The role of complement in arterial hypertension and hypertensive end organ damage

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Abstract

Increasing evidence indicates that hypertension and hypertensive end organ damage are not only mediated by hemodynamic injury but that inflammation plays an important role in the pathophysiology and contributes to the deleterious consequences of this disease. The complement system is an ancient part of innate immunity comprising multiple serum proteins and cellular receptors that protect the host from a hostile microbial environment and maintain tissue and cell integrity through the elimination of altered or dead cells. As an important effector arm of innate immunity, it plays also central roles in the regulation of adaptive immunity. Innate and adaptive immune responses have been identified as crucial players in the pathogenesis of arterial hypertension and hypertensive end organ damage. Thus, complement activation may drive the pathology of hypertension and hypertensive injury through its impact on innate and adaptive immune responses aside from direct effects on the vasculature. Indeed, recent experimental data strongly support a role for complement in all stages of arterial hypertension and hypertensive end organ damage. The remarkably similar clinical and histopathological features of malignant nephrosclerosis and atypical hemolytic uremic syndrome, which is driven by complement activation, suggest also a role for complement also in the development of malignant nephrosclerosis. We herein review the role of complement proteins in hypertension and hypertensive end organ damage.

Introduction

Hypertension remains a leading cause of global death and disability from cardiovascular disease and stroke. High blood pressure afflicts more than 1 billion people worldwide. The borders between normotension and hypertension are arbitrary. In the recent update of the "Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults" the border for stage 1 hypertension was lowered by 10 mmHg and starts now at 130 mmHg (Whelton et al., 2017). In consequence, the number of hypertensive patients increased i.e. in the United States over night from 32% to 43% of the American population (Wenzel et al., 2019).

In spite of this high prevalence of the disease and decades of research on the subject, the etiology of most cases of hypertension remains undefined (Montaniel & Harrison, 2016). An important development in the field of hypertension has been the realization that there is an immunologic contribution to the disease (Guzik et al., 2007; Norlander et al., 2018; Wenzel et al., 2016). The complement system has long been viewed as a complex network mainly serving innate immune functions. A growing body of evidence has accumulated demonstrating, that the functions of complement go beyond innate immunity. New discoveries in inflammatory diseases refine our appreciation of the close interdependency of "ancient" complement and "modern" adaptive immune mechanisms. Uncontrolled complement activation can cause autoimmunity, tissue inflammation and injury, and complement-inhibitory drugs are effective treatments for several inflammatory diseases. Arterial hypertension is a new field in complement research (Ward, 2016). Blood pressure elevation

induces the activation of complement system and production of complement factors. These factors accelerate the progression of vascular injury and promote the development of hypertension via two different kinds of regulation. First, complement factors directly promote vascular cell dysfunction through their specific receptors in effector cells. In addition, complement activation mediates immune cell recruitment and induces inflammatory factor production in these cells. This includes cells of the innate and adaptive immune system. These together aggravate hypertension-induced organ damage (Ruan & Gao, 2019).

Here, we update the current knowledge about the role of complement in arterial hypertension (Chen et al., 2020a; Ruan & Gao, 2019; Wenzel et al., 2017) and extend a summary towards what its known in the processes underlying hypertensive end organ damage.

Complement and hypertension

Complement is an ancient part of the host defense machinery that, together with the contact and coagulation systems and the various branches of innate and adaptive immunity, helps to maintain barrier functions and protect against microbial invasion after injury. The role of complement is to detect, tag and eliminate pathogenic intruders with almost immediate reactivity (Figure 1) but sufficient specificity to avoid damaging host cells (Koenderman et al., 2014; Ricklin et al., 2018; Zipfel & Skerka, 2009). The membrane-attack complex (MAC) forms transmembrane channels, which disrupt the cell membrane of target cells, leading to cell lysis and death as shown in figure 1. In addition, complement activation in blood and the interstitial fluids leads to the mobilization of scavenger cells and the induction of the general inflammatory reaction, all events aimed at removing the perceived threat and restoring homeostasis (Merle et al., 2015).

While the field is still working on defining the exact molecular mechanisms driven by complement in circulation during hypertension and end organ failure, there may now be an additional complement-mediated angle to think about in this disease pathology: For many decades, complement was perceived to be a blood-borne immune system that was solely located in the intravascular space, with the entire spectrum of its components synthesized (with few exceptions) almost exclusively in the liver. Reports in the 1980s documented the presence of a functionally intact intracellular pool of the core components C3 and C4 within lymphocytes and other cell types (Lubbers et al., 2017), but the broader implications of these findings remained obscure. However, these observations hinted at an extra-hepatic importance of complement already early on. Renewed interest in the roles of complement generated by non-liver cells in the past years led to the identification of C3-mediated and C5-mediated activation and signaling events driven by cell intrinsic complement expression and function in intracellular, autocrine and paracrine fashions (Lalli et al., 2008; Liszewski et al., 2013; Strainic et al., 2008). Whilst the role of tonic intracellular complement activity seems to be sustaining immune cell homeostasis via regulation of cell metabolism (Kolev et al., 2015), cell autonomous complement is further activated during the sensing of danger, including cellular stress, pathogen presence etc. Such sensing then leads to increased production and activation of cell-intrinsic complement and the autocrine and paracrine stimulation of complement activation fragment receptors on immune cells to induce their respective effector activity (Ricklin et al., 2018). In fact, all immune cells express either all or a high number of complement receptors and regulators and can hence easily integrate incoming local complement activation signals.

As shown in figure 1 polymorphic neutrophils, monocyte/macrophages, dendritic cells (DCs) and human $CD4^+$ and $CD8^+$ T cells express anaphylatoxin receptors and complement regulators. DCs in the kidney can be identified by expression of MHC II and CD11c, a component of complement receptor 4. More than 90% of renal dendritic cells also express the CD11b, a component of complement receptor 3 (Gottschalk & Kurts, 2015). Further, B cells express complement receptors 1 and 2 (CR1/CR2) that engage for optimal antibody production (Gottschalk & Kurts, 2015). Follicular helper cells sample and retain antigen also in a complement-receptor-dependent fashion (Panneton et al., 2019). It is now undisputed that complement and its receptors are fine-tuning and shaping adaptive immune responses. Since the adaptive immune system plays an important role in arterial hypertension, it is feasible to now explore complement also a potential regulator of arterial hypertension via an innate and 'adaptive immunity axis'. Therefore, as shown in figure 1 complement is engaged in three pathways inducing hypertension and hypertensive end organ damage: by

itself, by influencing innate immune cells like PMNs and monocyte/macrophages as well as by fine-tuning cells like DCs, T and B cells of the adaptive immune system. Hypertensive end organ damage most likely starts with endothelial dysfunction and injury (Figure 1).

We will initially focus on the better-defined connections between complement and hypertension and speculate on possible new connections in the outlook section.

The role of distinct complement pathways in hypertension

Classical pathway —the classical pathway may be considered a target when antibody mediated complement activation is involved in disease pathogenesis. Indeed, recent data suggest that also B cells/IgGs are crucial for the development of Ang II–induced hypertension and vessel remodeling in mice (Chan et al., 2015). Recent findings demonstrate a key role for complement C1-induced activation of β-catenin signaling in vascular remodeling and hypertension (Sumida et al., 2015). C1q activates beta-catenin signaling by binding to Frz and cutting Lrp5/6 with C1r/s, which is independent of Wnt ligands in aorta as well as skeletal muscle (Naito et al., 2012; Sumida et al., 2015). Ang II infusion raises blood pressure, promotes arterial remodeling characterized by vascular smooth muscle cell (VSMC) proliferation and upregulates the expression of Wnt/β-catenin target genes. Pharmacologic or genetic inhibition of β-catenin signaling suppresses VSMC proliferation without lowering blood pressure. Ang II infusion recruits macrophages into the aorta, and these macrophages secrete the complement component C1q. Depletion of macrophages, administration of a C1 inhibitor or genetic ablation of C1q suppresses Ang II-induced activation of β-catenin signaling and VSMC proliferation, identifying macrophage-secreted complement C1 as an inducer of β-catenin signaling and VSMC proliferation in hypertensive arterial remodeling. However, no effect on blood pressure is found (Wenzel et al., 2017).

Lectin pathway —the lectin pathway is evolutionarily older than the classical pathway and is more complex. Five lectin pathways-related pattern recognition proteins have been identified so far, including mannose-binding lectin (MBL), the ficolins 1 – 3 and collectin 11. These pattern recognition proteins detect different carbohydrate and/or acetylation patterns expressed generally by pathogens or stressed and dying host cells and circulate in complex with MBL-associated serine proteases (Ricklin et al., 2018). Hemodynamic factors including shear stress and hypertensive factors like angiotensin II, salt or aldosterone induce subtle injuries and damage cells, which then leads to the release of molecules called the Damage-Associated Molecular Patterns (DAMPs). These can the activate lectin pathway-derived sensors as well as other innate immune sensor systems such as the toll-like receptors (TLRs) and probably some inflammasome (Krishnan et al., 2014).

Alternative pathway – The alternative complement pathway has a critical role in amplifying the complement response independent of the initiating pathway and hence in exacerbating inflammatory pathologies. Healthy human cells are protected from the effects of the alternative pathway activation and subsequent C3/C5 convertase formation by a series of inhibitors of the regulator of complement activation family (Ricklin et al., 2018). The alternative pathway is primarily responsible for the rapid amplification of opsonisation on unprotected cells and provides a means of constant background activity. Deficiency of protective inhibitors plays a driving role in thrombotic microangiopathies that have similarities with malignant nephrosclerosis (Wenzel et al., 2017). It is therefore expected that the alternative pathway plays a role in hypertensive end organ damage and this is currently explored in the field.

The role of complement activation fragments in hypertension

Complement activation leads to generation of cleavage fragments like the opsonin and the anaphylatoxins C3a and C5a. The role of anaphylatoxins in hypertension has been reviewed recently by us (Wenzel et al., 2017) and we will only briefly summarize current findings. Zhang *et al.*reported increased levels of C5a in humans with high blood pressure. In line with this, Infusion of Ang II causing arterial hypertension led to increased systemic anaphylatoxin generation in mice (Zhang et al., 2014a). Conversely, C5a receptor 1 (C5aR1) deficient mice exhibited markedly reduced cardiac remodeling and inflammation after Ang II infusion. Similarly, pharmacological inhibition of C5a production by an anti-C5 monoclonal antibody or

C5aR1 targeting with an inhibitor (PMX53) recapitulated the effects of C5aR1 deficiency (Zhang et al., 2014a; Zhang et al., 2014b). Bone marrow chimera experiments revealed that C5aR1 expression on bone marrow–derived, and not vascular, cells was critical in mediating Ang II–induced cardiac injury and remodeling (Zhang et al., 2014a). The key role of C5aR1 as a disease driver was also confirmed in a rat model: Treatment of deoxycorticosterone acetate (DOCA) salt hypertensive rats with the C5aR1 antagonist PMX53 attenuated endothelial dysfunction and decreased hypertensive cardiac injury. PMX53 treatment resulted in less ventricular collagen deposition and hypertrophy as compared with untreated hypertensive rats, whilst C5aR1 antagonism did not change systolic blood pressure (Iyer et al., 2011). We also found such a blood pressure-independent nephroprotective effect of C5aR1 deficiency in an accelerated model of hypertension in mice (Weiss et al., 2016). Moreover, using a C5aR1 reporter mouse in a hypertension model, expression of C5aR1 in the kidney was shown on dendritic cells as well as in monocytes/ macrophages and granulocytes. No expression was detected on resident cells in the heart or the aorta of these animals. However, in contrast to the work of Zhang *et al.,* cardiac injury was accelerated in C5aR1 deficient mice with significantly increased cardiac fibrosis and heart weight in C5aR1 deficient mice after Ang II infusion. The reason for the difference between cardiac and renal injury is currently unclear. These observations indicate that the C5a:C5aR1 axis drives end organ damage in the kidney, while its exact role in cardiac injury remains controversial and needs to be further explored. The role of the alternative second C5a receptor (C5aR2) in hypertension is unknown. In C3a receptor (C3aR) deficient mice, Zhang *et al.* observed a trend for increased cardiac injury when compared to wild type mice after Ang II infusion (Zhang et al., 2014a; Zhang et al., 2014b) Similarly, preliminary data from our laboratory suggest an enhanced renal injury in C3aR deficient mice after Ang II infusion. A protective role for the C3aR in hypertension would align with the emerging concept that C3a has more dominant activity in the maintenance of cell homeostasis and/ or return to this homeostasis after tissue activation over C5a (West et al., 2020).

The presence of complement receptor expression infiltrating immune cells mostly in kidney suggests that these cells mediate some of the pathology. Indeed, an important role for T cells in hypertension was shown in 2007, when it was found that the increase in blood pressure caused by Ang II infusion was significantly blunted in mice – deficient for the recombinase-activating gene 1 (*Rag-1*$^{-/-}$ mice) – which are lacking for T and B cells. In further experiments, it was shown that the adoptive transfer of T cells restored the hypertensive response, indicating that T cells play an important role in generation of arterial hypertension. However, the contribution of T cells to hypertension has recently been challenged by work from different groups including ours that could not confirm increased resistance of *Rag-1* deficient mice to Ang II infusion (Ji et al., 2017; Seniuk et al., 2020). However, T cell response are highly diverse in nature and these contradictive findings could indicate that we need a better understanding of the exact nature of the T cell responses evoked during disease induction and progression. For example, an important subgroup of T cells are regulatory T cells (Tregs) a subpopulation of CD4$^+$ T cells with CD25 and Foxp3 positivity. The main function of Tregs is maintenance of immunological tolerance. Several groups have shown that adoptive transfer of Tregs lowers blood pressure and ameliorates cardiac and renal injury in different models of hypertension (summarized in (Wenzel et al., 2016)). The anaphylatoxins are known as regulators of Treg activity: C3a/C3aR and C5a/C5aR inhibit the function of CD4$^+$Foxp3$^+$ circulating Tregs (Kwan et al., 2013) and absence of C3aR and C5aR signals in CD4$^+$ T cells induces spontaneous Foxp3$^+$ Treg differentiation upon T cells activation. Also, activation of CD4$^+$ T cells from mice deficient in both C3aR and C5aR leads to a greater abundance of the anti-inflammatory cytokines like IL-10 and TGF-β1 produced by these cells (Strainic et al., 2013). Chen *et al* . have recently shown in a series of elegant experiments that Ang II–induced hypertension resulted in an elevated expression of C3aR and C5aR in Foxp3$^+$ Tregs. C3aR and C5aR double deficiency decreased blood pressure in response to angiotensin II compared with wild type mice via activating Foxp3$^+$ Tregs. Adoptive transfer of C3aR and C5aR double deficient Tregs showed a more profound protective effect against Ang II–induced blood pressure elevation and renal damage when compared to wild type Treg transfer. Depletion of Tregs with CD25 neutralizing antibodies abolished the protective effects (Chen et al., 2018). However, Chen *et al* . found that high blood pressure reduced the number of Tregs compared to normotensive mice. This is unusual since the number of Tregs in the kidney at least in models of accelerated hypertension is increased in our own work (Krebs et al 2014). Clearly, more work has to be done to explain the fascinating observation

and mechanisms that deficiency of C5aR1 and C3aR lowers blood pressure by upregulating Tregs.

Component C3

C3 is mainly produced by the liver, monocytes, and macrophages. Several other cells like fibroblasts, epithelial cells, endothelial cells, astrocytes, adipocytes, and myoepithelial cells have all been reported to be potential extrahepatic sources of complement molecules in tissues (Chen et al., 2020a). Evolutionary theory suggests that nature "preserves" its most vital components by multi-tasking them. This certainly applies to C3. It has been identified in sponges like phylum Porifera (Liszewski et al., 2017). A primitive version of C3 likely comprised the first element of the complements system and functioned intracellular. It can be speculated that the intracellular C3 also engaged in key metabolic processes, some of which are retained in modern times. With the advent of multi-cellularity and the circulatory system, C3 evolved its role as a secreted protein.

Elevated levels of serum C3 are associated with prehypertension in a study with over 7000 individuals and high C3 concentrations in plasma are associated with future increases in blood pressure and the development of hypertension (Bao et al., 2017; Engström et al., 2007). Concerning animal models, the role of C3 has been examined in spontaneously hypertensive rats and DOCA salt hypertension.

C3 in spontaneously hypertensive rats

The spontaneously hypertensive rat (SHR) is a widely used genetic model of hypertension. SHRs show exaggerated growth of cardiovascular organs in comparison with normotensive Wistar-Kyoto (WKY) rats (Sen et al., 1974). Enhanced DNA synthesis and organ hypertrophy were found to occur before the elevation of blood pressure in SHRs (Walter & Hamet, 1986). In addition, SHR-derived vascular smooth muscle cells (VSMC) as well as mesangial cells in culture show exaggerated growth in comparison with cells from WKY rats (Chen et al., 2020a). C3 expression is upregulated in VSMC from SHR compared to normotensive WKY rats. Exogenous C3a increases cell growth and suppression of C3a generation or C3aR activity in SHR-derived VSMCs and MCs inhibit cell growth (Ikeda et al., 2014; Lin et al., 2004). Negishi and colleagues showed that knockout of the *C3* gene by zinc-finger nuclease gene-editing in SHRs had a small but significant effect on systolic blood pressure, proliferation of mesangial cells and renal injury (Negishi et al., 2018). In contrast, mice deficient in C3 had similar basal blood pressure compared to wild type animals and showed no differences in hypertension or hypertrophy responses to *in vivo* infusion with angiotensin II (Coles et al., 2007). However, in this latter study a low dose of angiotensin (1.1 ng/g/min) was given to mice of the C57/BL6 black strain for only 7 days. 7 days of angiotensin infusion induce a mild increase in blood pressure and vascular hypertrophy but do not induce hypertensive injury. Therefore, a role for C3 in hypertensive injury cannot be ruled out based on this study.

C3 in the DOCA salt hypertension model

An increased expression of likely harmful C3 is also observed in another mouse-based hypertension model. The perivascular adipose tissue in the DOCA salt treated mice displays a clear increase in C3 expression (Ruan et al., 2015). Bone marrow transplantation from C3 knockout mice into wild type mice lowered vascular injury in DOCA salt exposed mice. This was accompanied by a greatly reduced deposition of C5a in the perivascular adipose tissue after transplantation of C3 deficient bone marrow. In addition, treatment with a C5aR peptide agonist aggravated vascular hypertrophy and fibrosis in DOCA salt treated mice. No effect on blood pressure was found (Ruan & Gao, 2019). These findings suggest that C3 from bone marrow derived cells contribute to the DOCA salt hypertensive vascular remodeling process largely via the downstream generation of C5a, most likely via C3/C5 convertase formation.

Complement C5

Raij *et al* . treated C5 deficient mice and C5 sufficient mice that differ in the single gene locus responsible for the presence or absence of the complement C5 component with DOCA salt. C5 deficient mice can neither generate C5a nor form the C5b-9 complex. Hypertensive C5 deficient mice developed more glomerular capillary loop dilatation and larger glomerular capillary tuft volumes than C5 sufficient mice. However,

DOCA C5 sufficient mice, compared to DOCA C5 deficient mice, had significantly more glomerular cell proliferation, cell necrosis, extra capillary proliferation and proteinuria. After 16 weeks, C5 sufficient mice, in comparison to C5 deficient mice, had more severe glomerulosclerosis, proteinuria and renal insufficiency. These changes occurred despite levels of hypertension that were similar in C5 sufficient and C5 deficient mice throughout the whole study period (Raij et al., 1989). These data suggest that C5 and/or C5b-9 play an important role in hypertensive glomerular injury but that these roles are complex and need to be carefully defined during the different stages of the disease.

This latter notion is also supported by the finding that mice lacking CD59a have elevated deposition of membrane attack complex. CD59 is the primary regulator of the membrane attack complex formation. However, and somewhat surprisingly, no increased blood pressure or cardiovascular injury was found in response to angiotensin II infusion (Coles et al., 2007).

Dahl SS rat

The Dahl salt sensitive (Dahl SS) rat is an established model of salt sensitive hypertension. High salt caused an increase in message for complement regulator CD59 in aortic tissue, with minimal change in the mouse-specific complement regulator Crry that controls the C3 convertase. Soluble complement receptor 1 (sCR1) is a soluble form of endogenous CR1 with demonstrated ability to inhibit complement activation at the C3 and C5 convertase levels of the complement pathway. Whilst application of sCR1 effectively inhibited total hemolytic complement activity as well as C3a generation in the Dahl SS rat model, sCR1 did not alter development of hypertension or albuminuria. This data indicate that complement activation is not critical for development of the salt-sensitive phenotype in Dahl rats or that the used approach to inhibit complement activation is not useful in this model of hypertension (Regal et al., 2018).

Non-canonical C3 activation in hypertension

Békássy et al. have recently shown that renin cleaves C3 into C3b and C3a, in a manner identical to the C3 convertase (Bekassy et al., 2018). Cleavage was inhibited by the renin inhibitor aliskiren. If this occurs also *in vivo,* it would be a missing link by which the renin-angiotensin system triggers the alternative pathway. This mechanism initiates complement activation, with specific tropism for the kidney, because renin concentrations are higher in the kidney than in the systemic circulation. Renin-angiotensin system blockade by an AT1 antagonist or an ACE Inhibitor induces a rise in renin. This could indicate that renin-angiotensin system blockade without renin inhibition could have an adverse effect in complement-mediated kidney disease. However, this intriguing possibility needs to be still proven for*in vivo* relevance.

Recently, a case of atypical hemolytic uremic syndrome (aHUS) with malignant hypertension and end-stage renal disease was reported in which high dosed renin inhibitor aliskiren added together with eculizumab improved clinical parameters of the patients suggesting that the use of high-dose aliskiren as an adjunct therapy in a patient treated with eculizumab for aHUS (Plasse et al., 2020). This is possibly an exciting therapeutic advance, but raises almost more questions than it does provide answers. For example, what is the relative contribution of renin to local kidney C3 activation? The C5 inhibitor eculizumab is currently used to treat complement-related kidney conditions such as aHUS. Does conventional renin-angiotensin system blockade interfere with optimal outcomes in complement-related disease? If renin-angiotensin system blockade results in renin release, why is there a large body of evidence supporting renin-angiotensin system blockade as a key nephroprotective maneuver even in nephropathies that may be complement-mediated? Do renin-secreting tumors develop complement activation? Do angiotensinogen and C3 compete for renin? And, above all, should aliskiren become the renin-angiotensin system blocker of choice for complement-mediated kidney disease (Perez-Gomez & Ortiz, 2020)? Future work will hopefully providing answers to these questions.

Role of complement in so-called benign and malignant nephrosclerosis

Patients presenting with severe hypertension in combination with hypertensive retinopathy, hypertensive encephalopathy and often renal injury with mild signs of thrombotic microangiopathy are labeled to have "malignant hypertension". Severe hypertension can induce thrombotic microangiopathy in the renal vascu-

lature, the occurrence of which has been linked to mechanical stress to the endothelium. In the majority of patients with arterial hypertension, so-called benign nephrosclerosis or hyaline arteriosclerosis can be found in the kidney. The structural changes of so-called benign nephrosclerosis are characterized by intimal fibroelastosis in arteries including interlobular caliber vessels and sub endothelial hyalinosis in afferent arterioles and pre-arterioles as shown in Figure 2a. The extent of hyalinosis usually correlates with duration and severity of arterial hypertension. Glomeruli show hypertensive injury most likely derived from barotrauma with segmental sclerosis and scarring (Figure 2b).

The histologic changes found in malignant nephrosclerosis are different and malignant nephrosclerosis is more correctly called stenosing arteriosclerosis. It is a form of thrombotic microangiopathy defined by progressive narrowing of interlobular arterial branches and afferent arterioles with intima fibrosis and onion-like appearance of intimal scarring as shown in Figure 2c. In renal biopsies with malignant nephrosclerosis, a pseudo neuroendocrine atrophy pattern can be found. The disease defining change is a progressive narrowing of interlobular arterial branches and afferent arterioles with intima fibrosis and onion-like appearance. The oxygen supply to the glomerulus is reduced resulting in ischemic wrinkling of the glomeruli and atrophic tubules (Figure 2c). The mechanisms causing transition from high blood pressure to malignant hypertension with malignant nephrosclerosis and renal failure are completely unknown. Patient presenting with malignant nephrosclerosis are often young and have a short history of uncontrolled hypertension. It is also unclear, why few patients with hypertension develop malignant nephrosclerosis and several others, with similar levels of blood pressure elevation over much longer time, do not (Wenzel et al. 2017). There must be a certain susceptibility and we argue that changes in the complement system could be among the causes. Malignant nephrosclerosis is hallmarked by complement deposition, inflammation and signs of thrombotic microangiopathy with a renal pathology resembling certain histological features of aHUS (Wenzel et al., 2015). A crucial involvement of the complement system in pathogenesis of thrombotic microangiopathy has been highlighted by numerous studies in the recent years and it has been shown that aHUS can be cured by therapeutic complement inhibition using the C5 cleavage inhibitor eculizumab (Noris & Remuzzi, 2009). The shared clinical and histopathological features between malignant nephrosclerosis and aHUS strongly suggest a role for complement also in the development of malignant nephrosclerosis and point to complement inhibition as a potential therapeutic strategy in malignant hypertension. One may speculate that over activity of complement factors or lack of complement inhibitors defines the subset of hypertensive patients that develop malignant hypertension and nephrosclerosis in response to arterial hypertension (Wenzel et al., 2017). A diagnosis of thrombotic microangiopathy on kidney biopsy in a patient presenting with hypertensive emergency has historically elicited the diagnosis of malignant hypertension-associated thrombotic microangiopathy. Recent studies, however, have raised awareness that a number of these patients may actually represent cases of aHUS. Timmermans and colleagues have published a series of data suggesting that complement defects may be the culprit of disease in patients who present with high blood pressure, severe renal disease, and often progress to end stage renal disease despite blood pressure control. In their first publication, they found genetic defects in the complement system in six out of nine patients with renal thrombotic microangiopathy attributed to severe hypertension from the Limburg Renal Registry. In contrast to patients without genetic defects, patients with complement defects invariably progressed to end-stage renal disease (Timmermans et al., 2017). Moreover, the group showed that serum samples collected at presentation from patients with hypertension associated thrombotic microangiopathy induced abnormal C5b-9 formation on microvascular endothelial cells indicating activation of complement in these patients (Timmermans et al., 2018). In a recent follow up study, the same authors present data showing that assessment of both *ex vivo* C5b-9 formation and screening for variants in complement genes may categorize patients with hypertensive emergency and thrombotic microangiopathy into two different groups, blood pressure mediated and complement mediated as shown in the upper part of figure 3. Thus, the first group requires blood pressure control whilst the second group should be treated with eculizumab and blood pressure control (Timmermans et al., 2020). However, malignant nephrosclerosis and aHUS presumably represent a continuum of two different diseases as shown in the lower part of figure 3. Laecke et al. recently proposed that variable degrees of the importance of blood pressure versus complement-mediated mechanisms could be found in malignant nephrosclerosis and aHUS (figure 3).

However, the fascinating data from the Limburg Renal Registry are not in agreement with data from Larsen *et al* .. The latter group performed next-generation sequencing to interrogate the coding regions of 29 genes encoding complement and coagulation cascade members that have been shown to be associated with aHUS.100 patients presenting with severe hypertension, renal failure and a kidney biopsy showing microangiopathic changes limited to the classic accelerated hypertension associated lesion of arterial intimal edema ('mucoid intimal hyperplasia') without accompanying glomerular microthrombi were analyzed. No pathogenic or likely pathogenic variants were identified in any of the genes analyzed. (Larsen et al., 2018). Data from the Spanish group for the study of glomerular diseases clearly show that severe and malignant hypertension are common among patients with aHUS. However, thrombotic microangiopathy is uncommon among patients presenting with malignant hypertension caused by diseases other than aHUS (Cavero et al., 2019).

Thus, and similar to the 'C5 situation', most data suggest a causative complement-nephrosclerosis relationship, but conclusive and irrefutable evidence is still missing.

Non-canonical complement and sensor system cross talks – evolutionary clues

Innate immune sensor systems have traditionally been viewed as primary protectors against infections. This view is now changing and it is becoming broadly acknowledged that all innate immune sensors, including the Toll-like receptors (TLRs) and inflammasomes are key to also detecting and rectifying deviations from cell homeostasis – and particularly impact on those that maintain cell physiology (Kolev & Kemper, 2017; Prochnicki & Latz, 2017; Yiu et al., 2017). Blood pressure control and host defense are such essential mechanisms of homeostasis and it therefore not surprising that evolution incorporated complement – among the TLRs and inflammasomes (McCarthy et al., 2014; Pasqua et al., 2018) – as active participant in the regulation of blood pressure. Infection can cause hypotension via fluid loss during fever, tachypnea and diarrhea. Septicemia induces inflammation-related vascular fluid losses. Thus, the risk of hypotension related to inflammation might have favored selection of mechanisms that link immune and complement activation to blood pressure increases for short-term survival benefits. Such an evolutionary force may explain why important antimicrobial effectors could have direct hypertensive effects by promoting vasoconstriction or sodium retention (Wenzel et al., 2016). First data suggest indeed that complement can directly activate the renin-angiotensin system since Chen *et al* . showed just recently that C3 binding to C3a upregulates renin transcription in vitro (Chen at al. 2020b).

The importance of the ancient sensor systems in the regulation of cell physiology became most apparent when their central roles in cell metabolism were discovered. For example, in the case of complement, it was found that low-level activation of human T cell intrinsic, intracellular C3 into C3a and C3b by the ancient protease cathepsin L (CTSL) leads to tonic lysosomal C3aR engagement. This, in turn, drives basal mammalian target of rapamycin (mTOR) activity and sustains homeostatic cell survival as shown in figure 1 (Liszewski et al., 2013). During immune cell activation and cell movement into tissues, intracellular C3 generation and activation is increased via LFA-1 mediated signals (Kolev et al., 2020) and autocrine engagement of the C3aR and CD46 (a receptor/regulator for C3b) drives then nutrient influx and metabolic programming (glycolysis, fatty acid metabolism and oxidative phosphorylation) needed for immune cell effector function (Arbore et al., 2018; Kolev et al., 2015). Importantly, intracellular and/or cell-autonomous complement engages in a heavy crosstalk with the NLRP3 inflammasome in immune cells to generate optimal and sustained tissue immunity (Arbore et al., 2016). In consequence, reduced or overactive intracellular complement contributes to the hypo- or hyperactive immune cells responses observed in primary immunodeficiency, in chronic infections and in autoimmunity, respectively (Arbore et al., 2017; West & Kemper, 2019). The finding that intracellular cell-autonomous complement is key to immune cell function now suggests that this could be a new avenue to explore in hypertension: it is possible that intracellular complement perturbation contributes the aberrant innate and adaptive immune cell behavior observed in this disease state (Figures 1 and 4). Indeed, changes in metabolic pathways are being functionally connected with pulmonary arterial hypertension (Ryan & Archer, 2015). Moreover, given that we have so far observed an intracellular complement in all cells analyzed, it may be worthy to explore a role for intracellular complement in the direct regulation of endothelial cell

metabolism during hypertension (Cao et al., 2019). Similarly, a 'second look' into the proteases that allow for intracellular non-canonical complement activation may also be informative. For example, a novel functional polymorphism in the*CTSL* gene alters blood pressure (Mbewe-Campbell et al., 2012) and changes in the CTSL gene promotor have been connected with hypertension (Chen et al., 2015) – potential changes, however, in C3 processing within the endothelium are unexplored.

Summary and outlook

The gravest consequence of chronic hypertension is end organ damage. In several experimental models in which complement proteins or their receptors are eliminated, target organ damage is reduced – however, in most studies blood pressure is not or only modestly reduced. Thus, albeit a wealth of work indicating strongly that the complement system is also involved the more immediate effects of hypertension, we know little about the underlying complement-mediated mechanisms (Figure 4). C1q, C3, C5, C3aR C5aR1 have been examined and their effect on blood pressure, cardiac, renal or vascular injury are solid, but again, how exactly their impact is generated, is unclear (Figure 4). Furthermore, many of the complement components have not been probed yet for potential disease involvement. For example, no data are available on the role of C5aR2 in hypertension, and nothing is known about the contributions of CD55, CD46 or other direct complement regulators to hypertension. Also, the impact of complement on other hypertension-related complications such hypertensive brain injury, malignant nephrosclerosis or endothelial dysfunction are either not explored yet or ill defined.

While blood pressure lowering is clearly important to reach this goal, prevention of local inflammation that accompanies hypertensive end organ damage should also to be addressed. Since complement has pro- as well as anti-inflammatory functions, complete inhibition of the canonical complement cascade may result in unwanted effects in arterial hypertension. Thus, if we want to harness the potential of the complement system in the design of novel therapeutic approaches to combat hypertension, renal, and cardiovascular diseases, we need to understand the exact spatial, temporal and cellular contributions of complement to these disease pathologies.

Based on what we know today this will likely require a holistic approach that ideally integrates large data set analyses about genetic complement variations in patients and the functional probing of intracellular and extracellular complement as well as its crosstalk with other (innate) immune sensors.

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Figure 1

Complement activation is initiated through three pathways: the classical, alternative or the lectin pathway. Full activation leads to the generation of several biologically active fragments including C5b-9, which 'seeds' membrane attack complex (MAC)-mediated direct lysis of pathogens. In addition, complement and extra- as well as intracellular complement receptors also fine shape cells of the innate and the adaptive immune system. Polymorphic neutrophils (PMN) and monocyte/macrophages express anaphylatoxin receptors. Dendritic cells can be identified by expression CD11c, a component of complement receptor 4. Dendritic cells also express the CD11b, a component of complement receptor 3. T cells express anaphylatoxin receptors in different intracellular compartments and on the surface. Intracellular C3 is converted by cathpepsin L to C3a that bind lysosomal C3aR. This drives mammalian target of rapamycin (mTOR) activity that sustains homeostatic cell survival. C5aR1 is found on the surface was well as on mitochondria whereas C5aR2 is only found on the surface. B cells express complement receptors 1 and 2 (CR1/CR2). Complement proteins, innate and adaptive immune cells are potential mediators of hypertension and hypertensive end organ damage. Endothelial dysfunction and injury are most likely the first step of hypertensive end organ injury.

Figure 2

The figure 2a shows hyaline arteriosclerosis, which is found in the kidney in so-called benign nephrosclerosis. Glomeruli show hypertensive injury most likely derived from barotrauma with segmental sclerosis and scarring (figure 2b). In cases of malignant nephrosclerosis, a pseudo neuroendocrine atrophy pattern can be found. The disease defining change of so-called malignant nephrosclerosis is a progressive narrowing of interlobular arterial branches and afferent arterioles with intima fibrosis and onion-like appearance. The oxygen supply to the glomerulus is reduced resulting in ischemic wrinkling of the glomeruli and atrophic tubules as shown in figure 2c. Histology pictures are kindly provided by T. Wiech, Hamburg, Germany.

Figure 3

The upper part of figure 3 summarizes the concept of Timmermans et al.. Measurement of *exvivo* formation of C5b-9 on endothelial cells and genetic screening can divide patients with hypertensive emergency and thrombotic microangiopathy into high blood pressure or complement mediated thrombotic microangiopathy. Malignant nephrosclerosis and aHUS are two diseases with an overlap as shown in the lower part. On the left hand, malignant nephrosclerosis driven primarily by high blood pressure and on the right hand aHUS driven by complement dysfunction. In between lies a continuum with increasing or decreasing importance of complement dysfunction and blood pressure. Histology shows onion-like narrowing of intrarenal vessels and ischemic glomeruli in malignant nephrosclerosis. In case of aHUS, fresh vascular and glomerular fibrin thrombi (arrows) are found as well as HUS glomerulopathy. Figure 3 modified from Timmermans et al. 2020 and van Laecke et al. 2017. MG=masson goldner staining. Histology pictures are kindly provided by T. Wiech, Hamburg, Germany.

Figure 4

Figures 4 summarizes the present knowledge on the role of complement in hypertension and hypertensive end organ damage. Shown is the complement cascade with the three pathways. In the classical pathway, C1q activates smooth muscle cell proliferation by the β catenin pathway. C3 knockout lowers blood pressure in spontaneously hypertensive rats but not mice with angiotensin II infusion. Renin has C3 convertase activity and can convert C3 independent of the three complement pathways. This effect can be inhibited by the renin inhibitor aliskiren. C5 probably by formation of C5b-9 aggravates glomerular sclerosis in DOCA salt hypertensive mice. C5 induces cardiac remodeling in angiotensin II infused since inhibition of C5 by an antibody is protective. Protectin (CD59) inhibits C5b-9. However, knockout of protectin has no effect on blood pressure. Treatment with soluble CR1 has no effect on blood pressure in Dahl salt sensitive rats. C3aR has protective effects in hypertension since knockout of the receptor seems to aggravate injury. The role of C5aR1 seems to be more complex. The C5a-C5aR1 axis aggravates cardiac injury since C5aR1 knockout as well as treatment with the C5aR1 antagonist PMX53 are cardio protective in hypertensive mice and rats. However, also cardio protective effects have been shown in an accelerated model of hypertension. The C5a/C5aR1 axis induces kidney injury. Treatment with a C5a agonistic peptide aggravates vascular remodeling in hypertension. Double knockout of C3aR and C5aR1 lowers blood pressure most likely by an increase of regulatory T cells. No data are available on the role of C5aR2 in hypertension or the effect of complement in hypertensive brain injury. Whether complement plays a role in the development of malignant nephrosclerosis is unclear.

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