Nephrol Dial Transplant (2005) 20: 2613–2619
doi:10.1093/ndt/gfi166
Advance Access publication 4 October 2005

Nephrology Dialysis Transplantation

Editorial Review

Complement and the kidney: What the nephrologist needs to know in 2006?

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Introduction

The renewed appreciation of the role of the complement system as a mediator and marker of renal damage has led to numerous recent investigations in the field of complement and renal disease. The aims of the present review are:

- to recapitulate the pathways of complement activation with an emphasis on the more recently described lectin pathway of complement activation
- to discuss some of the new data on the role of complement in renal disease and,
- to briefly provide information about new diagnostic techniques in the field of complement,

Pathways of complement activation

The complement system is not only an important component of the innate immune system but it also plays an essential role in the initiation and control of the adaptive immune response. The three pathways of complement activation converge at the level of C3. Activation of C3 leads to the formation of the membrane attack complex (MAC) on complement-activating surfaces (Figure 1).

(a) The *classical pathway of complement activation* is initiated via binding of its recognition molecule C1q to immune complexes or charged molecules. This leads to a conformational change resulting in the activation of the C1q-associated serine proteases C1r and C1s. Activated C1s cleaves both C4 and C2 which associate to form the classical pathway C3 convertase, the C4b2a enzyme complex. Next to activation by IgG and IgM immune complexes, C1q may also be activated by apoptotic and necrotic cells and by acute phase proteins such as CRP [1].

(b) The *lectin pathway of complement activation* utilizes the same C3 convertase as the classical pathway. It is initiated by binding of mannose-binding lectin (MBL) or ficolins which recognize patterns of carbohydrate ligands that are found on the surface of a wide variety of microorganisms [2]. MBL consists of up to six trimeric subunits and its structure resembles a bouquet-like shape similar to that of C1q. The plasma concentrations can vary up to 1000-fold. This variation is largely explained by single nucleotide polymorphisms within exon 1 of the MBL-2 gene. Polymorphisms of the promoter region contribute further to the variation in MBL levels. Binding of MBL to its ligands results in the activation of the associated serine protease MASP-2 and subsequent cleavage of C4 and C2 leading to the formation of C4b2a.

(c) The *alternative pathway of complement activation* depends on spontaneous hydrolysis of C3 in plasma leading to the formation of C3 (H_2O). This molecule binds to factor B. Subsequent activation by factor D results in the formation of C3 (H_2O) Bb. This complex cleaves additional C3 to C3a and C3b constantly and at a low rate. In the presence of an activating surface (e.g. a bacterial wall), C3b is protected from inactivation by regulatory proteins like factor I and H. As a result the more active alternative pathway C3 convertase C3bBb is formed, which is further stabilized by properdin.

The common *terminal pathway of complement activation* is similar for the classical, lectin and alternative pathways. The incorporation of C3b in the C3 convertases results in the formation of C3bBbC3b for the alternative pathway and C4b2a3b for the classical and lectin pathway. These C5 convertases initiate the

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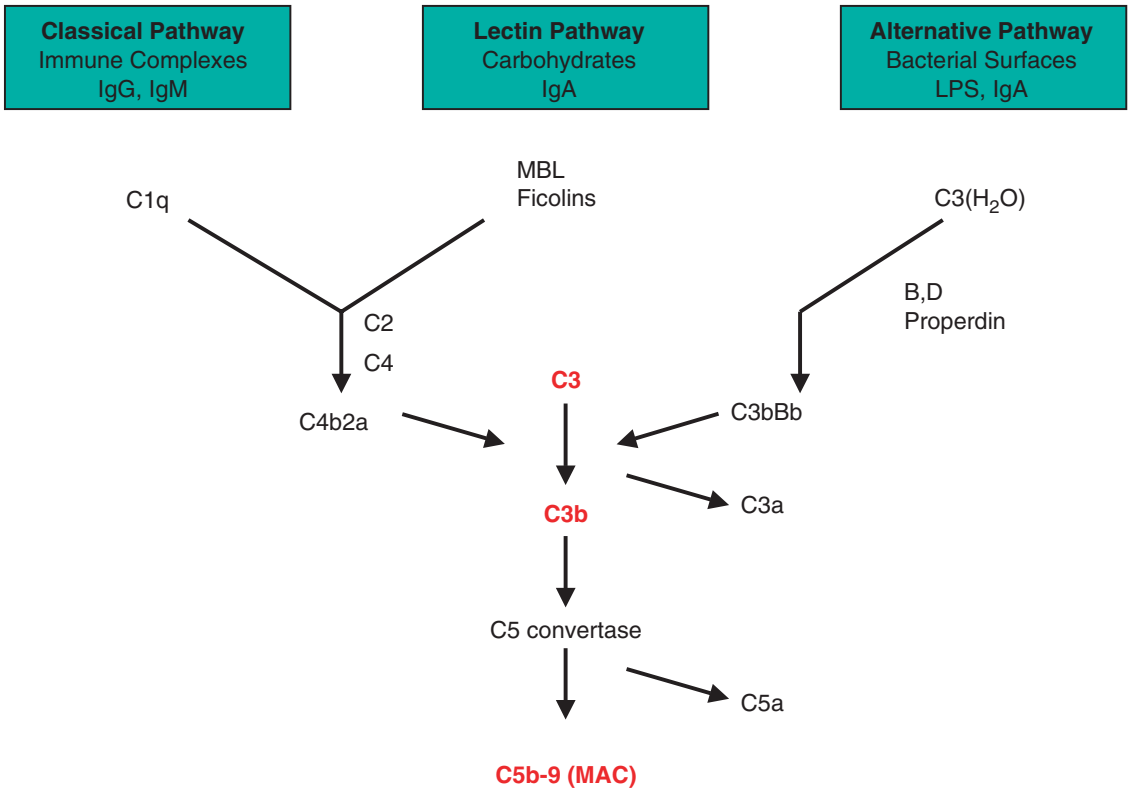

Fig. 1. Overview of the three pathways of complement activation.

formation of the membrane attack complex by cleavage of C5 to C5a and C5b. C5b forms a trimolecular complex with C6 and C7. After insertion into a cell membrane, C8 and multiple molecules of C9 bind to the complex. As a result the pore-forming MAC is assembled. While high pore densities cause cell death, sublytic doses of MAC with less dense pore insertion into the cell membrane may lead to cell activation [3] and enhancement of the innate immune responses.

Apart from the production of MAC with resulting lysis or activation of cells, complement activation can also lead to the production of the chemo-attractive anaphylatoxins, C3a and C5a. Complement split products such as C3b and C4b associate with immune complexes increasing their solubility and facilitating their clearance. Both MBL and C1q may bind to apoptotic cells and aid in their clearance [4–6].

Role of complement in renal disease

Glomerulonephritis

Complement may play a beneficial as well as a harmful role in renal disease. Complement deposition is detected in kidney biopsies obtained from patients with various forms of renal disease. Except for type II membranoproliferative glomerulonephritis, complement deposition is usually accompanied by the deposition of immunoglobulins. In the following section, we discuss some recent data on the role of complement in glomerular disease, taking lupus nephritis and IgA nephropathy as clinically relevant examples.

Lupus nephritis. The deposition of IgG, IgM IgA, C3 and C4 together with C1q is the hallmark of lupus nephritis and is referred to as the 'full house pattern' of immune deposition. The pattern of complement factors deposited in kidneys with lupus nephritis and the marked reduction of complement levels in most of these patients suggest an important role for damage, mediated by the classical pathway in lupus nephritis. The manipulation of the complement system in various mouse models has shed light on the complex role of complement in this disease. The disruption of both the C1q or the C4 gene in mice with a 129 × C57BL/6 genetic background leads to the spontaneous development of glomerulonephritis as well as to the production of autoantibodies and accumulation of apoptotic cells [7,8]. In line with these findings inherited deficiencies of C1q and C4 are strongly associated with the development of SLE in humans. Interestingly, C1q deficiency did not significantly influence the development of glomerulonephritis in the spontaneously lupus developing MLR/lpr mice [9]. If, on the other hand, lupus prone NZB/W mice were treated with an anti-C5 antibody, the development of glomerulonephritis was prevented [10]. Similar protective results were obtained when MLR/lpr mice were treated with the soluble rodent complement inhibitor rCrry-Ig [11]. Considering the data obtained both in human disease and animal models, it seems that the beneficial role of

the early components of the classical pathway in opsonization and clearance of apoptotic cells and immune complexes override the potentially damaging role mediated by downstream complement activation products. These considerations have important implications for the possible role of therapeutic interventions in the complement system. Complement inhibition further downstream may inhibit the production of the powerful anaphylatoxin C5a and MAC without impairing the protective role of the upstream components of the complement pathway.

Antibodies directed against C1q are detectable in 30–40% of SLE patients [12]. These antibodies correlate with the presence of active lupus nephritis with a sensitivity of 87% and a specificity of 92% [13]. The generation of homologous mouse anti-mouse C1q antibodies has provided a tool to study whether these antibodies actually play a role in the pathogenesis of lupus nephritis. Administration of these antibodies alone led to deposition of C1q in kidneys of naive mice with granulocyte influx without clinical expression of renal disease such as albuminuria. However, when mice were pre-treated with a subnephritogenic dose of rabbit anti-GBM antibodies, administration of mouse anti-C1q antibodies resulted in increased deposition of immunoglobulins and complement as well as marked renal damage [14]. The application of this model to mice genetically deficient for C4, C3 or all three Fc-γ receptors demonstrated that anti-C1q-mediated renal damage was dependent on both complement activation and the contribution of Fc-γ receptors.

IgA-nephropathy. Deposition of predominantly polymeric IgA of the IgA1-subclass is the hallmark of IgA nephropathy. Co-deposition of C3 is usually detected in renal biopsies. This is thought to result from activation of the alternative pathway, since IgA does not activate the classical pathway of complement. But as C4 deposition is detected in 30% of biopsies from kidneys with IgA nephropathy [15], complement activation via the mannose binding lectin (MBL) pathway has been suggested. Indeed co-deposition of IgA with MBL has been demonstrated in biopsies from patients with IgA nephropathy [16]. In line with these findings, our group has shown that MBL binds to IgA resulting in complement activation [17].

Ischaemia/reperfusion damage

Several studies have underscored the role of complement in ischaemia/reperfusion damage. Zhou *et al.* studied mice deficient for C3, C4, C5 or C6 in a kidney ischaemia/reperfusion model [18]. Deficiency of C3, C5 and C6 was associated with marked protection from ischaemia/reperfusion damage, whereas C4-deficient mice were not protected. These findings suggest an important role of C5b-9 activated by the alternative pathway in ischaemia/reperfusion damage. Classical pathway activation did not seem to play a role in this model. This concept has been supported by a study showing protection from renal ischaemia/reperfusion damage in mice deficient for factor B [19]. The same group has demonstrated C3b deposition without evidence of C4b deposition in human kidneys with acute tubular necrosis [20], showing that the alternative pathway may also be the dominant route of complement activation in ischaemia/reperfusion damage of the human kidney. Complement may also cause damage due to the formation of chemotactic molecules such as C5a [21].

An important role of mannose-binding lectin (MBL) has recently been demonstrated in ischaemia/reperfusion damage of the heart and intestine [22,23]. Mice deficient for MBL-A and MBL-C were protected from cardiac and gastrointestinal ischaemia/reperfusion injury whereas C1q-deficient mice were not protected. MBL deposition has been detected in mouse and human kidneys with ischaemia/reperfusion damage [24] and a possible contribution of MBL to ischaemia/reperfusion injury of the kidney has recently been proposed in a study using mice deficient for MBL A and C [25].

Kidney transplantation

The introduction of C4d staining in biopsies obtained from renal transplants has led to a new appreciation of the role of humoral rejection in renal transplantation. C4d binds covalently to basement membranes and therefore may remain detectable for weeks. The deposition of C4d onto peritubular capillaries indicates humoral rejection as shown by the strong correlation with panel-reactive [26] or donor-specific antibodies [27]. In several studies, staining for C4d has been shown to predict poorer graft survival [28,29]. These findings have resulted in the addition of antibody-mediated rejection to the Banff '97 classification of renal allograft rejection [30]. Numerous treatment modalities including intravenous immunoglobulins, plasmapheresis and anti-CD 20 have been tried successfully in patients with humoral rejection. No randomized trials are available at this moment to support this strategy. Apart from the obvious clinical implications of a timely diagnosis of humoral rejection, the detection of C4d in as many as 30% of kidney transplant biopsies has triggered an increased interest in the role of complement in mediating renal damage in rejection. The presence of C4d in renal biopsies suggests complement activation by the classical pathway. However, the lectin pathway may also interact with immunoglobulins, as has been shown for IgM and IgA [17,31]. With these findings in mind our group raised the issue whether MBL levels influence outcome in kidney transplantation. Indeed, higher pre-transplant MBL levels were associated with poorer graft survival [32]. The superior graft survival in patients with low MBL levels was explained by a lower rate of treatment-resistant rejection. These findings suggest that MBL plays an unfavourable role in renal transplantation.

Rejection and damage to renal allografts may not only be influenced by circulating complement, but also by complement produced locally in the kidney.

Pratt *et al*. studied the role of locally produced C3 in a mouse kidney transplantation model [33]. Whereas graft survival was not influenced when kidney recipients were C3 deficient, survival was markedly improved if the transplanted organ was obtained from C3-deficient mice. Possibly, locally produced C3 functions as a costimulator in the interaction between antigen presenting cells (APCs) and T-cells. This concept is supported by the recent report, that APCs lacking the complement inhibitor DAF (decay-accelerating factor) led to enhanced T-cell responses when compared with wild type APCs [34].

Atypical haemolytic-uraemic syndrome

Recent data suggest an important role for complement in atypical haemolytic-uraemic syndrome (HUS). Mutations in the complement regulatory protein factor H have been described in patients with sporadic and familial HUS in several studies [35–37]. The described mutations interfere with the capacity of factor H to control the activation of the alternative pathway on cellular surfaces. The determination of factor H serum levels is not sufficient to exclude factor H mutations, since mutant, dysfunctional factor H may circulate at normal concentrations [38]. A functional assay for factor H mutations has been described, which may facilitate screening for factor H mutations in patients with HUS [39]. More recently, mutations of the complement regulatory factor I and MCP have been proposed as predisposing factors in patients with atypical HUS [40–42]. Screening for these mutations may provide important information for risk assessment, since these patients have a high rate of recurrence of HUS after renal transplantation.

Progression of chronic renal disease

As complement molecules are detectable in urine from patients with non-selective proteinuria, it has been suggested that these components contribute to the tubulointerstitial damage in proteinuric renal disease [43]. Urinary C5b-9 excretion has been described in both animal models of membranous nephropathy and humans with this disease [44,45]. Interestingly, high levels of C5b-9 excretion have also been detected in patients with diabetic nephropathy, whereas low levels were detected in the relatively benign condition of minimal change disease [46].

Use of the C6-deficient PVG rat in various models of proteinuria-associated interstitial damage has provided strong evidence for a harmful role of complement in the progression of renal disease. In the puromycin model of proteinuric renal damage, complement-sufficient animals developed more severe tubulointerstitial damage than C6-deficient rats [47]. A similar protective role of C6-deficiency was demonstrated in the remnant kidney model. Once complement has entered the tubuli in the setting of unselective proteinuria, it may be activated on the tubular brush border by the high local ammonia concentrations [48].

Diabetic nephropathy

As mentioned above, high concentrations of C5b-9 are also found in the urine obtained from patients with diabetic nephropathy [46]. MAC deposition has been described in kidneys [49], nerves [50] and retinas [51] from patients with diabetes mellitus. Inactivation of the complement regulatory protein CD59 by glycation has been suggested as a possible mechanism underlying complement activation in diabetes [52]. A role for lectin pathway mediated damage in diabetic nephropathy is suggested by the association between high levels of MBL and microalbuminuria in diabetic subjects [53,54].

Taken together, these studies strongly suggest a role for complement in the amplification of vascular and tissue injury in diabetes.

Measurement of complement pathway activity: methods and indications

Circulating complement can be measured by both functional assays and by immunological methods measuring concentrations of the respective complement proteins' [55]. Functional assays of the complement pathway include the CH50 to assess the classical pathway and the AP50 to assess the alternative pathway.

The CH50 determines the capacity of the patient serum to lyse sheep erythrocytes coated with rabbit antibodies. It is a useful initial screening tool for the classical pathway, since an intact functional capacity of all nine components of the classical pathway is required for a normal result.

The AP50 measures lysis of unsensitized rabbit erythrocytes. Recently, a simple and standardized ELISA based assay of all three pathways of complement activation including the lectin pathway has been developed [56] and was shown to be valuable for the detection of primary and secondary complement deficiencies.

C4 and C3 levels are usually measured by radial immunodiffusion or nephelometry using polyclonal antibodies. Decreased levels of circulating C3 and C4 can be detected in several renal diseases and may help to narrow the differential diagnosis. Renal immune complex diseases associated with hypocomplementaemia include SLE, MPGN (all three types), cryoglobulinaemia, post-streptococcal glomerulonephritis and glomerulonephritis associated with chronic infection (e.g. endocarditis or abdominal abcesses). In post-streptococcal glomerulonephritis and MPGN type II, C3 is usually decreased more than C4 while a proportionate reduction in both C3 and C4 is generally

detected in the classical pathway mediated complement consumption of SLE and cryoglobulinamia.

We recommend the determination of both the classical pathway activity and alternative pathway activity as well as C3 and C4 levels for the initial screening of patients with a suspected complement deficiency. The combination of these determinations will help to identify the nature of complement consumption and to detect rare inherited complement deficiencies (e.g. C1q or C4 deficiency in SLE). In the setting of an unexplained propensity for infections or an increased risk for infections [57], the measurement of the lectin pathway may be appropriate.

In situations of increased complement catabolism in which complement depletion is not detected due to the replenishment by increased synthesis, complement turnover may de detected by the measurement of complement activation products such as C3a, C3d or C5a. Determination of factor H and I levels and function can be useful in atypical HUS.

To monitor patients with SLE serial determination of either C3 or C4 levels is sufficient. It is not clear whether one or both determinations is preferable above the other. Following either C3 or C4 may be helpful to monitor the response to treatment and to detect changes in activity. The interpretation of complement levels should always be done with consideration of the clinical context [58]. The addition of an anti-C1q antibody assay may help to predict the presence of nephritis in patients with SLE [13,59,60].

Inhibitors of complement activation in the treatment of renal disease

Following the increasing knowledge about the role of complement in the pathophysiology of various diseases, numerous options for therapeutic manipulation of the complement system have been proposed [61]. Therapeutic complement inhibition may be approached at various levels of the complement cascade. Inhibition at the initiation level may allow specific regulation of one or the three pathways without interfering with the protective function of the other pathways. An intervention at the level of C3 inhibits the entire complement system with the possibility of high efficacy, but the drawback of an increased risk of infections. Inhibition at the level of C5b-9 would prevent MAC mediated tissue damage without preventing complement-mediated clearance of immune complexes and apoptotic cells. Additionally, the anaphylatoxins C3a and C5a could be inhibited directly.

Many of these possible approaches have been tested in animal models of renal disease. The rodent C3 convertase inhibitor Crry has similarity with the human complement receptor 1 (CR-1). Both the overexpression of Crry and the application of recombinant Crry confer protection in a mouse model of anti-GBM glomerulonephritis [62,63]. Administration of soluble Crry to MLR/lpr mice resulted in a marked reduction in renal damage in this model of SLE [11]. A soluble form of human CR1 (sCR1) was protective in glomerular disease in rats [64]. Treatment with a membrane-binding complement regulator based on CR1 resulted in amelioration of ischaemia/reperfusion damage and rejection in a rat model of kidney transplantation [65]. A pharmaceutical preparation of sCR1 (TP-10; Avant Immunotherapeutics Inc., Needham, MA) has been developed but has not been tested in human renal disease.

Anti-C5 antibodies have been demonstrated to ameliorate lupus-like disease in mice [10] and pharmaceutical C5-inhibitors have been developed for use in humans. Results from ongoing trials with the fully humanized C5-inhibitor Eculizumab (Alexion Pharaceuticals, Cheshire, CT) in patients with membranous glomerulonephritis are being awaited. The efficacy of this antibody has been documented in paroxysmal nocturnal haemoglobinuria [66,67].

Concluding remarks

The complement system contributes to renal damage in many of the disease entities encountered by the nephrologist. A sound understanding of the complement system will aid the nephrologist in understanding the pathophysiology of renal disease and provide support in making the correct diagnosis. Monitoring complement may offer guidance in therapeutic decisions if interpreted with prudence in the clinical context. Whether therapeutic interventions in the complement system will result in meaningful improvements for our patients remains to be established. A sceptical position is justified in view of the large discrepancy between the huge volume of laboratory information and the modest progress in the implementation of complement-targeted therapy in clinical practice.

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