Minireview

The potential role of complement alternative pathway activation in hypertensive renal damage

Chongjian Wang*, Zhiyu Wang* and Wen Zhang

Division of Nephrology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
Corresponding author: Wen Zhang. Email: zhangwen255@163.com
*These authors contributed equally to this work.

Impact Statement

First, we discuss the evidence of the complement alternative pathway (AP) activation in subjects with hypertension and hypertensive renal damage. Next, we summarize the potential conditions that facilitate the activation of the AP in the kidney of patients with hypertension. In addition, we discuss the effect of the AP activation products on renal cells and immunocytes. Finally, we explore the efficacy of complement inhibition therapy in the context of hypertensive kidney damage. This minireview provides a systematic understanding of the role of the complement AP in hypertensive renal damage.

Abstract

Hypertensive renal damage is a common secondary kidney disease caused by poor control of blood pressure. Recent evidence has revealed abnormal activation of the complement alternative pathway (AP) in hypertensive patients and animal models and that this phenomenon is related to hypertensive renal damage. Conditions in the setting of hypertension, including high renin concentration, reduced binding of factor H to the glomerular basement membrane, and abnormal local synthesis of complement proteins, potentially promote the AP activation in the kidney. The products of the AP activation promote the phenotypic transition of mesangial cells and tubular cells, attack endothelial cells and recruit immunocytes to worsen hypertensive renal damage. The effects of complement inhibition on hypertensive renal damage are contradictory. Although clinical data support the use of C5 monoclonal antibody in malignant hypertension, pharmacological inhibition in hypertensive animals provides little benefit to kidney function. Therefore, the role of the complement AP in the pathogenesis of hypertensive renal damage and the value of complement inhibition in hypertensive renal damage treatment must be further explored.

Keywords: Hypertensive renal damage, complement alternative pathway, complement C3, C3a, C5a, C5b-9

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Pathogenesis of hypertensive renal damage

Hypertensive renal damage is a common secondary kidney disease caused by poor blood pressure control. The main clinical characteristics of hypertensive renal damage include long-term hypertension, minimal proteinuria (usually less than 0.5 g/day),[1] and progressive renal insufficiency. Some patients have sharply elevated blood pressure and severe kidney damage, a phenomenon termed malignant hypertension, which represents an emergency and is associated with future adverse renal outcomes. Our data showed that 60% of patients with hypertension and 100% of patients with malignant hypertension developed stage 3 chronic kidney disease (CKD) at 20 months.[2] The basic pathogenesis of hypertensive renal damage is hemodynamic injury and can be divided into two stages. On one hand, in response to long-term high blood pressure stimulation, renal arterioles are gradually altered, converting from hypertrophy to sclerosis and hyalinosis. On the other hand, secondary to narrowing of the arteriolar lumen and reduced flow, the glomerulus and renal tubules acquire ischemic damage, leading to glomerulosclerosis, tubular atrophy, and interstitial fibrosis.[3] In renal pathology, the above-mentioned features can be summarized as benign nephrosclerosis, which is observed in most patients with chronic hypertensive injury.[4] In patients with malignant hypertension, a sudden change in blood pressure directly affects glomeruli, arterioles, and interlobular arteries when the pressure exceeds the renal autoregulation range. Renal biopsy has revealed that the lesions of these patients included endothelial cell swelling, fibrinoid necrosis, capillary or arteriolar thrombi, and onion skin lesions.[4,5] Controlling and monitoring blood pressure in a timely manner are the basic treatments for hypertension. However, while blood pressure can be controlled within the normal range by antihypertension medications, these medications do not always prevent the development of albuminuria in hypertension patients.[6] Thus, researchers suspect that additional drivers of hypertensive renal damage exist, and a series of studies have been performed to elucidate the

underlying mechanisms. To date, various factors have been reported to contribute to the development of hypertensive renal damage, such as (1) endothelial dysfunction,[7] (2) activation of the renin–angiotensin–aldosterone system (RAAS),[8] (3) oxidative stress,[9] and (4) activation of the immune system. Evidence collected over the past years suggests that hypertensive renal damage is, at least in part, an immune-mediated inflammatory disorder.[10,11] The complement system is a collection of soluble and membrane-bound proteins that act as powerful amplifiers of the innate and adaptive immune systems. Among the three different complement activation pathways, the AP is not only an independent activation mode but also an amplification loop for the other two pathways converging on C3. Hence, in this review, we mainly concentrate on evidence supporting complement AP activation in subjects with hypertensive renal damage and discuss the effects of its products on renal cells and tissues.

Abnormal activation of the complement AP in hypertension and hypertensive renal damage

The complement system is a component of the innate immune system and comprises more than 30 soluble and membrane proteins. In normal cases, complement proteins are inactive and require activation to gain biological activity. Three activation pathways exist – the classical pathway, lectin pathway, and AP. Among them, the complement AP accounts for approximately 80–90% of complement activation.[12,13]

Activation process and function of AP

The complement AP activation starts with complement C3, which circulates in fluid at a high concentration and is hydrolyzed to form C3(H_2O) spontaneously but slowly.[14] Under the enzymatic digestion of factor D (FD), factor B (FB) is cleaved into Bb fragments and then combines with C3(H_2O). The resulting product, C3(H_2O) Bb, is a serine protease that can cleave C3 to generate C3b. Next, the C3b binds to membrane surfaces, resulting in another C3 convertase (C3bBb) via the recruitment of FB and FD. The process in which C3b mediates the production of additional C3b is called complement amplification. As the ratio of C3b to Bb increases, C5 convertase (C3bBbC3b) is formed, binds C5 with high affinity, cleaves C5 to C5b and C5a, and subsequently forms C5b-9 in the target membrane.[15] Because the complement AP is spontaneously initiated, accurate regulation of the activation degree is the main mechanism for maintaining its normal function and preventing tissue damage. These regulatory factors can be divided into the positive regulator properdin (complement factor P, CFP), liquid-phase negative regulators (CFH, CFI), and membrane-anchored negative regulators (CD46, CD55, and CD59). The types and concentrations of negative regulatory proteins are far greater than those of positive regulatory proteins, explaining why complement-mediated injury is rare under normal conditions. The activation and regulation process of the complement AP is shown in Figure 1. Once activated by pathogenic factors, complement proteins can perform various functions. For example, they play a critical role in the removal of pathogens, cellular debris, and dead cells and bridge the gap between innate and adaptive immunity to mediate inflammation.[16,17] Recent studies have found that complement proteins can also directly interact with tissue cells, including epithelial cells and endothelial cells, to participate in disease development.[18–20] Accumulating clinical and animal data confirm that the complement AP activation is associated with different types of hypertension and hypertensive renal damage.

Activation of the complement AP in subjects with hypertension

Compared with normotensive controls, complement fragments including C3a and C5a are significantly increased in hypertensive patients, which imply the overactivation of complement system.[21] Moreover, clinical studies showed the positive correlation between systolic blood pressure (SBP) and C3 but not C4.[22,23] C4 is a molecule involved in both classical and lectin pathways. This inconsistency suggests the involvement of the AP in the hypertensive pathogenetic process, although direct data on the activity of AP are still lacking. The relationship between hypertension and complement AP was further demonstrated by spontaneously hypertensive rats (SHRs) with *CFB* gene knockout. Complement FB is an indispensable participant in the AP amplification loop to produce C3 convertases. Researchers have found that these genetically manipulated rats had lower AP activity and blood pressure than normal SHRs.[24]

Activation of the complement AP in subjects with hypertensive renal damage

Abnormality of the complement AP is also deemed a risk factor for the progression of hypertension to hypertensive nephropathy. Based on urinary proteomic analysis, researchers have identified C3 as a pathogenic marker associated with albuminuria development in hypertensive patients with chronic renin–angiotensin system (RAS) suppression.[25] The hypertensive rat model also emphasizes the crucial role of the complement system in renal function reduction.[26] Recently, the activation of the complement AP has been a hot spot on the pathogenesis of malignant hypertension. Studies have found elevated complement activation products (C3a, C5a, and sC5b-9) in body fluids and excessive formation of C5b-9 *ex vivo* in malignant hypertensive patients.[5,27] In the renal biopsy, strong immunofluorescence of membrane-bound C3c and membrane-inserted C5b-9 revealed local activation.[5,28] Interestingly, the levels of FB, FD, FP, and FH in those patients are disordered and correlative with renal functions and thrombotic microangiopathy (TMA) lesions,[5] while the classical pathway activity and C4 level are normal.[27] Genetic abnormalities of complement alternative proteins, including C3, CFH, and CFI, are frequent in malignant hypertension. They are more likely to develop end-stage renal disease (ESRD) and disease recurrence than those without genetic variants.[28] The results signify that the AP is largely involved in hypertensive-associated complement activation.

Considering that hypertension is a heterogeneous disease, whether complement AP activation occurs in different hypertensive renal damage models and clinical cohorts should be carefully examined. In addition, the level change

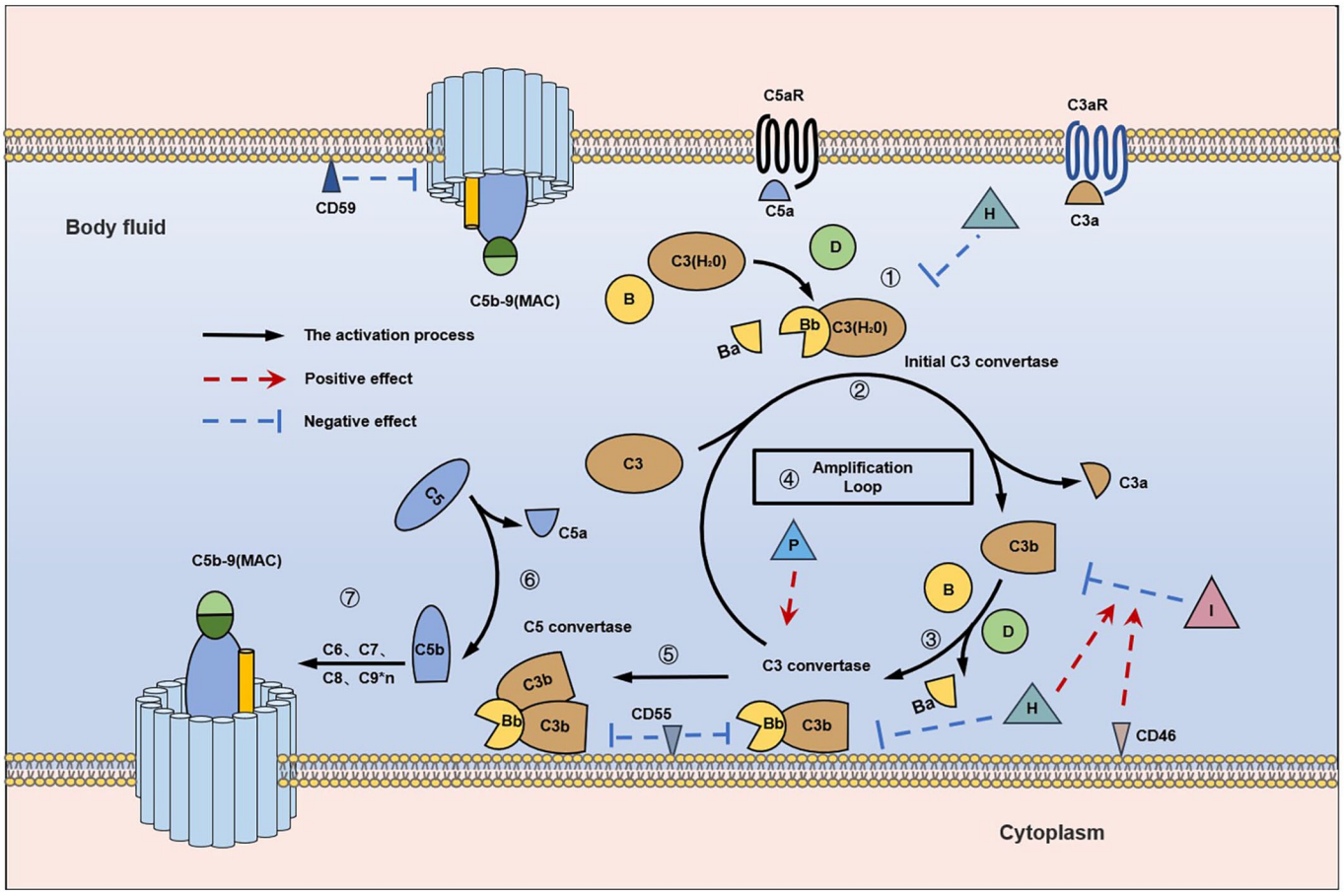

Figure 1. The activation and regulation process of the complement alternative pathway. The process could be divided into the following steps: (1) Hydrolyzed C3 (C3H2O) in body fluids generate initial C3 convertase under the action of factor D and factor B. (2) Initial C3 convertase then cleaves C3 into C3a and C3b. (3) C3b attaches to the membrane surface and recruits factors B and factor D to form a new C3 convertase. (4) The amplification loop is established and more C3b and C3 convertases are produced. (5) As the ratio of C3b to Bb increases, C3b binds to C3 convertase to form C5 convertase. (6) C5 convertase cleaves C5 into C5a and C5b. (7) C5b recruits C6, C7, C8, and C9 to form a membrane attack complex inserted into the cytomembrane. Multiple factors regulate the process: Factor P (properdin) stabilized the C3 convertases to promote AP activation; Factor I (FI) degrade C3b to block the process; Factor H inhibits the binding of C3b (or C3H2O) to factor B and facilitates the degradation of C3b by factor I; Membrane negative regulatory factor CD46 functions as a cofactor of FI; CD55 targets C3 convertase and C5 convertase to inhibit the activation of AP; CD59 prevents the formation of C5b-9 in cytomembrane. (A color version of this figure is available in the online journal.)

of complement proteins such as C3 and FB may not necessarily indicate the overactivity of AP. More specific complement AP activation markers must be detected, such as plasma levels of Ba and C3bBb, and functional studies of the AP.

Potential conditions facilitating activation of the complement AP in subjects with hypertensive renal damage

Some conditions potentially promote complement AP activation in the kidney of subjects with hypertension, including renin-induced C3 cleavage, decreased binding of the negative regulator CFH to heparin sulfate (HS) in the glomerular basement membrane (GBM), and disordered local complement synthesis. These potential conditions are presented in Figure 2.

Renin cleaves C3

The cleavage of complement C3 by renin can be an additional force to promote the activation of AP in the kidney. Renin is an aspartate protease and initiates the RAAS by cleaving angiotensinogen into angiotensin I.[29] High plasma renin activity is tightly correlated with the development and maintenance of hypertension.[30] Recently, Bekassy *et al*.[31] identified C3 as a novel substrate of renin. Renin-induced C3b can also bind to CFB and form functional C3 convertase in the presence of CFD. This new finding provides a possibility for the initiation of complement AP activation in the kidney of subjects with hypertension. An association also exists between the renin inhibitor aliskiren and complement AP activation. Patients treated with aliskiren showed normalized C3 levels in plasma and restored renal function.[31,32] Repeated kidney biopsies also suggested a reduction in complement C3 immunofluorescence in the kidney after treatment. Consistent with these findings, aliskiren ameliorated complement deposition in the nephron and renal vessels in transgenic hypertensive rats and performed slightly better than losartan.[26] Notably, renin is synthesized not only by the juxtaglomerular apparatus but also by the collecting duct in the renal medulla. The concentration of renin in the kidney tissue is higher than that in the circulation in subjects with hypertension.[33–36] Therefore, the complement AP may be activated under a high concentration of renin in the kidney of hypertensive patients. Unfortunately, the inability of mouse renin to cleave C3 limits further mechanistic studies

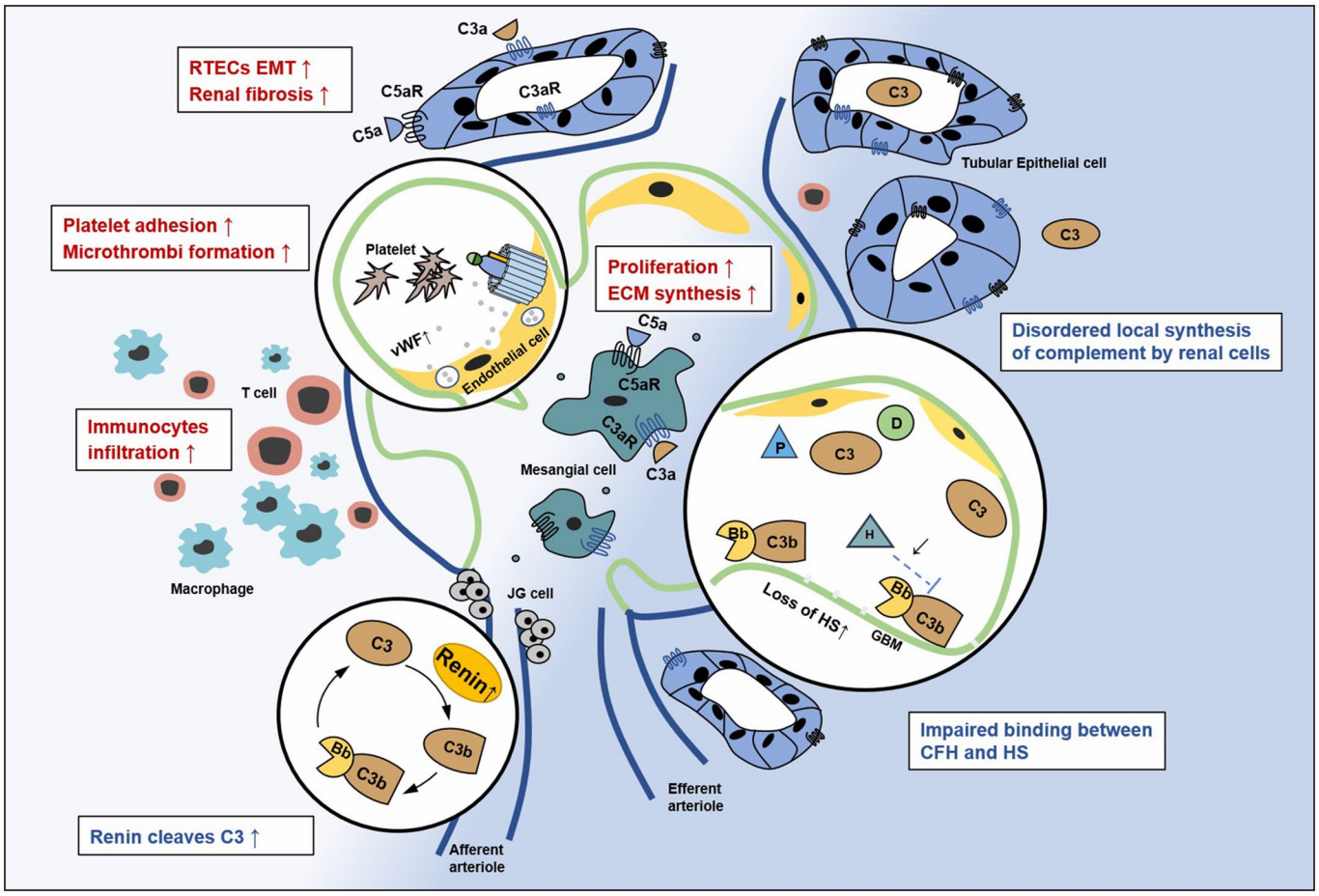

Figure 2. Potential conditions that facilitate the complement AP activation in the kidney (highlighted with the blue words in the textbox) and the effect of complement proteins on renal cells in hypertensive renal damage (highlighted with the red words in the textbox). In the kidney, high concentration of renin could cleave C3 to promote the formation of C3 convertase. The loss of heparin sulfate in GBM could reduce the recruitment of CFH and cause excessive C3bBb accumulation on GBM. Inflammatory cytokines could influence the local synthesis of complement proteins. All these factors ultimately induce the activation of complement AP. After the activation of complement AP, the products C3a, C5a, and C5b-9 exert an effect on mesangial cells, tubular epithelial cells, endothelial cells, and immunocytes to exacerbate the hypertensive renal damage. For mesangial cells, C3a and C5a could induce their proliferation and promote the excessive synthesis of extracellular matrix in glomeruli. For tubular epithelial cells, C3a and C5a could promote the epithelial–mesenchymal transition and renal fibrosis. For immunocytes, C3a and C5a could recruit them to the renal interstitium and aggravate inflammatory response. For endothelial cells, C5b-9 inserting into cytomembrane could cause the abnormal release of vWF, which then induces platelet adhesion and triggers the formation of renal microthrombi. (A color version of this figure is available in the online journal.)

in animal models. Whether the reaction exists *in vivo* in the presence of renin's general substrate angiotensin remains doubtful. Questions about the concentration of renin *in vivo* to function on C3 and concentration of aliskiren suppressing this novel cleavage are awaiting confirmation.[37]

Impaired binding between CFH and HS

Reduced binding of CFH (an important negative regulator of the AP) to HS (a component of the GBM or cell surface)[38] leads to dysregulation of the AP in the kidney. CFH binds to HS and enhances its negative regulatory capabilities, protecting host cells and tissue from complement attack.[39,40] Because GBM cannot synthesize membrane regulators like renal cells, the interaction between factor H and HS is vital for protection against complement deposition. Excessive loss of HS in the GBM decreases the capacity for CFH recruitment. The content of HS in the GBM, measured by immunohistochemical staining, is substantially reduced in hypertensive patients compared with normotensive subjects.[41] In addition, angiotensin II and aldosterone induce the glomerular expression of heparinase, which cleaves HS,[42–44] and may aggravate HS loss in hypertension patients with abnormal RAAS activation. As hypertension gradually progresses to hypertensive renal damage manifesting as albuminuria, the GBM is destroyed and exhibits lower HS levels. Then, the complement AP would be overactive following the reduced binding of CFH to the self-membrane.

Disordered synthesis of local complement proteins in the kidney

The kidney is a potential complement source.[18,45–47] Tubular epithelial cells can synthesize all complement AP proteins *in vitro*. Under the stimulation of various inflammatory cytokines, the expression of C3 is markedly increased. The high level of C3 synthesized by the kidney, rather than C3 in the circulation, is considered a critical mediator of kidney damage.[48] Apart from epithelial cells, glomerular endothelial cells (GECs) also synthesize various complement proteins. Researchers have found that compared with brain microvascular endothelial cells (BMECs) and human umbilical vein endothelial cells (HUVECs), GECs have higher levels of CFD and properdin synthesis.[49,50] CFD is the rate-limiting

step of complement AP activation, and properdin is the only factor that positively regulates the complement AP.[51] A significant increase in the concentration of Ba in the supernatant of GECs after tumor necrosis factor-alpha (TNFα) stimulation indicates the high sensitivity of renal cells to AP activation.[49,50] Hence, the kidney is vulnerable to complement AP activation because of the altered synthesis of local complement proteins by renal resident cells under the inflammatory background.

Effect of complement AP activation on specific cells contributing to hypertensive renal damage

After AP activation, three pathogenic complement proteins are produced – namely, anaphylatoxins (C3a, C5a), opsonin (C3b), and the terminal pathway product C5b-9 (also called the membrane attack complex). They function by binding to their corresponding receptor (C3aR, C5aR) or are directly deposited onto the target cell surface. In this section, we discuss the effects of complement proteins on different kidney cells in the context of hypertensive renal damage, and a brief outline of these effects is presented in Figure 2.

Role of complement AP activation in mesangial cells

Complement proteins can promote renal mesangial cell proliferation and extracellular matrix (ECM) synthesis. Mesangial cells are a cluster of mononuclear stellate cells localized in the glomerulus.[52] Under pathologic conditions, mesangial cells proliferate actively and produce excess ECM, which is the main cause of glomerulosclerosis. The proliferation of mesangial cells and glomerulosclerosis were significantly increased in C5-sufficient deoxycorticosterone acetate (DOCA)-salt mice compared with those in C5-deficient mice.[53] Anaphylatoxins are responsible for the change, and an *in vitro* study showed that C3a stimulates DNA synthesis and proliferation in mesangial cells in a dose-dependent manner. Mesangial cells produce more matrix Gla and collagen IV and convert to the synthetic phenotype, while C3aR antagonist treatment inhibits the phenomenon in mesangial cells from SHRs.[54]

Role of complement AP activation in tubular epithelial cells

Increasing studies have shown that complement AP activation promotes tubular epithelial–mesenchymal transition (EMT) and leads to abundant collagen deposition and renal fibrosis.[55,56] The phenomenon of tubular EMT has been observed in various animal models of hypertensive renal damage.[57,58] Interestingly, although tubular epithelial cells are the main group among resident renal cells that express anaphylatoxin receptors,[59] few studies have investigated the effect of anaphylatoxins on tubular EMT in the context of hypertensive renal damage. *In vitro* studies[60,61] and other *in vivo* kidney disease models[55,56,62] have shown that C3a/C5a promotes the EMT of renal tubular epithelial cells. Based on the evidence that the receptors of C3a and C5a are expressed abundantly in renal tubular epithelial cells, these proteins may facilitate the EMT of renal tubular cells in subjects with hypertensive renal damage. The downstream pathways may include multiple signals, such as NF-κB activation,[56] mitochondrial respiratory function disorder and reactive oxygen species generation.[63] Further studies remain to be performed.

Role of complement AP activation in endothelial cells

The obvious effect of the complement AP on endothelial cells derives from C5b-9. After excessive activation of the complement AP, C5b-9 is inserted into the GEC membrane and induces cytolytic damage directly. Renal biopsies of malignant hypertension patients have shown renal TMA and massive C5b-9 formation on GECs.[5,28] C5b-9 deposition was positively associated with arteriolar or capillary thrombi as well as GEC swelling, and this correlation was further investigated in a previous study. They knocked out the *C6* or *C9* gene in mice and found that it alleviated kidney TMA damage, while knockout of the *C5aR* gene did not.[64] This difference emphasizes the pathogenic role of C5b-9 in the formation of renal microthrombosis. However, the above study did not elucidate the mechanism by which C5b-9 caused this change. According to recent results obtained from nascent endothelial cells, the prothrombogenic effect of C5b-9 can be interpreted as a side effect of cellular self-protection. Endothelial cells initiate the repair of C5b-9 damage via membrane fusion of the Weibel–Palade body at the cost of releasing ultralarge von Willebrand factor (ulvWF).[65] The process *in vivo* can be reasonably speculated that after AP activation, excessive C5b-9 on GECs induces a high concentration of ulvWF in the local environment and promotes platelet adhesion and thrombus formation,[66] further aggravating renal ischemic damage.

Role of complement AP activation in immune cells

Complement overactivation recruits immune cells and promotes inflammation in the renal interstitium.[67] Immunocytes are the largest group of cells expressing anaphylatoxin receptors in the kidney and are the strong drivers of renal damage. Early recruitment of immune cells in the renal interstitium is a crucial mechanism of hypertensive renal injury.[68] The enhanced interstitial expression of C3 was significantly correlated with the densities of interstitial macrophages and T cells in hypertensive patients and animal models.[26,69] Anaphylatoxins stimulate the expression of monocyte chemoattractant protein-1 (MCP-1) in the kidney and are also responsible for the increase in T cells and decrease in Treg cells in the kidney.[21] C3a and C5a are also critical to the survival and proinflammatory phenotypic transition of immune cells.[70,71] Genetic knockout of both C3aR and C5aR not only prevents the elevation of BP induced by ANG II and refines immune cell infiltration but also downregulates interferon-γ, IL-1β, and IL-6 and upregulates the anti-inflammatory factor IL-10.[21] However, a study reported no changes in different immune cell groups in C5aR$^{-/-}$ hypertensive mice.[72] Because of the contradictory results, further studies should be conducted to determine whether the activation of the complement AP is the main factor underlying the infiltration of immune cells in the renal interstitium.

Complement inhibition is a potential treatment strategy for hypertensive renal damage

Considering that the complement AP is activated under the condition of hypertension and acts on different cells to aggravate kidney tissue damage, complement inhibition may be an optional therapy. The effectiveness of complement suppression therapy in patients with hypertension is controversial. Presently, the complement inhibitor exclusively used in patients with hypertension (with renal injury) is eculizumab, a humanized C5 monoclonal antibody that specifically binds to C5 to interrupt its cleavage. A clinical study demonstrated that early intervention with eculizumab can reduce C5 activation, restore renal function, and reduce TMA recurrence in subjects with malignant hypertension.[73] Drugs targeting the upstream region of the complement cascade, such as the C3 inhibitor compstatin,[74] have been gradually developed. In theory, these drugs can block the formation of C3 convertase to exert a stronger biological inhibitory effect. However, soluble CR1 treatment (target C3 and C5 convertases) neither downregulates blood pressure nor improves hypertension-related renal damage in rats.[75] SB290157,[76] the non-peptide antagonist of C3aR, has been widely used to explore the function of C3a/C3aR signaling. Cell experiments show that SB290157 can suppress the renin–angiotensin system, which is related to hypertensive renal damage,[54,77] but the effect of its global use in hypertensive animals remains uncertain. Medication targeting C3aR is not available in the clinic at present either. Hence, more research is urgently needed to clarify its efficacy.

Conclusions

Hypertensive renal damage is a common secondary kidney disease, and immune system abnormalities are considered an underlying pathogenic feature. Complement is a component of innate immunity, and an increasing number of studies have implied excessive activation of the complement AP in subjects with hypertension and hypertensive renal damage. Some conditions in the kidney also facilitate its activation, such as renin, HS loss, and local complement synthesis. After activation, products including anaphylatoxins and C5b-9 influence both renal cells and immune cells to aggravate inflammation and renal fibrosis in subjects with hypertension. Complement inhibitor therapy is a potential treatment, but its specific efficacy is uncertain. Therefore, substantial amounts of work must be performed to explore the role of the complement AP in hypertensive renal damage.

AUTHORS' CONTRIBUTIONS

CW, ZW, and WZ drafted the article. All the authors have read and approved the final article. CW and ZW contributed equally to the article.

DECLARATION OF CONFLICTING INTERESTS

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ORCID IDS

Chongjian Wang https://orcid.org/0000-0003-3810-5863
Zhiyu Wang https://orcid.org/0000-0002-4307-8014
Wen Zhang https://orcid.org/0000-0002-9011-361X

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