Innate immunity in CKD-associated vascular diseases

Stephen Zewinger, Timo Schumann, Danilo Fliser and Thimoteus Speer

Department of Internal Medicine, Nephrology and Hypertension, Saarland University Medical Centre, Homburg/Saar, Germany

Correspondence and offprint requests to: Thimoteus Speer; E-mail: timo.speer@uks.eu

Abstract

Chronic kidney disease (CKD) is associated with an increased risk for cardiovascular events. Therefore, the activation of the innate immune system plays an important role. In contrast to the adaptive immunity, unspecific recognition of conserved endogenous and exogenous structures by pattern recognition receptors (PRRs) represents a key feature of the innate immunity. Of these PRRs, Toll-like receptors (TLRs) as well as the inflammasome complex have been documented to be involved in the pathogenesis of cardiovascular diseases (CVDs). They are not only expressed in leukocytes but also in a variety of cell types such as endothelial cells or fibroblasts. While activation of TLRs on the cell surface leads to nuclear factor κB-dependent expression of pro-inflammatory mediators, the inflammasome is a cytosolic multimeric protein complex, which cleaves cytokines such as interleukin-1β into their biologically active forms. Several endogenous ligands for these PRRs have been identified as contributing to the development of a CKD-specific pro-inflammatory microenvironment. Notably, activation of TLRs as well as the inflammasome is associated with arterial hypertension, formation of atherosclerotic vascular lesions and vascular calcification. However, detailed molecular mechanisms on how the innate immune system contributes to CKD-associated CVDs are as yet poorly understood. Currently, several agents modulating the activation of the innate immune system are the focus of cardiovascular research. Large clinical studies will provide further information on the therapeutic applicability of these substances to reduce cardiovascular morbidity and mortality in the general population. Further trials including patients with CKD will be necessary to assess their effects on CKD-associated CVD.

Keywords: cardiovascular disease, chronic kidney disease, inflammasome, innate immunity, Toll-like receptors

Introduction

Chronic kidney disease (CKD) is frequent in Western populations. The incidence of patients with end-stage renal disease receiving dialysis therapy or kidney transplantation is 200 cases per million per year in many European countries [1]. Especially patients with end-stage renal disease, but also patients with slightly reduced kidney function exhibit a high cardiovascular burden leading to an increased risk of death, cardiovascular events and hospitalization [2]. Therefore, it is not surprising that recent studies identified CKD as a potent and independent risk factor for cardiovascular disease (CVD) in addition to traditional risk factors, e.g. arterial hypertension, smoking, obesity, dyslipidemia and diabetes [3]. Hereby, traditional risk factors as well as non-traditional risk factors, such as inflammation, contribute to the high cardiovascular burden in CKD patients [4].

Inflammation is mediated by an interaction of multiple components of the innate and adaptive immune systems including complement factors, white blood cells and cytokines. Moreover,
inflammation represents a well-known risk factor for CVD in the general population. CKD itself creates a pro-inflammatory microenvironment caused by infection, uraemic milieu or tissue ischaemia [4]. Several clinical entities of kidney diseases and nephropathies induced by hypertension, diabetes, ischaemia or toxic agents lead to sterile inflammation. This inflammation is often induced by intrinsic damage-associated molecular patterns (DAMPs) released by cells during cell death or tissue remodelling [5]. In addition, different parenchymal cell types express Toll-like receptors (TLRs) and components of the inflammasome. These stimulate the innate immune response causing microinflammation and vascular dysfunction [5].

In contrast to the adaptive immune system, the innate immune system is based on the unspecific recognition of distinct and conserved exogenous or endogenous pathogenic pattern by pattern recognition receptors (PRRs). These PRRs are expressed on a variety of cell types such as macrophages, dendritic cells, granulocytes and monocytes. The activation of these non-clonal receptors induces immediate cell activation resulting in activation of the complement system, opsonization and coagulation. Furthermore, they induce the release of pro-inflammatory cytokines and chemokines, and phagocytosis. The adaptive immune system, in contrast, is based on lymphocytes expressing highly specific T cell and B cell receptors that recognize unique major histocompatibility complex-bound antigens [6].

Both, the innate and the adaptive immune systems play important roles in the initiation and progression of CVD in the general population as well as in patients with CKD. The present review will focus on the specific role of the innate immune system in CKD-associated CVD.

**PATTERN RECOGNITION RECEPTORS**

The recognition of conserved molecular structures by PRRs represents a key feature of the innate immune system. These structures can either derive from exogenous (pathogen-associated molecular pattern, PAMP) or endogenous (DAMP) ligands [7]. Therefore, the innate immunity not only plays a crucial role in the detection of pathogens such as bacteria, viruses or fungi, but is also involved in sterile inflammation processes. Currently, four distinct classes of PRRs have been identified:

- **Toll-like receptors (TLRs)**
- **C-type lectin receptors (CLRs)**
- **Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs)**
- **Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs)**

These receptors are widely expressed, although their expression levels differ between distinct tissues. They are found not only on classical cells of the innate immune system (i.e. monocytes, macrophages, dendritic cells) but also on endothelial cells, epithelial cells and fibroblasts. Notably, expression of most of these receptors can also be detected in kidney tissue. They can either be located on the cell surface or in the intracellular compartment [7]. In addition to these classical PRRs, formyl peptide receptors (FPRs) have recently been suggested to be classified as non-classical PRRs [8]. Whereas CLRs and RLRs are mainly involved in the recognition of viruses and fungi, TLRs and NLRs play crucial roles in the recognition of endogenous ligands with associations to a variety of disease conditions such as atherosclerosis, arterial hypertension and CKD.

**Toll-like receptors**

TLRs comprise 10 functional receptors in humans (TLR1–10) and 12 in mice (TLR1–9 and 11–13), respectively. TLR1, TLR2, TLR4, TLR5 and TLR6 are typically localized in the plasma membrane and mainly interact with microbial membrane components including lipopolysaccharides (LPS), bacterial lipoproteins and fungal sugars [9]. TLR3, TLR7, TLR8 and TLR9, expressed in intracellular vesicles, act as sensors for bacterial/viral DNA and RNA. Interestingly, TLRs are also involved in detection of host endogenous substances [10] such as abnormal high-density lipoproteins (HDLs) isolated from patients with CKD [11]. Activation of TLRs initiates downstream signalling cascades culminating in increased expression of pro-inflammatory cytokines and/or type I interferons (IFNs) (Figure 1). TLR signalling can largely be classified MyD88 (myeloid differentiation primary response gene 88) dependent and TRIF (TIR-domain-containing adapter-inducing IFN-β) dependent [12]. With the exception of TLR3, all TLRs utilize MyD88 as an adaptor molecule connecting the receptor to activation of pro-transcriptional nuclear factor ‘kappa light chain enhancer’ of activated B cells (NF-κB) as well as mitogen-activated protein kinases (MAPKs). Upon activation, MyD88-dependent TLRs initiate assembling of the Myddosome, which contains MyD88 and interleukin (IL)-1 receptor-associated kinases (IRAK) 1, 2 and 4. IRAK1 then associates with tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6), thereby recruiting the transforming growth factor beta-activated kinase-1 (TAK1) complex leading to activation of MAPKs and NF-κB. This induces transcription of inflammatory cytokines. Employment of the TRIF-dependent pathway by TLR3 (or internalized TLR4) results in the activation of NF-κB and interferon regulatory transcription factor 3 (IRF3), eventually inducing the expression of type I IFN expression and pro-inflammatory cytokines. Besides classical TLR-dependent signalling pathways, it has been demonstrated that TLR activation may also induce non-canonical signalling events such as stimulation of NADPH oxidase (NOX)-dependent production of reactive oxygen species (ROS) [11]. While TLR3, TLR5, TLR7, TLR8 and TLR11 are classically activated by PAMPs, a variety of DAMPs have been described to activate TLR1, TLR2, TLR4 and TLR6 with particular relevance in CVD such as atherosclerosis and endothelial dysfunction [13]. Notably, it has been shown that activation of NF-κB and activator protein 1 (AP-1) by TLR2 requires the formation of a heterodimer between TLR2 and either TLR1 or TLR6 [14]. Besides these two receptors, scavenger receptors such as CD36 have also been shown to serve as co-receptor for TLR2 [15]. The TLR4 receptor, in contrast, forms homodimers [14].

**NLRs and inflammasomes**

In contrast to TLRs, NLRs are located in the cytoplasm. All of these receptors contain a highly conserved NACHT domain, which is required for self-oligomerization of the proteins upon ligand recognition. Additionally, most of them comprise C-terminal leucine-rich repeats (LRR), an N-terminal caspase
activation and recruitment domain (CARD) or a pyrin domain (PYD). Besides NOD1 and NOD2, NLRPs represent an important class of the NLR family [16]. In contrast to NOD1/2, they do not activate NF-κB and, thereby, induce the transcription of distinct pro-inflammatory genes. NLRs form a complex with the PYD-CARD adaptor protein ASC and caspase-1, which is then called ‘inflammasome’. Until now, several inflammasomes have been identified in humans, of which the NALP3 inflammasome is the best characterized. Figure 2A shows the composition and activation of the NALP3 inflammasome. Via caspase-1, the NALP3 inflammasome cleaves the pro-forms of the inflammatory cytokines IL-1β, IL-18 and IL-33 and converts them into their biologically active forms. The activation of the NALP3 inflammasome requires two distinct signals (Figure 2B). Signal 1 is mediated by activation of receptors (e.g. TLRs) inducing NF-κB-dependent expression of pro-IL-1β as well as the components of the NLRP3 inflammasome (i.e. NALP3, caspase-1, Asc). Subsequently, Signal 2 induces the assembly of the active NALP3 inflammasome and then mediates caspase-1-dependent cleavage of the pro-cytokines IL-1β, IL-18 and IL-33. These cytokines are then secreted to exert their effects [5, 7]. Until now, five distinct mechanisms are thought to deliver Signal 2:

- Cellular efflux of potassium ions
- Translocation to mitochondria
- Production of mitochondrial ROS
- Release of mitochondrial DNA (mtDNA) or cardiolipin
- (Phago-)Lysosomal destabilization

**Ligands of pattern recognition receptors**

Besides PAMPs, host-derived DAMPs that act as ligands for PRRs play important roles in CVD. Table 1 summarizes selected ligands for PRRs with particular relevance in CKD-associated CVD.

**ROLE IN CKD-ASSOCIATED CARDIOVASCULAR DISEASE**

**Arterial hypertension**

Arterial hypertension represents an important cause and consequence of CKD. During the past years, it became evident that...
the adaptive and innate immunities play important roles in the pathogenesis of arterial hypertension as well as hypertension-associated end-organ damage. In epidemiological studies, patients with hypertension had increased serum levels of TNF-α, IL-6 or C-reactive protein (CRP) [22]. Moreover, it has been documented that inhibition of the pro-inflammatory transcription factor NF-κB in endothelial cells ameliorated hypertension-induced renal and angiotensin II-induced inflammatory damages [23, 24]. Notably, as outlined above, NF-κB represents an important downstream target of TLRs. Therefore, it is not surprising that administration of a TLR4-neutralizing antibody reduced arterial blood pressure in spontaneous hypertensive rats [25]. Besides NF-κB up-regulation, activation of TLR4 stimulates the expression of transcription factor IRF3. It has been shown that infusion of angiotensin II into mice activates IRF3, which regulates development of cardiac fibrosis in response to angiotensin II [26]. The role of TLR4 in mediating arterial hypertension has been proved in a mouse model of arterial hypertension plays important roles in the pathogenesis of arterial hypertension as well as hypertension-associated end-organ damage. In epidemiological studies, patients with hypertension had increased serum levels of TNF-α, IL-6 or C-reactive protein (CRP) [22]. Moreover, it has been documented that inhibition of the pro-inflammatory transcription factor NF-κB in endothelial cells ameliorated hypertension-induced renal and angiotensin II-induced inflammatory damages [23, 24]. Notably, as outlined above, NF-κB represents an important downstream target of TLRs. Therefore, it is not surprising that administration of a TLR4-neutralizing antibody reduced arterial blood pressure in spontaneous hypertensive rats [25]. Besides NF-κB up-regulation, activation of TLR4 stimulates the expression of transcription factor IRF3. It has been shown that infusion of angiotensin II into mice activates IRF3, which regulates development of cardiac fibrosis in response to angiotensin II [26]. The role of TLR4 in mediating arterial hypertension has been proved in a mouse model of
NG-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. L-NAME administration induced a release of HMGB-1, a known TLR4 ligand, and promoted ROS production. Interestingly, the effect of L-NAME on blood pressure was blunted in Tlr4−/− mice [27]. Besides TLR4, activation of TLR2 by endogenous ligands might also affect the regulation of blood pressure. As will be discussed below, our group was recently able to show that intravenous injection of HDL from patients with CKD into mice increased blood pressure [11]. Notably, this adverse effect of HDL was mediated by TLR2 on the surface of endothelial cells (Figure 3).

Although these studies point to a certain role of TLR activation in the pathogenesis of arterial hypertension, the exact mechanistic details are not entirely clear. The association between inflammasome activation and the incidence of arterial hypertension is even more fragmentary. It has been shown that circulatory levels of the NLRP3-dependent cytokine IL-1β are increased in patients with arterial hypertension [28]. Moreover, when isolated monocytes from patients with hypertension were incubated with either the TLR4 agonist LPS or angiotensin II, they secreted more IL-1β than monocytes from healthy subjects [29]. Vice versa, valsartan, an angiotensin I (AT1) receptor blocker, reduced IL-1β secretion by monocytes from hypertensive patients [30]. These findings indicate an association between IL-1β-secreting and hypertension-

Table 1. Selected endogenous ligands of PRRs

<table>
<thead>
<tr>
<th>Substance</th>
<th>PRR</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Modified HDL</td>
<td>TLR2</td>
<td>[11]</td>
</tr>
<tr>
<td>OxLDL</td>
<td>TLR2, TLR4, NLRP3</td>
<td>[17]</td>
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<tr>
<td>Oxidized phospholipids (OxPL)</td>
<td>TLR4</td>
<td>[18]</td>
</tr>
<tr>
<td>Fatty acids (saturated or unsaturated)</td>
<td>TLR4</td>
<td>[18]</td>
</tr>
<tr>
<td>Versican</td>
<td>TLR2</td>
<td>[19]</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td>TLR4, NLRP3</td>
<td>[20]</td>
</tr>
<tr>
<td>Surfactant protein-A</td>
<td>TLR2, TLR4</td>
<td>[21]</td>
</tr>
<tr>
<td>Heat-shock protein 60 and 70</td>
<td>TLR2, TLR4</td>
<td>[19, 21]</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>TLR2, TLR4</td>
<td>[19, 21]</td>
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<tr>
<td>Fibronectin</td>
<td>TLR4</td>
<td>[19, 21]</td>
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<tr>
<td>Heparan sulphate</td>
<td>TLR4</td>
<td>[19, 21]</td>
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<tr>
<td>Soluble hyaluronan</td>
<td>TLR4</td>
<td>[19, 21]</td>
</tr>
<tr>
<td>ATP</td>
<td>NLRP3, P2X7</td>
<td>[19]</td>
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<tr>
<td>Uric acid</td>
<td>NLRP3</td>
<td>[19]</td>
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<tr>
<td>Oxalate crystals</td>
<td>NLRP3</td>
<td>[19]</td>
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<tr>
<td>Cholesterol crystals</td>
<td>NLRP3</td>
<td>[17]</td>
</tr>
<tr>
<td>Uromodulin</td>
<td>TLR4, NLRP3</td>
<td>[19]</td>
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**FIGURE 3:** Non-classical TLR2-dependent endothelial effects of HDL from CKD patients. Modified HDL from CKD patients activates TLR2 without its co-receptors TLR1 and TLR6, which inhibits Akt-dependent phosphorylation of eNOS. Moreover, it activates NOXs via SAPK/JNK, thereby stimulating endothelial production of ROS. This reduces endothelial NO bioavailability inducing endothelial dysfunction and hypertension. TLR, Toll-like receptor; Akt, protein kinase B; SAPK/JNK, stress-activated phospho kinase/c-Jun N-terminal kinase; NOX, NADPH oxidase.
promoting pathways. This is further underscored by the observation that IL-1β might directly modulate the vascular tone. Ex vivo incubation of aortae from hypertensive rats with IL-1β induced a stronger vasoconstriction when compared with aortae from normotensive rats [31]. Currently, we found only one study linking the inflammasome to hypertension in mice models [32]. Unilateral nephrectomy in mice and administration of deoxycorticosterone acetate and saline (1K/DOCA/salt) for 21 days increased mRNA expression of the inflammasome components NLRP3, ASC, caspase-1, IL-1β and IL-18 in the remaining kidney. While the aforementioned treatment increased systolic blood pressure in wild-type mice by ~40 mmHg, blood pressure in Asc−/− mice was ~40% lower. The absence of Asc also resulted in lower blood pressure levels in mice after angiotensin II infusion. Besides direct effects on the blood pressure, renal fibrosis associated with 1K/DOCA/salt-dependent hypertension was mitigated in Asc−/− mice. These results may provide some evidence for the role of the NLRP3 inflammasome and its related cytokines in the pathogenesis of arterial hypertension. However, it remains completely unclear which factors induce inflammasome activation in hypertension and how inflammasome activation increases vascular tone at the molecular level.

### Atherosclerosis

For years, atherosclerosis has been considered to be an inflammatory disease [33]. Besides resident cells of the vascular wall, circulating leukocytes are crucially involved in atherosclerotic lesion formation. They adhere to activated endothelial cells, transmigrate into the sub-endothelial layer, differentiate into macrophages and become foam cells upon internalization of pro-atherogenic lipids such as oxidized low-density lipoprotein (oxLDL) [34], a known stimulus of TLR2 and TLR4 [15].

Endothelial dysfunction, i.e. impaired release of vasoprotective substances by endothelial cells, represents an important initial step in the development of atherosclerosis. Besides classical cells of the innate immune system, endothelial cells also express functional TLRs on their surface. Interestingly, it has been shown that TLR2-deficient mice are protected from post-ischaemic coronary endothelial dysfunction [35]. Recently, our group was able to demonstrate that TLR2 might also be involved into the pathogenesis of CKD-associated endothelial dysfunction [11]. Whereas HDL from healthy donors is considered to be vasoprotective, we found that HDL from CKD patients is transformed into noxious particles that induce endothelial dysfunction and increase arterial blood pressure in mice. Moreover, we revealed that these adverse effects of HDL from CKD patients are mediated via an unknown intracellular pathway of TLR2 (Figure 3). As outlined above, signaling by TLR2 targeting NF-κB activation requires its association with TLR1 or TLR6 as co-receptor. Surprisingly, we found that abnormal HDL activates endothelial TLR2 in the absence of TLR1 and TLR6. This activation did not promote NF-κB activation but led to increased ROS production via SAPK/JNK-dependent activation of NOXs. The increased ROS production inhibited endothelial nitric oxide synthase (eNOS) and, thereby, reduced endothelial NO bioavailability.

While TLR-dependent inflammation contributes to vascular damage, a certain degree of inflammation might be required for vascular repair mechanisms. Accordingly, it has been documented that TLR2 ligands drive angiogenesis by promoting endothelial proliferation and migration [36]. Moreover, oxidative stress as induced by the accumulation of end products of lipid peroxidation has been shown to promote angiogenesis (i.e. de novo blood vessel growth) by activating TLR2 [10]. The relevance of these findings in CKD remains unknown, which raises the question to what extent the link between inflammation and repair of vascular lesions is impaired under CKD conditions.

Already in 2002, a significantly increased expression of TLR1, TLR2 and TLR4 in atherosclerotic vascular lesions was reported [37]. Several years later, it was documented that genetic deficiency of TLR2, TLR4 as well as the TLR adaptor protein MyD88 reduces atherosclerotic lesions in several mouse models of atherosclerosis [10, 38–40]. Besides TLRs, also the NLRP3 inflammasome might be involved in the pathogenesis of atherosclerosis. Activation of the NLRP3 inflammasome in endothelial cells might increase susceptibility to the development of atherosclerotic lesions [41]. Moreover, in an elegant study, Duewell et al. documented that cholesterol crystals—as present in atherosclerotic lesions—stimulate the NLRP3 inflammasome, thereby aggravating atherosclerosis [17]. Mechanistically, the effects of cholesterol crystals on NLRP3 activation were caused by (phago-)lysosomal damage. In this context, the scavenger receptor CD36 has emerged as an important ‘adjusting screw’ in translating the association of oxLDL with monocytes/macrophages into inflammasome activation [42].

Since there is currently no small rodent model to specifically study CKD-associated atherosclerosis, the role of the innate immune system in CKD-associated atherosclerosis remains widely elusive so far. However, the CKD-specific pro-inflammatory microenvironment with increased expression of PRRs on leukocytes of CKD patients [43, 44] might point to a pivotal role of innate immunity in promoting premature CKD-associated formation of atherosclerotic lesions.

### Vascular calcification

Arterial vascular calcification represents another frequent complication in patients with CKD. Calcification develops mainly in the media layer of blood vessels where osteochondrogenic differentiation of vascular arterial smooth muscle cells occurs. Thereby, the release of pro-osteochondrogenic mediators by the endothelium represents a crucial initial step in the development of vascular calcification [45].

It has been shown that systemic inflammation reduces endothelial expression of bone morphogenic protein (BMP) endothelial precursor cell-derived regulator (BMPER) [46]. This confers to a pro-inflammatory endothelial phenotype facilitating migration of leukocytes into the sub-endothelial space, where they promote pro-osteogenic differentiation of smooth muscle cells. Hereby, detailed mechanistic insights on how activated monocytes or macrophages interfere with pro-osteogenic differentiation of smooth muscle cells are missing. However, it has been suggested that direct cell–cell interaction as well as paracrine
effects by the release of inflammatory cytokines by monocytes/macrophages might play an important role [47]. Additionally, pro-inflammatory cytokines (e.g. TNF-α) activate endothelial NF-κB-dependent signalling pathways, which leads to endothelial secretion of BMP-2 and BMP-4 [48, 49]. Moreover, it has been documented that activation of TLR2 and TLR4 in endothelial cells by oxLDL also induces the expression and the release of BMP-2 and BMP-4 [50]. Endothelial-derived BMP-2 and BMP-4 subsequently bind to a complex of BMP receptor (BMPR) types I and II on the surface of vascular smooth muscle cells. This induces phosphorylation and nuclear translocation of distinct SMAD transcription factors, which then initiate transcription of pro-osteochondrogenic genes (e.g. Msx2, alkaline phosphatase, Oss) [48]. The role of TLR2 and TLR4 in osteogenic differentiation has been documented in human aortic valve interstitial cells, in which TLR2 and TLR4 agonists induced osteogenic phenotypical changes [51]. Notably, valve interstitial cells isolated from human stenotic valves showed an increased expression of TLR2 and TLR4.

For several years, it has been known that IL-1β may induce vascular calcification [52]. Accordingly, in a mouse model of familial hypercholesterolaemia, a monoclonal antibody directed against IL-1β diminished vascular calcification [53]. These findings might point to a role of the inflammasome as an IL-1β-activating platform in the process of vascular calcification. In 2005, it was documented that deposition of basic calcium phosphate crystals in macrophages triggers the release of pro-inflammatory cytokines such as IL-1β [54]. Several years later, it was shown that this release of IL-1β in response to basic calcium phosphate is mediated by the NLRP3 inflammasome [55]. Besides calcium phosphate crystals, calcium pyrophosphate dehydrate crystals activate the NLRP3 inflammasome, which might be of relevance in inflammatory joint diseases but also in vascular calcification [56]. Moreover, induction of calcification in vascular smooth muscle cells in vitro by β-glycerophosphate increased the expression of the NLRP3 inflammasome components NLRP3 and ASC as well as caspase-1. The expression of the same proteins was also enhanced in calcified human popliteal artery samples [57]. Although these observations may indicate a potential contribution of the NLRP3 inflammasome in the development of vascular calcification, a definite proof using appropriate knock-out models is lacking.

**THERAPEUTIC IMPLICATIONS**

Taken together, these studies provide a strong body of evidence that the innate immunity represents a crucial modulator of distinct CKD-associated CVD. However, information on a specific and causal relationship between CVD and the innate immune activation in CKD is sparse. Therefore, in most cases, findings from studies in the general population have to be translated to patients with CKD. Since the pathophysiology of CVD differs between the general population and CKD patients, this approach contains a degree of uncertainty and remains challenging.

Nevertheless, activation of the innate immune system might be involved in the pathogenesis of CVD. If this holds true, anti-inflammatory treatment strategies should be promising. Indeed, it has been shown that treatment with statins, which exert potent anti-inflammatory effects, reduces cardiovascular risk despite its lipid-lowering properties. In the JUPITER trial, statin treatment prevented cardiovascular events and lowered all-cause mortality in individuals with low LDL cholesterol but high levels of inflammatory markers [58]. Currently, there are many ongoing studies examining the effect of new agents targeting components of the innate immune system on cardiovascular mortality. In the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS), the effect of Canakinumab, a human monoclonal anti-human IL-1β antibody, on recurrent major cardiovascular events in patients with stable coronary artery disease at high inflammatory risk (i.e. CRP > 2 mg/L) is currently being assessed [59]. Notably, in a Phase IIb randomized, placebo-controlled trial, Canakinumab reduced systemic inflammation (i.e. CRP, IL-6, fibrinogen) in diabetic patients at high cardiovascular risk [60]. The human IL-1 receptor (a (IL-1Ra) antagonist Anakinra might represent an additional agent to reduce systemic inflammation. Anakinra, which is approved for the therapy of rheumatoid arthritis, has been shown to improve vascular and left ventricular function in these patients [61]. The same agent ameliorated glycaemia and beta cell secretory function and reduced systemic inflammation in patients with Type 2 diabetes [62]. To assess the definite efficacy of these new pharmacological approaches, the results of the ongoing large outcome trials must be awaited. In the case of positive results, further studies in CKD patients will be necessary.

Finally, based on several mouse models, associative epidemiological studies as well as in vitro experiments, the innate immunity represents a crucial gatekeeper in initiating and promoting CKD-associated CVD. However, future research is mandatory to definitely prove the link between the innate immune system and CKD-associated CVD.

**CONFLICT OF INTEREST STATEMENT**

The results presented in this article have not been published previously in whole or part.

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Received for publication: 6.9.2015; Accepted in revised form: 9.9.2015