Antibody-Mediated Renal Allograft Rejection: Diagnosis and Pathogenesis

Robert B. Colvin

Pathology Department, Massachusetts General Hospital, Boston, Massachusetts

Alloantibodies to HLA class I or II and other antigens expressed by endothelium cause a variety of effects on renal transplants, ranging from acute to chronic rejection, and even apparent graft acceptance (accommodation). Recognition of these conditions and appropriate therapy requires demonstration of C4d in biopsies, commonly confirmed by tests for circulating alloantibody. Substantial practical experience by pathologists in the interpretation and pitfalls of C4d stains are reviewed along with considerations of the clinical significance and pathologic mechanisms of the different effects of antibody on the endothelium of the renal allograft. Clinical trials will be needed to ascertain the optimal treatment for the newly appreciated conditions chronic humoral rejection and accommodation.

Homo transplants differ not only in the strength of rejection, but also in the nature and location of the phenomena induced by rejection. This conclusion must not be regarded as a cause for discouragement. On the contrary, it should spur further research.

—Jean Hamburger

Alloantibodies are now appreciated as important mediators of acute and chronic rejection, differing in pathogenesis, or "nature," from T cell–mediated rejection. Alloantibodies preferentially attack a different "location," namely the peritubular and glomerular capillaries, in contrast to T cells, which characteristically infiltrate tubules and arterial endothelium. Antibody-mediated rejection generally has a worse prognosis and requires a different form of therapy than the usual T cell–mediated acute rejection. Recognition of the clinical relevance of alloantibodies, beyond their historical role as mediators of hyperacute rejection, has rested on a new diagnostic technique (C4d) that permits a definitive diagnosis of antibody-mediated rejection in a renal biopsy the pioneering studies of Feucht *et al.* (1) in Munich showed that peritubular capillary (PTC) C4d deposition in renal transplant biopsies is strongly associated with a poor prognosis and raised the possibility that antibodies were responsible. Connection among the renal pathology, C4d deposition, and circulating donor-specific antibodies (2–4) clenched the evidence for acute antibody-mediated rejection, which is now widely accepted as a distinct clinicopathologic entity (5). C4d is a fragment of C4b, an activation product of the classic complement pathway (Figure 1). C4b (and C4d) contain an occult sulfhydryl group that forms a covalent, thioester bond with nearby proteins on activation by antibody and C1 (6). C4b/C4d remains bound in the tissue after

Ig and C1 have been released for several days (6). No functional role of C4d *per se* has been reported. C4d deposition is strongly associated with circulating antibody to donor HLA class I or class II antigens (2,7,8) and is currently the best single marker of complement-fixing circulating antibodies to the endothelium.

In the past decade, four forms of antibody-mediated graft injury have been defined (Table 1) (6,9–11). We review current concepts of these four conditions, the interpretation of C4d staining, and the biologic effects of antibody on the endothelium, all areas in which knowledge is evolving rapidly.

Acute Humoral Rejection

The diagnostic criteria for acute humoral rejection (AHR; acute antibody-mediated rejection) are given in Table 2. Patients with AHR present with an acute loss of graft function that often arises in the first few weeks after transplantation and cannot be distinguished from cell-mediated rejection on clinical grounds (4,11,12). AHR can also develop years after transplantation, often triggered by a decrease in immunosuppression (iatrogenic, noncompliance, or malabsorption). Presensitization is the major risk factor, but most of the patients with AHR had a negative cross-match. AHR has occurred with all immunosuppression regimens, even profoundly depleting therapy (13).

The first clue that circulating anti–class I HLA antibody caused a different pattern of acute rejection came from the studies of Halloran's group in Edmonton (12). These investigators showed that neutrophils in PTC and glomerular capillaries are strongly associated with circulating anti-donor HLA antibodies. Other features, such as fibrinoid necrosis of arteries and microthrombi, are also more common. However, none of these features is specific (*i.e.*, occurred only with circulating antibodies).

The pathology of AHR has a wide spectrum and can easily be missed by histologic criteria alone. Renal biopsies may show acute cellular rejection, acute tubular injury, or thrombotic microangiopathy (Figure 2). Neutrophils in capillaries are characteristically but not always found. Macrophages are now recog-
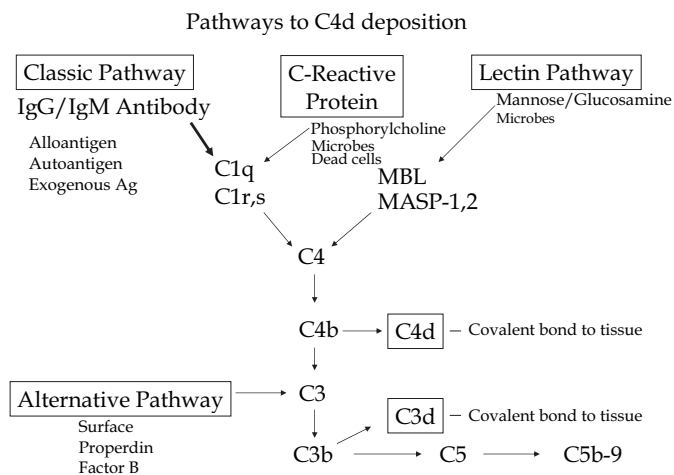
Pathways to C4d deposition

Figure 1. Complement activation pathways. The classic pathway is relevant to antibody-mediated rejection. The other pathways have not been shown to participate in acute or chronic rejection. C4d remains covalently bound in the tissue for several days after complement activation (see text). Adapted from reference (6), with permission.

nized as a common intracapillary cell in AHR in kidney (14) and heart (15) allografts. Typically, the PTC are dilated. Fibrinoid necrosis is found in a minority of cases (approximately 10 to 20%). A component of acute cellular rejection may also be present, as manifested by a prominent mononuclear infiltrate, tubulitis, or endarteritis. These lesions are generally not attributable to antibody alone. Treg cells (FOXP3^{+}) are rarer in the infiltrate than in cell-mediated rejection, perhaps contributing to the poorer prognosis in AHR (16). Microthrombi and interstitial hemorrhage also sometimes occur. The PTC and glomerular endothelium shows a variety of ultrastructural changes, including loss of fenestrations, detachment from the basement membrane, lysis, and apoptosis; complete destruction of capillaries can occur, leaving thickened laminated basement membranes (17).

Immunofluorescence (IF) curiously does not often show antibody or C3 deposition in the vessels. However, IF does show C4d in the majority of the PTC as a bright ring pattern, using a mAb in cryostat sections (2–4). Immunohistochemistry (IHC) works in formalin-fixed, paraffin-embedded tissues with a polyclonal antibody (13). By immunoelectron microscopy, C4d is detected on the surface of the endothelial cells and in intracytoplasmic vesicles (18). Antibodies that react to non-C4d portions of the C4 molecule do not show PTC deposition, arguing that what is detected in tissues is primarily C4d (19).

Antibodies to donor HLA class I or II antigens are present in 88 to 95% of the patients who have C4d deposition and acute graft dysfunction *versus* less than 10% in C4d-negative acute rejection (2,7,8). Antibodies to donor ABO antigens show a similar association. C4d deposition without detectable circulating antibody can be due to absorption by the graft, as demonstrated by elution of anti-HLA antibodies from rejected grafts in patients who had no detectable circulating antibody at the time, even from needle biopsies (20). Non-HLA, non-ABO antigens

are the target in a minority of cases, probably accounting for the rare C4d^{+} acute rejection in HLA-identical grafts (<2% of patients) (21). Autoantibodies to the angiotensin II type 1 receptor have also been associated with graft loss and fibrinoid necrosis that resembles alloantibody-mediated rejection except for the common absence of PTC C4d (22). Lack of C4d deposition in protocol biopsies in the presence of circulating donor-reactive antibody has been observed using flow cytometric assays and donor cells (7,8), perhaps in part because of antibodies of too low avidity or inability to fix complement. Fc receptors on NK cells (FcRIIA) may also play a role in acute rejection, and it is possible that some examples of AHR in biopsies that lack C4d are due to this mechanism.
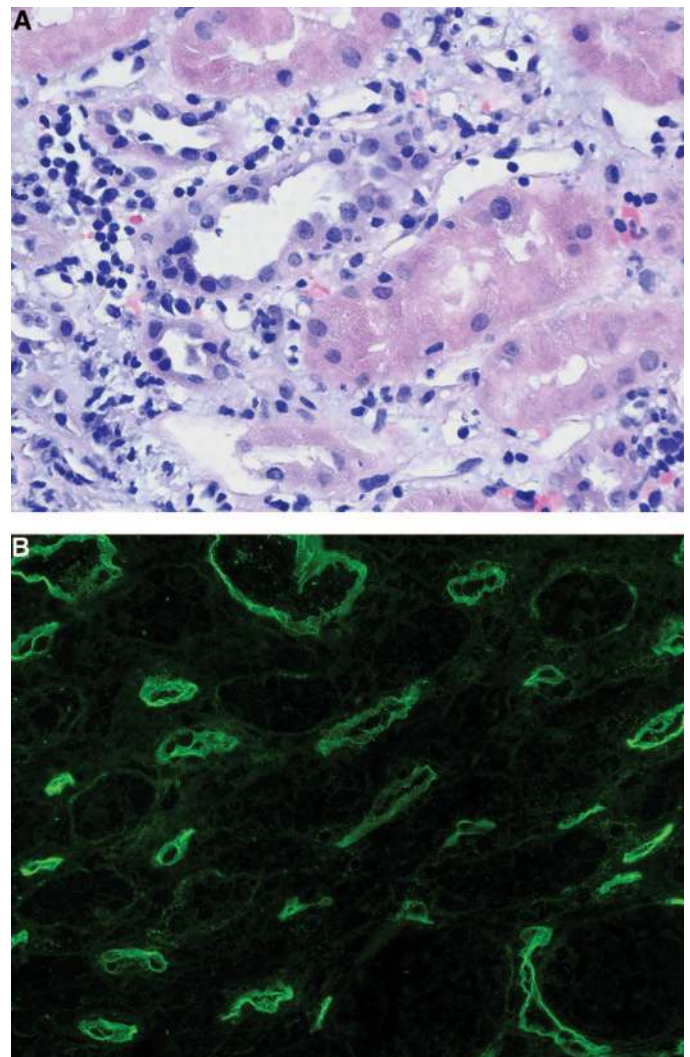

Figure 2. Acute humoral rejection. A 43-yr-old man developed rising creatinine (1.2 to 2.1 mg/dl) 6 mo after transplantation while he was on Prograf, CellCept, and prednisone. The patient had a high titer of IgG anti-donor class II antibodies. (A) Light microscopy shows interstitial edema, tubular injury, and neutrophils and mononuclear cells in peritubular capillaries (PTC). (B) Immunofluorescence (IF) microscopy demonstrates widespread, bright, linear staining of PTC for C4d.

Table 1. Antibody-mediated renal transplant pathology[a]

Diagnosis	Clinical Presentation	Histology	C4d	Serology	Outcome
Hyperacute rejection	Immediate graft failure (minutes to hours) after reperfusion	Neutrophils in glomeruli and PTC; hemorrhage, necrosis, thrombosis	Positive PTC; may be negative early	Anti-donor HLA or ABO positive in majority	Almost always irreversible
Acute humoral rejection	Rapid loss of graft function (days), any time after transplantation	Variable. Neutrophils and macrophages in glomeruli and PTC; fibrinoid arterial necrosis, acute tubular injury; ± cell-mediated rejection	Positive PTC; variable glomeruli	Anti-donor HLA (class I and/or II) or ABO positive in majority (approximately 90%)	Often reversible with appropriate treatment
Chronic humoral rejection	Slow, progressive loss of graft function (months to years), often with proteinuria, hypertension	GBM duplication; mononuclear cells in glomeruli and PTC; intimal fibrosis; tubular atrophy and interstitial fibrosis; PTC basement membrane multilamination (by EM)	Positive PTC (often patchy); glomeruli variably positive; occasionally only glomeruli positive	Anti-donor HLA positive in majority, especially to MHC class II antigens.	Outcome and optimal therapy not yet defined
Accommodation	Normal graft function	Normal, or minor changes	Positive PTC; variable glomeruli	Common with ABO incompatibility; occasionally with HLA antibodies	Outcome and optimal therapy not yet defined

[a]EM, electron microscopy; GBM, glomerular basement membrane; PTC, peritubular capillary.

Table 2. Diagnostic criteria for acute antibody-mediated rejection (AHR) (5,9)[a]

---

1. Morphologic evidence of acute tissue injury
   acute tubular injury
   neutrophils and/or mononuclear cells in PTC and/or glomeruli and/or capillary thrombosis
   fibrinoid necrosis/intramural or transmural inflammation in arteries
2. Immunopathologic evidence for antibody action
   C4d and/or (rarely) immunoglobulin in PTC
   Ig and complement in arterial fibrinoid necrosis
3. Serologic evidence of circulating antibodies to donor HLA or other anti-donor endothelial antigen

---

[a]Cases that meet only two of the three numbered criteria are considered suspicious for AHR. Acute cellular rejection may also be present.

Definition of Positive C4d Staining

The Banff schema defines positive C4d by IF as "widespread, strong linear circumferential PTC staining in cortex or medulla, excluding scar or necrotic areas" (9). This pattern has been associated with circulating alloantibodies. Many have taken 50% of the capillaries as the threshold. The dilemma has been what to do with those not uncommon cases with focally positive PTC that do not exceed 50%. In one study, Meehan and colleagues (23) found that diffuse C4d (≥50% of PTC) had a higher rate of graft loss 1 yr later (65%) than those with focal or no C4d (33% graft loss in both cases). In contrast, Magil and Tinckam (24) found that the outcome with diffuse or focal C4d was not distinguishable and was worse than those without C4d. Similarly, C4d staining of ≥25% of the PTC by IHC was associated with decreased 1-yr graft survival (13). More of the focal C4d cases had acute cellular rejection (93%) than did those with diffuse C4d (35%), suggesting that for C4d to be solely responsible for graft dysfunction, diffuse deposition is required (24). Alternatively, cellular rejection may interfere with C4d accumulation.

Comparison of C4d Techniques

The sensitivity of the C4d technique contributes to the extent observed. Decreased sensitivity and increased intra- and inter-observer variability have been reported for paraffin-IHC methods compared with frozen-IF (19). Of 26 cases that were diffusely positive by IF, 8% were minimally stained by IHC (three to 10 PTC total) and would be considered negative. Of those that were focally positive by IF, 52% were completely negative by IHC. The variability between observers (κ statistic) decreased from 0.9 to 0.6 for IHC. Similarly in another study, three of 15 of the diffusely positive cases by IF were only focally positive by IHC, and one of the five that were focally positive by IF became negative by IHC (25). IHC also has artifacts, such as staining of the plasma in the capillaries and interstitium as a result of fixation of soluble C4, which can render the sample uninterpretable. In one series, 8% of the samples showed this artifact, which was alleviated by decreasing the microwave

treatment time (23). However, IHC in fixed tissue has a great advantage over IF: Glomerular deposits can be assessed, because the normal mesangium does not stain for C4d in fixed tissue but does in frozen. Among the frozen-IF techniques, the triple-layer method is the most sensitive (4); for example, 50% of cases that were focally positive by double layer become diffusely positive by triple layer (25).

Pitfalls in Interpretation

False-negative C4d staining is found in areas of necrosis, and care must be taken in some cases to find areas of viable tissue, most easily done in fixed sections. Medulla is adequate for interpretation, because medullary capillaries also are targets of C4d. The tubules of the medulla may be confused capillaries, especially in frozen tissue. Arteries and arterioles not uncommonly have C4d, even in native kidneys with vascular disease. The reason is unknown, perhaps because of autoantibody or nonantibody complement activation.

Differential Diagnosis

C4d deposition in PTC is found in only a few instances of renal disease in native kidneys. Lupus nephritis may have bright, granular deposits along the PTC, corresponding to the immune complex deposits (6). One case of endocarditis was described with PTC deposits. Acute tubular necrosis does not generally show C4d in PTC, even in kidneys from non–heart-beating donors (6). Thrombotic microangiopathy is negative for C4d in PTC, as shown in five published cases of recurrent hemolytic uremic syndrome in a transplant (26), as well as unpublished experiences (9). This is particularly helpful, because AHR is always in the differential diagnosis of thrombotic microangiopathy. Thrombosis of the major renal vessels can lead to graft pathology that resembles AHR; however, no fibrinoid necrosis of arteries or C4d deposition in capillaries occurs. Glomerular C4d staining is commonly present in glomerular immune complex diseases and is not specific.

Other Complement Components Have Not Yet Proved Diagnostically Useful

C3 is the next component in the classic pathway sequence after C4; therefore, its cleavage products should indicate more complete complement activation (Figure 1). C3d (or C3c) was found in PTC in 39 to 60% of biopsies from HLA-mismatched grafts with diffuse PTC C4d (8,27–29). In general C3d and C4d were correlated. However, in one report, 19% of those with C3d had no C4d (28). This finding may be related to C3 activation *via* the alternative pathway, independent of C4. In the most comprehensive study, C3d was found only in conjunction with C4d in sensitized patients (8). Neutrophils in PTC or features of thrombotic microangiopathy correlated with C3d deposition in one study (8) but not in two others (8,29). The pathologic features of C3d$^+$C4d$^+$ biopsies were similar to those with C3d$^-$C4d$^+$ cases in ABO-compatible grafts (8). The presence of C3d was associated with increased risk for graft loss, compared with C3d$^-$ cases, but C3d provided no convincing additional risk compared with C4d$^+$. Macrophages in glomeruli correlated with C3c and C4d in glomeruli, which had a worse
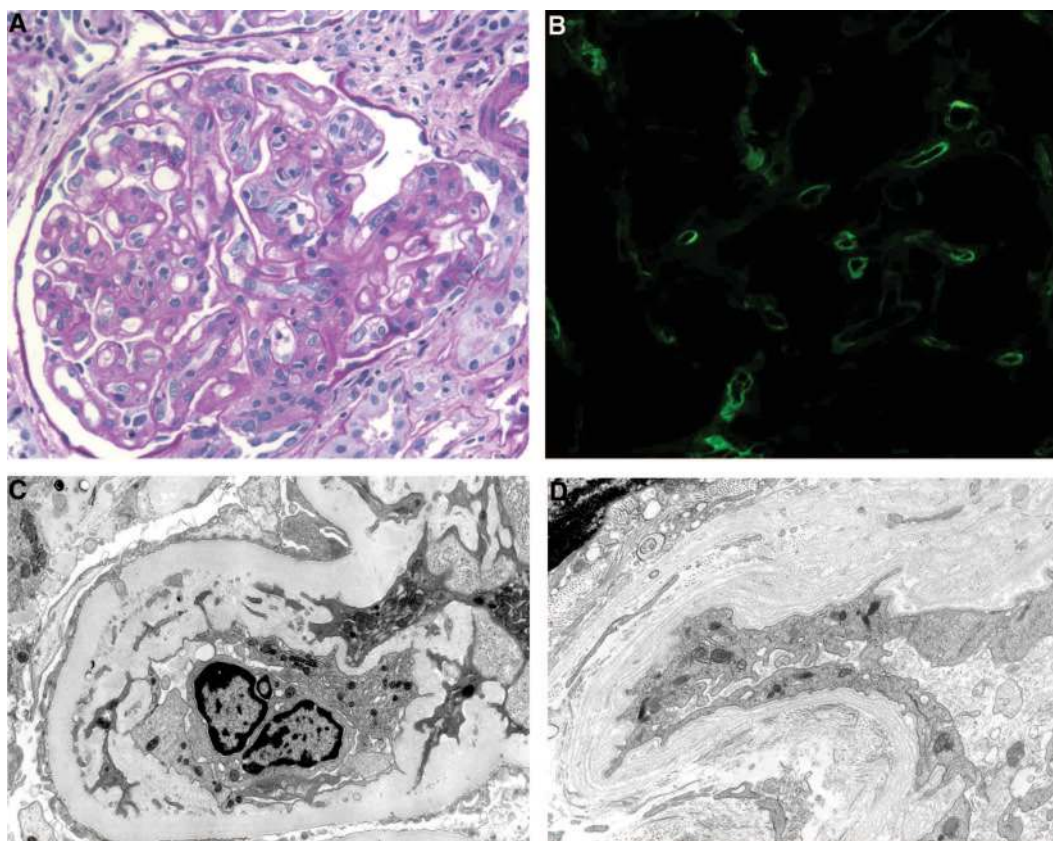
Figure 3. Chronic humoral rejection (CHR). An 18-yr-old woman 13 mo after transplantation had a rise in creatinine (1.2 to 1.6 mg/dl) and is on Prograf, CellCept, and prednisone; anti-donor HLA class II antibodies in serum. (A) Transplant glomerulopathy with duplication of the glomerular basement membrane (GBM) and accumulation of mononuclear cells in glomeruli (periodic acid-Schiff stain). (B) Immunofluorescence shows a patch distribution of C4d positivity in PTC. (C) Electron microscopy shows duplication of the GBM and reactive endothelial cells. (D) The PTC have prominent multilamination of the GBM.

prognosis than C4d alone (30). The interpretation of C3d is complicated by the common presence of C3d along the tubular basement membrane, commented on by Haas *et al.* (8). They concluded that C3d added little diagnostic value to C4d in positive cross-match grafts that showed histologic features of AHR (8). Similarly, Herman *et al.* (29) found that, in contrast to C4d, C3d was not associated with neutrophils in PTC, donor-reactive antibodies, or outcome. Therefore, at this time, no strong argument can be made for including C3d in the panel for ABO-compatible grafts.

Other complement components, such as C1q and C5b-9 (membrane attack complex [MAC]) are not conspicuous in PTC in acute rejection. MAC deposits in tubular basement membranes, rather than PTC (31), perhaps because of the expression of the inhibitor of MAC formation, CD59 in PTC. Lectin pathway components (Figure 1), which activate C4 by binding to microbial carbohydrates, are sometimes detected in conjunction with C4d. Mannose-binding lectin-associated serine protease-1 (MASP-1) was present in one of 11 protocol biopsies with C4d; no mannose-binding lectin was detected (27). Among 18 biopsies with C4d, 16 had diffuse H-ficolin along the PTC, whereas none of the 42 cases without C4d had H-ficolin. No MASP-1 or MASP-2 was detectable (32). The significance of these observa-

tions is not clear, because MASP proteins are required to activate C4 *via* the ficolins or mannose-binding lectin. C-reactive protein can also activate C4 but generally does not lead to full complement pathway activation (6).

Therapy

AHR requires different therapy than cell-mediated rejection. Therapy for AHR is plasmapheresis and intravenous Ig combined with intense immunosuppression (typically tacrolimus and mycophenolate mofetil). The use of these agents is empirical, because it is difficult to justify a randomized, controlled trial that leaves out any of these components. Immunoabsorption with protein A was tested in a randomized trial and reversed AHR in five of five cases, whereas four of five without early immunoabsorption lost their graft (33). For those that fail this treatment, other measures include anti-CD20 (rituximab) and splenectomy. Anti-complement (anti-C5) and inhibitors of plasma cell and B cell growth/survival (transmembrane activator and cyclophilin ligand interactor Ig) also deserve evaluation.

Hyperacute Rejection

The pathology of hyperacute rejection overlaps completely with AHR. Hyperacute rejection, by definition, arises within
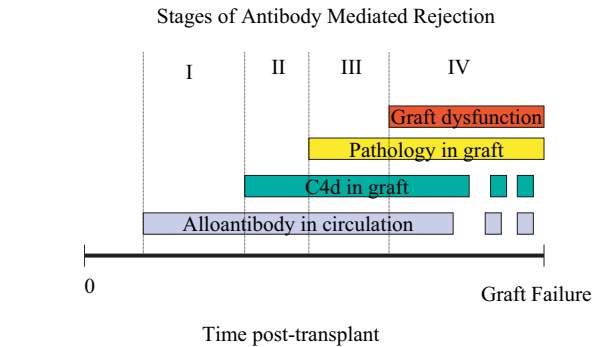
Stages of Antibody Mediated Rejection

Figure 4. Postulated stages of antibody-mediated rejection. Stages I to II represent accommodation, stage III represents subclinical humoral rejection, and stage IV represents CHR. The dashed lines for antibody and C4d deposition are meant to reflect the possibility of intermittent positivity over time. Inevitability of progression is not meant to be implied by the term "stages." The rate of progression is likely to be variable, and the early stages, I and II, are reversible. At any stage, the antibody/C4d may become negative, in which case the process is inactive.

minutes or hours in presensitized patients who have circulating HLA, ABO, or other alloantibody-to-donor endothelial surface antigens (9). Deposition of C4d occurs in PTC and glomeruli, just as in AHR. However, early biopsies may be negative for C4d, presumably because of lack of access of C4 to the site (vasoconstriction) or enough time for sufficient amount to be deposited. Furthermore, early biopsies may be C4d$^+$ without hyperacute rejection. Some presensitized patients with low titers of antibodies show C4d deposition in the capillaries immediately after reperfusion but without development of hyperacute rejection (34). Two such patients later developed AHR (5 to 35 d later). Finally, a few cases with hyperacute rejection have fibrinoid necrosis of arteries and no C4d deposition (or circulating anti-donor antibodies). The nature of this process is unknown but may involve antibody reacting to non-HLA antigens.

Table 3. Diagnostic criteria for chronic antibody-mediated rejection (CHR) (9,41)[a]

1. Histologic evidence of chronic injury (need 2 of 4)
   - arterial intimal fibrosis without elastosis
   - duplication of glomerular basement membrane
   - multilaminated PTC basement membrane
   - interstitial fibrosis with tubular atrophy
2. Evidence for antibody action/deposition in tissue (*e.g.*, C4d in PTC)[b]
3. Serologic evidence of anti-HLA or other anti-donor antibody

[a]If only two of the numbered criteria are present, then the diagnosis is considered "suspicious" for CHR.
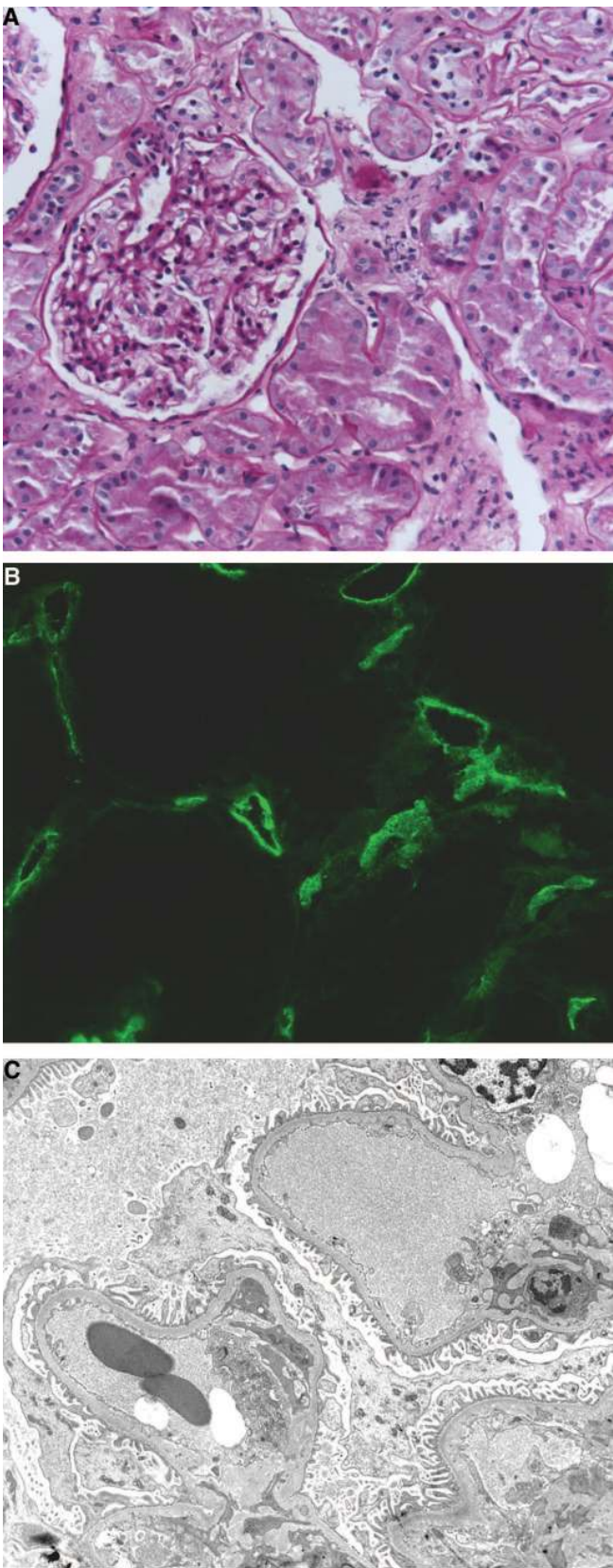[b]May be patchy in distribution.

Figure 5. Accommodation. Protocol biopsy 1 yr after renal transplantation in a recipient with normal renal function (creatinine 1.5 mg/dl) and antibodies to donor class II antigens. (A) Light microscopy is normal. (B) Prominent C4d deposition in PTC is present. (C) Electron microscopy shows no glomerular abnormality.

Chronic Humoral Rejection

In the past 5 years, evidence has accumulated that argues for a significant role of alloantibodies to MHC antigens in the pathogenesis of slowly progressive graft injury and dysfunction. Several studies have shown that circulating anti-HLA class I or II antibodies, either donor reactive (35,36) or *de novo*, non–donor reactive (36,37), are found in a substantial fraction of renal allograft recipients, and these are associated with later graft loss. Among 2278 recipients who were followed prospectively, graft failure 1 yr later was greater in those with alloantibodies than in those without (8.6 *versus* 3.0%; $P < 0.0001$). Increased risk for graft loss mainly affected those with intermediate graft dysfunction at the time of the antibody measurement (creatinine 2.0 to 3.9 mg/dl) (37). Antibodies to MICA (a polymorphic class I–related antigen) are also associated with late graft failure, even in the absence of measurable HLA antibodies (38).

Transplant glomerulopathy and arteriopathy are the pathologic features that usually are attributed to alloimmune mechanisms (9) (Figure 3). Transplant glomerulopathy is defined as widespread duplication or multilamination of the glomerular basement membrane (GBM), sometimes accompanied by mesangial expansion and accumulation of mononuclear cells in glomerular capillaries. C4d deposition in the graft is correlated with the lesions of transplant glomerulopathy and with circulating alloantibody (3,18,39,40). In a survey of graft biopsies that were taken 1 yr or more after transplantation, 34% had C4d in PTC (18). C4d was correlated with transplant glomerulopathy, multilamination of the PTC basement membrane, and mononuclear cells in capillaries (transplant glomerulitis). The combination of alloantibody, basement membrane multilamination, C4d, and duplication of the GBM has been termed the "ABCD tetrad" by Halloran and colleagues (41). Overall, approximately 50% of reported cases with transplant glomerulopathy have C4d in PTC (6). The Leiden group showed that glomerulopathy could occur with glomerular C4d in the absence of peritubular C4d deposition (40).

Transplant glomerulopathy is strongly associated with circulating antibodies to donor HLA antigens and a poor prognosis (3). Among 28 patients with transplant glomerulopathy, 64% had circulating antibodies to donor class I (28%), II (39%), or both I and II (33%) HLA antigens (42). Patients with a positive pretransplantation cross-match have a higher incidence of transplant glomerulopathy at 1 yr in protocol biopsies (22 *versus* 8%) (43). Previous AHR is also a risk factor for transplant glomerulopathy (odds ratio 17.5); 44% of the recipients with AHR showed transplant glomerulopathy in a later biopsy (43). Transplant glomerulopathy has a poor prognosis, even when subclinical, as detected in 1-yr protocol biopsies (graft survival approximately 50% 3 yr after diagnosis) (43). In a series of 14 protocol biopsies that were taken 10 yr after transplantation, three had C4d deposition, and all three had circulating anti-HLA antibodies and transplant glomerulopathy (44). Deterioration of renal function was greater in the C4d$^+$ group.

Because a substantial number of cases of transplant glomerulopathy have no detectable circulating HLA antibody or C4d in the graft at the time of diagnosis, the relationship among these factors is not simple, and some have questioned whether any relationship exists (45,46). In protocol biopsies 10 yr after transplantation, six of 11 of the C4d$^-$ patients also had transplant glomerulopathy, without C4d or anti-HLA antibodies (44). In another small study of biopsies that were taken for late graft dysfunction, C4d$^+$ PTC were commonly found in those with and without transplant glomerulopathy (four of five *versus* seven of 11) (47). In a comprehensive study, C4d was not correlated with transplant glomerulopathy among all patients (46). However, a closer examination of the data suggests that transplant glomerulopathy is more related to C4d than is interstitial fibrosis: Of 17 patients with transplant glomerulopathy, 53% were C4d$^+$, *versus* 14% of 21 patients with diffuse interstitial fibrosis ($P < 0.02$). In the Vienna series, C4d deposition preceded and predicted development of the glomerulopathy (18). These data are most compatible with the possibilities that (1) alloantibodies can cause transplant glomerulopathy and C4d$^+$ but are intermittent in action, (2) some transplant glomerulopathy may be non–antibody/non–complement-mediated (*e.g.*, thrombotic microangiopathy), and (3) antibodies can cause graft fibrosis without transplant glomerulopathy. A fourth alternative, that alloantibodies and C4d deposition are harmless and irrelevant to late graft injury, seems improbable.

We proposed that chronic humoral rejection (CHR; chronic, active antibody-mediated rejection) arises through a series of stages or states (Figure 4) (10). The serologic and pathologic evidence is most consistent with the hypothesis that the first common event is alloantibody production (stage I), followed by antibody interaction with alloantigens resulting in the deposition of C4d in PTC and possibly glomeruli (stage II), followed by pathologic changes (stage III) and graft dysfunction (stage IV) only later. We have observed the four stages in nonhuman primates with renal allografts (48); in this setting, with no immunosuppressive drugs, progression to chronic graft injury and loss is the rule. Whether it is similarly inexorable in immunosuppressed humans remains to be determined. The level of circulating antibody may fluctuate (knowledge on this is limited), in which case stages III and IV may persist after antibody and C4d disappear (inactive stage III or IV; stage IIIi or IVi). In any case, the hypothesized stages have offered a useful organizing structure for ongoing clinical trials to intervene in the early stages (I or II).

A consensus meeting at the National Institutes of Health proposed criteria for CHR (11) that have been incorporated into the Banff schema (41) (Table 3). In analogy to acute antibody-mediated rejection, three elements should be present: Histologic evidence of chronic injury, immunopathologic evidence of antibody action (*e.g.*, C4d), and evidence of antibody reactive to the donor in the circulation. CHR is distinct from AHR in that no acute inflammation (neutrophils, edema, necrosis, thrombosis) is present. However, cellular activity is often reflected by increased mononuclear cells in glomerular capillaries and PTC. Why donor-specific antibodies elicit AHR *versus* CHR is not clear. Perhaps factors such as titer, avidity, and effector functions are important, as well as the resistance of the graft endothelium (accommodation). The optimal therapy for CHR is undetermined, because the natural history is unknown and

drugs for long-term control of B cell and plasma cell function are not defined. Randomized trials are clearly indicated.

Accommodation

Transplantation across an ABO barrier, which normally precipitates hyperacute rejection, has been done successfully in many centers, using special protocols to deplete naturally occurring anti–blood group antibodies. Remarkably, antibody (primarily IgM) returns in the circulation, sometimes in high titers, yet no obvious graft rejection occurs, a state termed "accommodation" (49). At a cellular level, accommodation may occur *via* multiple mechanisms, including internalization, downregulation, inactivation, and inhibition of the target antigen (9,10). *In vitro*, antibodies induce nitric oxide synthase, Bcl-2, and Bcl-xl in endothelial cells, which confer resistance to apoptosis, as well as complement-regulatory proteins, such as CD59 (10,50,51). Stable ABO-incompatible grafts show differences in signaling pathways and cytokines by microarray gene expression analysis and notably increased levels of muc-1 in glomerular capillaries (49).

Accommodation in ABO-incompatible grafts is not due to a change in the nature of the antibody or loss of the target antigen in the graft, because C4d is deposited in the renal microcirculation. Protocol biopsies have revealed C4d along the PTC in 25 to 80% of ABO-incompatible grafts, with evidence of AHR in only 4 to 12% (8,52). Either the full complement pathway is not activated, or the endothelium develops resistance to its effects. A large study was designed to test whether C3d could distinguish those with accommodation from those with AHR (8). Approximately 40% of the C4d$^+$ biopsies (14 of 37) had C3d, and C3d correlated somewhat with histologic features of AHR; however, 70% of the C3d$^+$ biopsies also had no evidence of AHR, arguing that accommodation occurs distal to C3 activation (perhaps *via* inhibition of MAC). In any case, C4d deposition in ABO-incompatible grafts can be seen routinely without features of AHR and therefore is of limited value. Perhaps deposition of later complement components (C5b or MAC) or absence of heparan sulfate and syndecan-4-phosphate will show more correlation with injury, as has been reported in xenografts (53).

C4d deposition also occurs in 2 to 26% of histologically normal ABO-compatible grafts, the higher frequency found in HLA-presensitized patients (8,54) (Figure 5). In these patients, incidental C4d deposition does not necessary portend AHR; however, it may not be entirely benign. Among 17 patients who had C4d in PTC without histologic evidence of AHR or acute cellular rejection and who received no increased immunosuppression, graft loss at 3 yr was 32% compared with 0% among five patients who were treated with increased immunosuppression, suggesting that incidental C4d may represent a "smoldering rejection" (55).

Accommodation may have different degrees of effectiveness and stability, ranging from none (hyperacute rejection), to minimal (acute rejection), substantial (chronic rejection), or complete (stable accommodation). The minimal features that indicate transformation from accommodation to rejection have yet to be defined. For example, if the capillary endothelium is reactive by electron microscopy (loss of fenestrations) or if the GBM has mild segmental duplication, then does that change the diagnosis or the prognosis? The outcome will probably be determined primarily by the course and the pathogenicity of the alloantibody response itself. Even in ABO-incompatible grafts, accommodation may be incomplete: Transplant glomerulopathy is more frequent in 1-yr protocol biopsies of ABO-incompatible grafts than in ABO-compatible recipients (13 *versus* 8%) (43). Controlled trials and further follow-up will be needed to interpret the long-term significance of "incidental" C4d deposition (Figure 5).

Antibody and Complement Effects on Endothelium

The effects of antibody and complement on the endothelium range from lysis to activation, including production of growth factors, adhesion molecules, cytokines, and chemokines (10). Complement fixation is strongly associated with the ability of antibody to mediate AHR in animal models (56) and in humans (57,58). Recipients with anti-donor HLA class I antibodies that fix C4d to FlowPRA beads had inferior graft survival compared with those that did not, the latter having a similar outcome to patients with no anti-donor antibody (57). Complement-dependent cytotoxicity was also predictive of poorer outcome. Of note, complement-fixing HLA class II antibodies did not affect graft survival, even though they were associated with C4d deposition. Anti-donor antibodies of the strong complement-fixing subclass IgG3 were present in three patients with acute rejection but not in stable patients, whereas the latter had a significant rise only in the non–complement-fixing IgG4 subclass (58). In mice, non–complement-fixing IgG alloantibodies are associated with graft acceptance but can also activate endothelial cells to produce chemokines and promote rejection (56).

Direct linkage of circulating antibodies and chronic graft pathology was shown in experimental studies in which passive transfer of MHC antibodies could initiate the chronic arterial lesions in mice. Immunodeficient *scid* or RAG$^{-/-}$ mice that were given repeated doses of anti–class I alloantibodies developed fibrous intimal thickening of coronary arteries in cardiac allografts over 1 to 2 mo and C4d deposition in the capillaries (59).

In vitro anti–MHC class I antibodies promote endothelial proliferation *via* increased expression of basic FGF receptors, increased phosphorylation of Src, and NF-κB levels (60). Anti–class I antibodies increase endothelial resistance to apoptosis, as manifested by increased Bcl-xL, Bcl-2, and HO-1, and increased activity of phosphatidylinositol-3-kinase and Akt pathways and resistance to complement-mediated lysis (50,51). Anti–MHC class II antibodies also stimulate endothelial protein kinase C and Akt activation (61). S6 riboprotein (S6RP) is phosphorylated *via* the Akt pathway and is increased by either class I or class II antibodies (15). Some of these effects can be appreciated *in vivo* by study of biopsies. In human cardiac grafts, C4d deposition is correlated with microvascular expression of phosphorylated S6RP as detected by IHC (15). Curiously, increased S6RP was associated with antibodies to class II but not class I MHC molecules.

Are all antibodies pathogenetic? In some experimental settings, antibody can even promote graft acceptance (enhancing antibodies). One might predict that the "benign" antibodies would be deficient in their ability to fix complement or bind to Fc receptors (*e.g.*, IgG). IgG4 anti-donor HLA antibody is increased in recipients with long-term graft function (58). IgG4 does not fix complement and may inhibit immunologic injury. Avidity and titer also are probably major determinants. In the literature, there is little evidence for benign antibodies, provided that they react with donor HLA antigens. Until convincing evidence is reported, the default position is that development of donor-reactive HLA antibodies and/or C4d deposition should trigger a heightened state of clinical vigilance.

Conclusion

The detection of C4d deposition in capillaries of allografts has permitted the definition of two new forms of antibody-mediated rejection, acute and chronic, as well as an apparent clinicopathologic state in which the endothelium undergoes no obvious pathologic change (accommodation). Now that these conditions can be diagnosed reliably by the pathologist, randomized clinical trials can be initiated to ascertain the optimal therapy and prevention.

Disclosures

None.

References

1. Feucht HE, Schneeberger H, Hillebrand G, Burkhardt K, Weiss M, Riethmuller G, Land W, Albert E: Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 43: 1333–1338, 1993
2. Mauiyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Tolkoff-Rubin NE, Williams WW, Delmonico FL, Cosimi AB, Colvin RB: Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol* 13: 779–787, 2002
3. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB: Chronic humoral rejection: Identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 12: 574–582, 2001
4. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, Tolkoff-Rubin N, Cosimi AB, Colvin RB: Complement activation in acute humoral renal allograft rejection: Diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 10: 2208–2214, 1999
5. Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, Cecka MJ, Cosyns JP, Demetris AJ, Fishbein MC, Fogo A, Furness P, Gibson IW, Glotz D, Hayry P, Hunsickern L, Kashgarian M, Kerman R, Magil AJ, Montgomery R, Morozumi K, Nickeleit V, Randhawa P, Regele H, Seron D, Seshan S, Sund S, Trpkov K: Antibody-mediated rejection criteria: An addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 3: 708–714, 2003
6. Rotman S, Collins AB, Colvin RB: C4d deposition in allografts: Current concepts and interpretation. *Transplant Rev* 19: 65–77, 2005
7. Bohmig GA, Exner M, Habicht A, Schillinger M, Lang U, Kletzmayr J, Saemann MD, Horl WH, Watschinger B, Regele H: Capillary C4d deposition in kidney allografts: A specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol* 13: 1091–1099, 2002
8. Haas M, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, Segev DL, Simpkins CE, Warren DS, King KE, Zachary AA, Montgomery RA: C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: Correlation with histologic findings. *Am J Transplant* 6: 1829–1840, 2006
9. Colvin RB, Nickeleit V: Renal transplant pathology. In: *Heptinstall's Pathology of the Kidney*, 6th Ed., edited by Jennette JC, Olson JL, Schwartz MM, Silva FG, Philadelphia, Lippincott-Raven, 2006, pp 1347–1490
10. Colvin RB, Smith RN: Antibody-mediated organ-allograft rejection. *Nat Rev Immunol* 5: 807–817, 2005
11. Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, Kupiec-Weglinski J, Matas A, Montgomery RA, Nickerson P, Platt JL, Rabb H, Thistlethwaite R, Tyan D, Delmonico FL: National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant* 4: 1033–1041, 2004
12. Halloran PF, Schlaut J, Solez K, Srinivasa NS: The significance of the anti-class I antibody response. II. Clinical and pathologic features of renal transplants with anti-class I-like antibody. *Transplantation* 53: 550–555, 1992
13. Lorenz M, Regele H, Schillinger M, Exner M, Rasoul-Rockenschaub S, Wahrmann M, Kletzmayr J, Silberhumer G, Horl WH, Bohmig GA: Risk factors for capillary C4d deposition in kidney allografts: Evaluation of a large study cohort. *Transplantation* 78: 447–452, 2004
14. Tinckam KJ, Djurdjev O, Magil AB: Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status. *Kidney Int* 68: 1866–1874, 2005
15. Lepin EJ, Zhang Q, Zhang X, Jindra PT, Hong LS, Ayele P, Peralta MV, Gjertson DW, Kobashigawa JA, Wallace WD, Fishbein MC, Reed EF: Phosphorylated S6 ribosomal protein: A novel biomarker of antibody-mediated rejection in heart allografts. *Am J Transplant* 6: 1560–1571, 2006
16. Veronese FJ, Rotman S, Smith RN, Della Pelle P, Farrell ML, Kawai T, Cosimi AB, Colvin RB: Pathological and clinical correlates of FOXP3+ cells in renal allografts during acute rejection. *Am J Transplant* 2007, in press
17. Liptak P, Kemeny E, Morvay Z, Szederkenyi E, Szenohradszky P, Marofka F, Toldi J, Exner M, Ivanyi B: Peritubular capillary damage in acute humoral rejection: An ultrastructural study on human renal allografts. *Am J Transplant* 5: 2870–2876, 2005
18. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, Watschinger B, Kerjaschki D, Exner M: Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: A contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol* 13: 2371–2380, 2002
19. Seemayer CA, Gaspert A, Nickeleit V Mihatsch MJ: C4d staining of renal allograft biopsies: Comparative analysis of different staining techniques. *Nephrol Dial Transplant* 22: 568–576, 2006

20. Martin L, Guignier F, Bocrie O, D'Athis P, Rageot D, Rifle G, Justrabo E, Mousson C: Detection of anti-HLA antibodies with flow cytometry in needle core biopsies of renal transplants recipients with chronic allograft nephropathy. *Transplantation* 79: 1459–1461, 2005

21. Collins AB, Chicano S, Cornell LD, Tolkoff-Rubin N, Goes NB, Farrell ML, Cosimi AB, Colvin RB: Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO-compatible renal allografts. *Transplant Proc* 38: 3427–3429, 2006

22. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, Dechend R, Kintscher U, Rudolph B, Hoebeke J, Eckert D, Mazak I, Plehm R, Schonemann C, Unger T, Budde K, Neumayer HH, Luft FC, Wallukat G: Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 352: 558–569, 2005

23. Poduval RD, Kadambi PV, Josephson MA, Cohn RA, Harland RC, Javaid B, Huo D, Manaligod JR, Thistlethwaite JR, Meehan SM: Implications of immunohistochemical detection of C4d along peritubular capillaries in late acute renal allograft rejection. *Transplantation* 79: 228–235, 2005

24. Magil AB, Tinckam KJ: Focal peritubular capillary C4d deposition in acute rejection. *Nephrol Dial Transplant* 21: 1382–1388, 2006

25. Nadasdy GM, Bott C, Cowden D, Pelletier R, Ferguson R, Nadasdy T: Comparative study for the detection of peritubular capillary C4d deposition in human renal allografts using different methodologies. *Hum Pathol* 36: 1178–1185, 2005

26. Artz MA, Steenbergen EJ, Hoitsma AJ, Monnens LA, Wetzels JF: Renal transplantation in patients with hemolytic uremic syndrome: High rate of recurrence and increased incidence of acute rejections. *Transplantation* 76: 821–826, 2003

27. Sund S, Hovig T, Reisaeter AV, Scott H, Bentdal O, Mollnes TE: Complement activation in early protocol kidney graft biopsies after living-donor transplantation. *Transplantation* 75: 1204–1213, 2003

28. Kuypers DR, Lerut E, Evenepoel P, Maes B, Vanrenterghem Y, Van Damme B: C3D deposition in peritubular capillaries indicates a variant of acute renal allograft rejection characterized by a worse clinical outcome. *Transplantation* 76: 102–108, 2003

29. Herman J, Lerut E, Van Damme-Lombaerts R, Emonds MP, Van Damme B: Capillary deposition of complement C4d and C3d in pediatric renal allograft biopsies. *Transplantation* 79: 1435–1440, 2005

30. Molne J, Nyberg G, Blohme I, Rydberg L, Breimer ME, Svalander CT: Glomerular C3c deposition and intravascular macrophage accumulation in early humoral renal allograft rejection signifies a poor short-term outcome. *Apmis* 114: 700–711, 2006

31. Nishi S, Imai N, Ito Y, Ueno M, Fukase S, Mori H, Arakawa M, Bassam A, Saito K, Takahashi K, Gejyo F: Pathological study on the relationship between C4d, CD59 and C5b-9 in acute renal allograft rejection. *Clin Transplant* 18[Suppl 11]: 18–23, 2004

32. Imai N, Nishi S, Alchi B, Ueno M, Fukase S, Arakawa M, Saito K, Takahashi K, Gejyo F: Immunohistochemical evidence of activated lectin pathway in kidney allografts with peritubular capillary C4d deposition. *Nephrol Dial Transplant* 21: 2589–2595, 2006

33. Bohmig GA, Wahrmann M, Regele H, Exner M, Robl B, Derfler K, Soliman T, Bauer P, Mullner M, Druml W: Immunoadsorption in severe C4d-positive acute kidney allograft rejection: A randomized controlled trial. *Am J Transplant* 7: 117–121, 2006

34. Haas M, Ratner LE, Montgomery RA: C4d staining of perioperative renal transplant biopsies. *Transplantation* 74: 711–717, 2002

35. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW: Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. *Transplantation* 75: 1034–1040, 2003

36. Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, Dantal J, Giral M, Blancho G, Cantarovich D, Karam G, Follea G, Soulillou JP, Bignon JD: Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. *J Am Soc Nephrol* 16: 2804–2812, 2005

37. Terasaki PI, Ozawa M: Predictive value of HLA antibodies and serum creatinine in chronic rejection: Results of a 2-year prospective trial. *Transplantation* 80: 1194–1197, 2005

38. Mizutani K, Terasaki P, Rosen A, Esquenazi V, Miller J, Shih RNJ, Pei R, Ozawa M, Lee J: Serial ten-year follow-up of HLA and MICA antibody production prior to kidney graft failure. *Am J Transplant* 5: 1–8, 2005

39. Vongwiwatana A, Gourishankar S, Campbell PM, Solez K, Halloran PF: Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy. *Am J Transplant* 4: 124–129, 2004

40. Sijpkens YW, Joosten SA, Wong MC, Dekker FW, Benediktsson H, Bajema IM, Bruijn JA, Paul LC: Immunologic risk factors and glomerular C4d deposits in chronic transplant glomerulopathy. *Kidney Int* 65: 2409–2418, 2004

41. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nickeleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madriga L, Salomon DR, Seron D, Sheaff M, Weening JJ: Banff '05 meeting report: Differential diagnosis of chronic injury and elimination of chronic allograft nephropathy ("CAN") in the Banff schema. *Am J Transplant* 7: 518–526, 2007,

42. Sis B, Mueller T, Campbell P, Hunter C, Cockfield S, Solez K, Halloran PF: Transplant glomerulopathy, late antibody mediated rejection, and the ABCD tetrad. *Am J Transplant* [Suppl]: 469–470, 2006

43. Gloor JM, Cosio FG, Rea DJ, Wadei HM, Winters JL, Moore SB, DeGoey SR, Lager DJ, Grande JP, Stegall MD: Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. *Am J Transplant* 6: 1841–1847, 2006

44. Namba Y, Oka K, Moriyama T, Kyo M, Imamura R, Shi Y, Ichimaru N, Isaka Y, Takahara S, Okuyama A: Incidence of positive C4d deposition in long-term survival cases over 10 yr after renal transplantation. *Clin Transplant* 20[Suppl 15]: 20–25, 2006

45. Al Aly Z, Yalamanchili P, Cortese C, Salinas-Madrigal L, Bastani B: C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: An uncommon finding. *Transpl Int* 18: 800–805, 2005

46. Nickeleit V, Zeiler M, Gudat F, Thiel G Mihatsch MJ: Detection of the complement degradation product C4d in renal allografts: Diagnostic and therapeutic implications. *J Am Soc Nephrol* 13: 242–251, 2002

47. Akalin E, Dikman S, Murphy B, Bromberg JS, Hancock WW: Glomerular infiltration by CXCR3+ ICOS+ activated T cells in chronic allograft nephropathy with transplant glomerulopathy. *Am J Transplant* 3: 1116–1120, 2003

48. Smith RN, Kawai T, Boskovic S, Nadazdin O, Sachs DH, Cosimi AB, Colvin RB: Chronic antibody mediated rejection of renal allografts: Pathological, serological and immunologic features in nonhuman primates. *Am J Transplant* 6: 1790–1798, 2006

49. Park WD, Grande JP, Ninova D, Nath KA, Platt JL, Gloor JM, Stegall MD: Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant* 3: 952–960, 2003

50. Narayanan K, Jaramillo A, Phelan DL, Mohanakumar T: Pre-exposure to sub-saturating concentrations of HLA class I antibodies confers resistance to endothelial cells against antibody complement-mediated lysis by regulating Bad through the phosphatidylinositol 3-kinase/Akt pathway. *Eur J Immunol* 34: 2303–2312, 2004

51. Salama AD, Delikouras A, Pusey CD, Cook HT, Bhangal G, Lechler RI, Dorling A: Transplant accommodation in highly sensitized patients: A potential role for Bcl-xL and alloantibody. *Am J Transplant* 1: 260–269, 2001

52. Fidler ME, Gloor JM, Lager DJ, Larson TS, Griffin MD, Textor SC, Schwab TR, Prieto M, Nyberg SL, Ishitani MB, Grande JP, Kay PA, Stegall MD: Histologic findings of antibody-mediated rejection in ABO blood-group-incompatible living-donor kidney transplantation. *Am J Transplant* 4: 101–107, 2004

53. Williams JM, Holzknecht ZE, Plummer TB, Lin SS, Brunn GJ, Platt JL: Acute vascular rejection and accommodation: Divergent outcomes of the humoral response to organ transplantation. *Transplantation* 78: 1471–1478, 2004

54. Mengel M, Bogers J, Bosmans J-P, Seron D, Moreso F, Carrera M, Gwinner W, Schwarz A, De Broe M, Kreipe1 H, Haller H: Incidence of C4d stain in protocol biopsies from renal allografts: Results from a multicenter trial. *Am J Transplant* 5: 1050–1056, 2004

55. Dickenmann M, Steiger J, Descoeudres B, Mihatsch M, Nickeleit V: The fate of C4d positive kidney allografts lacking histological signs of acute rejection. *Clin Nephrol* 65: 173–179, 2006

56. Rahimi S, Qian Z, Layton J, Fox-Talbot K, Baldwin WM 3rd, Wasowska BA: Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am J Transplant* 4: 326–334, 2004

57. Wahrmann M, Exner M, Schillinger M, Haidbauer B, Regele H, Kormoczi GF, Horl WH, Bohmig GA: Pivotal role of complement-fixing HLA alloantibodies in presensitized kidney allograft recipients. *Am J Transplant* 6: 1033–1041, 2006

58. Gao ZH, McAlister VC, Wright JR Jr, McAlister CC, Peltekian K, MacDonald AS: Immunoglobulin-G subclass antidonor reactivity in transplant recipients. *Liver Transpl* 10: 1055–1059, 2004

59. Uehara S, Chase CM, Cornell LD, Madsen JC, Russell PS, Colvin RB: Chronic cardiac transplant arteriopathy in mice: Relationship of alloantibody, C4d deposition and neointimal fibrosis. *Am J Transplant* 7: 57–65, 2006

60. Jin YP, Singh RP, Du ZY, Rajasekaran AK, Rozengurt E, Reed EF: Ligation of HLA class I molecules on endothelial cells induces phosphorylation of Src, paxillin, and focal adhesion kinase in an actin- dependent manner. *J Immunol* 168: 5415–5423, 2002

61. Le Bas-Bernardet S, Coupel S, Chauveau A, Soulillou JP, Charreau B: Vascular endothelial cells evade apoptosis triggered by human leukocyte antigen-DR ligation mediated by allospecific antibodies. *Transplantation* 78: 1729–1739, 2004