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CKJ REVIEW

Leukocyte–endothelial interaction in CKD

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ABSTRACT

Chronic kidney disease (CKD) represents an independent risk factor for cardiovascular diseases (CVD). Accordingly, CKD patients show a substantial increased risk of cardiovascular mortality. Inflammation represents an important link between CKD and CVD. The interaction between endothelial cells and effector cells of the innate immune system plays a central role in the development and progression of inflammation. Vascular injury causes endothelial dysfunction, leading to augmented oxidative stress, increased expression of leukocyte adhesion molecules and chronic inflammation. CKD induces numerous metabolic changes, creating a uremic milieu resulting in the accumulation of various uremic toxins. These toxins lead to vascular injury, endothelial dysfunction and activation of the innate immune system. Recent studies describe CKD-dependent changes in monocytes that promote endothelial dysfunction and thus CKD progression and CKD-associated CVD. The NLR family pyrin domain containing 3–interleukin-1β–interleukin-6 (NLRP3–IL-1β–IL-6) signaling pathway plays a pivotal role in the development and progression of CVD and CKD alike. Several clinical trials are investigating targeted inhibition of this pathway indicating that anti-inflammatory therapeutic strategies may emerge as novel approaches in patients at high cardiovascular risk and nonresolving inflammation. CKD patients in particular would benefit from targeted anti-inflammatory therapy, since conventional therapeutic regimens have limited efficacy in this population.

LAY SUMMARY

Sterile inflammation, which refers to inflammation not caused by infections, plays an important role in the development and progression of chronic kidney disease and cardiovascular disease alike. Thereby, white blood cells circulating in the blood adhere to the wall of the blood vessels and transmigrate into the tissue. There, they release a plethora of substances, which further maintain an inflammatory response and which finally lead to irreversible tissue damage. Recently, anti-inflammatory therapies have emerged as novel therapeutic approaches in kidney and cardiovascular diseases.

Keywords: adhesion, cardiovascular disease, chronic kidney disease, inflammation

INTRODUCTION

Recent years have seen a substantial advance in our understanding of the development and progression of chronic kidney disease (CKD) and CKD-associated cardiovascular disease (CVD). CKD represents an independent risk factor for cardiovascular events, hospitalization and mortality risk [1].

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A better understanding of CKD and associated CVD is particularly important given that the prevalence of CKD is consistently rising. In the years between 1990 and 2017, there was an increase in the global prevalence of CKD of over 25%, with a global prevalence of 9.3% in 2017 [2]. Death from CKD and CVD attributable to impaired kidney function represented almost 5% of total mortality in 2017, rendering CKD the 12th leading cause of death [2]. In particular, the increased risk of CVD plays a crucial role [3]. Compared with the general population, patients with end-stage kidney disease (ESKD) aged 25–34 years show a 500- to 1000-fold increased annual mortality [4]. CKD represents one of the strongest independent cardiovascular risk factors [5].

Restoring homeostasis after damage is an important function of inflammation [6]. However, persistent inflammation causes adverse effects that eventually result in the progression of fibrosis and organ injury. The interaction of endothelial cells (ECs) and effector cells of the innate immune system plays a major role in the development and maintenance of inflammation. Endothelial injury leads to activation of ECs with increased expression of cytokines, chemokines and adhesion molecules. Thereby, inflammation induces binding of monocytes and neutrophils to the endothelium and promotes further stimulation of ECs through the release of additional cytokines [7].

Monocytes and macrophages are core cells of the innate immune system and express different pattern recognition receptors (PRRs) through which they recognize various damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) [8]. CKD leads to the accumulation of activators of the innate immune system. Recent studies highlight the crucial role of leukocytes, particularly monocytes, macrophages and neutrophils, which serve as key factors of sterile inflammation both in direct interaction with ECs and via the release of cytokines [9].

ENDOTHELIAL DYSFUNCTION

The endothelium is a monolayer of ECs covering the inner surface of blood vessels [10]. It exerts numerous effects in angiogenesis, antithrombotic action, provision of antioxidants, release of vasoactive substances and anti-inflammatory agents. The development of functional disorders of the endothelium, which is accompanied by an imbalance of vasodilatory and vasoconstrictive substances, is referred to as endothelial dysfunction. Endothelial dysfunction is thus associated with increased oxidative stress, chronic inflammation, leukocyte adhesion, hyperpermeability and vascular stiffening, and plays a pivotal role in the pathogenesis of several diseases including atherosclerosis, diabetes mellitus and CKD [7] (Fig. 1).

The "gold standard" for measuring endothelial (dys-)function in patients is determination of the arterial flow-mediated dilatation of the forearm (FMD). Using ultrasound imaging, the diameter of the brachial artery is determined at rest and after reactive hyperemia. The change in diameter provides information about endothelial function. Thereby, a reduced variability, as seen in patients with CKD, is indicative for endothelial dysfunction [11]. Several studies demonstrate an improvement of endothelial dysfunction, as measured by FMD, with angiotensin-converting enzyme inhibitors [12–14], angiotensin II receptor blockers [15, 16], aldosterone antagonists [17, 18], statins [19, 20] and allopurinol [21]. Moreover, it has been shown that patients with persistent endothelial dysfunction under heart failure therapy had a higher risk of cardiac events as compared with patients with improvement of endothelial dysfunction under the therapy [22].

The endothelium with its glycocalyx and cell junctions serves as a barrier against plasma proteins, cells and solutes [23]. Persistent inflammation is associated with endothelial injury and disruption of inter-endothelial junctions, leading to endothelial dysfunction [24].

Endothelial dysfunction due to injury represents a central step in the development of atherosclerosis accompanied by enhanced expression of adhesion molecules, leukocyte adhesion and increased permeability. Furthermore, the release of vasoactive molecules, cytokines and growth factors occurs. Immigration and activation of immune cells especially monocytes, macrophages and specific T cells, results in a sustained inflammatory response [25].

The investigation of transcriptional changes in different subtypes of ECs during various pathological processes provides new insights into the development of endothelial dysfunction and its impact [26]. For instance, it has been shown that kidney ECs are particularly vulnerable to transcriptional alterations induced by obesity. The data suggest that obesity-induced transcriptional changes in medullary and glomerular ECs are associated with cellular stress and endothelial dysfunction [27].

Leucocyte adhesion and adhesion molecules

Leukocyte adhesion represents a multi-step process mediated by different receptors. The first step towards rolling of leukocytes is initiated by L-selectin, E-selectin and P-selectin, which interact with P-selectin glycoprotein ligand-1 (PSGL1) and other glycosylated ligands [28]. Rolling leukocytes bind tightly with their integrins to members of the immunoglobulin superfamily, e.g. intercellular adhesion molecule 1 (ICAM1) or vascular cell adhesion protein 1 (VCAM1), on the endothelial surface [29]. This process is further supported by a broad spectrum of chemoattractants. The combination of different receptors and chemokines with different affinities enables targeting of specific leukocytes [30].

Transendothelial migration represents the final step in the cascade of leukocyte–endothelial adhesion, with the majority occurring through paracellular migration (90%) and only a small proportion through transcellular migration (10%) [31]. Adherent leukocytes can stimulate ECs via ICAM1 and VCAM1 to form a docking structure with high levels of adhesion molecules [6].

Inflamed ECs rearrange the distribution of functional molecules to support leukocyte transmigration [32], e.g. the adhesion molecules platelet and endothelial cell adhesion molecule 1 (PECAM1) and junctional adhesion molecule A (JAM-A), which are mobilized toward the luminal side after binding of leukocytes [6]. The process of transendothelial migration is mediated by a variety of endothelial junctional molecules and thereby affected by different cytokines such as interleukin-1β (IL-1β), which induces PECAM1-, ICAM2- and JAM-A-mediated transmigration [33].

This process of leukocyte adhesion is substantially altered in CKD and may contribute to the vulnerability of CKD patients for infections and CVD [34]. CKD patients show elevated plasma levels of monocyte chemoattractant protein-1 (MCP-1) and soluble VCAM as their kidney function declines. In addition, ECs stimulated with uremic plasma exhibit increased expression of MCP-1, sVCAM1 and IL-8 [35, 36]. Diabetic nephropathy, as one of the leading causes of CKD, promotes inflammation at least partially by the enhanced expression of adhesion molecules. *In vivo* experiments revealed that hyperglycemia results in increased expression of ICAM1 and VCAM1 on ECs [37]. Under physiological conditions, the luminary layer of

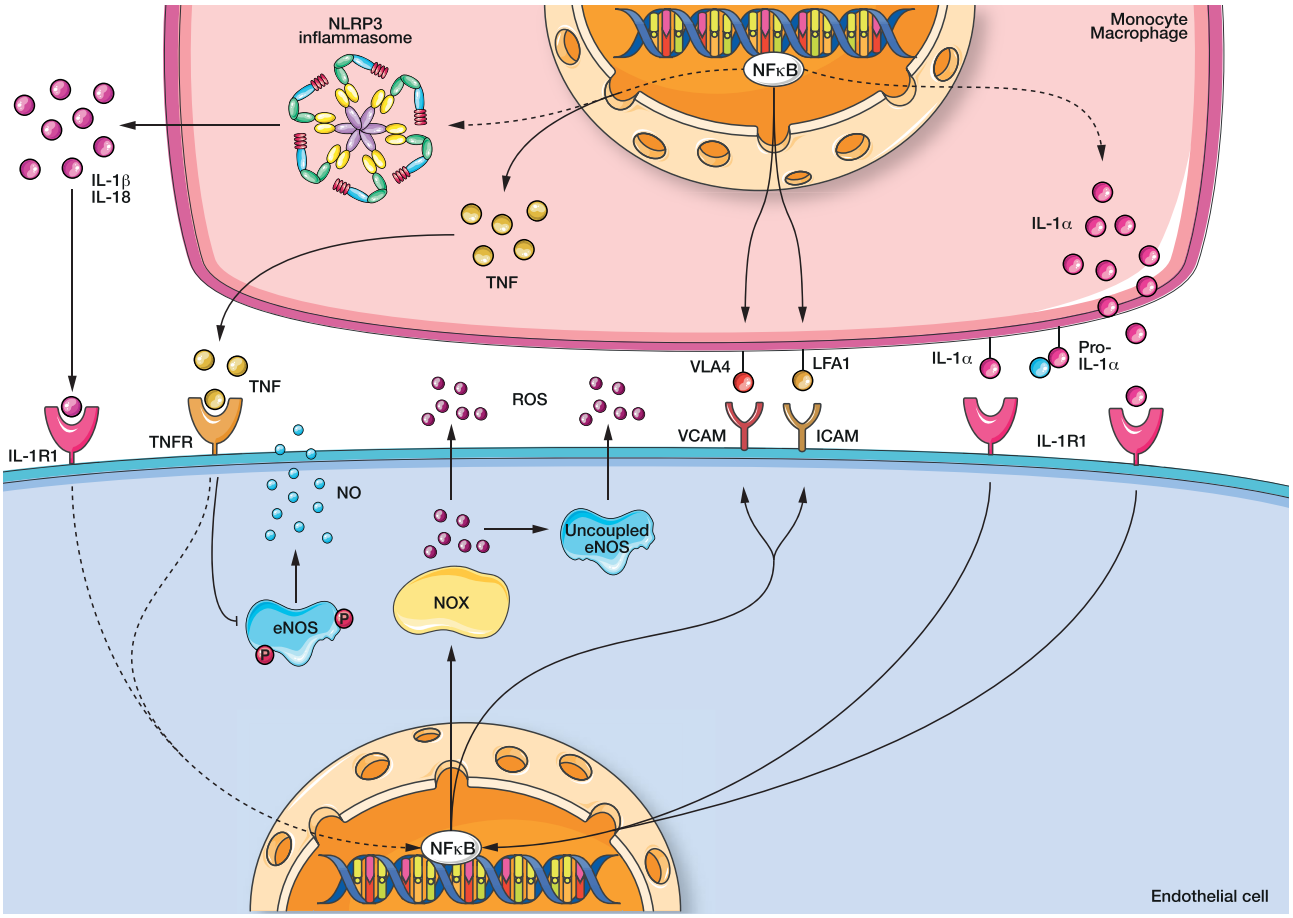

Figure 1: Interaction of monocytes with ECs. Monocytes and endothelial cells interact via several signaling pathways, including the release of various cytokines and chemoattractants, and direct interaction and binding of monocytes via adhesion molecules. Activation of NF-κB in monocytes/macrophages results in the increased expression of TNF, VLA4, LFA1 and IL-1α, and promotes the activation of the NLRP3 inflammasome. TNF induces a signaling cascade via TNFR leading to activation of NF-κB in ECs and, thereby, increased expression of multiple adhesion molecules (e.g. ICAM and VCAM) and NOX. NOX causes increased oxidative stress via the production of ROS. In addition, activation of TNFR and increased production of ROS lead to inhibition and uncoupling of eNOS. As a result, uncoupled eNOS secretes ROS instead of NO. This results in worsening of oxidative stress. ICAM and VCAM mediate monocyte/macrophage adhesion via binding to VLA4 and LFA1, respectively. Monocytes express IL-1α and its active form pro-IL-1α on their surface and can also secrete them. Interaction of IL-1α with ECs results in increased expression of VCAM1. IL-1α and pro- IL-1α potentially serve as adhesion molecules for monocytes by binding to IL-1R1. Monocytes/macrophages secret IL-1β, IL-18 and IL-33 after cleavage by the NLRP3 inflammasome. IL-1β binds to IL-1R1, which ultimately drives the activation of NF-κB in ECs. LFA1: Lymphocyte function-associated antigen 1; NOX1: NADPH oxidase 1; TNFR: TNF receptor; VLA4: very late antigen-4.

glomerular capillaries is covered by the glomerular endothelial glycocalyx (eGC), which consists of proteoglycan core proteins, glycosaminoglycan (GAG) chains and sialoglycoproteins [38]. eGC serves as a barrier to leukocyte adhesion. Accordingly, damage of the eGC induces increased leukocyte adhesion [39]. CKD is associated with the accumulation of the uremic toxin symmetric dimethylarginine (SDMA) in high-density lipoprotein (HDL) [40]. It has been shown that such modified HDL leads to eGC damage via a toll-like receptor 2 (TLR2)-mediated pathway [41] resulting in the expression of adhesion molecules and thereby inflammation. Another mechanism causing eGC damage are high-glucose-induced changes of eGC [38].

Reactive oxygen species and nitric oxide

The development of oxidative stress is based on an imbalance between pro-oxidant and anti-oxidant systems with formation of reactive oxygen species (ROS) by ECs. ROS play an important role in the progression of CKD and CVD [42]. The main sources of endothelial ROS production include xanthine oxidase, NADPH-oxidase (NOX), uncoupled endothelial nitric oxide synthase (eNOS) and mitochondrial dysfunction [7].

The physiological function of eNOS is the catalysis of nitric oxide (NO) production, which mediates vasodilation, and antithrombotic effects. Several signaling pathways contribute to impaired electron flow within eNOS, resulting in the transfer of electrons to superoxide instead of NO [43, 44]. This alteration is referred to as eNOS uncoupling and not only reduces NO production, but additionally induces the production of ROS [43]. Various uremic toxins that accumulate in CKD promote eNOS uncoupling and cause NOX4-dependent production of ROS. This leads to increased expression of adhesion molecules, endothelial dysfunction and inflammation [45–47]. Xanthine oxidase represents another relevant source of ROS, released during the metabolism of hypoxanthine via xanthine to uric acid. Xanthine oxidase thus contributes to oxidative stress and endothelial dysfunction [48]. In line with this, it has been shown [49] that the beneficial effect

of allopurinol on endothelial function may be partially caused by a reduction of oxidative stress through inhibition of xanthine oxidase. In the context of CKD and diabetic kidney disease, the disturbance of a variety of other physiological processes leads to the excessive production of ROS. This includes the disruption of mitochondrial function by a uremic and/or diabetic milieu [50]. Furthermore, ROS represent a crucial factor of another key component of the innate immune system, the NLR family pyrin domain containing 3 (NLRP3) inflammasome [51].

INNATE IMMUNE SYSTEM IN CKD

The association between the development and progression of CKD and CVD and inflammation has been known for decades [25]. Accordingly, it has been observed that C-reactive protein (CRP) levels and neutrophil cell counts are elevated in angina pectoris patients with coronary stenosis and that neutrophil counts correlate with angiographic stenosis complexity [52, 53].

Monocyte/macrophages

Monocytes are circulating white blood cells that can be classified into different subtypes based on their size, trafficking, innate immune receptor expression and ability to further differentiate after stimulation with cytokines or DAMPs [54–56]. In the context of sterile inflammation in the development of atherosclerosis, monocytes are recruited by a variety of adhesion molecules due to endothelial defects. P-selectin and E-selectin cause monocyte rolling on endothelium, VCAM subsequently leads to firm adhesion [55, 57]. Within CKD, a broad spectrum of modulations of monocyte/macrophages occurs to promote cardiovascular injury. For example, CKD induces increased expression of G protein–coupled receptor 68 (GPR68) on human monocytes, which enhances secretion of pro-inflammatory cytokines resulting in systemic inflammation and cardiac fibrosis [58]. Moreover, CKD causes altered lipid metabolism with reduced cholesterol efflux capacity and increased numbers of intermediate monocytes (CD14++CD16+) [59]. Intermediate monocytes are considered to be the subtype of monocytes with the highest inflammatory potential [60, 61]. A higher blood count of these monocytes is associated with worse cardiovascular outcomes [62]. A recent study demonstrated that the interaction of intermediate monocytes with ECs through CX3CR1 results in endothelial dysfunction. The interaction leads to up-regulation of endothelial expression of various chemokines and adhesion molecules such as ICAM1 and VCAM1, as well as increased expression of IL-1β. Thereby, intermediate monocytes promote inflammation and are associated with a higher risk for aortic stiffness and CVD [63].

Cytokines

In recent years, various biomarkers have been evaluated to improve prognostic assessment in CKD and CVD. Inflammatory biomarkers correlate with CVD in CKD patients [64] and are associated with higher long-term risk for CKD onset and progression [65, 66]. The use of nontraditional cardiovascular risk factors in determining cardiovascular risk of patients with CKD and especially hemodialysis patients plays a critical role, since traditional risk factors such as low-density lipoprotein-cholesterol (LDL-C) are increasingly discussed controversially in this population [67, 68]. Circulating tumor necrosis factor (TNF) represents an independent risk factor for CKD progression [66] and potentially offers the possibility to improve cardiovascular-risk estimates [69]. Furthermore, high-sensitivity CRP (hsCRP) is a relevant marker for cardiovascular risk [70] and higher IL-6 levels were independently associated with an increased risk of major adverse cardiovascular events (MACE) in a sub-study of STABILITY in all CKD stages [71]. In a cohort of patients with CKD stage 2–4, a polymorphism in the *IL6* promoter leading to elevated IL-6 levels was associated with history of CVD and predictive for the incidence of cardiovascular events [72]. Given the association of IL-6 with increased occurrence of cardiovascular events, IL-6 serves as a potential marker of residual inflammatory risk in patients on maximal secondary prophylaxis, who may benefit from additional anti-inflammatory therapy [73, 74]. Accordingly, a UK Biobank analysis showed that a genetic variant mimicking the effect of IL-6 inhibition reduced cardiovascular risk in CKD patients while having no effect in a non-CKD control group [75]. Furthermore, cytokines of the IL-1 superfamily such as IL-1β and IL-1α are of particular importance. An analysis of single nucleotide polymorphisms identified IL-1 as a potential link between inflammation and CKD [76]. Two polymorphisms in the *Il1* gene cluster were associated with a higher risk for developing ESKD.

IL-1α

Inflammation leads to the release of IL-1β, via activation of innate immune effector cells such as monocytes and macrophages, and promotes the development of atherosclerosis [77, 78]. Another link between metabolic stress, cellular response and inflammation is the release of IL-1α from monocytes after stimulation by free fatty acids. In this regard, IL1α deficiency was shown to be associated with reduced atherosclerotic lesion formations in mouse models [79].

Recently, it has been documented, that the IL-1α expression on the surface of monocytes from patients with acute myocardial infarction or CKD is significantly increased as compared with healthy subjects. IL-1α surface expression was associated with a higher risk of atherosclerotic cardiovascular disease (ASCVD) events. Monocyte interaction/adhesion with ECs is mediated by the interaction of IL-1α with IL-1 receptor 1 (IL-1R1). Moreover, IL-1α promotes the expression of the endothelial adhesion molecule VCAM1. *In vivo*, IL-1α deficiency inhibits the development of CKD induced by oxalate or adenine diet [80].

miRNA

MicroRNAs (miRNAs) are non-coding RNAs that play an important role in the regulation of gene expression. Dysregulation of miRNAs in CKD may contribute to CKD-associated inflammation and CVD as reviewed elsewhere in more detail [81]. Recent studies suggest that miRNAs may serve as potential biomarkers for endothelial dysfunction in the context of CKD [82, 83].

Inflammasome

Inflammasomes represent a group of multimeric cytosolic protein complexes capable of recognizing various PAMPs and DAMPs [84]. They are assembled after activation of innate immune sensors, e.g. NLRP3 and Absent in melanoma 2 (AIM2), which form a complex with the pyrin domain-caspase activation and recruitment domain (PYD-CARD) containing adaptor molecule apoptosis-associated speck-like protein (ASC) and effector protease pro-caspase-1. Activation of inflammasomes leads to maturation of distinct cytokines, such as IL-1β and IL-18 [85] (Fig. 2). They play a pivotal role in the pathogenesis of various

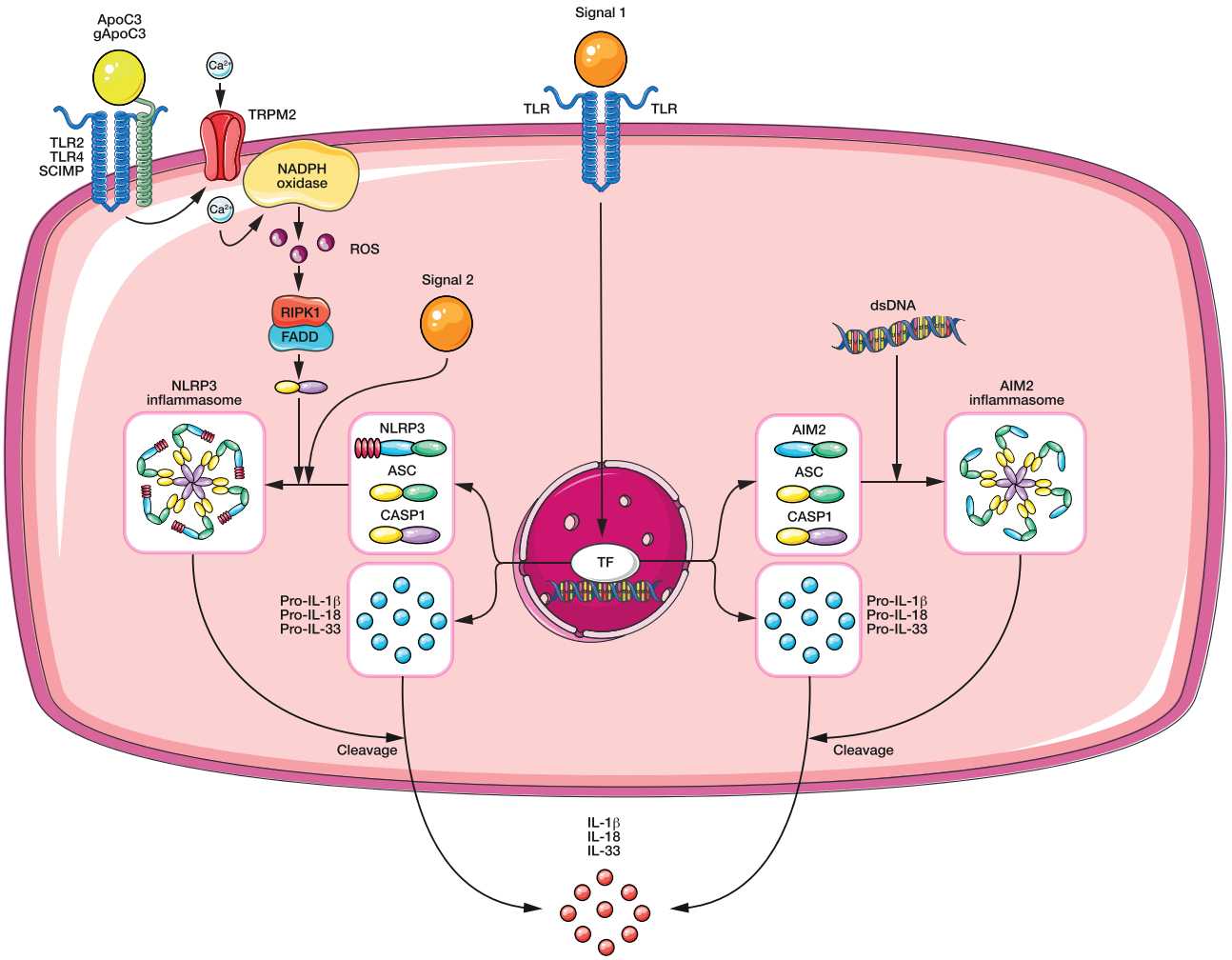

Figure 2: Activation of NLRP3 and AIM2 inflammasome. Canonical activation of the NLRP3 inflammasome proceeds in two steps. In the first step, signal 1 leads to the activation of TLRs and induces expression of NLRP3, caspase-1 and ASC. In the second step, an additional signal results in the formation of the NLRP3 inflammasome. The NLRP3 inflammasome converts pro-IL-1β, pro-IL-18 and pro-IL-33 to the active forms IL-1β, IL-18 and IL-33, respectively. Alternative activation of the NLRP3 inflammasome involves TLR2 and TLR4, which induce SCIMP-dependent cellular calcium influx through TRPM2. Calcium influx activates NOX leading to ROS production. Activation of the NLRP3 inflammasome follows via a RIPK1–FADD, CASP8 signaling pathway. AIM2 is stimulated by dsDNA and forms the AIM2 inflammasome with ASC and caspase-1. CASP8: Caspase 8: FADD: FAS-associated death domain protein; RIPK1: Receptor-interacting serine/threonine-protein kinase 1; SCIMP: SLP65/SLP76, Csk-interacting membrane protein; TRPM2:Transient Receptor Potential Cation Channel Subfamily M Member 2.

human diseases, e.g. infections, autoimmune diseases, acute and chronic inflammation, metabolic syndrome or atherosclerosis [84].

NLRP3 inflammasome

NLRP3 inflammasome activation is associated with several diseases, including hypertension, diabetes mellitus and atherosclerosis [86]. It is activated by a variety of stimuli that play a role in endothelial dysfunction, including ROS production, electrolyte shifts and mitochondrial dysfunction [87]. Development of atherosclerosis is accompanied by accumulation of cholesterol in the atherosclerotic plaques. Cholesterol crystals were found to act as activators of the NLRP3 inflammasome, thus providing a link between cholesterol metabolism and inflammation in the pathogenesis of atherosclerosis [88]. Accordingly, NLRP3 deficiency results in significantly reduced atherosclerotic plaque size after 8 weeks of Western diet in *Ldlr–/–* mice [89]. Another link between inflammation and CVD was demonstrated in a large-scale meta-analysis. The intronic variant rs10754555 within the *Nlrp3* gene is associated with increased NLRP3 expression and thereby, increased inflammation. Carriers of this variant showed a higher prevalence of coronary artery disease (CAD) and cardiovascular mortality [90]. Furthermore, pharmacological inhibition of the NLRP3 inflammasome *in vivo* results in reduced infarct size after myocardial ischemia reperfusion [91]. Vice versa, activation of the NLRP3 inflammasome seems to contribute to adverse cardiac remodeling after pressure overload *in vivo* [92]. ECs represent a target of IL-1β. IL-1β exposure leads to the expression of adhesion molecules and chemokines of ECs, and thereby promotes the adhesion of leukocytes [93, 94]. Distinct inflammasome components are upregulated after unilateral ureter obstruction (UUO). For instance, deficiency of the adapter molecule ASC was associated with a lower inflammatory response, less infiltration with inflammatory cells

Table 1: Activators of the innate immune system in CKD.

Activator of the innate immune system	Effects
APOC3/gAPOC3	NLRP3 inflammasome activation ↑
cHDL	Endothelial repair functions ↓
CHIP	DNA stress ↑ → AIM2 activation
	NLRP3 inflammasome activation
cLDL	LOX-1 activation ↑ → ROS production ↑ → eNOS uncoupling
	Autophagy of ECs ↑, ICAM expression ↑
HDL	SDMA accumulation ↑ → deterioration of the endothelial glycocalyx by TLR2 signaling
	SAA accumulation ↑ → cytokine secretion ↑
Indoxyl sulfate	TGF-β, TIMP-1, pro-collagen 1, PAI-1 expression ↑
	ROS production ↑
oxLDL	EC proliferation ↓, apoptosis ↑, ROS production ↑
	Cholesterol efflux ↓ → NLRP3 inflammasome activation ↑
p-Cresyl	EC proliferation ↓, endothelial repair function ↓
Phosphate	NO production ↓, ROS production ↑, vascular stiffness ↑
Uric acid	NLRP3 inflammasome activation ↑
Xanthine oxidase	ROS production ↑, uric acid ↑

cHDL: carbamylated HDL; cLDL: carbamylated LDL; oxLDL: oxidized LDL.

and secretion of cytokines, as well as less kidney fibrosis [95]. In *Nlrp3*$^{-/-}$ mice, reduced caspase-1 activation, decreased IL-1β and IL-18 release, and less fibrosis was observed after UUO [96]. Furthermore, *Nlrp3* knockout ameliorates proteinuria and CKD-associated inflammation after 5/6 nephrectomy [97]. These results are in line with previous publications demonstrating a relevant role of IL-1β and IL-18 in the development of proximal tubular damage and fibrosis [98]. Interestingly, pharmacological inhibition of the NLPR3 inflammasome by MCC950 reduced proteinuria and podocyte injury in lupus-prone NZM2328 mice [99]. Furthermore, the IL-1R antagonist anakinra exhibited a nephroprotective effect in diabetic mice [100].

AIM2 inflammasome

AIM2 is an inflammasome-forming receptor for double-stranded deoxyribonucleic acid (dsDNA), which forms a caspase-1-activating inflammasome together with ASC. The AIM2 inflammasome is involved in various CVD. *AIM2* deficiency promotes atherosclerotic plaque stability in atherosclerotic mice [101] and ameliorates adverse cardiovascular remodeling in diabetic rats [102]. Furthermore, excessive AIM2 inflammasome activation in type 2 diabetes contributes to post-myocardial infarction heart failure *in vivo* by promoting cell death and cytokine secretion [103]. Consistent with these observations, overexpression of AIM2 is observed in human heart failure, regardless of etiology, with AIM2 being primarily expressed by monocytes/macrophages in cardiac tissue [104].

ACTIVATORS OF THE INNATE IMMUNE SYSTEM IN CKD

CKD induces tremendous alterations in the metabolism and an impaired renal function itself causes accumulation of various substances in the blood of the patients. The resulting uremic milieu promotes inflammation through activation of the innate immune system (Table 1).

Lipoproteins

The uremic milieu in CKD leads to changes in the lipid metabolism and subsequently to uremic dyslipidemia. Modified lipoproteins play a key role in the pathogenesis of CKD and CVD [105].

LDL

Posttranslational modifications of LDL are an important component in mediating its adverse cardiovascular properties [106]. CKD leads to posttranslational carbamylation of LDL. Carbamylated LDL (cLDL) activates lectin-like oxidized LDL receptor 1 (LOX-1) resulting in increased ROS production, eNOS uncoupling and finally endothelial dysfunction [107]. Furthermore, cLDL induces autophagy of ECs causing the release of DNA fragments [108]. In addition, cLDL promotes endothelial expression of ICAM1 and subsequently tissue infiltration with monocytes/macrophages [109].

HDL

In patients with CKD, HDL loses its vasoprotective functions and turns into a noxious agent promoting endothelial dysfunction and inflammation. This is caused by the accumulation of SDMA within the HDL moiety. Modified HDL interacts with TLR2 on the surface of ECs, which results in a reduced bioavailability of NO and, thus, endothelial dysfunction [40, 110]. In addition, the CKD-associated accumulation of the acute phase protein serum amyloid A (SAA) in the HDL particle leads to a loss of its anti-inflammatory effects, enhances the release of cytokines [111] and is linked with inflammation and CVD [112]. CKD also promotes carbamylation of HDL. Carbamylated HDL inhibits endothelial repair functions and thereby promotes endothelial dysfunction [113].

Triglyceride-rich lipoproteins

Several studies demonstrate the association of triglyceride-rich lipoproteins with inflammation and CVD [114–116]. Zewinger

et al. [117] identified Apolipoprotein C3 (ApoC3) as an inducer of inflammation via alternative NLRP3 activation in human monocytes, resulting in an impaired repair of endothelial lesions and kidney injury. In the uremic milieu of CKD patients, post-translational guanidinylation of ApoC3 occurs. This causes increased binding of ApoC3 to monocytes and augmented inflammation. As a result, guanidinylated ApoC3 (gApoC3) promotes renal and vascular injury *in vivo* [118].

Uremic toxins

Uremic toxins interact with the innate immune system and initiate activation of monocytes and macrophages [119]. Alteration of the gut microbiome in CKD results in increased production of various toxic bacterial byproducts [120]. At the same time, reduced renal excretion enhances the accumulation of various uremic toxins such as indoxyl sulfate, p-crezyl, trimethylamine N-oxide (TMAO) and phenylacetylglutamine [121–124]. Elevated plasma concentrations of these toxins exhibit a dose-dependent association with the presence of CVD, endothelial dysfunction, cardiovascular events and mortality risk [120].

Declining renal function leads to accumulation of indoxyl sulfate, which causes increased expression of transforming growth factor (TGF-β), tissue inhibitor of metalloproteinase-1 (TIMP-1) and pro-collagen I, thereby promoting further deterioration of renal function [125]. Moreover, indoxyl sulfate promotes oxidative stress through the production of ROS in renal tubular cells and stimulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) contributing to progression of CKD via up-regulation of plasminogen activator inhibitor 1 (PAI-1) expression [126].

p-Cresyl likewise accumulates in CKD patients and is associated with an increased risk for the occurrence of CVD [127]. Among other effects, p-cresyl induces endothelial dysfunction through inhibition of endothelial proliferation and wound healing [128]. Furthermore, it causes oxidative stress through increased ROS production via multiple signaling pathways [129].

The uremic milieu in CKD can promote medial vascular calcification, which represents a hallmark of vascular ageing. In this regard, hyperphosphatemia, hypercalcemia, oxidative stress and uremic toxins contribute to the accumulation of hydroxyapatite in the vessel wall and thereby the development of vascular calcification [130]. Vascular calcification causes arterial stiffening and endothelial dysfunction [11]. The alterations of the vessel wall in CKD result in irregular flow patterns with mechanical stress on the endothelium, which enhances expression of adhesion molecules, cytokines and superoxide production by ECs [131–133]. *In vitro* stimulation of calcifying vascular cells derived from the artery wall with monocytes resulted in increased matrix mineralization through cell–cell interaction and through production of TNF [134]. Furthermore, a critical role of the NLRP3 inflammasome in vascular calcification is suggested [135, 136]. The aggregation of basic calcium phosphate crystals in CKD is able to activate monocytes [137] and the NLRP3 inflammasome [138]. Accordingly, administration of an anti-IL-1β antibody reduced aortic calcification in *Ldlr*-deficient mice fed with Western diet [139]. Different subtypes of monocytes and macrophages are able to promote or inhibit vascular calcification, which has been described elsewhere in detail [140].

Uric acid

Uric acid is the end-product of human purine metabolism produced by xanthine oxidase accumulating in the plasma of patients with CKD [141]. Several studies suggest that elevated uric acid levels are associated with an increased risk for progressive renal function deterioration [142, 143]. *In vivo* experiments demonstrate worsening of pre-existing CKD by hyperuricemia [144] and improvement of kidney disease by lowering uric acid [145]. However, clinical data to strongly advocate regular administration of uric acid–lowering therapy for delaying the progression of CKD in patients is limited [146]. By regulation of the AMPK–mTOR pathway, uric acid represents an activator of the NLRP3 inflammasome and promotes increased inflammation [147–149]. Lowering uric acid ameliorates the development of atherosclerotic plaques *in vivo* [147]. Another mechanism by which uric acid leads to activation of the NLRP3 inflammasome is the release of ROS. ROS cause the detachment of thioredoxin-interacting protein (TXNIP) from thioredoxin, which subsequently binds to NLRP3 and induces the assembly of the NLRP3 inflammasome [150]. Furthermore, ROS increase potassium efflux, which also results in the activation of the NLRP3 inflammasome [151].

Clonal hematopoiesis

Clonal hematopoiesis (CH) summarizes the accumulation of somatic mutations in specific genes of cells of the hematopoietic system. These mutations provide an advantage in self-renewal and/or proliferation, leading to clonal expansion [152]. The occurrence of somatic mutations that results in clonal hematopoiesis without causing cytopenia or dysplastic hematopoiesis is termed CH of indeterminate potential (CHIP) [153]. CHIP predisposes for hematological malignancies and is associated with a 12.9-fold higher risk of developing hematological disease [154].

CHIP mutations occur more frequently in higher age and are rare in individuals under 40 years of age [155]. Accordingly, approximately 5%–10% of subjects aged 65 years or older have these mutations [154]. The genes affected include *Dnmt3a*, *Tet2*, *Jak2*, *Asxl1*, *Sf3b1* and *Tp53* [156]. Several clinical and experimental studies have demonstrated the association between CHIP and a higher risk of all-cause mortality, CAD, myocardial infarction, heart failure and progression of atherosclerosis 155, [157–160].

Thereby, inflammation represents an important link between CHIP and CVD. NLRP3-mediated IL-1β secretion is increased in *Tet2*-deficient mice [160] and, vice versa, inhibition of NLRP3 reduces the effect of CHIP on atherosclerotic lesion formation [160]. In addition, Fidler *et al.* [161] described that the *Jak2* mutation V617F causes DNA stress in macrophages, leading to activation of the AIM2 inflammasome and IL-1β-dependent exacerbation of atherosclerosis.

A cohort study revealed an association between CHIP and an increased risk of deterioration of renal function in individuals without renal impairment [162]. Furthermore, CHIP was associated with a higher risk of progression to CKD and renal failure in a CKD cohort [163]. Finally, Dawoud *et al.* [164] presented an association between CHIP and worse outcomes in patients with CKD.

THERAPEUTIC APPROACHES

Establishing CKD-specific therapeutic approaches to prevent CVD represents a challenge, particularly since established therapies such as statins and the use of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors show limited efficacy in advanced CKD [165, 166]. CKD patients with atherosclerosis are at exceptionally high risk for the incidence of cardiovascular

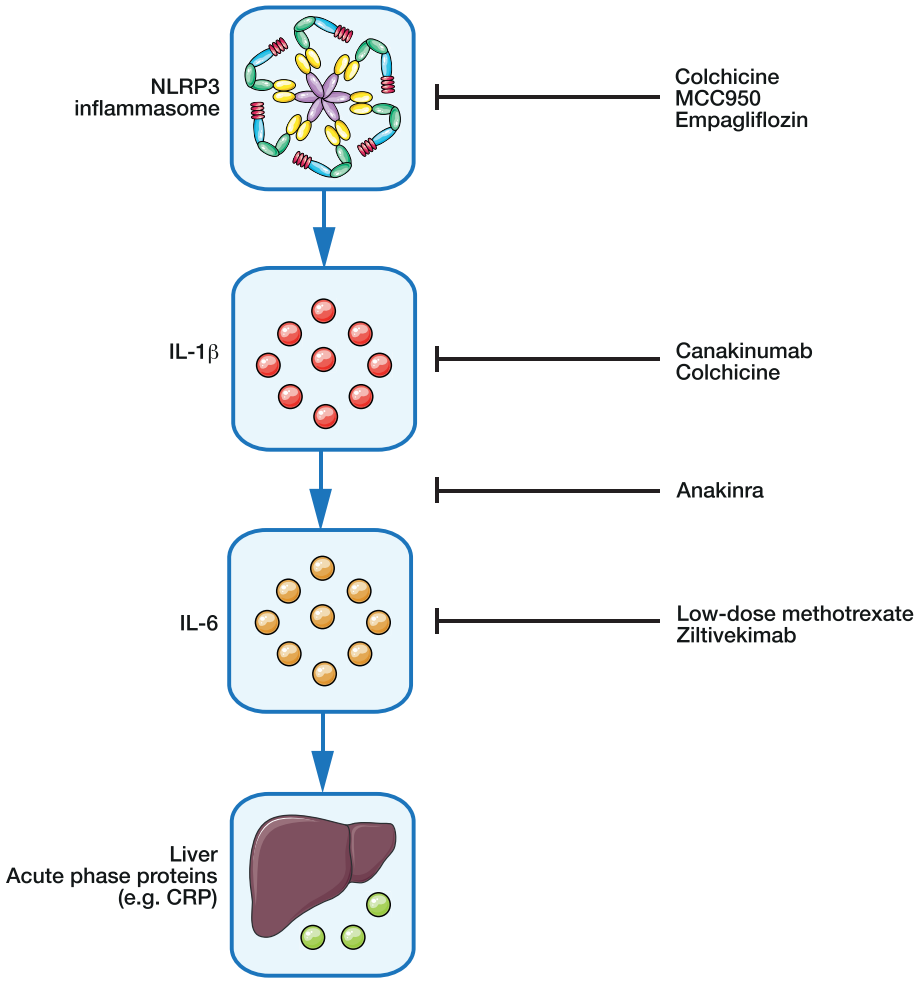

Figure 3: NLRP3–IL-1β–IL-6–CRP signaling pathway. NLRP3 activation leads to the maturation of IL-1β, which induces the release of other pro-inflammatory cytokines, such as IL-6. Notably, this pathway can be pharmacologically targeted by several agents. In the liver, IL-6 leads to the release of acute-phase proteins, e.g. CRP. ATP: adenosine triphosphate; BHB: β-hydroxylbutyrate.

events; in this regard, a crucial role is assigned to the innate immune system via the NLRP3–IL-1β–IL-6 axis [167]. Therefore, targeting inflammation may represent an additional therapeutic approach in patients with CKD and CVD (Fig. 3).

Canakinumab

The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial [168] was a randomized, double-blind trial that evaluated the efficacy of the monoclonal anti-IL-1β antibody canakinumab in the prevention of recurrent cardiovascular events. The trial included 10 061 patients with a history of myocardial infarction and residual inflammatory risk defined as hsCRP >2 mg/L. The vast majority of patients included received adequate secondary cardiovascular preventive treatment at the time of inclusion. The primary endpoint was the composite of nonfatal myocardial infarction, nonfatal stroke and cardiovascular death. Notably, canakinumab significantly reduced the risk for the primary endpoint as compared with placebo. This landmark study was the first in-men proof for the "inflammation hypothesis" in atherosclerosis.

A subgroup analysis of CANTOS demonstrates that CKD patients, particularly those with an adequate anti-inflammatory response, benefit from treatment with canakinumab. Canakinumab resulted in a reduced rate of MACE in patients with moderate to severe CKD [169]. Furthermore, another subgroup analysis indicates that residual inflammatory risk was associated with an increased incidence of MACE in CKD patients with an estimated glomerular filtration rate (eGFR) <60 mL/min. Elevated hsCRP and IL-6 served as markers for residual inflammatory risk despite adequate statin therapy. In comparison, LDL-C and non-HDL-C did not represent relevant predictors in this cohort [170].

The higher rate of (fatal) infections under canakinumab treatment represents a major limitation in targeting IL-1β [168]. New approaches address the specific targeting of injured heart tissue through the use of platelet microparticles (PM) designed to bind specifically to damaged tissue. This takes advantage of the natural infarct homing abilities of platelet membranes, which have been described in several previous publications [171, 172]. Anti-IL-1β-PM may offer the possibility of a targeted and intensified efficacy of IL-1β inhibition in the area of injury with a reduced incidence of side effects [173].

Colchicine

Colchicine, which is used as an anti-inflammatory medication in the treatment of gout and pericarditis, was tested for its

efficacy in cardiovascular prevention in several studies. Both the Colchicine Cardiovascular Outcomes Trial (COLCOT) trial [174] and the LoDoCo2 (Low-Cose Colchicine 2) trial [175] showed beneficial effects in CVD prevention after myocardial infarction or in patients with stable CAD, respectively.

Colchicine acts through several signaling pathways. It reduces the expression of adhesion molecules on inflamed ECs and neutrophils [176]. Furthermore, it decreases the expression of TNF receptors on macrophages and ECs [177], as well as the release of TNF by monocytes/macrophages [178]. In addition, colchicine decreases IL-1β and IL-18 by inhibiting the NLRP3 inflammasome [149].

When considering the data from previous studies on colchicine, it should be noted that patients with severe CKD were excluded from the previous studies. However, preanalytical data from LoDoCo2 suggest that colchicine may forfeit its cardiovascular-protective effects in patients with stage 3A or worse CKD [179]. In detail, a subgroup analysis of LoDoCo2 revealed a treatment benefit in subjects with an eGFR >60 mL/min [hazard ratio (HR) 0.67; 95% confidence interval (CI) 0.55–0.81], whereas no benefit was observed in the group of participants with an eGFR between 50 and 59 mL/min (HR 1.19; 95% CI 0.53–2.63) [175]. These data imply that the positive cardiovascular effects of colchicine decline with decreasing renal function. Nevertheless, a more precise statement requires further studies since only 5.5% of patients in LoDoCo2 had an eGFR <60 mL/min.

It should be mentioned that colchicine was associated with gastrointestinal side effects and infections in the described studies. Furthermore, colchicine was associated with an increased incidence of death from non-cardiovascular causes in the LoDoCo2 trial without reaching statistical significance [175]. A meta-analysis that combined the results of five trials that included 11 816 patients undergoing long-term colchicine treatment also showed a significantly increased incidence of non-cardiovascular deaths compared with placebo but without a change in all-cause mortality [180]. A sub-study of LoDoCo2 indicates that administration of low-dose colchicine is not associated with a change in renal function, but with a slight increase in alanine transaminase and creatine kinase [181].

Ziltivekimab

Based on the promising results in the inhibition of the NLRP3–IL-1β–IL-6 pathway, IL-6 represents another potential therapeutic target. The RESCUE trial, a randomized, double-blind, phase 2 trial included patients with stage 3–4 CKD and hsCRP >2 mg/L. Participants received the anti-IL-6 antibody ziltivekimab at various doses or placebo for up to 24 weeks. Administration of ziltivekimab resulted in a dose-dependent reduction in hsCRP after 12 weeks with a 92% reduction in the 30 mg dose group, compared with only 4% in the placebo group. The effects were maintained over the 24-week treatment period. In addition to the effects on hsCRP, a decrease of fibrinogen, serum amyloid A, haptoglobin, secretory phospholipase A2 and lipoprotein(a) was observed in the ziltivekimab group, while the total cholesterol to HDL-C ratio remained unchanged. Ziltivekimab was well-tolerated and no severe injection-related reactions were observed [182]. This phase-2 trial led to the design of the ongoing Ziltivekimab Cardiovascular Outcomes Study (ZEUS) trial. The goal is to enroll 6200 patients with stage 3–4 CKD with prevalent ASCVD and hsCRP >2 mg/L. Treatment duration is planned to be up to 4 years with monthly ziltivekimab or placebo administration. Primary endpoint is the occurrence of MACE. Moreover, the effect of ziltivekimab on kidney function will be evaluated [183].

Other anti-inflammatory agents

The nephro- and cardioprotective effects of sodium-glucose linked transporter 2 (SGLT2) inhibitors extend beyond their effect on glycemic control [184]. A potential anti-inflammatory effect of the SGLT2 inhibitor empaglifozin represents the maintenance and restoration of eGC [185]. Moreover, dapaglifozin ameliorates endothelial dysfunction in part by lowering oxidative stress [186]. A recent animal study indicates that empaglifozin improves renal function in diabetic mice through metabolic reprogramming and reducing oxidative stress [187]. Furthermore, SGLT2 inhibitors exhibit a dampening effect on the NLRP3 inflammasome [188].

Statins provide a wide range of effects in addition to their LDL-C-lowering capacity. They improve endothelial dysfunction by increasing NO bioavailability, anti-inflammatory effects and reduction of oxidative stress [189]. However, the pharmacological mechanisms underlying the effects of statins on the innate immune system are controversially discussed [190].

Dietary interventions and non-medical treatment

Besides pharmaceutical interventions, there is a growing interest in dietary and lifestyle interventions to reduce inflammation in CKD and CVD. In the EPPIC1 and EPPIC2 trials, patients with advanced CKD were treated with AST-120, which adsorbs uremic toxins in the gut. However, the therapy did not improve kidney outcomes [191].

Sustained weight reduction by dietary interventions was associated with lower hsCRP in the POUNDS LOST trial, independent of protein and carbohydrate proportions [192]. Moreover, increased intake of refined carbohydrates, sugary beverages and red/processed meats is associated with elevated levels of inflammatory biomarkers (e.g. hsCRP, IL-6, sICAM1, TNF), an unfavorable lipid profile and the occurrence of CVD. A Mediterranean diet supplemented with extra-virgin olive oil or nuts was associated with a reduced cardiovascular risk compared with a reduced-fat diet [193]. Consequently, dietary adjustments to reduce inflammation represent an interesting approach for future investigations [194].

Reduced physical activity resulting in decreased fitness and frailty are common in CKD patients and associated with increased risk of morbidity and mortality [195]. A small clinical trial showed a link between reduced inflammatory parameters (e.g. CRP, IL-6) and resistance training in patients with CKD [196]. Aerobic exercise training in particular seems to be associated with improved quality of life [197, 198]. Furthermore, a positive influence of physical activity on the gut microbiome is discussed, which in turn is associated with reduced inflammation, lower accumulation of uremic toxins and lower cardiovascular risk [199]. The influence of physical exercise on the gut microbiome with reduction of uremic toxins accumulation needs further investigation, as a recent smaller study could not demonstrate a significant decrease of uremic toxin levels in hemodialysis patients [200].

CONCLUSION

Endothelial dysfunction plays a critical role in the pathogenesis of CKD and CVD. After vascular injury, endothelial dysfunction is promoted by an interaction with the innate immune system and its immune effector cells. The processes involved are influenced by an altered metabolism in CKD. CKD is associated with an accumulation of various activators of the innate

immune system, which promote renal and cardiovascular injury alike. Thus, therapies targeting inflammatory signaling pathways represent an additional tool in the treatment of CKD and CVD. Recent studies demonstrate evidence of successful cardiovascular risk reduction through inhibition of the NLRP3–IL-1β–IL-6 signaling pathway. Subgroup analyses of these studies suggest promising results in cardiovascular risk reduction in patients with CKD. Further studies in CKD patients are needed to investigate the effects of anti-inflammatory therapies on CKD progression.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

No new data were generated or analysed in support of this research.

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