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 tubulo-interstitial (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

Key words: chronic kidney disease, complement activation, proteinuria

Complement Activation and Progression of Chronic Kidney Disease

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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]

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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 inhibitor 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


