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REVIEW

The complement system in pediatric acute kidney injury

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Abstract
The complement cascade is an important part of the innate immune system. In addition to helping the body to eliminate pathogens, however, complement activation also contributes to the pathogenesis of a wide range of kidney diseases. Recent work has revealed that uncontrolled complement activation is the key driver of several rare kidney diseases in children, including atypical hemolytic uremic syndrome and C3 glomerulopathy. In addition, a growing body of literature has implicated complement in the pathogenesis of more common kidney diseases, including acute kidney injury (AKI). Complement-targeted therapeutics are in use for a variety of diseases, and an increasing number of therapeutic agents are under development. With the implication of complement in the pathogenesis of AKI, complement-targeted therapeutics could be trialed to prevent or treat this condition. In this review, we discuss the evidence that the complement system is activated in pediatric patients with AKI, and we review the role of complement proteins as biomarkers and therapeutic targets in patients with AKI.

Keywords Acute kidney injury · Complement · Complement inhibitors

Introduction

Acute kidney injury (AKI) is associated with substantial morbidity and mortality in both acutely ill and critically ill children. The most common causes of AKI in pediatric patients are kidney ischemia/reperfusion injury (IRI), nephrotoxic medication exposure, and sepsis, although often AKI is multifactorial [1]. Recent epidemiologic studies show that AKI affects up to 20–25% of critically ill children, and affected patients frequently require kidney replacement therapy (KRT), prolonged invasive mechanical ventilation, and increased length of stay [2, 3]. In spite of these supportive measures, AKI is still associated with increased mortality [4]. Furthermore, even in patients who recover, AKI is associated with increased long-term risk of proteinuria, hypertension, chronic kidney disease (CKD), and decreased health-related quality of life scores [5–7].

Despite the high prevalence and burden of disease from AKI, there are few options for early diagnosis and disease progression monitoring. Importantly, clinical trials have been unsuccessful in developing effective strategies to prevent, treat, or mitigate AKI in children. Two main obstacles have hindered progress in this area. First, the diagnosis of AKI using the Kidney Disease: Improving Global Outcomes (KDIGO) criteria is based on changes to serum creatinine (sCr) levels and urine output [8]. sCr is an imprecise, late biomarker that varies widely in both chronically ill children and in children with critical illness. It can be difficult to interpret in patients with low muscle mass and must be adjusted for fluid volume status [9]. Additionally, it can take up to 48 h for sCr to increase after the glomerular filtration rate (GFR) has fallen by 50%. Urine output can also be difficult to analyze, especially in children who are incontinent and those without indwelling urinary catheters in place.

Second, AKI is typically multifactorial in etiology and the relative contribution of each mechanism varies from patient to patient, likely influencing the duration and severity of disease in each critically ill child. Thus, in addition to developing effective therapies for AKI, it is equally important that we develop biomarkers for stratifying patients to a particular treatment and monitoring the response.

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Overview of the complement system

The complement cascade is a vital component of the innate immune system, but uncontrolled complement activation also plays a critical role in the pathogenesis of numerous kidneys disorders. The complement system comprises soluble proteins, cell surface receptors, and regulatory proteins. Although most of the complement proteins in the plasma are produced in the liver, they can also be synthesized in other tissues. Elegant animal experiments have shown that complement proteins synthesized within the kidney contribute to kidney IRI [10].

The complement system is activated through three different pathways (classical pathway, lectin pathway, and alternative pathway) which all converge upon C3, cleaving it to form C3a and C3b as seen in Fig. 1A. Complement activation through the classical and lectin pathways involves pattern recognition molecules, such as immunoglobulin and mannose-rich cellular surfaces. C3b can bind with factor B. Factor B is then cleaved by factor D to form C3bBb, the alternative pathway convertase (activating enzyme complex). C3bBb then cleaves additional C3, creating an amplification loop. Consequently, C3b generated by the classical or lectin pathways can feed into the alternative pathway amplification loop, leading to further activation. In fact, even when the system is initially triggered through the classical pathway by immune-complexes, amplification through the alternative pathway may account for the majority of downstream activation fragments that are generated [11]. The C3bBb convertase is stabilized by the addition of properdin, positively regulating alternative pathway activation. Conversely, the C3bBb convertase is negatively regulated by regulatory proteins that either inactivate the C3b molecule or accelerate the decay of the convertase (see below).

The alternative pathway is also activated in plasma through the spontaneous hydrolysis of C3 to form C3(H_20), a process called "tick-over" [12]. Like C3b, C3(H_20) can combine with factor B to form a convertase [C3(H_20)Bb]. C3b generated by the C3(H_20)Bb convertase can react with amine and hydroxyl groups on nearby surfaces, potentially forming the alternative pathway convertase described above (C3bBb). Ordinarily, however C3b generated by tick-over is inactivated by soluble regulators (factors H and I) [12]. Because of this efficient regulation, C3b generated through tick-over is probably too short-lived to initiate alternative pathway activation on host tissues. In the setting of impaired complement regulation, however, tick-over may initiate alternative pathway activation on nearby surfaces.

Initiation of the system through any of the activation pathways leads to common downstream effector functions. C3 fragments covalently bind to nearby surfaces ("opsonization") and serve as ligands for several different receptors. Activation also causes formation of a multimeric pore on target surfaces (C5b-9, or the "membrane attack complex"). C5b-9 can lyse target cells and also causes various sub-lytic effects, including cell stimulation [13]. Activation also generates soluble fragments of C3 and C5: C3a and C5a, the "anaphylatoxins" (Fig. 1A). The anaphylatoxins cause chemoattraction of myeloid cells, release of additional proinflammatory mediators via leukocyte activation, and vascular leak from increased vascular permeability [14]. These downstream effects are important for the clearance of pathogens, but they can also cause bystander injury to host tissues.

There is significant variation in normal complement levels in individuals and normative values differ by age and gender. In a study of healthy adults, increasing age was associated with increased classical and alternative pathway activity [15]. Additionally, increasing age was associated with higher levels of C5, C8, and C9 (terminal pathway proteins), whereas it was inversely associated with factor D levels [15]. In healthy infants, complement levels are typically 50–75% of normative adult values. Some factors (C2, C4, C5, C6, factor B) reach normal adult values by 6 months of age, while others (C1q, C3) remain significantly lower than adult norms [16]. The effect of these variations in complement protein levels on complement-mediated inflammation remain unclear, but it is possible that lower levels of complement proteins attenuate complement-mediated inflammation in pediatric patients.

Given the potentially harmful effects of the complement activation fragments, the system is normally controlled by various regulatory proteins expressed on cell surfaces and in plasma [17]. Decay accelerating factor (DAF, or CD55) dissociates the convertases, acting as a negative regulator (Fig. 1B) [18]. A second mechanism of regulation is provided by factor I, a soluble protease that inactivates C3b by cleaving it to form iC3b. iC3b can no longer associate with factor B, so it is unable to form more convertase. Factor I requires cofactor proteins in order to inactivate C3b. Membrane cofactor protein (MCP, or CD46) and complement receptor 1 (CR1) are cell surface proteins that can serve as cofactors. Factor H has both decay accelerating function and cofactor function for the alternative pathway convertase. It is a soluble protein that can also bind to host surfaces [19].

The importance of the regulatory proteins is illustrated by the strong association of defective complement regulation with inflammatory diseases [13]. The kidney appears to be particularly susceptible to injury in the setting of impaired complement regulation, even when the underlying defects affect complement regulation throughout the body. Factor H, for example, is an alternative pathway regulator present in plasma and other body fluids. Genetic and acquired defects

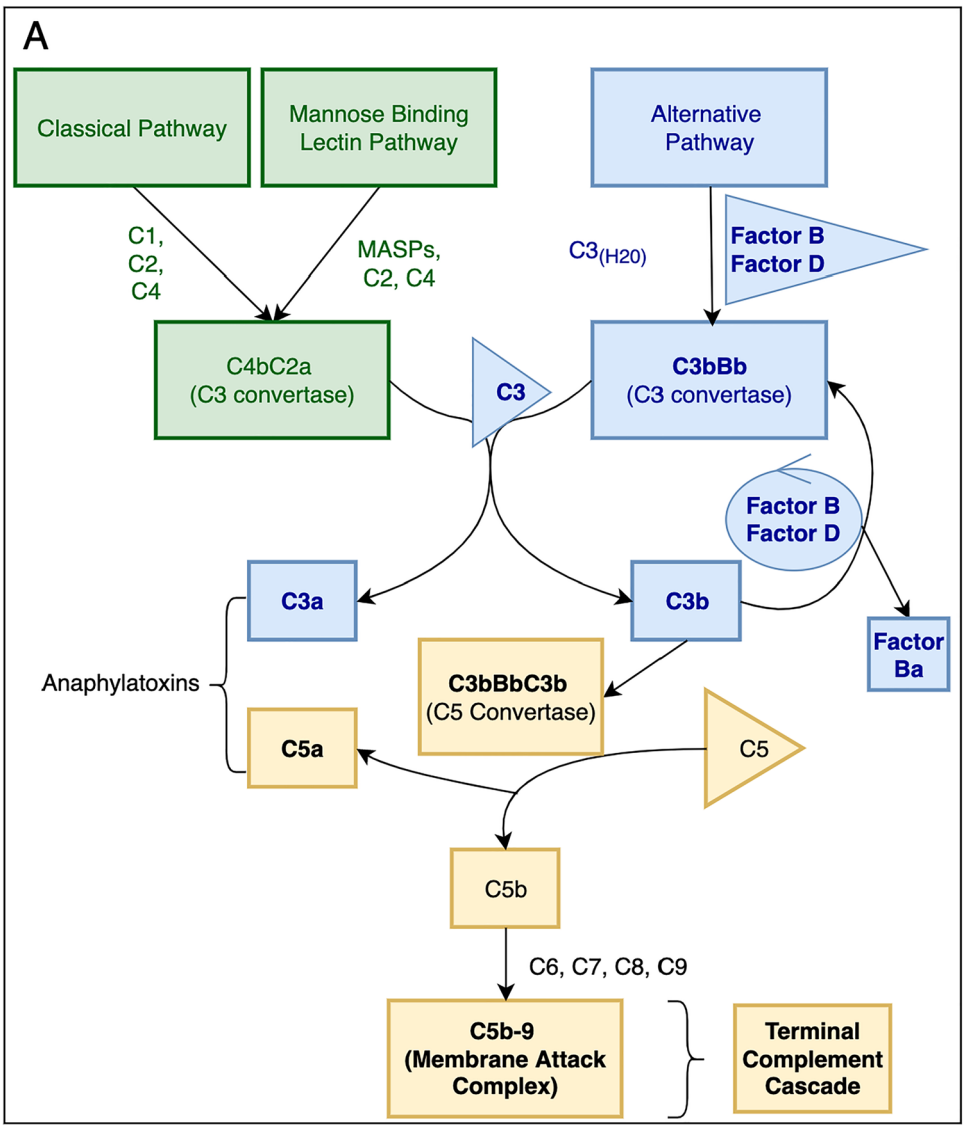

Fig. 1 Overview of the complement cascade. **A** Activation pathways. The classical pathway, mannose-binding lectin pathway, and alternative pathway converge on C3, cleaving C3 into activation fragments C3a and C3b. C3b joins with factor B, which is then cleaved by the rate-limiting enzyme factor D. This generates the Ba fragment, which can be measured as a marker of this process. It also creates the C3 convertase (C3bBb). C3bBb is involved in the amplification loop of the alternative pathway, increasing the generation of downstream activation fragments. C3b also joins with C3bBb to create the C5 convertase (C3bBbC3b), which converts C5 into C5a and C5b. C3a and C5a function as anaphylatoxins which cause chemoattraction of myeloid cells, leukocyte activation leading to release of proinflammatory mediations, and increased vascular permeability causing vascular leak. C5b joins with C6, C7, C8, and C9 to form C5b-9, also termed the membrane attack complex (MAC) which lyses target cells. **B** Complement regulatory proteins. Regulatory proteins are integral in controlling the complement cascade and preventing pathologic activation within tissues. Factor H is a regulator of the alternative pathway that inactivates C3b, competes with factor B for C3b binding (and prevents formation of C3 convertase), and accelerates C3 convertase decay. Factor H is a soluble protein that controls alternative pathway activation in the fluid phase, but it can also bind to cells and extracellular matrix to control activation at those locations. CD46 is another cofactor (for factor I) that mediates inactivation of C3b. CRIg (complement receptor of immunoglobulin family) acts on C3b and inhibits alternative pathway activation. CD59 binds C8 and C9, thereby preventing the formation of the membrane attack complex C5b-9. Decay accelerating factor (DAF or CD55) increases the breakdown of the C3 and C5 convertases within the pathway

in factor H function are strong risk factors for development of atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G), and disease in these patients is often limited to the kidneys [20]. Mutations in CD46, a co-factor to factor I, have also been identified in patients with aHUS and C3G [21]. It is unknown why the kidney is so frequently the target of complement-mediated inflammation, but the complement activating proteins may become concentrated

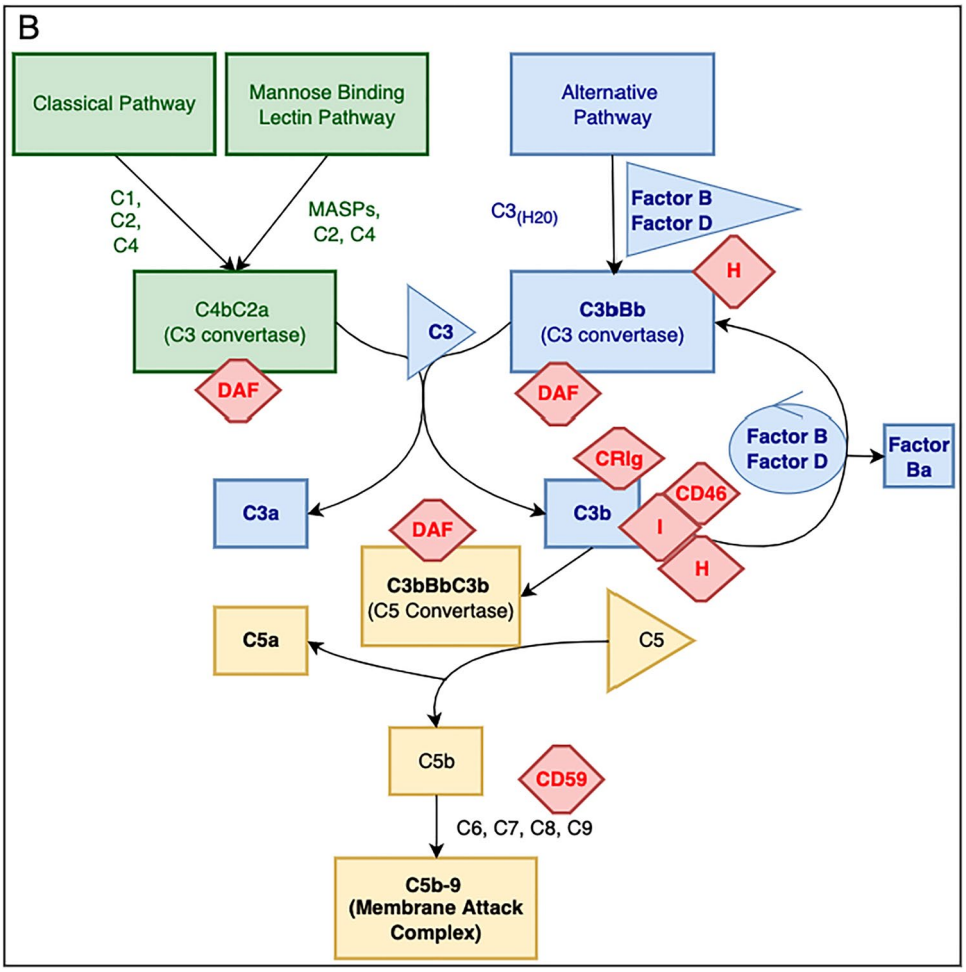

Fig. 1 (continued)

in the capillaries due to glomerular filtration, and the acidic environment and ammonia synthesis within the kidney may also promote alternative pathway activation [22].

AKI pathogenesis

Despite a large number of different etiologies of AKI, many disparate mechanisms converge on a common pathological lesion of tubular injury. Tubular epithelial cells are susceptible to injury after a wide range of insults, including sepsis, toxins, and IRI (e.g., after cardiovascular surgery or kidney transplantation). IRI and toxins directly injure tubular epithelial cells [23–26]. Patients with sepsis or systemic inflammatory response syndrome (SIRS) are often hypotensive and treated with nephrotoxic medications, and sepsis also causes several microvascular derangements that reduce kidney perfusion, including capillary leak, microthrombi formation, and endothelial injury [27–34]. Although patients are often diagnosed as having "acute tubular necrosis" (ATN), this is a histologic diagnosis and biopsies are usually not performed in these clinical settings. Nevertheless, tubular epithelial cells and granular casts (which contain degrading epithelial cells) can often be detected in the urine, providing evidence of tubular injury.

Interestingly, even though the primary insult to the kidney is often non-immune (i.e., nephrotoxic exposures or IRI), AKI is associated with significant tissue inflammation. When tissue damage occurs, expression of "damage associated molecular patterns (DAMPS)" increases on cell surfaces, which increases vascular permeability and ischemic hypoperfusion [35]. DAMPS are recognized by toll-like receptors (TLRs), of which TLR4 has increased expression after IRI on tubular epithelial cells and on leukocytes infiltrating the kidney [36]. Together, DAMPs and TLRs act after endothelial and tubular injury to release inflammatory cytokines and chemokines, which recruit leukocytes and contribute to further kidney injury [26, 37, 38]. There is also bi-directional crosstalk between TLRs, complement, and cytokines: TLRs induce expression of complement

components, and complement receptors may regulate TLR-dependent responses [39].

TLR4 activation also leads to enhanced complement factor B synthesis after sepsis [40]. Mice treated with an anti-factor B inhibitor were protected from apoptotic and necrotic tubular injury [41]. TLRs also prime cells to undergo pyroptosis, which is programmed cell death leading to cellular lysis and release of proinflammatory intracellular contents [42].

These kidney-specific proinflammatory events are transmitted systemically and lead to cytokine anomalies, perturbations in other immune cells, and dysfunction in the lungs, heart, and other vital organs [43, 44].

Complement activation in AKI — lessons from pre-clinical models

Complement has been implicated in AKI pathogenesis in numerous animal models, including bilateral IRI, nephrotoxin-induced injury, and sepsis-induced injury. It is noteworthy that the complement system plays a pathogenic role in animal models that employ diverse types of injury and a wide range of species. In contrast to models of glomerular disease, complement activation in models of AKI primarily occurs within the tubulointerstitium and peri-tubular capillaries. These studies shed light onto the pathways by which complement is activated in the injured kidney, the mechanisms by which the system contributes to injury, and the role of complement regulatory proteins in limiting/permitting complement activation in AKI.

Activation pathways in AKI

The mechanisms by which complement is activated have been carefully investigated in models of kidney IRI. One seminal study compared IRI in mice with genetic deficiency of several different complement proteins [45]. Mice lacking C3 ($C3^{-}/^{-}$ mice) cannot activate complement through any of the activation pathways and were protected from injury [45]. In contrast, mice genetically deficient in C4 ($C4^{-/-}$ mice) cannot activate complement through the classical and lectin pathways, and these mice had no protection from IRI. In another study using a similar IRI model, mice with genetic deletion of factor B ($fB^{-/-}$ mice, unable to activate complement through the alternative pathway) were protected from IRI [46]. Together, these studies point to an important role for the alternative pathway, and similar patterns of injury have been observed in other models of AKI. In sheep, for example, non-steroidal anti-inflammatory drug (NSAID)-induced AKI is associated with alternative pathway complement activation in the kidney [24]. Alternative pathway activation was also seen in the tubulointerstitium of mice with ciclosporin-induced AKI [47].

The complement system is also activated in several animal models of sepsis. Sepsis from cecal ligation and puncture led to TLR activation and increased levels of factor B and factor C3 gene expression in the liver, heart, and kidneys. Activation fragments of these complement proteins were increased in the serum, liver, heart, and kidneys [40, 48], suggesting alternative pathway activation. Neutralization of complement activation using an antibody that prevents cleavage of C5, antagonists of C5a receptors 1 and 2, or a C5a receptor 1 knockout model all demonstrated improvement in mortality and end-organ failure [49–53].

Factor $B^{-/-}$ mice had improved survival post-sepsis, further indicating a role for the alternative pathway [40]. This study also showed that alternative pathway deficiency retained the ability of the immune system to clear infection. Importantly, the immune response generated lower levels of proinflammatory cytokines that have been implicated in the pathogenesis of sepsis.

More recently, studies in mice have revealed unique mechanisms of complement activation in the tubulointerstitium, and that complement regulation is disrupted after injury of tubular epithelial cells (Fig. 2). The lectin pathway activating protein collectin-11 (CL-11) is released from post-ischemic stressed renal tubule cells and then binds to L-fucose that is expressed on the epithelial cell surface [54] (Fig. 2B). CL-11 can engage mannose associated serine protease (MASP)-2, a lectin pathway enzyme that cleaves C3. The process is then presumably amplified through the alternative pathway, explaining the role of factor B in this process. Interestingly, a model of rhabdomyolysis-induced AKI was also recently shown to activate complement through the same mechanisms [55]. This suggests that CL-11-induced complement activation in the tubulointerstitium may be a common response of the tubules to various stressors. Furthermore, expression of complement regulatory proteins on the epithelial cell surface is reduced after ischemia (Fig. 2C) [56]. Thus, while injury of the tubular epithelial cells increases the local concentration of activating proteins, it simultaneously reduces the concentration of regulatory proteins.

In the study mentioned above, mice lacking the terminal complement protein C6 ($C6^{-/-}$ mice) showed protection from IRI comparable to that seen in $C3^{-/-}$ mice [45]. The $C6^{-/-}$ mice can generate C3a, C3b, and C5a, but cannot form C5b-9. Thus, protection in this strain implicates C5b-9 in kidney IRI. Other studies have also investigated the role of C5a, however, demonstrating that mice deficient in C5a receptor (C5aR) expression or wild-type mice treated with a C5aR antagonist are protected in models of IRI [57, 58]. It is important to understand the relative contributions of these various complement-mediated

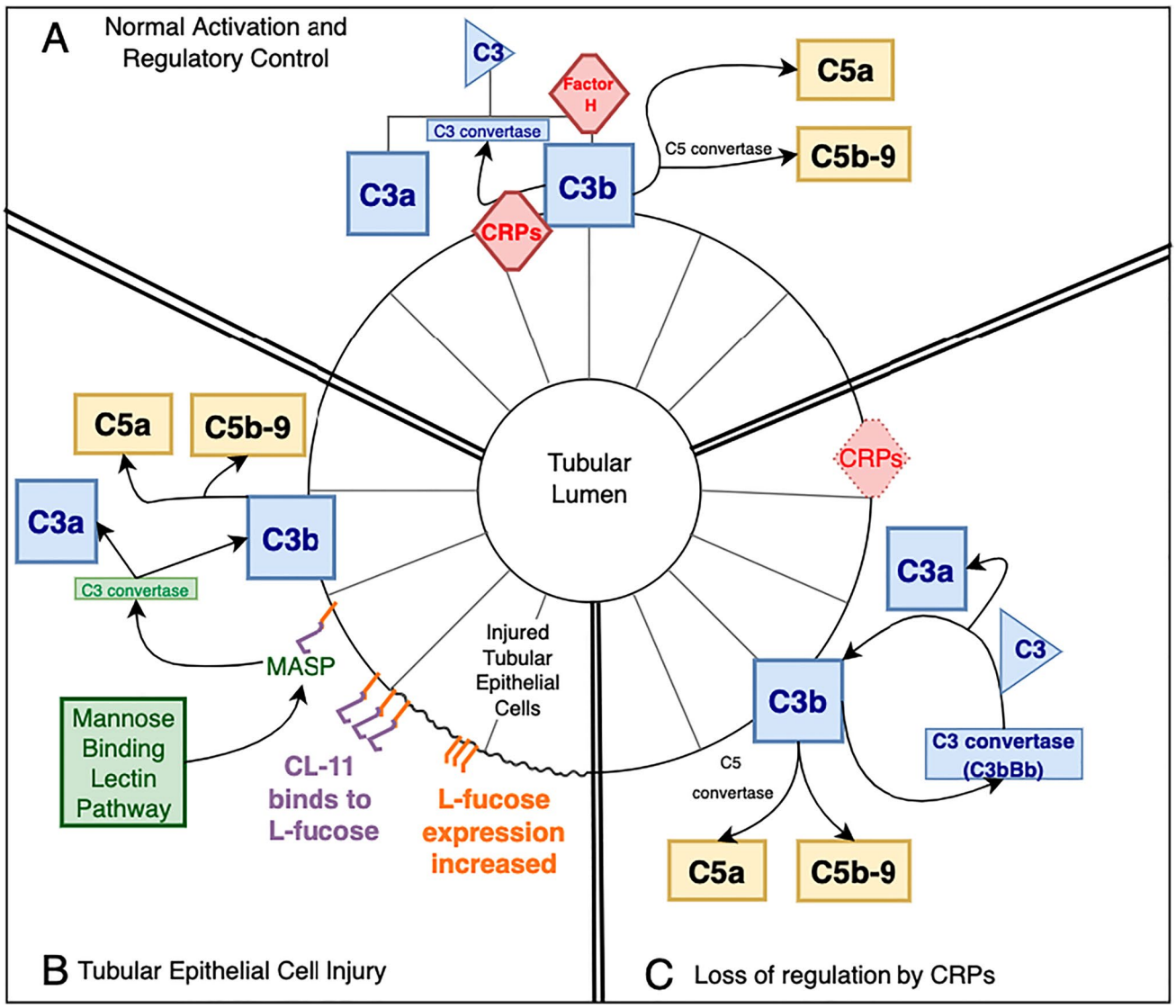

Fig. 2 Site of complement activation within the renal tubulointerstitium. **A** Normal complement activation and regulatory control. Complement activation through any inciting pathway activates the conversion of the fluid-phase C3 into C3a and C3b. C3b deposits on the renal tubular epithelial cell in a process normally controlled by factor H and cell surface complement regulatory proteins (CRPs). **B** Complement activation in the setting of tubular epithelial cell injury. Stressed or injured tubular epithelial cells increase expression of L-fucose on the cell surface. Collectin-11 (CL-11) functions as a pattern recognition molecule within the mannose-binding lectin pathway. CL-11 binds to L-fucose. CL-11/L-fucose/MASP complexes then promote complement activation via cleavage of C3. **C** Expression of cell-surface regulatory proteins (CRPs) is disrupted after tubular epithelial cell injury. In this situation, C3b and the C3 convertase are no longer efficiently inactivated, and activation proceeds

mechanisms to kidney disease, as drugs that block specific complement pathways and activation fragments are in clinical development [59, 60]. Nevertheless, these studies demonstrate that complement activation in the tubulointerstitium can directly injure target epithelial cells and also trigger a broader inflammatory response that exacerbates injury. In addition to these acute effects, activation of the complement system may also contribute to the long-term sequelae of AKI. Several studies have shown, for example, that complement deficiency protected mice from development of kidney fibrosis after induction of ischemic or toxic acute tubular injury [61–63].

The role of complement regulatory proteins in AKI

As mentioned above, defective alternative pathway regulation is a strong risk factor for aHUS and C3G. This begs the question as to whether these same defects (e.g., mutations in factor H) predispose patients to kidney injury in the setting of IRI or toxin exposure, particularly given the

prominent role played by the alternative pathway in these settings. Mice with partial deficiency of complement regulatory proteins, including mice with heterozygous deficiency of factor H, develop worse IRI than control mice [56, 64]. Similar findings have not been reported in humans, although it is possible that the glomerular process dominates the clinical assessment of patients with aHUS and C3G.

Mice with genetic deletion of the cell surface complement regulators CD55 (also known as decay accelerating factor or DAF) and CD59 (*Daf-1*$^{-/-}$*CD59a*$^{-/-}$ mice) also develop more severe IRI than control mice [65, 66]. Interestingly, complement is activated on the peritubular capillaries after IRI in this strain, whereas activation after IRI typically occurs on the tubular epithelial cells. Furthermore, healthy endothelial cells ordinarily control complement activation on the cell surface very effectively [67]. Thus, these regulatory proteins probably limit the severity of vascular injury in IRI when they are expressed normally. Complement receptor of the immunoglobulin superfamily (CRig) is a cell surface receptor expressed by macrophages. CRig binds to C3b, blocking the formation of C3bBb (C3 convertase) and preventing activation of the alternative pathway [17, 68] (Fig. 1B). A specific role of CD55, CD59, and CRig in human AKI has not, as far as we are aware, been demonstrated.

Nevertheless, the above studies highlight the role of complement regulatory proteins in limiting kidney injury, and also some of the features that distinguish AKI from other complement-mediated kidney diseases, such as glomerular disease and antibody-mediated transplant rejection. Complement activation in the kidney is pathologic in all of these settings, but the mechanisms of activation, the histologic patterns of activation, and the downstream mediators of injury appear to be distinct [69].

Evidence of complement activation in patients with AKI

Studies of adult patients

Evidence of complement activation has been detected in urine and biopsy samples from adult patients with AKI, demonstrating that complement is also activated in human disease. C3d is deposited along the tubular basement membrane of biopsies with morphologic evidence of tubular injury, and the pattern is similar to that seen in animal models [70]. C4d was not seen in the tubulointerstitium, arguing against involvement of the classical or lectin pathways. As mentioned above, however, some mechanisms of lectin pathway activation can bypass C4. Complement fragments were also measured in urine samples from a case–control study that evaluated AKI in adults after cardiac surgery. In patients with AKI, urine factor Ba increased as AKI severity increased, and the change in Ba levels preceded the rise of serum creatinine [71]. This shows an ability to use urine factor Ba as a mechanistic biomarker predicting AKI development in this patient population. These findings are also consistent with preclinical models showing the causative role of factor B in AKI pathogenesis.

Complement as primary driver as disease presenting as AKI

It is worth noting that a child presenting with AKI often has an unclear diagnosis, and complement-mediated diseases (e.g., aHUS and C3G) may present with similar findings. The cardinal signs of aHUS, for example, may overlap with other causes of AKI in critically ill children, such as sepsis with disseminated intravascular coagulation (DIC) (causing thrombocytopenia and AKI), or pre-renal AKI caused by diarrhea and hypovolemia. Furthermore, C3G may mimic a post-infectious glomerulonephritis, and as the clinical manifestations are often preceded by infection, sepsis may still be on the differential. The diagnosis of transplant-associated thrombotic microangiopathy (TA-TMA) can be even more challenging. These patients have often been treated with nephrotoxic drugs or radiocontrast, and they are immunocompromised and at high risk of infection. Thus, many patients with TA-TMA likely have concomitant tubular injury. Even though aHUS, C3G, and TA-TMA are rare disorders, they represent a treatable subset of patients with AKI. These disorders, if untreated, are associated with high morbidity and mortality rates, and therefore, clinicians must have a high index of suspicion for the diagnoses. They also serve as models for treatment with complement-targeted therapeutics. Overall, C3G/aHUS may have overlapping presentations and pathophysiology and have been discussed in previous excellent reviews [72, 73]. Further descriptions of these diseases are beyond the scope of this review.

Evidence of complement activation in pediatric patients with AKI

In addition to complement as the primary disease driver, complement may be activated secondarily after tubular injury from ischemia, nephrotoxins, or via a systemic inflammatory response. A pilot study evaluated plasma and urine complement factors in critically ill children who required invasive mechanical ventilation [74]. Children with stage 3 AKI (based on KDIGO criteria) were matched to patients without AKI based on illness severity scores (PELOD-2) [75]. As this was a post hoc analysis, there was variable timing in specimen acquisition with some patients in whom AKI was diagnosed prior to urine and plasma collection. However, all specimens were obtained prior to KRT initiation. Urine factor Ba and plasma

factor C4a levels increased in proportion to AKI severity, with the highest levels occurring in patients who ultimately required KRT. Plasma C4a levels were independently associated with major adverse kidney outcomes at 30 days (MAKE30). Severe KDIGO stage 2–3 AKI at day 3 of Pediatric Intensive Care (PICU) admission was associated with urine Ba, plasma Bb, plasma C4a, and plasma C3a levels. This adds to the evidence that complement is involved in AKI pathogenesis. The relative contributions of each factor (and thus the differing pathways) remain unclear, however, as well as the different implications of changes in plasma compared to urine complement measurements. It is also unknown whether the role of complement activation in pediatric AKI differs from the role in adult AKI, and this question warrants more research.

An important question is whether the elevation in plasma complement activation fragments in a patient's AKI is caused by complement activation as part of the disease process or whether it is simply that clearance of the fragments is reduced in the setting of a lower glomerular filtration rate (GFR). The increase in the levels of multiple different fragments in both plasma and urine argues that the changes are due to increased activation of the system, not simply reduced clearance through the kidney. A related question is how to determine whether fragments detected in plasma and urine were generated within the kidney. For patients with AKI in the setting of systemic illness, such as sepsis, there is likely both intrarenal and extrarenal complement activation. In the study cited above, however, patients with and without AKI were matched via PELOD-2 scores (and thus illness severity) [75], so differences in systemic inflammation were probably not the only reason for elevated plasma complement levels.

Although increases in plasma complement fragments are probably not due to lower clearance by the kidney, a reduced GFR does contribute to increased alternative pathway activation. Normally factor D is filtered through the glomerulus and nearly fully reabsorbed within the tubules, then rapidly catabolized [76, 77]. In vitro experiments show that supplementation of factor D to normal sera resulted in an increase in alternative pathway activity, with similar function compared to sera of patients with stage 5 CKD [78, 79]. In patients with stage 5 CKD, factor D in plasma is increased nearly tenfold due to impaired glomerular filtration, and elevated levels of Ba in plasma suggest that there is also an increase in AP turnover [80, 81]. Factor D levels have not been studied in AKI but may also increase in this setting due to decreased GFR.

Complement proteins as biomarkers

The pitfalls of serum creatinine and urine output as the sole biomarkers used to predict, diagnose, and monitor treatment effectiveness of AKI are well known, and thus the search for ongoing biomarkers continues. Ideally, an earlier marker of kidney injury would lead to improved timeliness of diagnosis/risk identification and optimized initiation of potential therapeutic interventions. Table 1 shows complement factors that have shown potential as future biomarkers in a variety of kidney diseases. Of these, urine Ba, plasma C3a, and plasma C4a have shown promise in pilot studies of AKI [71, 75], and other potentially relevant AKI biomarkers include c5b-9 and Bb levels.

Various other biomarkers are also being evaluated for use in pediatric AKI diagnosis, including neutrophil gelatinase-associated lipocalin (NGAL), IL-18, KIM-1, IGFBP-7, TIMP-2, and a "renal angina index" (RAI) [101–108]. Complement activation may be an indication of tissue damage and/or inflammation, but the predictive value of complement fragments relative to these other biomarkers requires further study. Even if complement biomarkers are not superior to these other analytes for the early detection of AKI, the complement measurements could still be particularly useful for identifying patients most likely to benefit from therapeutic complement inhibitors. They could also be used as pharmacodynamic/response biomarkers to show the biological response in patients who receive such treatments.

Complement inhibitors for prevention or treatment of AKI

Complement inhibitory drugs are currently being used to treat several kidney diseases, and ongoing clinical trials are testing many additional drugs in pediatric patients (Table 2) [109, 110]. Eculizumab and ravulizumab, for example, are monoclonal antibodies to C5 that are approved for treatment of aHUS. Avacopan is a C5aR antagonist that was recently approved for treatment of ANCA vasculitis and recently completed a phase 2 trial in patients with C3G. Although there are not currently any clinical trials of complement inhibition for prevention or treatment of AKI per se, insights can be gained from studies in other diseases. Importantly, eculizumab and ravulizumab have been used in pediatric patients with aHUS, and they are safe and effective when given in conjunction with meningococcal vaccination and/or prophylactic antibiotics [111–116]. A factor B inhibitor has been tested in Phase II trials evaluating adult patients with aHUS, C3G, and paroxysmal nocturnal hemoglobinuria (PNH) [117]. Similarly, a C3 inhibitor and factor D inhibitors have been explored in patients with PNH and C3G [118, 119]. Given the evidence that the alternative pathway is involved in AKI pathogenesis, these drugs may hold particular promise as a treatment for AKI, and the current studies will provide important data regarding the safety of alternative pathway inhibition in patients with kidney disease.

Table 1 Complement factors with potential use as AKI biomarkers

Biomarker	Population/disease state	Significant findings
Urine Ba	Adults post-cardiac surgery [71]	↑ Urine Ba = ↑AKI severity
	Critically ill children [74]	↑ Urine Ba = ↑AKI severity
	FSGS [82]	↑ Urine Ba at diagnosis ctc
Urine Bb	ANCA-associated vasculitis [83]	↑ Urine Bb in active disease vs. disease remission
	FSGS [84]	↑ Urine Bb ctc
Plasma Ba	Adults with TA-TMA [85]	↑ Plasma Ba → 2 weeks later = TA-TMA diagnosis
Plasma Ba	FSGS [82]	↑ Plasma Ba at diagnosis ctc
Plasma Bb	Adults with primary membranous nephropathy [84]	↑ Plasma Bb compared to control
Plasma Bb	FSGS [82]	↑ Plasma Bb = more severe disease
Urine C3	Critically ill adults with sepsis [86]	Urine C3a/C3 ratio is an inverse acute phase reactant
Urine C3a	FSGS [84, 87]	↑ Urine C3a ctc Urine C3a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
Urine C3a	ANCA-associated vasculitis [83]	↑ Urine C3a in active disease vs. disease remission
Urine C3b	FSGS [88]	↑ Urine C3b ctc
Urine C3d	Lupus nephritis (LN) [89]	↑ Urine C3d elevated in active LN compared to inactive or chronic LN
	Tubulo-interstitial nephritis [90]	↑ Urine C3d ctc
Plasma C3a	Critically ill children [74]	↑ Plasma C3a = ↑AKI severity
	Adults with primary membranous nephropathy [84]	↑ Plasma C3a compared to control
	FSGS [84, 87]	Plasma C3a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
Plasma C4a	Critically ill children [74]	↑ Plasma C4a = MAKE30 outcomes
Urine C4a	FSGS [82]	↑ Urine C4a at diagnosis ctc
Urine C5a	Kidney transplant [91]	↑ Donor urine C5a associated with recipient's delayed graft function
	FSGS [84, 87]	Urine C5a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
	ANCA-associated vasculitis [83]	↑ Urine C5a in active disease vs. disease remission
Urine factor H	IgA nephropathy [92]	↑ Urine factor H ctc
	Cisplatin nephropathy [93]	↑ Urine factor H after cisplatin, correlated with lower eGFR
	Nephritis [94]	↑ Urine factor H ctc
Urine properdin	IgA nephropathy [92]	↑ Urine properdin ctc
	Kidney transplant recipients [95]	↑ Urine properdin → ↑ risk of graft failure
Urine CD59	Type 2 DM [96]	↑ → Lower risk of stage 5 CKD and death
	Membranous glomerulonephritis [97]	↑ Urine CD59 ctc
Plasma sC5b-9	Deceased donor kidney transplant recipients [98]	↑ Perioperative plasma sC5b-9 = worse graft function 1 year later
	FSGS [84]	↑ Plasma sC5b-9 ctc
Urine sC5b-9	Membranous nephropathy [84, 99, 100]	↑ Urine sC5b-9 ctc Urine sC5b-9 levels correlated with worse outcome with potential for dynamic marker of ongoing injury Urine sC5b-9 levels may identify patients with a membranous lesion
	FSGS [84]	↑ Urine sC5b-9 ctc
	IgA nephropathy [92]	↑ Urine sC5b-9 ctc
	Cisplatin nephropathy [93]	↑ Urine sC5b-9 after cisplatin, correlated with lower eGFR
	Kidney transplant recipients [95]	↑ Urine sC5b-9 → ↑ risk of graft failure
	ANCA-associated vasculitis [83]	↑ Urine sC5b-9 in active disease vs. disease remission
	FSGS [82, 87, 88]	↑ Urine sC5b-9 at diagnosis ctc Urine C5a correlated with renal dysfunction, proteinuria, and interstitial fibrosis
	Membranous glomerulonephritis [97]	↑ Urine sC5b-9 ctc

Ctc = compared to control, FSGS = focal segmental glomerulosclerosis, TA-TMA = transplant-associated thrombotic microangiopathy, DM = diabetes mellitus

Table 2 Complement therapeutics for pediatric kidney diseases

Complement therapeutics				
Name	Disease	Target	Status	Pediatric age or weight range
Eculizumab	aHUS	C5	FDA approved	1 month–18 years
Eculizumab	Kidney transplantation	C5	Single-center trial completed	1 year–18 years
Eculizumab	TA-TMA	C5	Phase 2 clinical trial	Birth–18 years
Eculizumab	Primary membranoproliferative glomerulonephritis	C5	Phase 2 clinical trial	Children ≥ 30 kg
Eculizumab	STEC-HUS	C5	Phase 2, open-label multicenter trial	2 months–18 years
Ravulizumab	aHUS	C5	Phase 3, open-label multicenter study	Birth–18 years
Ravulizumab	TA-TMA	C5	Phase 3, open-label single-arm multicenter trial	1 month–18 years
Crovalimab	aHUS	C5	Phase 3, single-arm study, multicenter	Birth–18 years
Cemdisiran	aHUS	C5 mRNA liver production suppression	Phase 2, randomized, double-blind, placebo-controlled trial	12–18 years
Avacopan	C3 glomerulopathy	C5aR	Randomized double-blind placebo phase 2 clinical trial	12–18 years
Danicopan	C3 glomerulopathy Immune-complex-mediated membranoproliferative glomerulonephritis	Factor D	Phase 2 clinical trial	17–18 years
Pegcetacoplan	C3 glomerulopathy Immune-complex-mediated membranoproliferative glomerulonephritis	C3	Phase 3, randomized, placebo-controlled multicenter study	12–18 years

The use of complement inhibitory drugs in patients with sepsis and in kidney transplant recipients may also provide insight into the ability of these drugs to prevent or treat AKI. For example, delayed graft function (DGF) in transplant recipients is primarily caused by IRI. Eculizumab has been trialed in 57 pediatric kidney transplantation recipients [120]. Patients were randomized to a single dose of eculizumab prior to transplantation, and treatment with eculizumab was associated with better early graft function. There was also lower arteriolar hyalinosis at subsequent biopsies in the eculizumab-treated group. Of concern, however, four patients in the eculizumab group developed flu-like illnesses within 60 days of transplantation and lost their allografts. This interesting study supports the use of complement inhibitors for preventing AKI, but also underscores the infectious risk of complement inhibition. In contrast to these results, a study of eculizumab in adult transplant recipients was well tolerated but did not reduce the incidence of DGF [121].

Even though the complement system helps the body to fight infections, there is evidence that complement activation contributes to the pathogenesis of sepsis [122]. Complement inhibitors are being tested in patients with sepsis and trauma and could potentially reveal whether complement inhibition prevents AKI in these high-risk settings [123]. Along the same lines, clinical trials are currently underway studying anti-complement therapy in COVID-19-induced sepsis, and preliminary results show that they are safe, tolerable, and there was a trend towards less AKI in treated patients [124, 125].

Conclusion

AKI in children is associated with a high burden of morbidity and mortality. Unfortunately, there are currently no effective therapies for preventing or treating this disease. Complement activation appears to play a significant role in AKI pathogenesis in pre-clinical animal models, and there is evidence that the system is activated in pediatric and adult patients with AKI. Multiple complement inhibitory drugs are in clinical development, and many of these agents have already been used in patients with kidney disease. These drugs hold promise for preventing AKI in high-risk patients, or for treating patients with established disease. Equally important, complement-related biomarkers predict the development of AKI, and may be useful for guiding and/or monitoring treatment with complement inhibitory drugs.

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Declarations

Conflict of interest Dr. Stenson and Dr. Kendrick declare they have no financial interests. Dr. Dixon is a consultant for Apellis and Alexion Pharmaceuticals, Inc. Dr. Thurman received royalties from Alexion Pharmaceuticals, Inc. and is a consultant for Q32 Bio, Inc., a company developing complement inhibitors. He also holds stock and will receive royalty income from Q32 Bio, Inc.

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