Antibodies against antigens that are expressed on renal and other solid-organ allografts have long been known to be a potential barrier to successful transplantation. However, it is only in the past 15 years that we have started to understand the full spectrum of antibody effects on the allograft, which include hyperacute rejection, early and late acute rejection, and chronic rejection. This increased understanding is based in large part on pathologic observations and has contributed to the development of effective treatments for acute antibody-mediated rejection (AMR), which once was associated with almost certain graft loss. Experience with these treatments has allowed in turn for successful renal transplantation across ABO blood groups and in recipients with preexisting antibodies to donor MHC antigens, thereby providing an avenue to transplantation for highly sensitized patients who would otherwise have to remain dialysis dependent. Finally, we are just beginning to understand the phenomenon of graft accommodation, which allows for long-term function of allografts despite the presence of antidonor antibodies.

In this article, we review the various types of AMR in renal allografts, including the special case of grafts from ABO-incompatible donors. As pathologists, our primary focus is on pathologic aspects of these processes, including diagnosis of AMR in renal allograft biopsies, although some pertinent points regarding therapy and outcomes also are included.

Hyperacute Rejection
Reports from the 1960s identified cases of hyperacute rejection (HAR) of cadaveric kidneys that were transplanted into recipients with preexisting antibodies against donor ABO (1,2) and MHC (3) antigens. In the latter study, five of seven cases of HAR occurred in recipients who had received one or more previous renal transplants (3). A typical clinical scenario described in cases of HAR is the development of cyanosis within the graft minutes to a few hours after completion of the vascular anastomosis, with subsequent arteriographic evidence of progressive cortical necrosis, necessitating removal of the graft (3). Histologically, the major findings that are associated with HAR are neutrophil and platelet margination in glomerular and peritubular capillaries; red blood cell stasis, fibrin deposition, and thrombosis within the microvasculature with sparing of larger blood vessels (at least early in the process); and acute tubular injury and variable degrees of cortical necrosis, depending on the interval between transplantation and biopsy or removal of the graft (2,3). Immunofluorescence (IF) studies demonstrated IgG (but not IgM) in glomerular and peritubular capillaries (3). Kincaid-Smith et al. (4) found that the presence of four or more neutrophils in most glomeruli on a biopsy taken 20 to 30 min after grafting was predictive of a high likelihood of preexisting antidonor antibodies and development of HAR.

Fortunately, with the serologic technologies and expertise now available, HAR has become an extremely rare occurrence. However, the histologic changes described above for HAR qualitatively resemble those seen in acute AMR, as is discussed below.

Acute AMR
In the early 1990s, Halloran et al. (5,6) described an atypical or accelerated form of acute renal allograft rejection that occurred
in some presensitized recipients who had inadvertently received a transplant despite a positive T cell cross-match or who had developed antibody against donor HLA class I antigens after transplantation. This form of acute rejection was associated with rapid deterioration of graft function and a high incidence of graft loss but was clearly distinct from HAR in that its onset was days to weeks after transplantation in a previously well-functioning graft (5). However, Halloran et al. (5,6) clearly recognized that the pathologic features of this form of acute rejection, which we now recognize as acute AMR, more closely resembled those of HAR than those of acute cellular rejection, with the central feature being endothelial injury within the microvasculature. Unlike previously reported experience with HAR (3), however, antibody could be demonstrated within the microvasculature by IF in only a minority of the cases (5,6).

The histopathologic spectrum of early (mean 11 days after transplantation) acute rejection episodes associated with the presence of donor-specific anti-HLA antibodies (DSA) was reported by Trpkov et al. (7) several years later. Features that were found to best distinguish acute rejection in the presence of DSA from acute rejection in the absence of DSA were (in order of greatest significance) arterial fibrinoid necrosis, cortical infarction, neutrophil margination in peritubular (but not glomerular) capillaries, acute glomerulitis (and, more specific, glomerular monocyte/macrophage but not T cell infiltration) (8), and glomerular and vascular fibrin thrombi. Notably, moderate or severe lymphocytic tubulitis and mild intimal arteritis were seen significantly more often in acute rejection in the absence of DSA than in the presence of such antibodies (7). Although these features have proved helpful in identifying cases of AMR on renal allograft biopsies, none is specific. For example, endothelial injury within the microvasculature with associated neutrophil margination and/or thrombotic microangiopathy may be a manifestation of acute calcineurin inhibitor toxicity (9,10), and acute glomerulitis often is seen in acute cellular rejection with intimal arteritis (11). Importantly, Trpkov et al. (7) found that IF staining for Ig and C3 as well as electron microscopic findings were similar in DSA-positive and DSA-negative acute rejection and therefore not of value for diagnosis of acute AMR.

A major breakthrough in the diagnosis of AMR has been the use of immunohistologic (IF or immunoperoxidase) staining for C4d, a cleavage product of the complement component C4 that becomes covalently bound to tissues at the site of C4 activation. Feucht et al. (12,13) first demonstrated C4d in peritubular capillaries (PTC) of biopsies of renal allografts with early dysfunction. C4d staining was correlated with an increased risk for graft loss and also was significantly associated with recipient history of pretransplantation sensitization and high levels of panel-reactive antibodies (12,13). Others have since extended these findings. Collins et al. (14) found PTC C4d staining in each of 10 renal allograft biopsies that showed acute AMR, characterized by neutrophil margination in PTC and demonstration of circulating DSA at the time of biopsy, and in none of 14 cases of DSA-negative acute cellular rejection (although four of the latter biopsies showed neutrophils in PTC, indicating the lack of specificity of this finding for AMR). Mauiyyedi et al. (15), from the same research group, found that 18 of 20 cases of C4d-positive acute rejection were associated with the presence of DSA, as opposed to just one of 47 cases of C4d-negative acute rejection.

Bohmig et al. (16) found a similar high specificity (93%) of PTC C4d for acute rejection episodes associated with circulating DSA, although the sensitivity of C4d staining in this study was much lower than in the studies of Collins et al. (14) and Mauiyyedi et al. (15). Perhaps the reason for this relates to the staining method used to detect C4d deposition. Collins et al. (14) and Mauiyyedi et al. (15) performed indirect IF studies on frozen sections of unfixed tissue, using a commercially available mAb against human C4d. By contrast, Bohmig et al. (16) performed immunoperoxidase staining on sections of formalin-fixed, paraffin-embedded tissue using a polyclonal, peptide-specific anti-C4d antibody developed by their research group (17). Although the latter method may be less sensitive than the indirect IF method, it does have the advantage of allowing the entire biopsy specimen to be included in the paraffin block for histologic examination and allowing for the examination of archived tissue. However, it should be noted that PTC C4d staining is observed in the renal medulla as well as in the cortex (14), and we believe that for laboratories that perform IF staining for C4d on frozen sections, freezing a sample of the medulla for IF may in fact be preferable in cases in which there is no clinical evidence to suggest a recurrent or de novo glomerular disease, in that it allows all of the cortical tissue present (including arteries) to be embedded in paraffin for optimal histologic evaluation.

Two independent (albeit with some overlap in participants) working groups of renal pathologists, nephrologists, and transplant surgeons have recommended sets of criteria for the diagnosis of acute AMR on renal allograft biopsies (18,19). Features that were agreed on by these groups, summarized in Table 1, are the need for morphologic (Figure 1), immunopathologic (Figure 2), and serologic features of AMR. There is a disagreement between the working groups with respect to a requirement for clinical evidence of graft dysfunction, which is included as a diagnostic criterion by the National Institutes of Health working group (18) but not by the Banff working group (19). The latter group thus acknowledges the distinct possibility of subclinical acute AMR. Indeed, there is evidence, based on detection of C4d staining and leukocyte (neutrophil and monocytic) margination in PTC, for subclinical acute AMR in protocol biopsies of stably functioning grafts (20,21).

In patients who undergo one or more renal allograft biopsies for graft dysfunction, a number of studies have shown that 35 to 54% will have at least one biopsy with PTC C4d (13,17,22,23). In the majority of these studies, one or more C4d-positive biopsies were significantly associated with an increased rate of graft loss (13,17,22). This association between PTC C4d and graft loss was independent of the presence or absence (17) or histologic type (purely interstitial versus vascular) (22) of concurrent acute cellular rejection. In contrast, Nickeleit et al. (23) found no significant difference in graft survival in patients with or without a C4d-positive biopsy on retrospective staining of banked frozen tissue. They noted, however, that rejection epi-
sodes that were found retrospectively to be C4d positive had been treated aggressively with antilymphocytic preparations.

Findings from two recent studies suggest that additional staining of renal allograft biopsies for C3d, a cleavage product of complement component C3 that also binds covalently to tissue, may be useful in identifying particularly aggressive cases of acute AMR (24,25). Sund et al. (24), in 37 protocol biopsies that were taken a median of 7 d after transplantation, found C4d in 11, with concurrent C3d deposition in three. Graft loss within 2 months (mo) occurred in two of the C3d-positive cases, whereas only one C3d-negative graft was lost (at 15 mo). Each C3d-positive biopsy also showed strong (3+/H11001;0t o3+/H11001) scale) PTC C4d staining, whereas six of the C4d-positive, C3d-negative biopsies showed ≤1+ C4d staining. Furthermore, all three C3d-positive biopsies showed prominent neutrophil margination in PTC; by contrast, such margination was limited in five of the C3d-negative biopsies and absent in one. Kuypers et al. (25) compared outcomes of 30 renal allograft recipients who developed acute rejection with PTC C3d during the first year after transplantation with those of 82 patients who developed C3d-negative acute rejection and found that C3d-positive rejection was associated with a higher rate of graft loss (23 versus 7%) at 1 year.

Pathologic evidence indicates that acute AMR, although often considered a potential cause of early graft dysfunction, may occur de novo at any time after transplantation. In a retrospective study of sequential biopsies, Regele et al. (26) found that in many cases, PTC C4d deposits first were detected several years after transplantation, with previous biopsies that were C4d negative, and in one patient, C4d deposition first was noted 17 years after transplantation! Furthermore, eight of 21 patients with C4d-positive biopsies were C4d negative on follow-up biopsies, even though im-

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Table 1. Diagnostic criteria for acute AMR in renal allograft biopsies

1. Morphologic evidence
   - neutrophils and/or monocytes/macrophages in PTC and/or glomeruli (acute glomerulitis)
   - arterial fibrinoid necrosis
   - thrombi in glomerular capillaries, arterioles, and/or small arteries
   - acute tubular injury
2. Immunohistologic evidence
   - C4d in PTC
   - Ig and/or complement in arterial fibrinoid necrosis
3. Serologic evidence
   - circulating antibodies to donor HLA or other specific antidonor antibodies at the time of biopsy

*At least one finding in each of the three categories must be present for a biopsy to be diagnostic of acute AMR. Biopsies that meet two of the three criteria may be regarded as suspicious for acute AMR on the basis of criteria established by National Institutes of Health (18) and Banff (19) working groups; the National Institutes of Health criteria also require clinical evidence of graft dysfunction (see text). AMR, antibody-mediated rejection; PTC, peritubular capillaries.

*A small fraction (probably ≤10%) of cases of acute AMR will show acute tubular injury as the only significant morphologic change (15).

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Figure 1. Histologic expressions of acute antibody-mediated rejection (AMR). (A) Margination of neutrophils in peritubular capillaries. (B) Arterial fibrinoid necrosis. (C) Thrombotic microangiopathy. The glomerulus contains intracapillary fibrin thrombi and also shows red blood cell stasis, suggesting poor perfusion. (D) Acute tubular injury. The proximal tubules appear dilated with flattening of the epithelium. Glomeruli appear unremarkable, and there is no significant interstitial inflammation or leukocyte margination in peritubular capillaries. Magnifications: ×400 in A and C; ×200 in B; ×100 in D (all hematoxylin and eosin [H&E] stain).

Figure 2. Immunohistologic findings in acute AMR. (A) C4d staining in peritubular capillaries. Staining is strong, linear, and diffuse (indirect immunofluorescence with mouse monoclonal anti-human C4d, followed by FITC-conjugated anti-mouse IgG). (B) IgG deposition in the wall of a small artery showing early fibrinoid necrosis (direct immunofluorescence with FITC-conjugated anti-human IgG). Magnifications: ×400 in A; ×200 in B.
munosuppressive therapy was modified after the initial biopsy in only one of these cases. In our own renal biopsy practice, we have seen a number of late (>1 yr after transplantation) C4d-positive acute rejection episodes, with histologic features of acute AMR (usually concurrent with acute cellular rejection) and documented DSA at the time of biopsy.

Improvements in diagnosis have led to more frequent recognition of acute AMR and have helped foster the development of new treatment protocols that have greatly improved graft survival in patients with acute AMR. The most commonly used protocols involve high-dose intravenous Ig, plasmapheresis, immunoadsorption, or a combination of low-dose intravenous Ig and plasmapheresis, together with more traditional antirejection agents, particularly when there is concurrent acute cellular rejection (27–31). Rates of reversal of AMR of approximately 90% have been reported using such protocols (28,30); by contrast, with traditional immunosuppression alone, reversal rates for AMR were <50%, with 1-yr graft survival rates in patients who developed AMR between 62 and 16% (6,32,33).

Role of Antibody in Chronic Rejection

In retrospect, the first evidence that antibodies may be involved in the pathogenesis of chronic rejection comes from the early study of HAR of Williams et al. (3). In this study, one graft with HAR regained partial function. The patient died 3 mo later, and at autopsy, the graft showed obliterator vascular changes that were typical of chronic rejection. More than 3 decades later, Mauiyyedi et al. (34) reported that 23 (61%) of 38 renal allograft biopsies that showed histologic features of chronic rejection, namely glomerular basement membrane duplication (chronic transplant glomerulopathy) and/or arterial intimal fibrosis with intimal mononuclear cell infiltration, showed PTC C4d staining. Of 25 cases for which serum specimens from the day of biopsy were available for testing, 15 (88%) of 17 cases of C4d-positive chronic rejection but none of eight C4d-negative cases with similar histologic changes were associated with DSA. By contrast, only one of 21 biopsies that showed chronic cyclosporine nephrotoxicity was C4d positive (34). Regele et al. (26) detected PTC C4d in 73 (34%) of 213 biopsies that were performed >1 yr after transplantation because of graft dysfunction. C4d deposition was found to be significantly associated with changes of chronic rejection, namely chronic transplant glomerulopathy and PTC basement membrane multilayering, as well as with margination of mononuclear leukocytes in PTC. Furthermore, in biopsies with normal glomerular morphology, PTC C4d deposition was found to be associated with development of chronic transplant glomerulopathy in follow-up biopsies (26). These findings strongly suggest that humoral immunity contributes to the development of morphologic changes that are believed to signify chronic rejection.

The National Institutes of Health working group (18) suggested diagnostic criteria for chronic AMR in renal allografts, which like criteria for acute AMR include immunopathologic (C4d in PTC), serologic (anti-HLA or other antidonor antibody), and histologic components. The last requires three of the following four lesions to be present: Arterial intimal fibrosis, interstitial fibrosis/tubular atrophy, duplication of the glomerular basement membrane, and lamination of PTC basement membranes, although the first two of these are nonspecific findings and require comparison with a perioperative biopsy to ensure that these changes developed after transplantation. Furthermore, accurate demonstration of PTC basement membrane lamination requires electron microscopy, which is not routinely performed on renal allograft biopsies at most centers.

C4d in ABO-Incompatible Allografts: Rejection or Accommodation?

Protocols that are similar to those used for treating AMR after transplantation also have proved useful in eliminating antibodies to donor HLA and ABO blood group antigens that existed before transplantation in sensitized patients, thereby allowing for successful transplantation of kidneys across these previously unscaleable immunologic barriers (31,35–40). In biopsies of initially HLA-incompatible renal allografts, including biopsies that were performed for graft dysfunction and protocol biopsies of stably functioning grafts, we have found a strong association between PTC C4d staining and neutrophil margination, similar to findings in cases with a negative pretransplantation cross-match. Twenty-seven (26%) of 103 protocol biopsies of initially HLA-incompatible grafts and 78 (60%) of 129 biopsies of such grafts that were performed for dysfunction were C4d positive. By contrast, in 55 protocol biopsies of ABO-incompatible allografts with stable function, we found PTC C4d staining in 44 (80%), with strong and diffuse staining in the majority of these. Furthermore, there was no significant correlation between PTC C4d staining and neutrophil margination (unpublished observations). Other studies in biopsies of ABO-incompatible grafts have yielded variable results. Onitsuka et al. (41) found PTC C4d in 15 of 19 biopsies (diffuse staining in 10) of ABO-incompatible grafts that were performed for graft dysfunction during the first 3 mo after transplantation. However, all 19 biopsies showed neutrophil margination in PTC and/or thrombotic microangiopathy, with these changes being most severe in biopsies that showed diffuse PTC C4d, including those of three grafts that subsequently were lost to AMR (41). Fidler et al. (42) found PTC C4d in each of nine biopsies of ABO-incompatible grafts with clinical dysfunction and glomerular thrombi and/or PTC neutrophil margination and also found PTC C4d in 15 of 16 biopsies of ABO-incompatible grafts with stable renal function. Of these 15 C4d-positive biopsies, 10 showed focal glomerular thrombi, although only two of these and no others showed PTC neutrophil margination. Finally, Kanetsuna et al. (43) found PTC C4d staining in eight of 14 biopsies of ABO-incompatible grafts that were performed 1-h after transplantation. This C4d likely reflects the presence of anti–blood group antibody, because we previously observed PTC C4d in only two of 47 1-h postperfusion biopsies, both positives being in recipients who had a positive flow cytometric cross-match for anti-HLA DSA at the time of transplantation and subsequently developed AMR (44). In the study of Kanetsuna et al. (43), four of 14 patients, three with C4d on their 1-h postperfusion biopsy and one without, developed AMR during the first 30 d after transplantation. Together, the findings in
these studies suggest that in biopsies of ABO-incompatible grafts that are performed for early dysfunction, PTC C4d may be associated with AMR and graft injury, whereas in stably functioning grafts, PTC C4d staining is frequently not associated with AMR and may, as suggested by Platt (45) and Kanetsuna et al. (43), reflect graft accommodation.

The phenomenon of accommodation, in which the graft acquires resistance to humoral injury and continues to function well despite the continued presence of antibody against a target antigen expressed on graft endothelium, is well documented in ABO-incompatible kidney transplants (45,46). It has been proposed that in these cases, complement regulatory proteins and/or other control mechanisms may interrupt the complement cascade distal to the generation of C4d, so the persistence of C4d on graft endothelium represents a marker for the arrest of the complement cascade rather than ongoing complement-mediated graft injury (47). Whether accommodation also may occur in instances when there is circulating antibody against HLA antigens expressed on the graft remains to be determined, although our studies of PTC C4d staining and neutrophil margination in initially HLA-incompatible renal allografts noted above suggest that if this does occur, then it is considerably less common than with ABO-incompatible transplants. As suggested by Platt (45), careful histologic and immunohistologic study may help to answer this question and address any potential role of complement in the accommodation process.

References

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