

Complement and Renal Transplantation: From Donor to Recipient

Jeffrey Damman,^{1,3} Theo A. Schuurs,¹ Rutger J. Ploeg,¹ and Marc A. Seelen²

Long-term kidney graft survival is affected by different variables including donor condition, ischemia-reperfusion injury, and graft rejection during the transplantation process. The complement system is an important mediator of renal ischemia-reperfusion injury and in rejecting allografts. However, donor complement C3 seems to be crucial in renal transplantation-related injury as renal injury is attenuated in C3 deficient kidney grafts. Interestingly, before ischemia-reperfusion induced C3 expression, C3 is already induced in donors suffering from brain death. Therefore, strategies targeting complement activation in the brain-dead donor may increase graft viability and transplant outcome.

Keywords: Brain death, Donor, Complement, Kidney.

(*Transplantation* 2008;85: 923–927)

Long-term graft survival of renal transplants is determined by different variables including donor condition, kidney preservation, human leukocyte antigen matching, duration of cold and warm ischemia, and injury caused by reperfusion during transplantation (1). For years, several research groups have focused on the impact of ischemia-reperfusion injury (IRI) on long-term kidney graft survival. Besides the importance of IRI, also the condition of the donor does determine long-term graft survival. Terasaki et al. showed that kidneys obtained from living donors, despite a high degree of human leukocyte antigen mismatching, had higher survival rates compared with deceased grafts obtained from brain-dead or non-heart beating donors. These results did not depend on the duration of cold ischemia, so other causes had to be considered (2). As the majority of deceased grafts are derived from brain-dead donors, the hypothesis was formulated and subsequently confirmed during the past years that brain death (BD) should be regarded as a risk factor for long-term graft survival. Our group and others have shown the negative influence of donor BD on organ viability and survival rates after transplantation in various organs (3–6).

Recently, several groups investigated the role of complement in clinical renal transplantation. Complement component C3 is the central complement component on which

all complement pathways converge. Local renal production of complement C3 seems important in graft rejection, because the absence of locally synthesized C3 significantly reduces renal allograft rejection (7). These studies emphasize the pathogenic role of complement in renal transplantation-related injury and indicate that strategies targeting complement induction during the transplantation process could improve transplant outcome. In this review, we will discuss three moments of complement activation during the transplant process: at the time of rejection, IRI, and as a result of donor condition.

The Complement System

The complement system is an important part of the innate immune response and has diverse roles in defensive immunity. Essentially, the complement system is involved in the elimination of pyogenic bacteria, the interaction between innate and adaptive immunity, and the clearance of cellular debris and immune complexes after inflammatory injury. Three different complement pathways can be distinguished: the alternative pathway, the classical pathway, and the lectin pathway. The alternative pathway cascade is activated when hydrolyzed C3, which is continuously formed through cleavage of systemic C3 (“C3 tickover”), binds to microbe cell surface components in vicinity. The classical pathway is activated after the binding of C1 to antibody–antigen complexes, damaged cells, or cell particles. The lectin pathway is activated when circulating lectins such as mannose binding lectin (MBL) and ficolins bind to carbohydrate mannose residues on certain microbes. Activation of the three pathways results in the formation of a C3- and subsequently a C5 convertase eventually leading to the formation of the membrane attack complex (MAC) C5b–C9. In addition to the capacity to generate MAC, the split products of C3, C4, and C5 have immunomodulating functions (8, 9).

¹ Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands.

² Department of Nephrology, University Medical Center Groningen, Groningen, The Netherlands.

³ Address correspondence to: Jeffrey Damman, Department of Surgery, University Medical Center Groningen, University of Groningen, CMC V, Y2144, Hanzplein 1, 9713 GZ Groningen, The Netherlands.

E-mail: j.damman@chir.umcg.nl

Received 2 November 2007. Revision requested 26 November 2007.

Accepted 24 December 2007.

Copyright © 2008 by Lippincott Williams & Wilkins

ISSN 0041-1337/08/8507-923

DOI: 10.1097/TP.0b013e3181683cf5

Complement and Renal Transplantation

Rejection

Schematically, renal allograft rejection can be divided in humoral (i.e., B-cell dependent) and cellular (i.e., T-cell dependent) rejection. Interaction between complement and the B-cell response in renal transplantation has been extensively described; however, the pathogenesis of complement mediated T-cell responses still remains unclear (10–13). Recently, it is shown that donor proximal epithelium cell-bound C3 and dendritic C3 are able to potentiate the T-cell response in vitro, whereas macrophages deficient for C3 have an impaired potency to stimulate alloreactive T-cells (14–16). These results suggest that C3 is able to interact with alloreactive T-cells, enhancing the alloimmune response. Evidence exists that C3 is activated through the alternative pathway, as increased synthesis of alternative pathway components are observed at the interaction between antigen presenting cells (APCs) and T-cells (17). Moreover, absence of donor factors B and D showed impaired T-cell responses whereas absence of C4 in vivo or in vitro does not influence, respectively, T-cell responses or allograft rejection (16–18).

Once C3 is activated, C3a and C5a are generated that are able to potentiate the T-cell response through their receptors. In this context, Heeger et al. showed that decay accelerating factor, which dissociates C3- and C5-convertases on the cell surface, is a potent regulator of T-cell differentiation. Absence of decay accelerating factor on APCs enhances C5a dependent IL-12 secretion by APCs thereby stimulating T-cell differentiation (19). Besides, also C3a is able to regulate the alloimmune response through its receptor on APCs (20). Extensive review articles on the role of T-cells and complement in allograft rejection are recently published by Sacks et al. and Zhou et al. (21, 22).

Ischemia-Reperfusion Injury

Renal IRI occurs when a reduced or deprived blood flow exists through the renal artery followed by recovery of blood perfusion, provoking an inflammatory response. The process is complex and poorly understood but is, among other factors, characterized by infiltration of neutrophils, induction of proinflammatory proteins on vascular endothelial cells, reactive oxygen species formation, and complement activation (23–25). Regarding complement, Zhou et al. showed that C3-, C5-, and C6-deficient mice were protected from renal IRI, whereas C4 deficient mice remained susceptible for IRI. Substitution of C6-deficient mice with C6 did indeed reestablish sensitivity for IRI whereas treatment with C5a antibodies did not show additional renal protection (26). These findings indicate that the injury seen after renal IR is probably regulated by the MAC and less by C5a mediated neutrophil recruitment. However, de Vries et al. showed that a C5a-receptor-antagonist (C5aRA) was capable to attenuate renal IRI. Protective effects of C5aRA were shown to be independent of neutrophil influx pointing at other mechanisms of C5a through the C5a receptor (C5aR) (27). Possibly, there is a direct effect of C5a on the renal tubular epithelium after induction of several proinflammatory genes. Also, Arumugam et al. (28) showed a marked reduction of renal IRI after treatment with a C5aRA.

Thus it seems that C5a and the MAC are both involved in the pathogenesis of IRI in the kidney. Although Zhou et al.

showed full protection against renal IRI in C3 deficient mice, other studies showed only partial protection (29). The differences might be explained by the use of different durations of IRI. Although Zhou et al. and de Vries et al. used models with 45 to 60 min of IRI, IRI induced by Park et al. took only 20 to 30 min. Evidence exists that the injury caused by short time periods of ischemia followed by reperfusion are mediated by neutrophils whereas longer time periods are mediated by apoptosis (30, 31). Therefore, in the studies using longer ischemia times, contribution of complement, and especially contribution of the MAC, to IRI seems to be more significant. Besides differences in duration of IR also different mouse strains, different models of IRI and the unknown secondary effects of knock-outs might explain the differences in observed outcomes.

It is important to know through which complement pathways IRI is mediated as it disguises its initiation. The study carried out by Zhou et al. (26), showed that mice deficient in C4 were not protected against renal IRI. In addition, absence of C4 in the donor or recipient fails to prevent renal allograft rejection in mice (18). These data indicate that renal IRI is most probably not mediated through the classical pathway as C4 is an essential component of the classical complement pathway. Furthermore, Park et al. demonstrated that renal IRI is independent from immunoglobulins and T-lymphocytes, although others determined an important role for T- and B-cells in IRI (29, 32, 33). However, B-cell mediated IRI seems to occur independent of complement (32). Confirming the importance of the alternative pathway, additional studies show less IRI, a decline of tubulointerstitial C3 deposition, and neutrophil influx in factor B deficient mice (34). Also the increased alternative pathway activation in kidney biopsies from patients with acute tubular necrosis was observed (35). Recently, involvement of the MBL-pathway in renal IRI was demonstrated in a mouse renal IRI model and in clinical posttransplant acute renal failure. A strong colocalization was seen between MBL-A and -C depositions and C6 deposition, indicating MBL involvement of renal complement activation (36). Furthermore, knock-out mice for MBL-A and MBL-C were protected from kidney damage after IR. Reconstitution of knock-out mice with recombinant human MBL before surgery restored renal damage after IR, confirming the importance of the MBL-pathway in renal IRI (37). Probably, MBL-mediated renal IRI acts through direct splicing of C3 by mannan-binding lectin serine peptidase 1 and not through C4, as C4 knock-out mice were not protected against renal IRI. Thus several studies indicate a prominent role of both the alternative- and MBL pathway and less for the classical pathway in renal IRI.

Renal IRI mainly involves tubular epithelium and thus, if circulating complement components are important in renal IRI, penetration through the vascular endothelium into the extravascular compartment is needed. However, some of the major complement components have such a large molecular size that penetration through the basal membrane is probably very poor if not impossible. Therefore, involvement of locally produced complement in the extravascular compartment seems more likely, whereas circulating complement components possibly have a more prominent role in vascular endothelial damage after reperfusion of an ischemic kidney. Although most complement components are synthesized in

the liver by hepatocytes during the acute phase response, the kidney itself is a prominent producer of complement. Endothelial cells, mesangial cells, glomerular epithelial cells, and tubular epithelial cells are capable of producing complement components *in vitro* (38–44). One reason that locally extravascular produced C3 is such a major factor responsible for renal IRI, might be the lack of regulator proteins on the tubular epithelium. Normally, the complement system is tightly regulated because of the presence of complement regulator proteins (45, 46). These proteins protect complement-mediated injury in the host and deficiency of these proteins is associated with several renal diseases (47–50). CR1/related gene-protein *y* (*Crry*) is expressed on rodent tubular epithelial cells being most susceptible for IRI (51). *Crry* is a complement inhibitor, binding C3 and C5 convertases, thereby reducing both classical and alternative complement pathway activity. Decreased *Crry* expression leads to increased sensitivity for IRI and poorer graft function (51, 52). Moreover, mice overexpressing *Crry* are protected from acute renal failure. In contrast, treatment with *Crry*-Ig did not influence renal IRI outcome, which can be explained by the short time period of IR and the use of heparin, a potent complement inhibitor, obscuring possible benefits (53). In summary, these results indicate that endogenous *Crry* expression regulates complement activation on the tubular epithelium. As complement is highly expressed on the renal tubular epithelium in IRI, it is likely that *Crry* complement regulation is overwhelmed by tubular complement deposition or tubular *Crry* is lost because of ischemia.

Donor Condition

During the past years more and more evidence has been found that not only posttransplantation factors, but also the condition of the donor is of major importance for long-term kidney graft survival (2). In kidney transplantation, grafts are retrieved from living, heart beating, and non-heart-beating donors. The majority of donor kidneys are retrieved from heart beating or brain-dead donors who suffer from hemodynamic and hormonal instabilities resulting in immune activation, thereby affecting organ viability before transplantation. The nature of organ immune activation in brain-dead patients was first investigated using rat brain-dead models. A series of animal studies by different groups have shown upregulation of proinflammatory molecules and influx of polymorphonuclear cells and MØ in the kidney (5, 6, 54). Also in human brain-dead donors, an identical immune activation was observed in donor kidneys (55). Furthermore, kidneys having suffered from BD show poorer function and lower graft survival after transplantation (56, 57). Therefore, appropriate treatment in the donor will be effective to improve donor organ quality and subsequently transplant outcome. A better insight however in the processes of BD is essential to determine the target of intervention. Successful intervention strategies to reduce the proinflammatory response induced by BD have been reported. Dopamine has been shown to reduce major MHC class II and P-selectin expression and prevented upregulation of TNF- α and MCP-1 (58). Furthermore, P-selectin blockade therapy has been demonstrated to improve renal allograft function from brain-dead donors (59). Interestingly, enhanced complement production is required for upregulation of P-selectin and CXC chemokines

(such as MCP-1) in IR models (60, 61). Finally, recent findings suggest that interventions may be even more efficient when given already to brain dead donors. Local production of complement already in the donor kidney might have devastating consequences for kidney viability (7). Interestingly, also donor C3 allotype influences long-term renal transplant outcome (62). Kusaka et al. (6) were the first to demonstrate the presence of complement in kidneys of rat brain-dead donors. Recently, our group observed comparable results in a time course experiment of BD. Briefly, BD was induced in rats and organs were retrieved after 0.5, 1, and 4 hr of BD. Kidneys showed an eight times upregulation of complement C3 gene expression after 4 hr of BD compared with sham operated animals (data not published). These data suggest involvement of the complement system in the pathogenesis of renal injury caused by BD. To evaluate the clinical validity of complement expression in rat brain-dead donors, we examined C3 expression in human kidney biopsies obtained from human brain-dead and living donors during organ procurement. Gene expression of C3 was significantly higher (eightfold) in kidney biopsies obtained from brain-dead donors compared with living (un)related donors. Furthermore, additional experiments suggested no enhanced renal C3 expression resulting from injury related to cold ischemia or reperfusion after the pre-existing upregulation after BD. Most interestingly, we also demonstrated an association between C3 gene expression and renal transplant function in allograft from brain-dead donors early after transplantation: High C3 expression in kidneys from brain-dead donors after transplantation is negatively associated with early posttransplant renal function.

These findings suggest that after reperfusion of the renal allograft, large amounts of complement, already induced by the BD insult before transplantation, are present in vicinity of damaged tissue. Besides, no additional C3 expression is found because of reperfusion after BD. For that reason, the intervention in complement activation in allografts from brain-dead donors before transplantation is potentially more effective to improve allograft function than at the time point of reperfusion injury after transplantation.

Targeting the Complement System

Because involvement of complement in renal transplantation becomes more clear, strategies targeting the complement system might become a promising approach in reducing renal allograft injury with subsequent improvement of transplant outcome. The complement activation cascade can be targeted at different levels to attenuate complement mediated injury using, for example, soluble complement receptor 1 (sCR1), C5 antibodies, or a C5aRA.

sCR1 is a complement regulator protein and has two important functions in diminishing complement cascade activation: acting as a cofactor for C3b and C4b breakdown and acceleration of C3- and C5-convertase degradation (63). In a rat model of intestinal IRI, treatment with sCR1 significantly reduced intestinal myeloperoxidase activity and mucosal injury (64). sCR1 administration also ameliorated the protection of the rat liver from IRI (65, 66). In renal transplantation, sCR1 has been shown to prevent acute rejection and to extend kidney allograft survival (67, 68). In clinical practice, sCR1 has already been successfully used in the treatment for myocardial IRI thereby reducing myocardial infarct

size by at least 44% (69–71). Moreover, TP10 (an sCR1) did significantly decrease incidence of mortality and myocardial infarction in high-risk male patients undergoing cardiac surgery or cardiopulmonary bypass, although primary endpoints of the study were not improved (myocardial infarction, prolonged intraaortic balloon pump support, prolonged intubation) (72).

Other complement regulator proteins (e.g., CD59, CD55, or CD46) or agents upregulating these proteins are possible mechanisms for intervention. Zhang et al. (73), for example, managed to produce a fusion protein of an immunoglobulin with CD59 (CD59-Ig), which has the ability to inhibit MAC-formation in a site-specific manner. Because MAC formation plays a role in renal IRI, this might be a tool preventing MAC-induced renal injury during IR.

Another approach to inhibit complement activation more downstream in the cascade is by blocking the conversion of C5 and thereby inhibiting formation of C5a and the MAC. Amsterdam et al. were the first to investigate the influence of monoclonal antibody administration against C5a during myocardial infarction in pigs. They found an inhibition of neutrophil cytotoxic activity after treatment with mAb against C5a (74). Furthermore, Vakeva et al. observed decreased myocardial IRI when rats were treated with mAb against complement C5 (75). Also in a mouse model of renal IRI, treatment with a monoclonal antibody against C5 prevented late inflammation and apoptosis (76). Concerning renal IRI, as described before, several groups managed to reduce kidney injury (28) and even renal function (27) after treatment with C5aRA in different models of renal IRI.

Another strategy to target complement-mediated injury is by using small interfering RNA thereby silencing C3 or C5, consequently attenuating or inhibiting further complement-mediated injury (77, 78). Furthermore, blocking the alternative pathway of complement-reduced serum urea nitrogen levels and extent of apoptosis after IRI in treated mice compared with controls (79).

Thus, several strategies can be effectively used to target the complement system in different models of IRI. Targeting complement activation should be focused on alternative pathway and MBL-pathway dependent activation of the terminal pathway, and production of chemokines such as C5a and C3a. Finally, recent findings suggest that interventions may be even more efficient when given already to BD donors.

CONCLUSION

Absence of C3 in transplanted kidneys greatly improves kidney survival after transplantation, indicating the importance of local complement production by the donor kidney. It is proven that complement has important mediator functions during kidney rejection and renal IRI. However, complement is already induced after the onset of BD in the donor, before kidney transplantation. Therefore, if contribution of BD-induced complement seems to be substantial, intervening in complement induction has to occur before or shortly after the onset of BD.

REFERENCES

- Pirsch JD, Ploeg RJ, Gange S, et al. Determinants of graft survival after renal transplantation. *Transplantation* 1996; 61: 1581.
- Terasaki PI, Cecka JM, Gjertson DW, et al. High survival rates of kidney transplants from spousal and living unrelated donors. *N Engl J Med* 1995; 333: 333.
- van der Hoeven JA, Molema G, Ter Horst GJ, et al. Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased immunologic activation of donor kidneys. *Kidney Int* 2003; 64: 1874.
- van der Hoeven JA, Ploeg RJ, Postema F, et al. Induction of organ dysfunction and up-regulation of inflammatory markers in the liver and kidneys of hypotensive brain dead rats: A model to study marginal organ donors. *Transplantation* 1999; 68: 1884.
- Schuurs TA, Morariu AM, Ottens PJ, et al. Time-dependent changes in donor brain death related processes. *Am J Transplant* 2006; 6: 2903.
- Kusaka M, Pratschke J, Wilhelm MJ, et al. Activation of inflammatory mediators in rat renal isografts by donor brain death. *Transplantation* 2000; 69: 405.
- Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med* 2002; 8: 582.
- Walport MJ. Complement. First of two parts. *N Engl J Med* 2001; 344: 1058.
- Walport MJ. Complement. Second of two parts. *N Engl J Med* 2001; 344: 1140.
- Carroll MC. The complement system in B cell regulation. *Mol Immunol* 2004; 41: 141.
- Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu Rev Immunol* 2000; 18: 393.
- Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. *Clin Exp Immunol* 1991; 86: 464.
- Feucht HE, Schneeberger H, Hillebrand G, et al. Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; 43: 1333.
- Li K, Patel H, Farrar CA, et al. Complement activation regulates the capacity of proximal tubular epithelial cell to stimulate alloreactive T cell response. *J Am Soc Nephrol* 2004; 15: 2414.
- Peng Q, Li K, Patel H, et al. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol* 2006; 176: 3330.
- Zhou W, Patel H, Li K, et al. Macrophages from C3-deficient mice have impaired potency to stimulate alloreactive T cells. *Blood* 2006; 107: 2461.
- Heeger PS, Lalli PN, Lin F, et al. Decay-accelerating factor modulates induction of T cell immunity. *J Exp Med* 2005; 201: 1523.
- Lin T, Zhou W, Farrar CA, et al. Deficiency of C4 from donor or recipient mouse fails to prevent renal allograft rejection. *Am J Pathol* 2006; 168: 1241.
- Lalli PN, Strainic MG, Lin F, et al. 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.
- Peng Q, Li K, Anderson K, et al. Local production and activation of complement up-regulates the allostimulatory function of dendritic cells through C3a–C3aR interaction. *Blood* 2007 [Epub ahead of print].
- Sacks SH, Chowdhury P, Zhou W. Role of the complement system in rejection. *Curr Opin Immunol* 2003; 15: 487.
- Zhou W, Medof ME, Heeger PS, et al. Graft-derived complement as a mediator of transplant injury. *Curr Opin Immunol* 2007; 19: 569.
- Takada M, Nadeau KC, Shaw GD, et al. The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. *J Clin Invest* 1997; 99: 2682.
- Kelly KJ, Williams WW Jr, Colvin RB, et al. Interleukin-1-deficient mice are protected against ischemic renal injury. *J Clin Invest* 1996; 97: 1056.
- Rabb H, O'Meara YM, Maderna P, et al. Leukocytes, cell adhesion molecules and ischemic acute renal failure. *Kidney Int* 1997; 51: 1463.
- Zhou W, Farrar CA, Abe K, et al. Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J Clin Invest* 2000; 105: 1363.
- de Vries B, Kohl J, Leclercq WK, et al. Complement factor C5a mediates renal ischemia-reperfusion injury independent from neutrophils. *J Immunol* 2003; 170: 3883.
- Arumugam TV, Shiels IA, Strachan AJ, et al. A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. *Kidney Int* 2003; 63: 134.

29. Park P, Haas M, Cunningham PN, et al. Injury in renal ischemia-reperfusion is independent from immunoglobulins and T lymphocytes. *Am J Physiol Renal Physiol* 2002; 282: F352.
30. Iwata A, Harlan JM, Vedder NB, et al. The caspase inhibitor z-VAD is more effective than CD18 adhesion blockade in reducing muscle ischemia-reperfusion injury: Implication for clinical trials. *Blood* 2002; 100: 2077.
31. Daemen MA, van't V, Denecker G, et al. Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. *J Clin Invest* 1999; 104: 541.
32. Burne-Taney MJ, Ascon DB, Daniels F, et al. B cell deficiency confers protection from renal ischemia reperfusion injury. *J Immunol* 2003; 171: 3210.
33. Huang Y, Rabb H, Womer KL. Ischemia-reperfusion and immediate T cell responses. *Cell Immunol* 2007; 248: 4.
34. Thurman JM, Ljubanovic D, Edelstein CL, et al. Lack of a functional alternative complement pathway ameliorates ischemic acute renal failure in mice. *J Immunol* 2003; 170: 1517.
35. Thurman JM, Lucia MS, Ljubanovic D, et al. Acute tubular necrosis is characterized by activation of the alternative pathway of complement. *Kidney Int* 2005; 67: 524.
36. de Vries B, Walter SJ, Peutz-Kootstra CJ, et al. The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. *Am J Pathol* 2004; 165: 1677.
37. Moller-Kristensen M, Wang W, Ruseva M, et al. Mannan-binding lectin recognizes structures on ischaemic reperfused mouse kidneys and is implicated in tissue injury. *Scand J Immunol* 2005; 61: 426.
38. Sacks SH, Zhou W, Pani A, et al. Complement C3 gene expression and regulation in human glomerular epithelial cells. *Immunology* 1993; 79:348.
39. Sacks S, Zhou W, Campbell RD, et al. C3 and C4 gene expression and interferon-gamma-mediated regulation in human glomerular mesangial cells. *Clin Exp Immunol* 1993; 93: 411.
40. Zhou W, Campbell RD, Martin J, et al. Interferon-gamma regulation of C4 gene expression in cultured human glomerular epithelial cells. *Eur J Immunol* 1993; 23: 2477.
41. Hong Y, Zhou W, Li K, et al. Triptolide is a potent suppressant of C3, CD40 and B7h expression in activated human proximal tubular epithelial cells. *Kidney Int* 2002; 62: 1291.
42. Sheerin NS, Zhou W, Adler S, et al. TNF-alpha regulation of C3 gene expression and protein biosynthesis in rat glomerular endothelial cells. *Kidney Int* 1997; 51: 703.
43. Liszewski MK, Farries TC, Lublin DM, et al. Control of the complement system. *Adv Immunol* 1996; 61: 201.
44. Seelen MA, Brooimans RA, van der Woude FJ, et al. IFN-gamma mediates stimulation of complement C4 biosynthesis in human proximal tubular epithelial cells. *Kidney Int* 1993; 44: 50.
45. Ichida S, Yuzawa Y, Okada H, et al. Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int* 1994; 46: 89.
46. Pickering MC, Cook HT, Warren J, et al. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet* 2002; 31: 424.
47. Goodship TH, Liszewski MK, Kemp EJ, et al. Mutations in CD46, a complement regulatory protein, predispose to atypical HUS. *Trends Mol Med* 2004; 10: 226.
48. Richards A, Kemp EJ, Liszewski MK, et al. Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. *Proc Natl Acad Sci USA* 2003; 100: 12966.
49. Holt DS, Botto M, Bygrave AE, et al. Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. *Blood* 2001; 98: 442.
50. Li B, Sallee C, Dehoff M, et al. Mouse Crry/p65. Characterization of monoclonal antibodies and the tissue distribution of a functional homologue of human MCP and DAF. *J Immunol* 1993; 151: 4295.
51. Thurman JM, Ljubanovic D, Royer PA, et al. Altered renal tubular expression of the complement inhibitor Crry permits complement activation after ischemia/reperfusion. *J Clin Invest* 2006; 116: 357.
52. Bao L, Wang Y, Chang A, et al. Unrestricted C3 activation occurs in Crry-deficient kidneys and rapidly leads to chronic renal failure. *J Am Soc Nephrol* 2007; 18: 811.
53. Park P, Haas M, Cunningham PN, et al. Inhibiting the complement system does not reduce injury in renal ischemia reperfusion. *J Am Soc Nephrol* 2001; 12: 1383.
54. Takada M, Nadeau KC, Hancock WW, et al. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation* 1998; 65: 1533.
55. Nijboer WN, Schuurs TA, van der Hoeven JA, et al. Effect of brain death on gene expression and tissue activation in human donor kidneys. *Transplantation* 2004; 78: 978.
56. Pratschke J, Wilhelm MJ, Kusaka M, et al. Accelerated rejection of renal allografts from brain-dead donors. *Ann Surg* 2000; 232: 263.
57. Pratschke J, Wilhelm MJ, Laskowski I, et al. Influence of donor brain death on chronic rejection of renal transplants in rats. *J Am Soc Nephrol* 2001; 12: 2474.
58. Schaub M, Ploetz CJ, Gerbaulet D, et al. Effect of dopamine on inflammatory status in kidneys of brain-dead rats. *Transplantation* 2004; 77: 1333.
59. Gasser M, Waaga-Gasser AM, Grimm MW, et al. Selectin blockade plus therapy with low-dose sirolimus and cyclosporin a prevent brain death-induced renal allograft dysfunction. *Am J Transplant* 2005; 5(4 Pt 1): 662.
60. Atkinson C, Zhu H, Qiao F, et al. Complement-dependent P-selectin expression and injury following ischemic stroke. *J Immunol* 2006; 177: 7266.
61. Thurman JM, Lenderink AM, Royer PA, et al. C3a is required for the production of CXC chemokines by tubular epithelial cells after renal ischemia/reperfusion. *J Immunol* 2007; 178: 1819.
62. Brown KM, Kondeatis E, Vaughan RW, et al. Influence of donor C3 allotype on late renal-transplantation outcome. *N Engl J Med* 2006; 354: 2014.
63. Kirschfink M. Controlling the complement system in inflammation. *Immunopharmacology* 1997; 38: 51.
64. Hill J, Lindsay TF, Ortiz F, et al. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia-reperfusion in the rat. *J Immunol* 1992; 149: 1723.
65. Chavez-Cartaya RE, DeSola GP, Wright L, et al. Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. *Transplantation* 1995; 59: 1047.
66. Lehmann TG, Koepfel TA, Kirschfink M, et al. Complement inhibition by soluble complement receptor type 1 improves microcirculation after rat liver transplantation. *Transplantation* 1998; 66: 717.
67. Pratt JR, Hibbs MJ, Laver AJ, et al. Allograft immune response with sCR1 intervention. *Transpl Immunol* 1996; 4: 72.
68. Pratt JR, Hibbs MJ, Laver AJ, et al. Effects of complement inhibition with soluble complement receptor-1 on vascular injury and inflammation during renal allograft rejection in the rat. *Am J Pathol* 1996; 149: 2055.
69. Weisman HF, Bartow T, Leppo MK, et al. Soluble human complement receptor type 1: In vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* 1990; 249: 146.
70. Smith EF III, Griswold DE, Egan JW, et al. Reduction of myocardial reperfusion injury with human soluble complement receptor type 1 (BRL 55730). *Eur J Pharmacol* 1993; 236: 477.
71. Lazar HL, Bao Y, Gaudiani J, et al. Total complement inhibition: An effective strategy to limit ischemic injury during coronary revascularization on cardiopulmonary bypass. *Circulation* 1999; 100: 1438.
72. Lazar HL, Bokesch PM, van LF, et al. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. *Circulation* 2004; 110(11 suppl 1): I1274.
73. Zhang HF, Yu J, Bajwa E, et al. Targeting of functional antibody-CD59 fusion proteins to a cell surface. *J Clin Invest* 1999; 103: 55.
74. Amsterdam EA, Stahl GL, Pan HL, et al. Limitation of reperfusion injury by a monoclonal antibody to C5a during myocardial infarction in pigs. *Am J Physiol* 1995; 268(1 Pt 2): H448.
75. Vakeva AP, Agah A, Rollins SA, et al. Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: Role of the terminal complement components and inhibition by anti-C5 therapy. *Circulation* 1998; 97: 2259.
76. de Vries B, Matthijsen RA, Wolfs TG, et al. Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: Inhibition of late apoptosis and inflammation. *Transplantation* 2003; 75: 375.
77. Zheng X, Feng B, Chen G, et al. Preventing renal ischemia-reperfusion injury using small interfering RNA by targeting complement 3 gene. *Am J Transplant* 2006; 6: 2099.
78. Zheng X, Zhang X, Sun H, et al. Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. *Transplantation* 2006; 82: 1781.
79. Thurman JM, Royer PA, Ljubanovic D, et al. 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.