,39] Fortunately, since the introduction of the crossmatch technique, hyperacute AMR has become a rare event in transplantation.

Acute Antibody-Mediated Rejection

The role of humoral mechanisms in allograft rejection was not appreciated for a long time until the work by Halloran et al[40] restimulated the interest in noncellular effector mechanisms in the early 1990s. They described a new type of clinical entity: acute allograft rejection associated with the development of de novo anti-HLA antibodies after transplantation. Patients in whom these features occurred carried a particularly poor prognosis for graft survival. In a subsequent report, they added the description of pathologic features observed in those patients with an anti–class I antibody response.[41] Around the same time, Feucht[22] showed that the presence of C4d in peritubular capillaries early after transplantation was associated with an inferior graft survival at 1 year, a finding later confirmed in many other studies.

These findings formed the basis for the new proposal for the diagnosis of acute AMR rejection in kidney allografts by the working group updating the Banff 2001 classification.[25] Clinically acute AMR is associated with organ dysfunction and may occur with or without signs of cellular rejection. Typical features of acute AMR are neutrophils in the peritubular capillaries or glomeruli and fibrinoid necrosis of arteries. It is now accepted that 3 cardinal features are essential for making the diagnosis of acute AMR and these are summarized in Table 2. Patients should have C4d+ and circulating DSAs, along with the findings of graft destruction. Figure 1A shows diffuse C4d staining of peritubular capillaries in a paraffin section of an allograft with acute AMR. Acute AMR is classified into 3 types according to the type of tissue injury. Type I, acute tubular necrosis (ATN)-like, represents a small fraction of patients (<10%), and acute tubular injury with a few tubulointerstitial neutrophil infiltrates are the only morphologic changes (Fig. 1B). Type II mainly involves glomeruli with neutrophils and monocyte infiltration (glomerulitis), and fibrin microthrombi, resembling thrombotic microangiopathy (Fig. 1C). Arterial inflammation with or without fibrinoid changes are the main features of type III acute AMR (Fig. 1D).

Acute AMR may occur any time after transplantation but mostly common occurs early after transplantation in sensitized patients with pretransplant DSAs. The incidence of acute AMR varies in different centers depending on the methods used to define DSAs and the policy for performing transplantation in sensitized patients. Acute AMR is probably very unusual in centers using more sensitive tests to define anti-

Table 2. Classification of Acute Antibody-Mediated Rejection

Morphologic evidence of tissue injury
Accumulation of polymorphonuclear neutrophils and monocytes/macrophages in cortical peritubular capillaries
Glomerulitis with neutrophils and/or monocyte infiltration, glomerular and arteriolar fibrin microthrombi, and severe vasculitis with fibrinoid necrosis, as well as acute tubular injury all have been described as additional pathologic findings
Sixth Banff Conference on Allograft Pathology in 2001 classified AHR into 3 types
Type I: ATN-like
Type II: capillary-glomerulitis, polymorphonuclear, and/or mononuclear leukocytes in peritubular capillaries
Type III: arterial-transmural inflammation/fibrinoid change
C4d deposits as immunopathologic evidence for antibody-mediated action
Serologic evidence of circulating antibodies to donor HLA or to other donor endothelial antigens at the time of biopsy examination

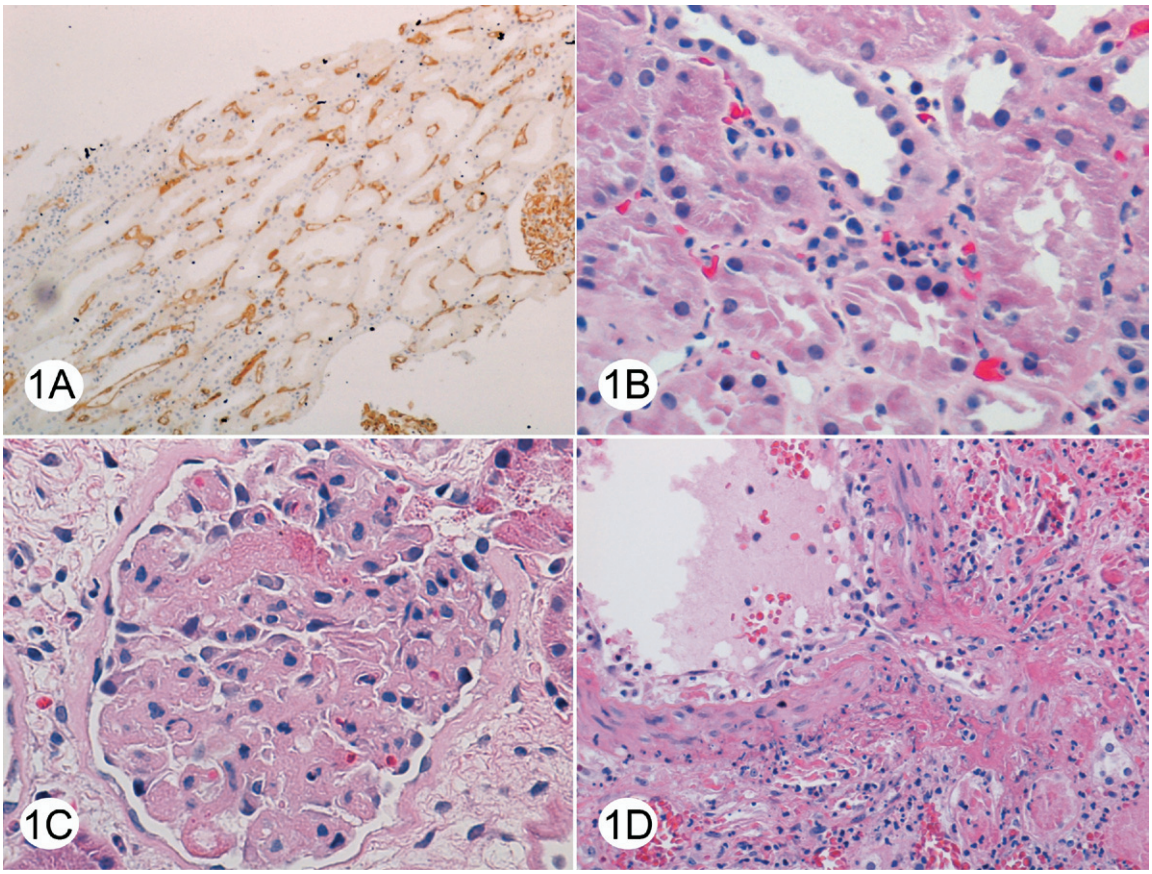

Figure 1. Histologic features of acute AMR. (A) Diffuse C4d distribution along peritubular capillaries (immunoperoxidase method). (B) Type I acute AMR: ATN-like with a few tubulointerstitial neutrophils (hematoxylin and eosin stain). (C) Type II acute AMR: glomerulus with flocculent material in subendothelial and mesangial regions, patchy loss of endothelial cells, and fibrin microthrombi resembling thrombotic microangiopathy (hematoxylin and eosin stain). (D) Type III acute AMR: small artery branch with transmural fibrinoid changes (hematoxylin and eosin stain).

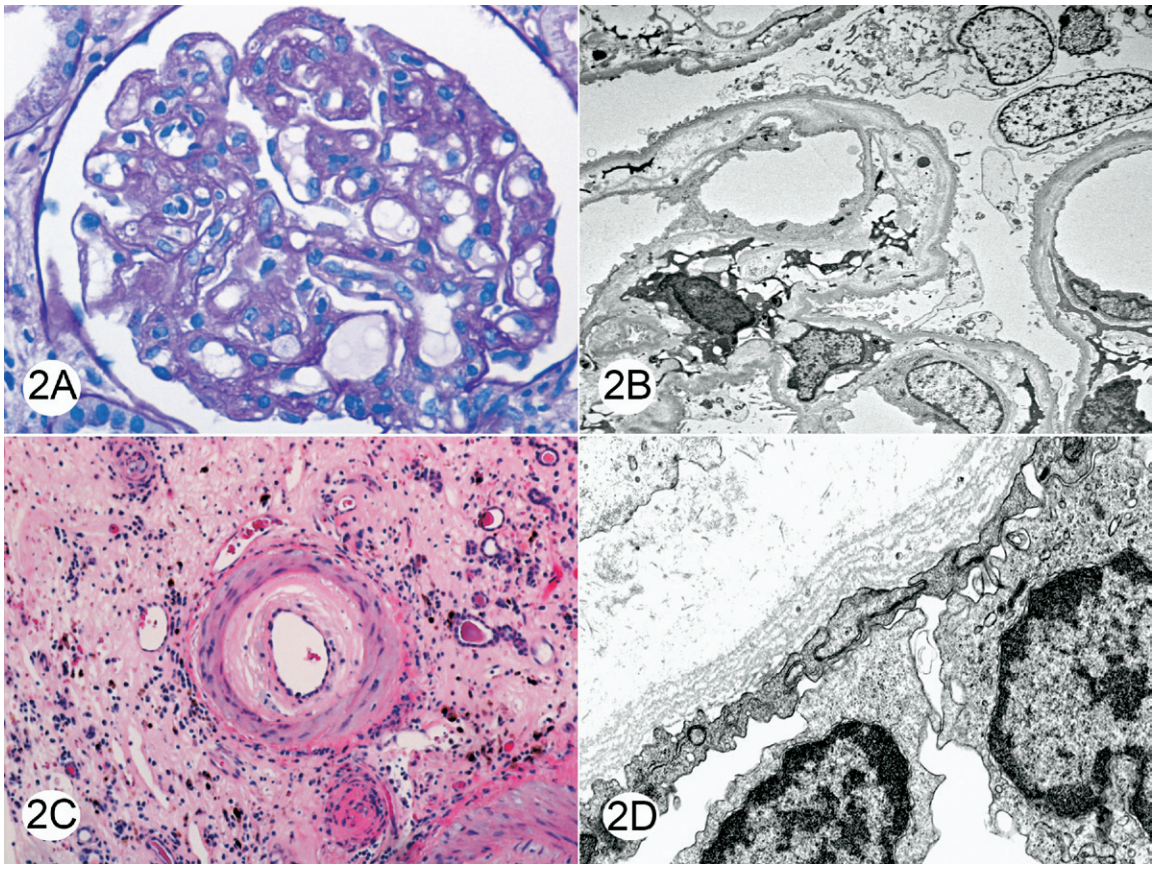

Figure 2. Transplant glomerulopathy. (A) Double capillary wall contours (light microscopy, periodic acid–Schiff stain) secondary to widening of the (B) subendothelial region by subendothelial basement membrane lamella enclosing flocculent material and focal mesangial interposition (electron microscopy). (C) Chronic allograft arteriopathy: fibrous intimal thickening with superimposed lymphoid infiltrates (hematoxylin and eosin stain). (D) Peritubular capillary basement membrane multi-layering (electron microscopy).

HLA antibodies, such as single-antigen bead flow assays, and exclude all patients with DSAs from receiving transplantation. However, it may be more common in centers performing transplantation in DSA-positive patients with desensitization protocols.

Chronic Antibody-Mediated Rejection

Chronic allograft nephropathy (CAN) is a multifactorial process in which immunologic and nonimmunologic factors contribute to the progressive demise of renal graft function. Histopathologic features of CAN are nonspecific, including interstitial fibrosis, tubular atrophy, and fibrous intimal thickening in the arteries, with variable glomerular lesions. Recent studies have suggested that 3 pathologic features, chronic transplant glomerulopathy (TGP), chronic allograft arteriopathy (CAA), and peritubular capillary basement membrane multilayering detected by electron microscopy, may indicate immune-mediated mechanisms. TGP, seen in 10% to 20% of biopsies with CAN, is characterized by reduplication of the glomerular basement membrane, widening of the subendothelial space, interposition of mesangial matrix, and endothelial swelling (Figs. 2A and 2B). CAA is characterized by intimal proliferation of arteries with mononuclear infiltrates (Fig. 2C). Mauiyyedi et al[29] showed that 61% of biopsy samples with chronic rejection (TGP and/or CAA) had C4d+ staining, and 88% of these patients had DSAs. Regele et al[42] reported that 34% of the biopsies taken 1 year after transplantation had C4d deposits in peritubular capillaries, which was found to be associated significantly with TGP and peritubular capillary basement membrane multilayering (Fig. 2D),

Table 3. Proposed Diagnostic Criteria for Chronic Antibody-Mediated Rejection

Serologic: evidence of anti-HLA or other antidonor antibody
Immunopathologic: evidence for antibody action/deposition in tissue (C4d in peritubular capillaries)
Histologic: evidence of chronic injury (3 out of 4 required)
Arterial intimal fibrosis
Duplication of glomerular basement membrane
Laminated peritubular capillary basement membrane
Interstitial fibrosis/tubular atrophy
Clinical: evidence of chronic graft dysfunction

Data from Takemoto et al.[43]

indicating a role for the humoral alloimmune response in the pathogenesis of TGP. In the light of these findings, a consensus meeting at the National Institutes of Health proposed criteria for the diagnosis of chronic antibody-mediated graft injury (Table 3).[43] If the patient has all 4 criteria, the term *chronic rejection* can be used instead of CAN. Despite earlier studies showing a strong relationship between TGP, C4d+, and circulating DSAs, a recent study by Al Aly et al[44] reported that none of the 20 patients with TGP had C4d staining. At our center all clinically indicated transplant kidney biopsies are studied routinely by C4d staining. We recently reviewed the CAN and TGP biopsy samples for C4d staining.[45] Only 2 of 46 biopsies with CAN (4%) and 2 of 20 TGP biopsies (10%) were C4d+. Twenty-three CAN and 16 TGP patients were studied for DSAs, and 6 (26%) and 4 (25%) patients had DSAs, respectively. The low prevalence of C4d+ and DSAs in our TGP patients can be explained by the following factors.

First, patients may have C4d+ in the allograft before TGP develops. Therefore, biopsy procedures performed at later stages of the disease may not show C4d staining. Second, DSAs are absorbed in the allograft, so patients did not have circulating DSAs. Martin et al[46] investigated anti-HLA antibodies by FlowPRA in 20 kidney transplant recipients who underwent transplant nephrectomy. Interestingly, although 42% and 32% had DSAs in their sera at 1 year after transplantation, and at the time of nephrectomy, respectively, 74% of nephrectomy eluates and postnephrectomy serum samples showed DSAs, showing that in some cases anti-HLA antibodies were bound to the allograft and were not detectable in serum.

Third, TGP might develop as a result of mechanisms other than DSAs, such as cellular immunity. We recently undertook immunohistologic analysis of human renal transplant biopsies with CAN with or without TGP for the presence of chemokines and chemokine receptors, and inducible costimulator. We found expression of inducible costimulator and the chemokine receptor CXCR3, both characteristic of activated effector T cells, plus staining for the CXCR3 ligands Mig and IP-10, by intraglomerular and periglomerular leukocytes in biopsies with CAN and TGP, but not CAN alone, suggesting that cellular immunity may underlie TGP versus CAN.[47]

Future prospective studies with serial protocol biopsy procedures and monitoring anti-HLA antibodies may define the development of TGP and chronic AMR.

EFFECTOR MECHANISMS OF ANTIBODY-MEDIATED REJECTION

The endothelial cell is the primary target of alloantibody-mediated injury. The binding of alloantibodies to MHC or ABO antigens expressed on endothelial cells initiates a cascade of events involving the activation of complement that leads to the formation of the membrane attack complex causing cell lysis. Complement further triggers the recruitment of inflammatory cells, activates endothelial cells, and promotes the production of proinflammatory molecules. The endothelial damage is followed by activation of platelets, the formation of thrombi (sometimes resembling throm-

botic microangiopathy), and proliferation of endothelial and smooth muscle cells. Alloantibodies also can mediate antibody-mediated cellular cytotoxicity, in which natural killer cells and macrophages bind to the Fc region of antibodies, promoting lysis of target cells.[48,49] Although B cells play the main role in AMR by secreting antibodies and can be activated in the absence of T cells, especially in response to blood group antigens, it is believed that in responses to HLA antigens they still require help from CD4+ T cells for full activation, differentiation, and antibody production. CD40/CD40-ligand interaction is particularly important in T/B cell help. Although T-cell activation is suppressed by current immunosuppressive medications and the AMR may occur without findings of cellular rejection, it is not clear how anti-HLA antibodies may develop through T-cell–independent mechanisms. Some transplant recipients develop de novo DSAs later after transplantation without previous recognized acute rejection episodes.

The degree of allosensitization is linked closely to long-term graft function. The half-life of the organ is significantly longer in patients with low levels of PRA. A recent report showed that the titer of the alloantibody and the density of HLA molecule expression on endothelial cells are linked directly to the differential aspects of class I signaling.[50,51] Low levels of DSAs support the expression of anti-apoptotic proteins, whereas high concentrations of anti– class I antibodies favor cell proliferation. The importance of antibody titer on favorable versus detrimental effects for the allograft remains to be elucidated further, as do other protective mechanisms contributing to accommodation (eg, the ability of a graft to function despite the presence of antibodies).

TREATMENT OF ANTIBODY-MEDIATED REJECTION

Therapeutic strategies for acute AMR include combinations of the following: (1) removal of antibodies by plasmapheresis (PP) or immunoadsorption (IA); (2) prevention of further alloantibody synthesis by intravenous immunoglobulins (IVIG) or rituximab (anti-CD20); (3) inhibition of B-cell proliferation, and T-cell activity to inhibit T-cell– dependent B-cell immune responses by current immunosuppressive medications including mycophenolate mofetil, steroids, and calcineurin inhibitors; and (4) splenectomy in severe rejection episodes resistant to the first 3 treatment approaches.

Table 4 summarizes the studies about the treatment of acute AMR that used a combination of different treatment approaches.[27,52-64] We included the studies showing the existence of acute AMR by circulating DSAs, positive cross-match, or C4d staining, and reported at least 3 patients. However, all studies other than the last study in Table 4 by Bohmig et al[64] were uncontrolled case series. The low incidence of AMR makes single-center, prospective, and controlled studies difficult, and multicenter trials are required to compare the effectiveness of different treatment modalities. PP or IA are effective in removing the circulating alloantibody load and are applied successfully in the treatment of AMR. A randomized prospective controlled study comparing IA with tacrolimus and mycophenolate mofetil were terminated at the first interim analysis as a result of significant outcome difference in IA-treated patients (80% vs 20%).[64] Both PP and IA also are used as a pre-emptive strategy in desensitization protocols to abrogate cross-match positivity and to prevent the development of AMR in highly sensitized patients with DSAs.

IVIG preparations have immunomodulatory properties and have been used in the treatment of autoimmune and inflammatory disease. IVIG has been used in the field of transplantation since the 1990s, after in vitro studies showed the inhibition of anti-HLA lymphocytotoxicity of sera from highly sensitized patients, and later in vivo studies that showed decreased titers of anti-HLA antibodies.[65,66] IVIG currently is used in desensitization protocols of cross-match–positive or ABO-incompatible kidney transplant recipients.[67] There are many proposed mechanisms of IVIG involving different parts of the immune response, including inhibition of the activation and effector functions of complement, cytokine cascades, T- and B-lymphocyte functions, and modulation of dendritic cells. Anti-idiotypic antibodies binding to anti-HLA antibodies might be the immediate mechanism of IVIG, but the immunomodulatory effects of IVIG treatment persist well beyond its half-life,

Table 4. Treatment of Acute Antibody-Mediated Rejection

Study	Number of Patients Treated	Therapy	Success Rate
Persson et al,[60] 1995	12	IA, IVIG	50%
Pretagostini et al,[61] 1996	23	IA, ALS/OKT3	70%
Hickstein et al,[53] 1998	11	IA	64%
Pascual et al,[59] 1998	5	PP, IVIG	100%
Jordan et al,[54] 1998	7	IVIG, OKT3/ATG	100%
Montgomery et al,[58] 2000	3	PP, IVIG	100%
Bohmig et al,[27] 2001	10	IA	90%
Abraham et al,[52] 2003	18	PP	78%
Koller et al,[55] 2004	3	IA, PP	100%
Shah et al,[62] 2004	7	PP, ATG	86%
White et al,[63] 2004	9	PP, IVIG	89%
Min et al,[57] 2005	6	IA	100%
Lehrich et al,[56] 2005	23	PP, IVIG, ATG/OKT3	94%
Bohmig et al,[64] 2006	5	IA	80%
	5	Tacrolimus/MMF	20%

ALS, antilymphocyte serum; MMF, mycophenolate mofetil.

indicating ongoing active inhibitory mechanisms. IVIG interacts with Fcγ receptor IIB, which is a negative signaling receptor on B cells and inhibits the expression of CD19 on activated B cells.[68]

Montgomery et al[58] reported 7 kidney transplant recipients with AMR who were treated with PP and low-dose IVIG (100 mg/kg). The number of PP sessions varied from 2 to 31. All patients responded to treatment. A retrospective analysis of 22 patients with AMR who were treated with IVIG and PP were reported in 2 consecutive reports.[56,69] AMR was confirmed with C4d+ staining and/or DSAs. Three to 6 sessions of PP were used with 5% human albumin replacement. IVIG was given at 2.0 g/kg after the last PP session. The 2-year graft survival rate was 78% in AMR patients, which is lower than in patients with acute cellular rejection (85%), but the difference was not statistically significant. A similar protocol using 4 to 6 sessions of PP, followed by IVIG (500 mg/kg for the first 3 days, followed by 250 mg/kg for the last 2 treatments) successfully treated 8 of 9 patients with AMR.[63] Jordan et al[70] treated 18 C4d+ AMR with high-dose IVIG (2 g/kg), with or without PP, in patients who received a desensitization protocol with high-dose IVIG to abrogate cross-match positivity. Thirteen patients (72%) responded to treatment.

We have used IVIG and Thymoglobulin (Genzyme, Cambridge, MA) induction treatment in CDC B-cell and/or flow cytometry T- and/or B-cell cross-match kidney transplant recipients.[71-73] Twenty patients had pretransplant DSAs anti-HLA antibodies by Flow Beads (Luminex, Austin, TX). Four patients had class I, 4 patients had class II, and 12 patients had both class I and II DSAs by Flow Beads. Posttransplant follow-up evaluation of these antibodies at 6 months to 1 year after transplantation showed that 7 patients lost their DSAs.

Rituximab, a chimeric murine/human monoclonal antibody, binds to CD20 on pre-B and mature B lymphocytes. It has been approved for the treatment of refractory or relapsed B-cell lymphomas. Several mechanisms for the elimination of B cells by rituximab have been proposed, including CDC, antibody-dependent cellular cytotoxicity, and stimulation of apoptotic pathways.[74] The approved dose of rituximab for the treatment of lymphoma is 375 mg/m^2 as an intravenous infusion for 4 consecutive weeks. Rituximab can be detected for months and B-cell recovery takes 6 to 12 months after the completion of treatment. Rituximab has

been used off label in transplant patients for the following: (1) treatment of posttransplant lymphoproliferative disease; (2) treatment of AMR; (3) to decrease PRA levels in highly sensitized patients awaiting kidney transplantation; and (4) as part of desensitization protocols in cross-match–positive or ABO-incompatible kidney transplantation.

Becker et al[75] used rituximab in 27 patients for refractory rejection along with additional steroids (24 patients) and antithymocyte globulin (22 patients). Twenty-four patients responded to treatment. However, it was not clear if those patients had AMR or refractory cellular rejection because of the lack of information about C4d staining and DSAs. We treated 7 AMR patients, diagnosed with C4d+ staining and DSAs, with IVIG, PP, and rituximab. Five patients responded to treatment, but 2 patients lost their allografts. Plasma cells primarily produce antibodies and do not express CD20, which raises the question of the complete effectiveness of rituximab treatment in the management of AMR. There are no clinical prospective studies on the use of rituximab in rejection or desensitization, and the exact dose and the frequency of rituximab treatment for transplant recipients in comparison with lymphoma patients are not certain.

Polyclonal rabbit antithymocyte globulin (ATG) contains antibodies against activated B-cell and plasma cell surface antigens. Most patients with AMR require pulse steroids and/or antilymphocyte agents in addition to B-cell–directed treatment to suppress T-cell–dependent B-cell responses and/or ongoing cell-mediated rejection. In 1 study, 7 AMR kidney transplant patients were treated with rat ATG and PP, and 6 patients responded to treatment.[62]

Splenectomy removes a major source of lymphocytes, including antibody-secreting B cells, and may be a last option in refractory AMR that does not respond to treatments discussed earlier. However, it has not been well studied and long-term outcomes can be compromised because of the increased risk of sepsis.

The treatment for chronic AMR is unknown. We recently reported a patient with CAN, C4d+ staining on biopsy specimen, and de novo DSAs. She was treated with IVIG and her creatinine level returned to her baseline level a month after the last IVIG treatment and repeat measurements of DSAs were negative.[76] Prospective studies involving large numbers of chronic AMR patients are required to investigate the effects of IVIG.

GRAFT OUTCOME IN ANTIBODY-MEDIATED REJECTION

The clinical significance of AMR in long-term allograft survival has not been reported in detail. Sensitized patients with pretransplant DSAs and/or cross-match positivity have increased acute and chronic rejection and decreased graft survival. Karpinski et al[77] reported that 6 of 18 T-cell FC cross-match–positive patients had early graft loss, with histopathologic findings of AMR in 5 of 6 patients. The 12 patients with positive T-cell FC cross-match who maintained graft function experienced more adverse posttransplant events, including more early, steroid-resistant, and recurrent rejections. Furthermore, in a subgroup of patients undergoing protocol biopsy examinations, those with a positive T-cell FC cross-match showed more subclinical rejection. Positive FC cross-match also was shown to be associated with the development of chronic rejection. Gebel et al[78] reviewed previous studies that investigated the effect of positive FC cross-match results on allograft survival and reported that 20% of primary grafts and 60% of regrafts were lost within 3 months if the FC cross-match was positive, compared with only 5% and 15%, respectively, in FC cross-match negatives. Bray et al[3] showed 100% one-year graft survival in patients with pretransplant negative FC cross-match and negative FlowPRAs, and also in those with positive FC cross-match but 0% FlowPRA, and therefore no DSAs. However, the 1-year graft survival was only 40% in patients with both FC cross-match positivity and DSAs by FlowPRA.

We recently reviewed the effect of de novo DSAs on allograft outcome.[79] The frequency of anti-HLA antibodies detected after kidney transplantation is extremely variable, ranging between 1.6% and 60%. Most studies have shown a significant relationship between the development of de novo anti-HLA antibodies and acute rejection episodes, and patients with alloanti-

bodies showed lower graft survival, poorer allograft function, and more proteinuria.

CONCLUSIONS

The introduction of both C4d staining in renal allograft biopsies and sensitive methods to detect anti-HLA antibodies, such as single antigen bead flow assays, into tissue typing techniques helped to define the diagnosis of antibody-mediated rejection. The use of sensitive and specific solid-phase assays to identify the anti-HLA antibodies before transplantation is important to determine sensitized patients, and should be the standard test before transplantation. All transplant kidney biopsies should be stained with C4d and patients undergoing a biopsy procedure should be tested for circulating DSAs to determine the antibody-mediated mechanisms in allograft failure. The treatment of AMR involves the combination of different treatment modalities involving PP, IA, IVIG, rituximab, and splenectomy. Future prospective and randomized studies are required to define the most appropriate treatment for AMR.

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