Review Article

Complement Activation and Progression of Chronic Kidney Disease

Ziyong Tang,[1] Neil Sheerin[2]

Proteinuria is a strong predictor of progression in chronic kidney disease. Complement proteins are a major constituent of the urine of proteinuric patients. Complement is activated in the tubular lumen through the alternative pathway, and complement activation products are deposited on the apical surface of tubular epithelial cells. Recent animal studies have suggested that complement activation in the tubular compartment plays an important role in proteinuria-associated tubulointerstitial injury. Complement deficiency, depletion or inhibition all reduce the tubular cell damage and interstitial fibrosis that develops in proteinuric animals. In particular, inhibition of anaphylatoxin receptors protects the kidneys from proteinuria-associated damage. In this review, we discuss the evidence for a role of complement activation in the progression of chronic kidney disease. [*Hong Kong J Nephrol* 2009;11(2):41–6]

Key words: chronic kidney disease, complement activation, proteinuria

補體是尿蛋白的主要成分之一，而蛋白尿是慢性腎臟病進展的主要預測指標。補體在腎小管腔中經替代途徑活化，其活化產物沉積在小管上皮細胞的腔面。最近的動物實驗證據表明這種補體活化在蛋白尿相關的腎間質纖維化中起著很重要的作用。補體缺失，清除或抑制都能減輕蛋白尿動物的小管上皮細胞損傷和間質纖維化的程度。特別地，抑制過敏毒素的受體也能保護蛋白尿時的腎臟。在本綜述中，我們討論了補體活化在慢性腎臟病進展的作用。

Introduction

The routine use of estimated glomerular filtration rate to measure kidney function has resulted in improved diagnosis of chronic kidney disease (CKD) and a better understanding of its epidemiology. In developed countries, CKD stages 3–5 affects approximately 10% of the population [1,2]. CKD, even in its mildest forms, is associated with increased morbidity and mortality [3] and is therefore now recognized as a major public health problem. Not all patients with CKD will progress to renal failure and a requirement for renal replacement therapy, but an increasing number of patients are reaching this stage [4]. This has major significance for both the individual and for health care providers. There is thus considerable interest in ways to slow the progression of CKD, and angiotensin-converting enzyme inhibitors and angiotensin receptor blockers are now part of routine practice in an attempt to achieve this. However, progress in this area has been hampered by an incomplete understanding of the biological processes that lead to progression of CKD.

The histological features of the kidney in CKD—namely glomerular sclerosis, tubular atrophy and tubulointerstitial (TI) fibrosis—are similar irrespective of the initial disease. This suggests that common injurious pathways are activated in the damaged kidney that lead to a further decline in function. Nephrologists have known for many decades that the immune system, including activation of the complement system of proteins, is responsible for many kidney diseases. The deposition of complement proteins in the glomerulus in inflammatory glomerulonephritis is well known. However, there is increasing evidence that the immune system may also be involved in the TI injury that occurs in CKD. In this review, we discuss the involvement of

[1]Department of Nephrology, Peking University Third Hospital, Beijing, China, and [2]Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.
Correspondence to: Dr. Neil Sheerin, School of Clinical Medical Sciences, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK.
Fax: (+44) 0191-2220723; E-mail: neil.sheerin@ncl.ac.uk

ELSEVIER

the complement system in the development of interstitial renal injury.

Pathology of CKD

Many forms of progressive, noncystic CKD originate in the glomerulus, but they are almost invariably associated with histological injury to the tubulointerstitium. In fact, it is the severity of TI injury that predicts overall renal prognosis, better than the degree of histological damage to the glomerulus [5,6]. Therefore, there has been increasing interest in the mechanisms underlying the development of TI injury [7].

An increase in fibroblast number is a crucial event in mediating CKD progression. In healthy kidneys, fibroblasts are in a quiescent state and are responsible for the maintenance of extracellular matrix homeostasis with a balance between matrix synthesis and degradation. However, during renal injury, activated fibroblasts are present in increased numbers and with increased capacity to produce matrix. The imbalance between the synthesis and degradation of matrix proteins leads to irreversible changes in renal structure and function [8]. The accumulation of fibroblasts, particularly activated myofibroblasts, could be due to proliferation of resident fibroblasts or migration of fibroblast precursors from the bone marrow [9]. More recently, accumulating data suggest that fibroblasts may be derived from tubular epithelial cells through the process of epithelial to mesenchymal transition [10].

Proteinuria and CKD Progression

These observations raise one key question: Why does glomerular disease result in pathology at a distant site within the tubulointerstitium? Several explanations have been proposed for this, including:

- changes in post-glomerular blood flow leading to interstitial hypoperfusion and ischemia;
- downstream effects on the tubulointerstitium of cytokines and growth factors released from the injured glomerulus;
- loss of immune tolerance to kidney antigens due to glomerular injury;
- changes in glomerular permselectivity with leak of serum proteins into the urine that damage the tubular epithelium.

Proteinuria is common in CKD and occurs by two distinct but not exclusive mechanisms. It may be a manifestation of the original renal disease or can occur in any kidney disease with a significant reduction in the nephron number due to hyperfiltration in surviving nephrons.

The weight of clinical and experimental evidence has led to the hypothesis that the development of proteinuria is a major cause of TI injury [11] rather than simply a marker of the severity of glomerular disease. The amount of proteinuria is a strong predictor of prognosis in renal disease, with high levels of proteinuria predicting an ominous outcome [12]. Interventions that reduce the amount of protein in the urine, for example with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, improve the renal prognosis in proteinuric nephropathies [13–15]. There is also a clear link between proteinuria and TI damage and, in animal models, induction of glomerular proteinuria leads to TI damage [16]. A number of factors present in the urine of proteinuric patients have been proposed as mediators of progressive injury in CKD. Albumin is a major component of the protein that leaks into the urine and direct toxicity of albumin is therefore possible. A significant correlation was found between glomerular hyalinosis and the level of albuminuria in rats with 5/6th reduction in renal mass [17]. *In vitro* studies have shown that exposure of proximal tubular epithelial cells (PTECs) to albumin results in the production of a variety of chemoattractant, profibrotic agents and vasoconstrictive agents that may contribute to TI inflammation [18]. It has been suggested that the fatty acids carried by albumin may be responsible for the cytotoxicity of albumin to PTECs [19]. However, the effect of albumin on PTECs *in vitro* has not been confirmed *in vivo*. In minimal change disease, there is a high level of albuminuria but without TI injury and loss of kidney function. Lipoproteins [20] and growth factors [21] in the urine have also been proposed to be mediators of CKD progression.

In nonselective proteinuria, the urine contains all the proteins that are in the serum [22]. Complement proteins are a major constituent of serum protein (10% of total serum protein) and are found in the urine of proteinuric patients. It is conceivable that persistent leakage of complement proteins into the urine may be injurious to the cells of glomeruli and tubules, and this may contribute to the progression of CKD.

Complement in Proteinuria-associated TI Injury

The complement system is made up of over 30 serum and membrane-bound proteins and is an integral part of the innate immune system, but also has a key role in augmenting adaptive responses. It is activated by three pathways: the classical, alternative and mannose-binding lectin pathways. These lead to the assembly of the pivotal C3 and C5 convertases that generate proteins with chemotactic, opsonic, inflammatory and cytolytic activity. In proteinuria, the tubular epithelial cells are exposed to complement proteins,

the activation of which can alter tubular cell function and contribute to chronic TI injury.

Complement is activated in the tubules during proteinuria

There is evidence from clinical studies that complement is activated within the lumen of tubules in proteinuric patients. The membrane attack complex, C5b-9, can be detected in the urine of patients with glomerular disease at a much higher concentration than in urine from healthy individuals [23]. This is true even if the primary disease is not associated with glomerular complement activation, e.g. diabetic nephropathy. In a second study, complement activation products (iC3b, Bb, MAC) were almost undetectable in the urine of healthy subjects, but there was a significant increase in the urinary concentration of these products in proteinuric patients that was proportional to the level of proteinuria [24]. This was true of most diseases studied, including focal glomerular sclerosis, IgA nephropathy, membranous nephropathy and diabetic nephropathy. Interestingly, this association was not seen in minimal change disease, which is not usually associated with TI injury despite high levels of proteinuria.

Immunohistochemical analysis of 46 human kidney biopsies with various glomerulopathies found that, irrespective of the type of glomerular disease, there is a strong correlation between tubular C5b-9 deposition and interstitial monocyte infiltration and interstitial volume expansion [25]. This observation does not prove that C5b-9 has a causal role in the development of injury but is certainly supportive of this hypothesis. As the C5b-9 complex is about 1,000 kDa, it is unlikely to be filtered into the urine, again suggesting that complement is activated in the tubular compartment.

Activation of complement by PTECs

PTECs have the capacity to activate the complement system. When acetone-fixed rat or human kidney sections were incubated with fresh normal human serum (NHS), fixation of C3 occurred along the luminal border of proximal tubules. This fixation was not found on other tubular segments or in glomeruli. In addition to C3, properdin deposition occurred (but not C1q, C4, IgG, IgA or IgM) [26]. The addition to fresh serum of Mg-EGTA, which blocks the classical but not the alternative pathway, did not influence the binding of complement to the tubules [27]. Both observations suggest that complement is activated through the alternative pathway on the brush border of proximal tubules. Incubation of cultured, heat-killed, human kidney cells with NHS activates complement. This complement activation is largely dependent on factor B [28]. Human PTECs incubated with NHS fixed C3, properdin and C5b-9 on their surface, but not C1q or C4. Complement fixation was abrogated if PTECs were incubated with EDTA-treated NHS or C3-deficient human serum, but not with Mg-EGTA-treated NHS or C1q-deficient human serum, again confirming the important role of the alternative pathway [29].

The mechanism of complement activation

Protein overload in tubular cells is associated with ammonium production. It has been shown that C3 treated with ammonium hydroxide has properties comparable to those of soluble C3b. C3 modified by ammonia is called amidated C3, and can form the alternative pathway convertase of the complement cascade [30] and may contribute to TI injury [31]. Acidification of the urine within the tubular lumen may also enhance complement activation [32]. Nucleated cells express complement regulatory proteins to protect themselves against inappropriate complement activation. However, Ichida et al reported that there was a paucity of complement regulators on the apical surface of tubular cells [33]. Decay accelerating factor was not expressed in proximal tubules and membrane cofactor protein was only expressed on basolateral membranes. CD59 was weakly expressed in the brush border of proximal tubules [33]. This deficiency of complement regulatory proteins on the luminal side of proximal tubules may allow uncontrolled activation of complement.

Complement Activation Influences TI Injury

Animal models have been used to demonstrate the causal link between complement activation and TI injury. Several approaches have been employed.

The use of complement-deficient animals

C6-deficient rats have been used to study the effects of C5b-9 in disease development. Equivalent levels of proteinuria were induced in wild type or C6-deficient rats by puromycin. However, wild type rats developed more severe histological TI injury than C6-deficient rats, suggesting that C5b-9 formation during proteinuria contributes to TI damage [34]. This may be due to C5b-9 causing accumulation of interstitial myofibroblasts [35], although the mechanism by which this occurs is unknown. Similarly, in rats with proteinuria induced by 5/6 nephrectomy, C6 deficiency was protective, reducing TI damage and functional loss [36]. In contrast to these results, the C6-deficient rats were not protected from other non-proteinuric renal injury [37].

Turnberg et al found that mice deficient in C3 were protected from TI injury following induction of proteinuria by adriamycin [38]. Factor D-deficient mice were also protected and no C3 deposition was seen on

tubular cells in adriamycin-induced proteinuria in the absence of factor B [39]. The absence of complement regulator CD59 exacerbated disease [39]. This is consistent with the study in the C6-deficient rats suggesting a pathogenic role for C5b-9 [34], and with the human biopsy study that demonstrated CD59 on the luminal surface of tubular epithelial cells [33]. The adriamycin nephropathy model has also been used to study the role of locally synthesized C3. Using a transplantation strategy, mice with absent renal synthesis of C3 developed milder TI injury than mice with the capacity to synthesize C3 in their kidneys [40]. This suggests a role for local C3 synthesis in the development of TI inflammation and fibrosis. However, in contrast, the results of a protein overload model suggest a predominant role for ultrafiltered C3 [41].

The use of complement depletion or inhibition to prevent injury

Depletion of complement with cobra venom factor and the use of biological inhibitors have both been used to study the role of complement in the development of TI injury. Cobra venom factor treatment reduced proteinuria-associated TI injury in puromycin-induced nephropathy [42] and anti-Thy-1 nephritis [43]. Soluble complement receptor 1, a complement inhibitor based on the human receptor, had a similar effect on TI injury in these two models [42,43]. A more recent study coupled a single-chain antibody fragment directed against a tubular antigen to Crry (complement receptor-related protein, the rodent analog to decay accelerating factor and membrane cofactor protein), thereby targeting the inhibitor to tubules. This inhibitor-targeting approach reduced injury and preserved renal function after the induction of puromycin nephropathy [44].

As an alternative to inhibiting complement, Hori et al used antisense oligodeoxynucleotides against Crry to reduce expression and thereby increase susceptibility to complement-mediated injury. When injected into a rat, anti-Crry oligodeoxynucleotides reduced expression of Crry protein by PTECs. After the induction of proteinuria, more severe TI injury was found in anti-Crry oligodeoxynucleotide-treated animals compared with controls [45].

Complement inhibitors are important in maintaining tissue integrity

The studies discussed above demonstrate that complement activation causes TI injury in proteinuric disease. However, there is also evidence that spontaneous complement activation can cause TI injury in an otherwise normal kidney. If a rat kidney is perfused with monoclonal antibodies that block the activity of the complement inhibitors Crry and CD59, tubular injury occurs [46]. Damage occurs if Crry alone is inhibited but is worsened if CD59 is also inhibited, implicating a role for C5b-9 [47]. This is prevented by the depletion of C3. Crry knockout is embryolethal, but mice can be salvaged by generating double knockouts of Crry and C3. If a kidney from a Crry/C3 knockout is transplanted into a mouse with an intact systemic complement system, spontaneous complement activation occurs within the kidney with tubular injury and interstitial fibrosis, leading to renal failure and death from renal failure within 3 weeks [48].

The role of anaphylatoxins in TI injury

The release of anaphylatoxins as a consequence of complement activation can also cause renal injury. Bao et al found that either C3a receptor antagonist or C5a receptor antagonist blocked TI injury in experimental lupus nephritis in mice [49,50]. However, it is not possible to distinguish between a direct effect of anaphylatoxins on the kidney and the development of autoimmunity. Although no role for C5b-9 was demonstrated in non-proteinuric renal disease, Boor et al demonstrated an important role for C5a in the development of TI injury [51]. C5 deficiency and C5a receptor antagonist treatment reduced the degree of TI fibrosis in mice that had undergone unilateral ureteral obstruction [51]. Our recent results also suggest that anaphylatoxins contribute to injury in proteinuric nephropathy [52,53]. A similar key role for C5a in the development of proteinuria-associated TI injury in the horse apoferritin model has also been reported [54].

How does complement cause TI injury?

Complement activation on tubular epithelial cells can induce the production of a range of proinflammatory and profibrotic mediators. C5b-9, C3a and C5a have all been shown to increase the production of chemokines [55], cytokines [56], growth factors [57] and matrix proteins [58–60], and also to increase the immunogenic potential of PTECs [61]. In addition, our experiments have shown that C3a can stimulate cultured PTECs *in vitro* to undergo epithelial to mesenchymal transition, including loss of E-cadherin, acquisition of α-smooth muscle actin, and production of collagen I [52,53]. Alternatively, the chemotactic effects of anaphylatoxins and the extensively reported ability of these proteins to activate cells of myeloid origin may be important [62]. At present, we do not know which effect of complement contributes most to the development of injury, and it may be that many, and perhaps all, of these functions are important.

Concluding Remarks

Proteinuria is associated with TI injury and both predict a poor renal prognosis. Evidence of complement

activation can be found in proteinuric urine and this may contribute to the progression of TI fibrosis. Inhibition of complement activation or the actions of anaphylatoxins can block the progression of CKD. At present, however, most of the evidence to support this is based on studies in experimental animals. The next and most challenging step is to translate these experimental observations into treatments that influence patient outcome.

Acknowledgments

Dr. Ziyong Tang was supported by a K.C. Wong fellowship from the Chinese government.

References

1. Atkins RC. The epidemiology of chronic kidney disease. *Kidney Int Suppl* 2005;(94):S14–8.
2. Stevens PE, O'Donoghue DJ, de Lusignan S, Van Vlymen J, Klebe B, Middleton R, et al. Chronic kidney disease management in the United Kingdom: NEOERICA project results. *Kidney Int* 2007; 72:92–9.
3. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;351:1296–305.
4. Li PK, Kwan BC, Leung CB, Kwan TH, Wong KM, Lui SL, Tsang WK, Mak CC, Mak SK, Yu AW, Tang S. Prevalence of silent kidney disease in Hong Kong: the screening for Hong Kong Asymptomatic Renal Population and Evaluation (SHARE) program. *Kidney Int Suppl* 2005;(94):S36–40.
5. Risdon RA, Sloper JC, De Wardener HE. Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968;2:363–6.
6. Nath KA. The tubulointerstitium in progressive renal disease. *Kidney Int* 1998;54:992–4.
7. Harris RC, Neilson EG. Toward a unified theory of renal progression. *Annu Rev Med* 2006;57:365–80.
8. Muller GA, Zeisberg M, Strutz F. The importance of tubulointerstitial damage in progressive renal disease. *Nephrol Dial Transplant* 2000;15 Suppl 6:76–7.
9. Wada T, Sakai N, Matsushima K, Kaneko S. Fibrocytes: a new insight into kidney fibrosis. *Kidney Int* 2007;72:269–73.
10. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 2002;110:341–50.
11. Zandi-Nejad K, Eddy AA, Glassock RJ, Brenner BM. Why is proteinuria an ominous biomarker of progressive kidney disease? *Kidney Int Suppl* 2004;(92):S76–89.
12. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest* 2006;116:288–96.
13. GISEN Group. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). *Lancet* 1997;349:1857–63.
14. Lea J, Greene T, Hebert L, Lipkowitz M, Massry S, Middleton J, et al. The relationship between magnitude of proteinuria reduction and risk of end-stage renal disease: results of the African American study of kidney disease and hypertension. *Arch Intern Med* 2005;165:947–53.
15. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, et al; RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001;345:861–9.
16. Eddy AA. Interstitial nephritis induced by protein-overload proteinuria. *Am J Pathol* 1989;135:719–33.
17. Olson JL, de Urdaneta AG, Heptinstall RH. Glomerular hyalinosis and its relation to hyperfiltration. *Lab Invest* 1985;52:387–98.
18. Lai KN, Leung JC, Chan LY, Guo H, Tang SC. Interaction between proximal tubular epithelial cells and infiltrating monocytes/T cells in the proteinuric state. *Kidney Int* 2007;71:526–38.
19. Arici M, Brown J, Williams M, Harris KP, Walls J, Brunskill NJ. Fatty acids carried on albumin modulate proximal tubular cell fibronectin production: a role for protein kinase C. *Nephrol Dial Transplant* 2002;17:1751–7.
20. Ong AC, Jowett TP, Moorhead JF, Owen JS. Human high density lipoproteins stimulate endothelin-1 release by cultured human renal proximal tubular cells. *Kidney Int* 1994;46:1315–21.
21. Honkanen E, Teppo AM, Tornroth T, Groop PH, Gronhagen-Riska C. Urinary transforming growth factor-beta 1 in membranous glomerulonephritis. *Nephrol Dial Transplant* 1997;12:2562–8.
22. Woo KT, Lau YK, Yap HK, Lee GS, Chiang GS, Lim CH. Protein selectivity: a prognostic index in IgA nephritis. *Nephron* 1989; 52:300–6.
23. Ogrodowski JL, Hebert LA, Sedmak D, Cosio FG, Tamerius J, Kolb W. Measurement of SC5b-9 in urine in patients with the nephrotic syndrome. *Kidney Int* 1991;40:1141–7.
24. Morita Y, Ikeguchi H, Nakamura J, Hotta N, Yuzawa Y, Matsuo S. Complement activation products in the urine from proteinuric patients. *J Am Soc Nephrol* 2000;11:700–7.
25. Mosolits S, Magyarlaki T, Nagy J. Membrane attack complex and membrane cofactor protein are related to tubulointerstitial inflammation in various human glomerulopathies. *Nephron* 1997; 75:179–87.
26. Camussi G, Rotunno M, Segoloni G, Brentjens JR, Andres GA. *In vitro* alternative pathway activation of complement by the brush border of proximal tubules of normal rat kidney. *J Immunol* 1982; 128:1659–63.
27. Camussi G, Tetta C, Mazzucco G, Vercellone A. The brush border of proximal tubules of normal human kidney activates the alternative pathway of the complement system *in vitro*. *Ann N Y Acad Sci* 1983;420:321–4.
28. Baker PJ, Osofsky SG. Activation of human complement by heat-killed, human kidney cells grown in cell culture. *J Immunol* 1980; 124:81–6.
29. 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.
30. von Zabern I, Nolte R, Vogt W. Treatment of human complement components C4 and C3 with amines or chaotropic ions. Evidence of a functional and structural change that provides uncleaved C4 and C3 with properties of their soluble activated forms, C4b and C3b. *Scand J Immunol* 1981;13:413–31.
31. Nath KA, Hostetter MK, Hostetter TH. Pathophysiology of chronic tubulo-interstitial disease in rats. Interactions of dietary acid

load, ammonia, and complement component C3. *J Clin Invest* 1985; 76:667–75.
32. Peake PW, Pussell BA, Mackinnon B, Charlesworth JA. The effect of pH and nucleophiles on complement activation by human proximal tubular epithelial cells. *Nephrol Dial Transplant* 2002;17:745–52.
33. Ichida S, Yuzawa Y, Okada H, Yoshioka K, Matsuo S. Localization of the complement regulatory proteins in the normal human kidney. *Kidney Int* 1994;46:89–96.
34. Nangaku M, Pippin J, Couser WG. Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. *J Am Soc Nephrol* 1999;10:2323–31.
35. Rangan GK, Pippin JW, Couser WG. C5b-9 regulates peritubular myofibroblast accumulation in experimental focal segmental glomerulosclerosis. *Kidney Int* 2004;66:1838–48.
36. Nangaku M, Pippin J, Couser WG. C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. *J Am Soc Nephrol* 2002;13:928–36.
37. Rangan GK, Pippin JW, Coombes JD, Couser WG. C5b-9 does not mediate chronic tubulointerstitial disease in the absence of proteinuria. *Kidney Int* 2005;67:492–503.
38. Turnberg D, Lewis M, Moss J, Xu Y, Botto M, Cook HT. Complement activation contributes to both glomerular and tubulointerstitial damage in adriamycin nephropathy in mice. *J Immunol* 2006; 177:4094–102.
39. Lenderink AM, Liegel K, Ljubanovic D, Coleman KE, Gilkeson GS, Holers VM, et al. The alternative pathway of complement is activated in the glomeruli and tubulointerstitium of mice with adriamycin nephropathy. *Am J Physiol Renal Physiol* 2007; 293:F555–64.
40. Sheerin NS, Risley P, Abe K, Tang Z, Wong W, Lin T, et al. Synthesis of complement protein C3 in the kidney is an important mediator of local tissue injury. *FASEB J* 2008;22:1065–72.
41. Abbate M, Zoja C, Corna D, Rottoli D, Zanchi C, Azzollini N, et al. Complement-mediated dysfunction of glomerular filtration barrier accelerates progressive renal injury. *J Am Soc Nephrol* 2008;19:1158–67.
42. Nomura A, Morita Y, Maruyama S, Hotta N, Nadai M, Wang L, et al. Role of complement in acute tubulointerstitial injury of rats with aminonucleoside nephrosis. *Am J Pathol* 1997;151:539–47.
43. Morita Y, Nomura A, Yuzawa Y, Nishikawa K, Hotta N, Shimizu F, et al. The role of complement in the pathogenesis of tubulointerstitial lesions in rat mesangial proliferative glomerulonephritis. *J Am Soc Nephrol* 1997;8:1363–72.
44. He C, Imai M, Song H, Quigg RJ, Tomlinson S. Complement inhibitors targeted to the proximal tubule prevent injury in experimental nephrotic syndrome and demonstrate a key role for C5b-9. *J Immunol* 2005;174:5750–7.
45. Hori Y, Yamada K, Hanafusa N, Okuda T, Okada N, Miyata T, et al. Crry, a complement regulatory protein, modulates renal interstitial disease induced by proteinuria. *Kidney Int* 1999;56:2096–106.
46. Nomura A, Nishikawa K, Yuzawa Y, Okada H, Okada N, Morgan BP, et al. Tubulointerstitial injury induced in rats by a monoclonal antibody that inhibits function of a membrane inhibitor of complement. *J Clin Invest* 1995;96:2348–56.
47. Watanabe M, Morita Y, Mizuno M, Nishikawa K, Yuzawa Y, Hotta N, et al. CD59 protects rat kidney from complement mediated injury in collaboration with crry. *Kidney Int* 2000;58:1569–79.
48. Bao L, Wang Y, Chang A, Minto AW, Zhou J, Kang H, et al. Unrestricted C3 activation occurs in Crry-deficient kidneys and rapidly leads to chronic renal failure. *J Am Soc Nephrol* 2007; 18:811–22.
49. Bao L, Osawe I, Puri T, Lambris JD, Haas M, Quigg RJ. C5a promotes development of experimental lupus nephritis which can be blocked with a specific receptor antagonist. *Eur J Immunol* 2005;35:2496–506.
50. Bao L, Osawe I, Haas M, Quigg RJ. Signaling through up-regulated C3a receptor is key to the development of experimental lupus nephritis. *J Immunol* 2005;175:1947–55.
51. Boor P, Konieczny A, Villa L, Schult AL, Bucher E, Rong S, et al. Complement C5 mediates experimental tubulointerstitial fibrosis. *J Am Soc Nephrol* 2007;18:1508–15.
52. Tang Z, Sacks S, Sheerin N. Complement acts as a mediator of renal tubulointerstitial injury. *Mol Immunol* 2007;44:248. [Abstract]
53. Tang Z, Sacks S, Sheerin NS. Innate immunity acts as a mediator of renal tubulointerstitial injury. *Mol Immunol* 2007;44:3932. [Abstract]
54. Welch TR, Frenzke M, Witte D, Davis AE. C5a is important in the tubulointerstitial component of experimental immune complex glomerulonephritis. *Clin Exp Immunol* 2002;130:43–8.
55. Thurman JM, Lenderink AM, Royer PA, Coleman KE, Zhou J, Lambris JD, et al. C3a is required for the production of CXC chemokines by tubular epithelial cells after renal ischemia/ reperfusion. *J Immunol* 2007;178:1819–28.
56. David S, Biancone L, Caserta C, Bussolati B, Cambi V, Camussi G. Alternative pathway complement activation induces proinflammatory activity in human proximal tubular epithelial cells. *Nephrol Dial Transplant* 1997;12:51–6.
57. Peake PW, O'Grady S, Pussell BA, Charlesworth JA. C3a is made by proximal tubular HK-2 cells and activates them via the C3a receptor. *Kidney Int* 1999;56:1729–36.
58. Braun MC, Reins RY, Li TB, Hollmann TJ, Dutta R, Rick WA, et al. Renal expression of the C3a receptor and functional responses of primary human proximal tubular epithelial cells. *J Immunol* 2004;173:4190–6.
59. Abe K, Li K, Sacks SH, Sheerin NS. The membrane attack complex, C5b-9, up regulates collagen gene expression in renal tubular epithelial cells. *Clin Exp Immunol* 2004;136:60–6.
60. Burger A, Wagner C, Hug F, Hansch GM. Up-regulation of intracellular calcium, cyclic adenosine monophosphate and fibronectin synthesis in tubular epithelial cells by complement. *Eur J Immunol* 1999;29:1188–93.
61. Li K, Patel H, Farrar CA, Hargreaves RE, Sacks SH, Zhou W. Complement activation regulates the capacity of proximal tubular epithelial cell to stimulate alloreactive T cell response. *J Am Soc Nephrol* 2004;15:2414–22.
62. Boulay F, Mery L, Tardif M, Brouchon L, Vignais P. Expression cloning of a receptor for C5a anaphylatoxin on differentiated HL-60 cells. *Biochemistry* 1991;30:2993–9.