

## Complement in Renal Transplant Rejection

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The complement system plays multiple pathogenetic roles in rejection of renal allografts and one of the components, C4d, is an important diagnostic marker of antibody mediated rejection (1, 2). Complement proteins (e.g., C3) arose as an ancient, innate defense system in invertebrates that preceded the adaptive immune system in phylogeny. It is not surprising that during evolution these complement proteins and their receptors were incorporated into both the antibody and T cell mediated responses. C4 first evolved in conjunction with immunoglobulin. The Belgian microbiologist Jules Bordet (Nobel Prize 1919) discovered and defined complement as the factor(s) in fresh normal serum necessary for lysis of antibody coated red cells. The present discussion focusses on four topics: Ischemia/reperfusion injury, T cell mediated rejection, antibody mediated rejection and accommodation.

### I. Ischemia/Reperfusion

All renal transplants begin their sojourn in a new host after suffering ischemia and reperfusion injury (IRI). This is manifested by delayed graft function (DGF), defined as post-transplant requirement for dialysis, which occurs in 15-20% of deceased donor grafts and >90% of asystolic donor grafts (3). DGF is an adverse complication primarily due to the associated increased risk of acute rejection (4). The link between ischemia and increased immunogenicity may involve the complement system.

The complement system is an important mediator of IRI, as shown in the protection from IRI in mice that are genetically deficient in any of several complement components (2). In mouse renal models of ischemia, the alternative pathway is most important. Mice deficient in C3-, C5-, or C6 are protected from renal IRI, whereas C4-deficient mice were not (5). The lack of requirement for C4 does not exclude the MBL pathway, since MBL can bypass C4 (6). Tubular epithelial cells, rather than vessels, are the main site of injury. Antibody to C5a had little effect. Thus renal ischemia differs from other organs in which C5a is important and the vessels are the main target.

Autoantibodies contribute to IRI in non-renal organs (heart, muscle, intestine), where complement fixation is dependent on autoantibodies to intracellular antigens which are externalized upon ischemia, such as non-muscle myosin heavy chain type II A and C (7, 8). The role of autoantibodies in renal IRI is controversial. In one report, RAG1<sup>-/-</sup> mice were just as susceptible to IRI as wild type (WT) mice (9). In contrast, B cell knockout mice ( $\mu$ MT) were resistant to IRI, which was restored by serum. C3d deposition occurred even in the absence of Ig (10), arguing the classical pathway is not involved and consistent with a role for MBL. Indeed, further studies showed that MBL-deposition occurs in the early reperfusion phase and C3, C6 and C9 later along the tubules and in the peritubular capillaries, with MBL in the same sites. In non-renal IRI, The lectin pathway has also been implicated, as MBL deficient mice are protected from IRI (11). MBL binds to IgM autoantibodies, independent of C1q, which is not required for IRI.

Deficiency of cell surface complement regulators exacerbates renal IRI. Mice genetically deficient in CD59, the major regulator of the membrane attack complex (C5b-9), were more susceptible to IRI and had more C9 deposition along the tubules than WT mice (12), although another CD59 deficient strain were not (13). Deficiency of CD55 (Decay Accelerating Factor) promoted renal IRI, and this deficiency was synergistic with CD59 deficiency (13). Partial deficiency of *Crry* (*CR1*-related gene/protein  $\gamma$ ) increases susceptibility to IRI and increases C3 deposition along the TBM (14).

In biopsies of ATN in humans, C3d, but not C4d, is increased along the TBM (15). Human proximal tubular cells cultured with normal human serum activate the alternative pathway and fix C3 on their surface, along with properdin, terminal complement components and C5b-9, but not C1q or C4 (16). This is probably the reason that C3, C5, C6, C7, C9 and C5b-9 neoantigen are deposited in the TBM in tubulo-interstitial disease (17). In ischemic allografts in humans, MBL deposition occurs early in peritubular capillaries and tubular epithelial cells, similar to the mouse (18). Thus there is evidence that both the alternative and the MBL pathways accounts for the focal C3 along the TBM.

Therapies for ATN based on these observations have had some success in mice, including anti-C5 (19), membranophilic myristoylated CR1(20), C5aR antagonist (21), anti-factor B (22), and inhibition of local C3 synthesis with perfusion with C3 siRNA (23). Both anti-factor B and C3 siRNA decreased C3b accumulation in tubules, as did, curiously, anti-C5 (19, 22, 23). Clinical trials have started with myristoylated CR1, which at last report showed that treatment of donor organs was both feasible and safe (2). Monoclonal anti-C5 has had some success in severe cardiac ischemia (24), and but has not been tested in transplantation. Infusion of myristoylated CR1(20) not only ameliorates IRI in mice, it also inhibits acute cellular rejection (cellular infiltrate, T cell proliferative response and renal function), arguing that early complement activation from ischemia promotes the immune response. This is quite compatible with the observations in humans that DGF increases the risk of acute rejection (4).

## II. T cell mediated rejection

A characteristic feature of acute cellular rejection in humans (which persists in later biopsies) is abundant C3 deposition along the TBM in a segmental linear pattern, which exceeds the "normal" pattern (4). C3 is often pronounced in atrophic tubules. C5b-9 is also deposited along the TBM in about 30% of cases (25). Most of the C3 derives from local synthesis, which increases in rejection (26, 27). Allografts contribute about 4% of the circulating C3, increasing to 10% during rejection (28). Peritubular C3 is largely derived from proximal tubular cells, as judged by C3 allotype antibodies; donor specific C3 mRNA can be detected in rejecting renal allografts by PCR (29). Tubular synthesis of C3 in vitro is promoted by exposure to IFN $\gamma$  (30), IL-17 (31) or IL-2 (32) and in biopsies correlates with local IFN $\gamma$  production (27). Synthesis of other complement components by the kidney has been demonstrated, such as C2, C4, and factor B (see ref (32)). Furthermore other cell types such as endothelial cells can make C3.

In mice intragraft synthesis of both C3 and C4 also rises substantially during acute rejection (33). That the intragraft C3 synthesis might be pathogenetically important was established in a seminal publication in mice (30). In these studies congenic C3 gene-disrupted C57BL/6<sup>H-2b</sup> (C3<sup>-/-</sup>) or normal B6 kidneys were transplanted into normal B10.Br<sup>H-2k</sup> recipients. Median graft survival was increased from 12.5 days with WT kidneys to >100 days in the C3<sup>-/-</sup> allografts. The C3 status of the recipient was irrelevant; prolonged survival occurred only when the allograft was C3 deficient. C3 and C5b-9 were deposited along the TBM in WT but not C3<sup>-/-</sup>kidneys.

Rejection appeared to be T cell-mediated, since the lesions were tubulitis and endothelialitis. Acute rejection was not affected by blocking the classical pathway by local or systemic deficiency in C4 (33), suggesting that the mechanism was not via antibody mediated rejection.

Recipients of C3-/- kidneys have a reduced alloantigen driven proliferative response of T cells, leading to the conclusion that C3 in graft cells promotes antigen presentation to T cells. Human T cells express CR1 and CR2 and are able to bind to C3b and C3d. In mice the combined gene product (CR1/2) is restricted to <5% of CD4+ cells, which expands during rejection (30). Dendritic cells synthesize C3 which is required for normal T cell priming (34). Conversely, absence of a complement regulator DAF from dendritic cells increases the immune response (35). The complex molecular mechanisms appear to involve alterations in IL-12 production, IFN $\gamma$ , C5a, and Foxp3 cells, but the mechanisms are not yet established (35).

That local C3 synthesis in the graft is also relevant to the human has been suggested by the unexpected finding that the C3 allotype of the graft, but not the donor influences outcome (36). C3 has two main allotypes, F (fast) and S (slow) in the human, caused by a single nucleotide substitution that leads to a change from glycine (C3F) to arginine (C3S) at position 80. Among 513 recipients, graft survival was significantly lower with a C3S/S donor allotype than with C3F/F or C3F/S donor allotypes (hazard ratio 2.2). Graft function was also significantly worse (P<0.001). The effect was restricted to recipients who did not themselves possess the F allele, suggesting that the benefit of the F allotype can be achieved with C3 from the recipient. The C3F/S mutation is on the surface of the molecule and may affect interactions with receptors; alternatively the beneficial effect is due to a linkage with other unidentified genes. The C3F allotype has been linked to other immunologically mediated renal disease such IgA nephropathy and MPGN (see ref (36))

### **III. Antibody Mediated Rejection**

Complement is of course involved in antibody mediated graft injury, as Bordet would have predicted. Demonstration of C4d in peritubular capillaries remains the most robust indicator of circulating anti-donor HLA or ABO antibodies a topic that has been extensively and recently reviewed (1). In the last decade, four forms of antibody mediated graft injury have been defined (4, 37-39): hyperacute, acute and chronic rejection and accommodation. Thus diagnosis of these conditions depends on the combination of C4d deposition and specific histologic or ultrastructural lesions.

Antibodies to donor HLA class I or II antigens are present in 88-95% of the patients who have C4d deposition and acute graft dysfunction vs. less than 10% in C4d negative acute rejection (40-42). 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 (43). 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) (44).

Whether complement fixation is necessary for the pathogenesis of antibody mediated rejection is of some interest. Complement fixation is strongly associated with the ability of antibody to mediate AHR in animal models (45). Antibodies of different isotypes vary in their ability to fix complement. In humans, IgG1,2,3 and IgM fix complement by the classical pathway; IgG4 and IgA do not). Anti-donor antibodies of the strong complement fixing subclass, IgG3, were

present in patients with acute rejection, but not in stable patients, whereas the latter had a significant rise only in the non-complement fixing, IgG4 subclass (46).

Wahrmann and colleagues in Vienna first described a method to measure complement fixing antibodies by testing FlowPRA beads coated with HLA antigens for their ability to stain for C4d by flow cytometry after incubation in plasma (47, 48). C1q and C3d/b could also be detected, but the C3 was taken up independent of DSA. This test has been used to determine the clinical significance of complement fixing DSA. 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 (48). Complement dependent cytotoxicity (CDC-PRA) was also predictive of poorer outcome. Of note, complement-fixing HLA class II antibodies did not affect graft survival, even though associated with C4d deposition in the graft. These studies were extended to cardiac grafts by Smith and colleagues who tested pre-transplant sera on Luminex beads coated with HLA antigens for their C4d fixing ability (49). They found that DSA that fixed complement was associated with a decreased graft survival compared to those with non-complement fixing DSA (graft survival at one year decreasing from 54% to 20%).

**Other complement components** (1). C3 is the next component in the classic pathway sequence after C4, and therefore its cleavage products should indicate more complete complement activation. C3d (or C3c) was found in PTC in 39-60% of biopsies from HLA mismatched grafts with diffuse PTC C4d (42, 50-52). In general C3d and C4d were correlated. However, in one report, 19% of those with C3d had no C4d (51). 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 (42). C3d deposition correlated with AHR in all studies of ABO compatible grafts (42, 50-52). Neutrophils in PTC or features of thrombotic microangiopathy correlated with C3d deposition in one study (42) but not two others (42, 52). The pathologic features of C3d+C4d+ biopsies were similar to those with C3d-C4d+ cases in ABO compatible grafts (42). The presence of C3d was associated with increased risk of graft loss, compared to C3d negative 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 prognosis than C4d alone (53). The interpretation of C3d is complicated by the common presence of C3d along the TBM (42). C3d added little diagnostic value to C4d in positive crossmatch grafts showing histologic features of AHR (42). Similarly, Herman et al found that, in contrast to C4d, C3d was not associated with neutrophils in PTC, donor reactive antibodies or outcome (52). Thus at this time no strong argument can be made for including C3d except in the panel for ABO incompatible 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 (54), perhaps because of the expression of the inhibitor of MAC formation, CD59 in PTC. Lectin pathway components, 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 1/11 protocol biopsies with C4d; no MBL was detected (50). 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 (55). The significance of these observations is not clear, since MASP proteins are required to activate C4 via the ficolins or MBL. C-reactive protein (CRP) can also activate C4 but generally does not lead to full complement pathway activation (37).

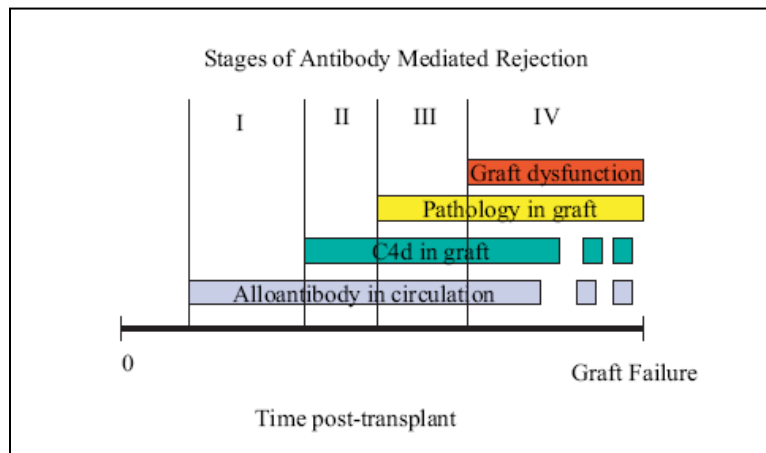
In addition to acute humoral rejection, over the last 5 years, evidence has accumulated that argues for a significant role of alloantibodies to MHC antigens in the pathogenesis of slowly progressive graft injury and dysfunction (56), a process now defined in the Banff system as chronic, active antibody mediated rejection (57). The components are listed below:

### Diagnostic Criteria for Chronic Antibody Mediated Rejection (CHR) (4, 58)

1. Histologic evidence of chronic injury: need 2 of 4
  - Arterial intimal fibrosis without elastosis
  - Duplication of glomerular basement membrane
  - Multi-laminated PTC basement membrane
  - Interstitial fibrosis with tubular atrophy
2. Evidence for Ab action/deposition in tissue (e.g., C4d in PTC)
3. Serologic evidence of anti-HLA or other antidonor antibody

The most tightly associated pathological feature is transplant glomerulopathy (duplication of the GBM) (59), although some have also found an association with chronic transplant arteriopathy (56), or even just interstitial fibrosis (60). C4d is deposited in glomerular capillaries (as best shown in paraffin sections) and well as in PTC, which may be sparse. Overall in transplant glomerulopathy, about 30% will have C4d, and about 70% will have DSA, most often with class II specificity (61). The C4d, DSA negative cases may be a late stage of CHR or due to non-antibody mediated injury (TMA from calcineurin inhibitor toxicity or T cell mediated glomerular damage). CHR progresses through 4 stages as shown in non-human primates (62) and in rare clinical cases (unpublished observations) (1). Murine studies have demonstrated that complement fixing antibodies to graft class I MHC antigens are sufficient to induce chronic transplant arteriopathy in RAG-1<sup>-/-</sup> mice (63).

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**Complement effects on the endothelium** (38). The primary target of antibody mediated rejection is in the capillary and arterial endothelium. Complement fixation on the endothelium can lead to cytolysis, via C5b-9 (MAC) (64) and complement can activate the endothelial cells, the latter relevant to both acute and chronic rejection. Exposure to MAC in sublytic concentrations, as soluble C5b-9, or C3a and C5a, increases expression of E-selectin, ICAM-1, and VCAM-1 on cultured endothelial cells (65). MAC elicits signals for endothelial-cell proliferation, as shown by the release of PDGF and bFGF (66), and for the production of the chemokines CCL2, IL-8 and CCL5, through stimulation of IL-1 $\alpha$  production (67). C3a and C5a increase the endothelial expression of cytokines and chemokines (such as IL-6, IL-8, IL-1 $\alpha$  and

CCL5), and promote signaling through the MAP kinase pathway (68, 69). Antibodies also have effects on the endothelium independent of antibody. In vitro anti-MHC class I antibodies promote endothelial proliferation via increased expression of basic FGF receptors, increased phosphorylation of Src, and NF- $\kappa$ B levels (70). In mice, non-complement fixing IgG alloantibodies are associated with graft acceptance, but can also activate endothelial cells to produce chemokines and promote rejection (45). Further studies will be needed to determine whether complement fixation is necessary for chronic rejection. Recent studies in the mouse indicate that the chronic arteriopathy can be induced in C3 deficient RAG1<sup>-/-</sup> mice give passive antibody (Hirohashi et al unpublished).

**Complement effects on B cells(38).** The magnitude of the IgG response to alloantigens are dependent on C3 and C4, but not C5, as shown using skin grafts in C3, C4 or C5 knockout mice (71). B cells and dendritic cells express the complement receptors CD35 (CR1, C3b -C4b) and CD21 (C3d receptor), and are therefore able to retain antigen covalently linked to C3 or C4. Engagement of CD21 lowers the threshold for B-cell activation and thereby acts as a natural adjuvant (72). This also promotes B cell antigen presentation to T cells for those antigens also able to bind to that B cell Ig (73). The prominent accumulation of C4d and C3d in normal germinal centers can be taken as evidence for complement participation in B cell activation (74).

**Practical aspects of C4d staining.** The Banff03 defined positive C4d by immunofluorescence as "widespread, strong linear circumferential peritubular capillary (PTC) staining in cortex or medulla", often interpreted as >50% of the capillaries. Authors have used a variety of cutoffs for the <50% cases. Banff 2007 has added more specific definitions of C4d extent, with the intention that comparisons will be easier between publications (Solez et al, submitted).

#### Banff 2007 C4d Scores (% of biopsy with PTC+)

C4d0: Negative:	0%
C4d1: Minimal C4d stain/detection:	1<10%
C4d2: Focal C4d stain/positive:	10-50%
C4d3: Diffuse C4d stain/positive:	>50%

As stated in the recent Banff meeting report (Solez et al, submitted), the interpretation of C4d staining should be adjusted for the applied technique. IHC on paraffin section is usually less sensitive by about one grade level (i.e. diffuse staining on IF (cryosections) can be seen as focal on IHC (paraffin sections) (75, 76). Therefore, the report should indicate the actual % of tissue involved and the potential clinical significance. For example, diffuse positive C4d by IF or IHC is highly correlated with circulating anti-donor antibody. Focal positive C4d by IHC is possibly equivalent to diffuse positive IF, and should be re-tested on IF, if possible. The clinical significance of focal positive C4d by IF or minimal C4d by IHC is unknown. Meehan and colleagues found that focal C4d in PTC had one year graft losses no different from C4d negative cases (77). In contrast, Magil and Tinckam found that the outcome of focal C4d was worse than those without C4d (78). C4d staining of  $\geq 25\%$  of the PTCs by immunohistochemistry was associated with decreased one year graft survival (79).

#### IV. Accommodation

Accommodation is defined as normal graft function (and histology) in the presence of antibodies to donor antigens (80). This has been observed most commonly in transplants across an ABO barrier after pre-transplant reduction of antibodies. Remarkably, even if the ABO antibodies

return after 4-6 weeks, no obvious graft rejection occurs. 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 (80). Accommodation in ABO incompatible grafts is not due to a change in the nature of the antibody or to loss of the target antigen, since C4d is deposited in the renal microcirculation. Protocol biopsies have revealed C4d along the PTC in 25-80% of ABO incompatible grafts, with evidence of AHR in only 4-12% (42, 81). 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 acute humoral rejection (42). About 40% of the C4d+ biopsies (14/37) had C3d, and C3d correlated somewhat with histologic features of acute humoral rejection, however 70% of the C3d+ biopsies also had no evidence of injury, arguing that accommodation occurs distal to C3 activation (perhaps via inhibition of MAC). In any case, C4d deposition in ABO incompatible grafts and therefore is of limited diagnostic value.

C4d deposition also occurs in 2-26% of histologically normal ABO compatible grafts, the higher frequency found in HLA-presensitized patients (42, 82). In these patients incidental C4d deposition does not necessarily portend acute humoral rejection, however, it may not be entirely benign. Among 17 patients who had C4d in PTC without histologic evidence of acute humoral or cellular rejection, and who received no increased immunosuppression, graft loss at 3 years was 32% compared with 0% among those 5 patients treated with increased immunosuppression, suggesting that incidental C4d may represent a “smoldering’ rejection” (83).

The most recent Banff meeting (2007) agreed to include a new diagnostic category, “ C4d deposition without morphologic evidence of active rejection” (Solez et al, submitted). The criteria are:

- C4d+ peritubular capillaries
- Circulating anti-donor antibodies
- No histologic sign of active rejection, including g0, cg0, ptc0 and no ptc lamination

Cases with simultaneous borderline changes or acute tubular necrosis are considered as indeterminate. The term accommodation was not used, because the long term stability of this condition has not been established.

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. Controlled trials and further follow-up will be needed to interpret the long-term significance of “incidental” C4d deposition. 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 (1).

## REFERENCES

1. Colvin RB, Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol*, 2007; 18: 1046-56.
2. Zhou W, Medof ME, Heeger PS, Sacks S, Graft-derived complement as a mediator of transplant injury. *Curr Opin Immunol*, 2007; 19: 569-76.
3. Brook NR, White SA, Waller JR, Veitch PS, Nicholson ML, Non-heart beating donor kidneys with delayed graft function have superior graft survival compared with conventional heart-beating donor kidneys that develop delayed graft function. *Am J Transplant*, 2003; 3: 614-8.
4. Colvin RB, Nickleit V, *Renal transplant pathology*, in *Heptinstall's Pathology of the Kidney*, J.C. Jennette, et al., Editors. 2006, Lippincott-Raven: Philadelphia. p. 1347-490.
5. Zhou W, Farrar CA, Abe K, Pratt JR, Marsh JE, Wang Y, Stahl GL, Sacks SH, Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J Clin Invest*, 2000; 105: 1363-71.
6. Selander B, Martensson U, Weintraub A, Holmstrom E, Matsushita M, Thiel S, Jensenius JC, Truedsson L, Sjöholm AG, Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2. *J Clin Invest*, 2006; 116: 1425-34.
7. Austen WG, Jr., Zhang M, Chan R, Friend D, Hechtman HB, Carroll MC, Moore FD, Jr., Murine hindlimb reperfusion injury can be initiated by a self-reactive monoclonal IgM. *Surgery*, 2004; 136: 401-6.
8. Zhang M, Alicot EM, Chiu I, Li J, Verna N, Vorup-Jensen T, Kessler B, Shimaoka M, Chan R, Friend D, Mahmood U, Weissleder R, Moore FD, Carroll MC, Identification of the target self-antigens in reperfusion injury. *J Exp Med*, 2006; 203: 141-52.
9. Park P, Haas M, Cunningham PN, Bao L, Alexander JJ, Quigg RJ, Injury in renal ischemia-reperfusion is independent from immunoglobulins and T lymphocytes. *Am J Physiol Renal Physiol*, 2002; 282: F352-7.
10. Burne-Taney MJ, Ascon DB, Daniels F, Racusen L, Baldwin W, Rabb H, B cell deficiency confers protection from renal ischemia reperfusion injury. *J Immunol*, 2003; 171: 3210-5.
11. Zhang M, Takahashi K, Alicot EM, Vorup-Jensen T, Kessler B, Thiel S, Jensenius JC, Ezekowitz RA, Moore FD, Carroll MC, Activation of the lectin pathway by natural IgM in a model of ischemia/reperfusion injury. *J Immunol*, 2006; 177: 4727-34.
12. Turnberg D, Botto M, Lewis M, Zhou W, Sacks SH, Morgan BP, Walport MJ, Cook HT, CD59a deficiency exacerbates ischemia-reperfusion injury in mice. *Am J Pathol*, 2004; 165: 825-32.
13. Yamada K, Miwa T, Liu J, Nangaku M, Song WC, Critical protection from renal ischemia reperfusion injury by CD55 and CD59. *J Immunol*, 2004; 172: 3869-75.
14. Thurman JM, Ljubanovic D, Royer PA, Kraus DM, Molina H, Barry NP, Proctor G, Levi M, Holers VM, Altered renal tubular expression of the complement inhibitor Crry permits complement activation after ischemia/reperfusion. *J Clin Invest*, 2006; 116: 357-68.
15. Thurman JM, Lucia MS, Ljubanovic D, Holers VM, Acute tubular necrosis is characterized by activation of the alternative pathway of complement. *Kidney Int*, 2005; 67: 524-30.
16. Biancone L, David S, Della Pietra V, Montrucchio G, Cambi V, Camussi G, Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. *Kidney Int*, 1994; 45: 451-60.
17. Khan TN, Sinniah R, Role of complement in renal tubular damage. *Histopathology*, 1995; 26: 351-6.
18. de Vries B, Walter SJ, Peutz-Kootstra CJ, Wolfs TG, van Heurn LW, Buurman WA, The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. *Am J Pathol*, 2004; 165: 1677-88.
19. De Vries B, Matthijsen RA, Wolfs TG, Van Bijnen AA, Heeringa P, Buurman WA, Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: inhibition of late apoptosis and inflammation. *Transplantation*, 2003; 75: 375-82.
20. Pratt JR, Jones ME, Dong J, Zhou W, Chowdhury P, Smith RA, Sacks SH, Nontransgenic hyperexpression of a complement regulator in donor kidney modulates transplant ischemia/reperfusion damage, acute rejection, and chronic nephropathy. *Am J Pathol*, 2003; 163: 1457-65.

21. Arumugam TV, Shiels IA, Strachan AJ, Abbenante G, Fairlie DP, Taylor SM, A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. *Kidney Int*, 2003; 63: 134-42.
22. Thurman JM, Royer PA, Ljubanovic D, Dursun B, Lenderink AM, Edelstein CL, Holers VM, Treatment with an inhibitory monoclonal antibody to mouse factor B protects mice from induction of apoptosis and renal ischemia/reperfusion injury. *J Am Soc Nephrol*, 2006; 17: 707-15.
23. Zheng X, Feng B, Chen G, Zhang X, Li M, Sun H, Liu W, Vladau C, Liu R, Jevnikar AM, Garcia B, Zhong R, Min WP, Preventing renal ischemia-reperfusion injury using small interfering RNA by targeting complement 3 gene. *Am J Transplant*, 2006; 6: 2099-108.
24. Smith PK, Carrier M, Chen JC, Haverich A, Levy JH, Menasche P, Shernan SK, Van de Werf F, Adams PX, Todaro TG, Verrier E, Effect of pexelizumab in coronary artery bypass graft surgery with extended aortic cross-clamp time. *Ann Thorac Surg*, 2006; 82: 781-8; discussion 88-9.
25. Endo T, Nakao S, Koizumi K, Nishio M, Fujimoto K, Sakai T, Kumano K, Obara M, Koike T, Successful treatment with rituximab for autoimmune hemolytic anemia concomitant with proliferation of Epstein-Barr virus and monoclonal gammopathy in a post-nonmyeloablative stem cell transplant patient. *Ann Hematol*, 2004; 83: 114-6.
26. Andrews PA, Pani A, Zhou W, Sacks SH, Local transcription of complement C3 in human allograft rejection. Evidence for a pathogenic role and correlation to histology and outcome. *Transplantation*, 1994; 58: 637-40.
27. Serinsoz E, Bock O, Gwinner W, Schwarz A, Haller H, Kreipe H, Mengel M, Local complement C3 expression is upregulated in humoral and cellular rejection of renal allografts. *Am J Transplant*, 2005; 5: 1490-4.
28. Tang S, Zhou W, Sheerin NS, Vaughan RW, Sacks SH, Contribution of renal secreted complement C3 to the circulating pool in humans. *J Immunol*, 1999; 162: 4336-41.
29. Andrews PA, Finn JE, Lloyd CM, Zhou W, Mathieson PW, Sacks SH, Expression and tissue localization of donor-specific complement C3 synthesized in human renal allografts. *European Journal of Immunology*, 1995; 25: 1087-93.
30. Pratt JR, Basheer SA, Sacks SH, Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med*, 2002; 8: 582-7.
31. Van Kooten C, Boonstra JG, Paape ME, Fossiez F, Banchereau J, Lebecque S, Bruijn JA, De Fijter JW, Van Es LA, Daha MR, Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *Journal of the American Society of Nephrology*, 1998; 9: 1526-34.
32. Brooimans RA, Stegmann AP, van Dorp WT, van der Ark AA, van der Woude FJ, van Es LA, Daha MR, Interleukin 2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. *J Clin Invest*, 1991; 88: 379-84.
33. Lin T, Zhou W, Farrar CA, Hargreaves RE, Sheerin NS, Sacks SH, Deficiency of C4 from donor or recipient mouse fails to prevent renal allograft rejection. *Am J Pathol*, 2006; 168: 1241-8.
34. Peng Q, Li K, Patel H, Sacks SH, Zhou W, Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol*, 2006; 176: 3330-41.
35. Lalli PN, Strainic MG, Lin F, Medof ME, Heeger PS, Decay accelerating factor can control T cell differentiation into IFN-gamma-producing effector cells via regulating local C5a-induced IL-12 production. *J Immunol*, 2007; 179: 5793-802.
36. Brown KM, Kondeatis E, Vaughan RW, Kon SP, Farmer CK, Taylor JD, He X, Johnston A, Horsfield C, Janssen BJ, Gros P, Zhou W, Sacks SH, Sheerin NS, Influence of donor C3 allotype on late renal-transplantation outcome. *N Engl J Med*, 2006; 354: 2014-23.
37. Rotman S, Collins AB, Colvin RB, C4d deposition in allografts: Current concepts and interpretation. *Transplantation Reviews*, 2005; 19: 65-77.
38. Colvin RB, Smith RN, Antibody-mediated organ-allograft rejection. *Nat Rev Immunol*, 2005; 5: 807-17.
39. 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*, 2004; 4: 1033-41.
40. 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*, 2002; 13: 779-87.
41. Bohmig GA, Exner M, Habicht A, Schillinger M, Lang U, Kletzmayer 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*, 2002; 13: 1091-9.
  42. 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*, 2006; 6: 1829-40.
  43. 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 transplant recipients with chronic allograft nephropathy. *Transplantation*, 2005; 79: 1459-61.
  44. 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. *Transplantation Proc*, 2006; in press.
  45. 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*, 2004; 4: 326-34.
  46. Gao ZH, McAlister VC, Wright JR, Jr., McAlister CC, Peltekian K, MacDonald AS, Immunoglobulin-G subclass antidonor reactivity in transplant recipients. *Liver Transpl*, 2004; 10: 1055-9.
  47. Wahrmann M, Exner M, Regele H, Derfler K, Kormoczi GF, Lhotta K, Zlabinger GJ, Bohmig GA, Flow cytometry based detection of HLA alloantibody mediated classical complement activation. *J Immunol Methods*, 2003; 275: 149-60.
  48. 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*, 2006; 6: 1033-41.
  49. Smith JD, Hamour IM, Banner NR, Rose ML, C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. *Am J Transplant*, 2007; 7: 2809-15.
  50. 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*, 2003; 75: 1204-13.
  51. 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*, 2003; 76: 102-8.
  52. 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*, 2005; 79: 1435-40.
  53. 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*, 2006; 114: 700-11.
  54. 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*, 2004; 18 Suppl 11: 18-23.
  55. 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*, 2006; 21: 2589-95.
  56. Mauyyedi 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. *Journal of the American Society of Nephrology*, 2001; 12: 574-82.
  57. 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, Nickleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madruga L, Salomon DR, Seron D, Sheaff M, Weening JJ, Banff

- '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant*, 2007; 7: 518-26.
58. Solez K, Colvin RB, Racusen L, Sis B, Halloran PH, Birk P, Cascalho M, Collins AB, Campbell PM, Demetris A, Banff '05 meeting report: Differential diagnosis of chronic injury and elimination of chronic allograft nephropathy ("CAN") in the Banff schema. *American J. Transplantation*, in press.
  59. 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*, 2002; 13: 2371-80.
  60. David-Neto E, Prado E, Beutel A, Ventura CG, Siqueira SA, Hung J, Lemos FB, de Souza NA, Nahas WC, Ianhez LE, David DR, C4d-positive chronic rejection: a frequent entity with a poor outcome. *Transplantation*, 2007; 84: 1391-8.
  61. Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, Cruz J, Meng C, Wishart D, Solez K, Halloran PF, Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant*, 2007; 7: 1743-52.
  62. 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*, 2006; 6: 1790-8.
  63. 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*, 2007; 7: 57-65.
  64. Nakashima S, Qian Z, Rahimi S, Wasowska BA, Baldwin WM, 3rd, Membrane attack complex contributes to destruction of vascular integrity in acute lung allograft rejection. *J Immunol*, 2002; 169: 4620-7.
  65. Saadi S, Holzknrecht RA, Patte CP, Platt JL, Endothelial cell activation by pore-forming structures: pivotal role for interleukin-1alpha. *Circulation*, 2000; 101: 1867-73.
  66. Benzaquen LR, Nicholson-Weller A, Halperin JA, Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *Journal of Experimental Medicine*, 1994; 179: 985-92.
  67. Selvan RS, Kapadia HB, Platt JL, Complement-induced expression of chemokine genes in endothelium: regulation by IL-1-dependent and -independent mechanisms. *J Immunol*, 1998; 161: 4388-95.
  68. Albrecht EA, Chinnaiyan AM, Varambally S, Kumar-Sinha C, Barrette TR, Sarma JV, Ward PA, C5a-induced gene expression in human umbilical vein endothelial cells. *Am J Pathol*, 2004; 164: 849-59.
  69. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC, Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *Faseb J*, 2003; 17: 1003-14.
  70. 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*, 2002; 168: 5415-23.
  71. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH, The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation*, 2001; 72: 1310-8.
  72. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT, C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science*, 1996; 271: 348-50.
  73. Prechl J, Baiu DC, Horvath A, Erdei A, Modeling the presentation of C3d-coated antigen by B lymphocytes: enhancement by CR1/2-BCR co-ligation is selective for the co-ligating antigen. *Int Immunol*, 2002; 14: 241-7.
  74. Zwirner J, Felber E, Schmidt P, Riethmuller G, Feucht HE, Complement activation in human lymphoid germinal centres. *Immunology*, 1989; 66: 270-7.
  75. 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*, 2005; 36: 1178-85.

76. Seemayer CA, Gaspert A, Nিকেleit V, Mihatsch MJ, C4d staining of renal allograft biopsies: comparative analysis of different staining techniques. *Nephrol Dial Transplant*, 2006.
77. 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*, 2005; 79: 228-35.
78. Magil AB, Tinckam KJ, Focal peritubular capillary C4d deposition in acute rejection. *Nephrol Dial Transplant*, 2006; 21: 1382-8.
79. Lorenz M, Regele H, Schillinger M, Exner M, Rasoul-Rockenschaub S, Wahrmann M, Kletzmayer J, Silberhumer G, Horl WH, Bohmig GA, Risk factors for capillary C4d deposition in kidney allografts: evaluation of a large study cohort. *Transplantation*, 2004; 78: 447-52.
80. 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*, 2003; 3: 952-60.
81. 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*, 2004; 4: 101-7.
82. Mengel M, Bogers J, Bosmans J-P, Serón D, Moreso F, Carrera M, Gwinner W, Schwarz A, De Broe M, Kreipe H, Haller H, Incidence of C4d stain in protocol biopsies from renal allografts: Results from a multicenter trial. *Am J Transplant*, 2004; in press.
83. Dickenmann M, Steiger J, Descoeurdes B, Mihatsch M, Nিকেleit V, The fate of C4d positive kidney allografts lacking histological signs of acute rejection. *Clin Nephrol*, 2006; 65: 173-9.

