Molecular mechanisms of crystal-related kidney inflammation and injury. Implications for cholesterol embolism, crystalline nephropathies and kidney stone disease

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ABSTRACT

Crystals are particles of endogenous inorganic or organic composition that can trigger kidney injury when deposited or formed inside the kidney. While decades of research have focused on the molecular mechanisms of solute supersaturation and crystal formation, the pathomechanisms of crystal-induced renal inflammation remain largely unknown. The recent discovery of the intracellular NLRP3 inflammasome as a pattern recognition platform that translates crystal uptake into innate immune activation via secretion of IL-1β and IL-18 revised the pathogenesis of gout, silicosis, asbestosis, atherosclerosis and other crystal-related disorders. As a proof of concept, the NLRP3 inflammasome was now shown to trigger inflammation and acute kidney injury (AKI) in oxalate nephropathy. It seems likely that this and potentially other innate immunity mechanisms drive crystalline nephropathies (CNs) that are associated with crystals of calcium phosphate, uric acid, cysteine, adenine, certain drugs or contrast media, and potentially of myoglobin during rhabdomyolysis and of light chains in myeloma. Here, we discuss the proven and potential mechanisms of renal inflammation and kidney injury in crystal-related kidney disorders. In addition, we list topics for further research in that field. This perspective may also provide novel therapeutic options that can help to avoid progressive tissue remodeling and chronic kidney disease in patients with kidney stone disease or other CNs.

Keywords: cystinosis, gout, myeloma cast nephropathy, nephrolithiasis, rhabdomyolysis

INTRODUCTION

A number of acute and chronic kidney disorders are related to crystals deposition or formation inside the kidney referred to here as crystalline nephropathies (CNs). CNs are a heterogeneous group of kidney diseases ranging from vascular crystal embolism to supersaturation-driven crystallization within tubules and kidney stone formation inside the renal pelvis. The molecular mechanisms of crystal formation and crystal attachment to epithelial cell matrix molecules have been extensively studied during the last few decades [1, 2]. Less research efforts, however, have addressed the question of how intrarenal crystal formation actually leads to kidney injury. The traditional concept of crystal-induced kidney injury focusses on urinary tract obstruction. No doubt, bilateral obstructive urolithiasis can cause acute kidney injury (AKI), but tubular crystal plugs and casts rarely obstruct enough nephrons at the same time to explain AKI. In view of the evolving evidence that toxic and post-ischemic AKIs are largely driven by the associated inflammatory response raises the question whether inflammation is also the driving factor in crystal-induced AKI. Recent discoveries on the molecular mechanisms of crystal-induced inflammation now enforce a new view on this old disease and may answer questions that could not sufficiently be explained by the traditional concepts and that may launch novel treatment options for CN. Therefore, we discuss here the recently discovered molecular mechanisms of crystal-induced inflammation in view of the clinical and experimental knowledge on CN and propose areas of research to further develop this field.
causes ischemic kidney injury of cortical areas encompassing entire nephrons (Figure 1). Renal cholesterol embolism is associated with a local as well as with a systemic inflammatory response [3]. Cholesterol crystals are surrounded by macrophages and a mixed inflammatory cell infiltrate [4]. The inflammatory response is probably mostly induced by the ischemic injury rather than by immune recognition of cholesterol crystals. Later stages of the disease are associated with widespread interstitial fibrosis [4].

**Luminal crystal plugging and cast formation**

The most frequent mechanism of nephrolithiasis is solute supersaturation in the distal tubule, a process often triggered by transient dehydration [2, 5]. The distal tubule is the site of proton secretion and intratubular acidosis promotes the precipitation of certain solutes and drugs, while lack of acidification promotes the precipitation of others [2, 5, 6]. A lack of crystallization inhibitors promotes crystal plugging and tubular obstruction, while the secretion of desialylated Tamm–Horsfall protein in the thick ascending limb of the distal tubule supports obstruction by crystal cast formation, e.g. in myeloma cast nephropathy (Figure 1) [7, 8]. Free light chains and myoglobin also form crystal-like particles within the proximal tubule where they elicit toxic effects upon the uptake by proximal tubular cells, a process that can, e.g. cause light chain Fanconi syndrome [9]. Monosodium urate crystals form in collecting ducts and even tophi in the surrounding interstitium of the renal medulla where urine osmolarity reaches its maximum [10].

**Diffuse crystallization**

Cystine in humans and calcium oxalate in mice can also localize diffusely to the renal interstitium (Figure 1) [1, 11]. In addition, hydroxyapatite deposition in the interstitium at the thin loop of Henle, referred to as Randall’s plaque, can cause a loss of the urothelial lining of the renal pelvis [5].

**Stone formation in the renal pelvis**

Randall’s plaque is a site of crystal adhesion and growth of calcium oxalate stones in the calices of the renal pelvis (Figure 1) [1, 12]. This illustrates the role of an intact epithelium in preventing crystallization and, vice versa, implicates epithelial injury as a trigger for crystallization. Apatite plugs within the orifice of the ducts of Bellini are another site of pelvic stone growth consisting of brushite, urate, apatite or a mixture of these minerals (Figure 1). In contrast to many other stones do magnesium ammonium phosphate (struvit) stones grow in alkaline urine within the renal pelvis often driven by ammonium production from uropathogenic bacteria. Recurrent urinary tract infections also contribute to CKD progression in such cases [13, 14].

**MOLECULAR MECHANISMS OF CRYSTAL-INDUCED INFLAMMATION**

The molecular mechanisms of crystal-induced inflammation have only been discovered recently. Crystals act as a ‘danger signal’ that activates innate immunity via the NACHT, LRR and PYD domains-containing protein (NLRP3) inflammasome. For example, monosodium urate crystals induce gouty arthritis [15], calcium pyro-phosphate dihydrate crystals pseudo-gout [15], cholesterol crystals drive vessel wall inflammation in atherosclerosis [16], hydroxyapatite crystals induce arthropathy [17], calcium oxalate crystals trigger oxalate nephropathy [18], fibrillar peptide amyloid β contributes to Alzheimer’s disease [19], silica crystals and asbestos particles to pulmonary fibrosis [20], hemozoin particles to malaria [21] and uromodulin/Tamm–Horsfall protein particles to renal inflammation [22]. The NLRP3 inflammasome is the best-studied among all inflammasomes from the nucleotide-binding oligomerization domain-like receptors, which belong to the family of pattern recognition receptors [23]. This family also includes the Toll-like receptors (TLRs) and retinoic acid inducible gene-like helicases. These intracellular (cytoplasmic) receptors that sense the microbial as well as non-microbial danger signals lead to generation of active caspase-1, which process various cellular substrates including the cytokines IL-1β and IL-18, especially in macrophages and dendritic cells. However, the generation of active IL-1β and IL-18 depends on two distinct signals (Figure 2):

**Signal 1**

The extracellular foreign particles as well as the intracellular danger associated molecular patterns are recognized by TLRs. All TLRs have an extracellular sensing leucine-rich repeat domain and a highly conserved cytoplasmic Toll- and IL-1R domain, which mediates the intracellular signaling pathways [24]. Following the ligand binding, various adaptors engaged. The recruitment of these adaptors triggers a cascade of signaling molecules and eventually activates pro-inflammatory transcription factors including NF-κB. These induce the expression and secretion of various pro-inflammatory cytokines and chemokines, while certain factors remain within the cell as they need a second signal for being secreted, i.e. pro-IL-1β, pro-IL-18.

**Signal 2**

The NLRP3 inflammasome is a multiprotein complex composed of a sensor protein, the adaptor protein ASC and the inflammatory pro-caspase-1. Upon activation, NLRP3 changes its conformation and recruits ASC to assemble the NLRP3 inflammasome, which then activates caspase-1. Active caspase-1 subsequently cleaves the pro-IL-1β and pro-IL-18 causing their maturation to IL-1β and IL-18, which can be secreted [23].

Until now three distinct models of inflammasome activation have been studied in detail [25]: (i) lysosomal disintegration and release of its content by phagocytosed material; (ii) induction of reactive oxygen species production at mitochondrial membranes and (iii) potassium efflux by membrane channels or ionophoric compounds. Recent studies document that most crystals activate NLRP3 via all three mechanisms, while alum and amyloid-β involve lysosomal degradation and release of cathepsin B after phagocytosis only (Table 1).
**IL-1β/IL-1R1 and IL-18/IL-18R signaling**

The activity of secreted mature IL-1β and IL-18 is via the IL-1 receptor (IL-1R) 1 and IL-18 receptors, respectively, which are present on both immune and non-immune cells [26]. Either receptor belongs to the IL-1R family. Activation of TLR/IL-1R leads to the activation of MyD88 resulting in the recruitment of IL-1R-associated kinase (IRAK) 1 and IRAK4 to the receptor. Following IRAK1 phosphorylation by IRAK4, TRAF6 interacts with IRAK1 leading to dissociation and relocation of the IRAK1-IRAK4-TRAF6 complex to the plasma membrane.
membrane. This process subsequently leads to the phosphorylation and degradation of IκB with subsequent activation of NF-κB setting up further inflammation [26].

TUBULAR INJURY IN CN

Many forms of CNs display significant tubular injury, but how do crystals harm tubular cells? Currently, it is assumed that crystals harm tubular cells by either indirect mechanisms involving inflammation or directly, i.e. crystal-induced tubular cell death [27].

Indirect mechanisms

The inflammation established by inflammasome activation in renal mononuclear phagocytes and subsequent TLR/IL-1R signaling contributes to tubular injury (Figure 2). The activated NF-κB within both immune and non-immune cells increases the expression of pro-inflammatory cytokines and chemokines that results in infiltration of inflammatory cells (e.g. neutrophils, lymphocytes, macrophages, NK cells and Treg cells etc.) into the kidney interstitium during CN. Moreover, the secondary complications like tubular obstruction arose from the renal crystals deposits also add to tubular injury during CN.

Direct mechanisms

Crystals are known to be cytotoxic when they come in direct contact with the tubular epithelium. For example, Chen et al. have demonstrated that the CaOx monohydrate crystals (COM) adhere tightly to the MDCK cells with subsequent detrimental effects on the cells [28]. The internalization of COM crystals by MDCK cells results in alteration of

FIGURE 2: Mechanisms of crystal-induced renal inflammation. (i) Intraluminal crystal deposition (light chain casts, calcium oxalate, monosodium urate, calcium phosphate, myoglobin, drugs, cysteine and adenine) can lead to tubular obstruction and functional loss of single nephrons. Tubular obstruction alone should not cause AKI unless far >50% of tubules are completely obstructed. (ii) Crystal uptake and tubular cell injury can be triggered by crystals of around 1 μm size that can be endocytosed (light chains, calcium oxalate, monosodium urate, calcium phosphate, myoglobin, drugs and contrast media, cysteine, adenine, free light chains). Phagosomal destabilization leads to passive necrosis and potentially to programmed forms of necrosis. Necrotic tubular cells release DAMPs such as ATP, histones or HMGB1 that have the potential to activate pattern recognition receptors on other tubular cells. This indirect effect should account for crystal-induced cytokine and chemokine release by cultured tubular cells in vitro. A direct NLRP3-mediated activation of IL-1/IL-18 release by tubular cells remains speculative. (iii) Deposition or translocation of crystals into the interstitial compartment has mainly been reported for calcium oxalate, calcium phosphate, adenine, monosodium urate and cysteine. Here, crystals can be picked up by endothelial cells, fibroblasts and dendritic cells (DCs). Crystal endocytosis by these mononuclear phagocytes will activate the NLRP3 inflammasome to trigger IL-1β/IL-18 secretion and potentially pyroptotic cell death. ATP and histone release from dying tubular cells as well as Tamm–Horsfall protein leakage from the thick ascending limb of the distal tubule into the interstitium also activate the NLRP3 inflammasome in DCs and macrophages. Cytokine and chemokine release by all these cell types triggers the serial recruitment of neutrophils and pro-inflammatory macrophages that amplify the inflammatory tissue environment by producing additional cytokines and chemokines, which largely contributes to crystal-induced AKI. Persistent crystal deposition will raise additional effects as regenerative pathways join in and drive concomitant mesenchymal repair processes that foster interstitial fibrosis in addition to the persistent inflammation-driven epithelial atrophy.
several proteins involved in energy production which contribute to mitochondrial dysfunction and subsequent increase in the ROS production [29]. Therefore, it can be assumed that these ROS generation may activate NLRP3 inflammasome augmenting the renal inflammation. Moreover, it is known that the dying cells release several DAMPs which activate PPRs. Among them, DAMPs like ATP and histones (own unpublished data) can also activate NLRP3 in vitro. More specifically, it is known that the dying cells release several DAMPs which activate NLRP3 inflammasome augmenting the renal inflammation. Moreover, it is known that the dying cells release several DAMPs which activate PPRs. Among them, DAMPs like ATP and histones can also activate NLRP3 inflammasome. Also, we have shown that upon exposure, CaOx crystals directly kill tubular epithelial cells and release ATP which activates the NLRP3 inflammasome and contribute to tubular injury. Furthermore, inhibition of ATP protects the kidney from (Figure 2) tubular injury during acute oxalate nephropathy [18].

### INFLITRATING LEUKOCYTES AMPLIFY INFLAMMATION AND INJURY

Crystal-induced intrarenal chemokine expression initiates a serial recruitment of different leukocyte subsets, which is probably similar to that of other triggers of AKI. Leukocyte recruitment is triggered by chemokines, which mediate their chemotactic activity via chemokine receptors on the neutrophil surface [30]. Neutrophils dominate the early leukocytic cell infiltrate like in post-ischemic or toxic AKI [31]. The rapid recruitment of neutrophils seems to represent an unspecific danger response program that was positively selected throughout evolution for its benefits in host defense upon traumatic injury and pathogen entry [32]. In sterile injuries such as most forms of kidney disease, however, the bactericidal properties of activated neutrophils rather contribute to collateral immunopathology by the release of reactive oxygen species and other toxic mediators [33], which largely determines tubular injury and renal functional impairment during AKI [27, 34]. This, however, has not yet been formally demonstrated in animal models of CN.

The early neutrophil influx is followed by the recruitment of circulating monocytes that mature into macrophages upon tissue entry [27, 31]. Like in other forms of AKI, the pro-inflammatory tissue environment of crystal-related AKI should prime monocyte polarization into pro-inflammatory macrophages, that express Ly6C<sup>hi</sup>, iNOS and TNF but this needs to be confirmed for models of CN. The recruitment of pro-inflammatory macrophages involves chemokines such as CCL2, CCL5 and CXCL10, which mediate their chemotactic activity via chemokine receptors on the neutrophil surface such as CCR2, CCR5 and CXCR3, respectively [35]. The pattern of renal inflammatory cells described in human renal biopsy studies performed on patients experiencing CNs depends greatly upon the elapsed time between the initial exposure to the crystals and the time at which the biopsy was performed. In general, most reports document the presence of monocytes and macrophages with an occasional eosinophil near cast and crystalline plugs at the time of biopsy. Biopsies performed later in the disease process reveal chronic inflammatory changes like multinucleated giant cells surrounding interstitial sites of crystalline deposits and inside tubules surrounding cast formations. While numerous leukocytes are not commonly reported early in the stages of CNs, neutrophils

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### Table 1. Crystal- or particle-related nephropathies

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Crystal/particle</th>
<th>Deposition site</th>
<th>AKI or CKD?</th>
<th>Extent of inflammation in AKI/CKD</th>
<th>Extent of fibrosis in AKI/CKD</th>
<th>Crystal activating NLRP3 in vitro?</th>
<th>In vivo data for NLRP3 in kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol embolism</td>
<td>Cholesterol, Monosodium urate</td>
<td>Embolism, Luminal CD, medulla</td>
<td>AKI</td>
<td>+</td>
<td>N/A</td>
<td>[16]</td>
<td>–</td>
</tr>
<tr>
<td>Oxalate nephropathy</td>
<td>Calcium oxalate</td>
<td>Luminal PT, DT, diffuse</td>
<td>AKI/CKD</td>
<td>+</td>
<td>+</td>
<td>[18]</td>
<td>[18, 41]</td>
</tr>
<tr>
<td>Nephrocalcinosis/</td>
<td>Calcium phosphate, Hydroxyapatite</td>
<td>Luminal DT, interstitial (at papilla)</td>
<td>AKI/CKD</td>
<td>+</td>
<td>+</td>
<td>[15]</td>
<td>–</td>
</tr>
<tr>
<td>Adenine nephropathy</td>
<td>2,8-Dihydroxyadenine</td>
<td>Luminal DT diffuse</td>
<td>AKI/CKD</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>LC Fanconi syndrome</td>
<td>Light chains</td>
<td>Inside PT</td>
<td>AKI/CKD</td>
<td>+/-</td>
<td>+/-</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Myeloma cast nephropathy</td>
<td>Light chains, uromodulin</td>
<td>Luminal DT</td>
<td>AKI/CKD</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Rhabdomyolysis</td>
<td>Myoglobin, uromodulin</td>
<td>PT toxicity Luminal AKI DT</td>
<td>+</td>
<td>N/A</td>
<td>?</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Contrast nephropathy</td>
<td>Hyperosmolar contrast media</td>
<td>Luminal DT, intratubular</td>
<td>AKI</td>
<td>+</td>
<td>N/A</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Drug-induced kidney injury</td>
<td>Indinavir, acyclovir, sulfadiazine, ...</td>
<td>Luminal DT and CD</td>
<td>AKI, rarely +</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Cystinosis</td>
<td>Cystine</td>
<td>Interstitial, PT, podocyte</td>
<td>CD</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>–</td>
</tr>
<tr>
<td>Pelvic struvit stone</td>
<td>Magnesium ammonium phosphate</td>
<td>Renal pelvis</td>
<td>CKD/AKI</td>
<td>N/A with UTI</td>
<td>N/A</td>
<td>?</td>
<td>–</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury; CKD, chronic kidney disease; PT, proximal tubule; DT, distal tubule; CD, collecting duct; N/A, not applicable; LC, light chain; UTI, urinary tract infection.
have been reported to be common in renal tubules of patients with uric acid nephropathy [36].

Together, crystal-induced tissue injury serves as a trigger for intrarenal expression of pro-inflammatory cytokines and chemokines that trigger the serial recruitment of distinct leukocyte subsets into the kidney. Probably being a reminiscence of host defense, this mechanism (Figure 2) causes additional ‘collateral’ tissue injury that contributes to the clinical syndrome of crystal-induced AKI like in other sterile forms of AKI. Hence, this process is currently understood as an unspecific innate immunity response program that should not depend on the type of crystal involved.

RESOLUTION OF CRYSTAL-RELATED RENAL INFLAMMATION VERSUS PROGRESSIVE SCARRING

The recruitment of phagocytes holds the potential of crystal clearance from the interstitial compartment, while intratubular plugs rather re-dissolve or clear via urinary flow. Several studies have documented that intrarenal crystallization can be a transient phenomenon and that crystal deposits disappear at later time points. For example, Vervaet et al. reported calcium oxalate and calcium phosphate crystal translocation into the interstitial space of rat and human kidneys where infiltrating mononuclear cells contributed to crystal disintegration and clearance [37]. In fact, transcriptome analysis of transient oxalate nephropathy in mice supports a role of macrophages for crystal clearance [38, 39]. Immunostaining and transmission electron microscopy can spot interstitial macrophages that ingest crystals and form multinucleate giant cells around larger crystals [38, 40, 41]. Macrophages have the capacity to dissolve internalized calcium oxalate crystals, but the molecular mechanisms of crystal breakdown remain obscure [40]. A similar breakdown of calcium phosphate crystals or apatite would require an osteoclast-like macrophage phenotype which has not yet been reported in the kidney.

Crystal removal eliminates the trigger for kidney injury and is a prerequisite for the resolution of renal inflammation. Hence, transient crystallization and crystal clearance allow recovery from acute CN, but the molecular and cellular mechanisms have not yet been clarified. Until now, we can only speculate that this involves similar mechanisms of AKI recovery and repair like in other forms of transient AKI, e.g. a macrophage phenotype switch toward anti-inflammatory M2 macrophages [31, 42, 43], the secretion of anti-inflammatory cytokines and lipid mediators [44, 45], and epithelial recovery by tubular cell proliferation [46]. When de novo crystallization, plug or cast formation persist or the crystals cannot be removed (like calcium phosphate crystals from the interstitium), inflammation will persist and cause tubular atrophy [41]. In addition, the concomitant aberrant tissue repair manifests as interstitial fibrosis [41]. At the thin loops of Henle within the papilla, this process leads to Randall’s plaque formation, which can become a nidus for calyx stones [12]. The various combinations of these factors render patients with CN or kidney stone disease at higher risk for CKD and eventually end-stage renal disease [13]. In patients with struvite stones, recurrent urinary tract infections impose an additional risk for CKD progression [14]. Calculi in the ducts of Bellini, i.e. the confluent collecting ducts of the papilla, may obstruct the outflow of many nephrons as another potential mechanism of CKD progression [47].

Table 2. Upcoming questions about crystal-induced kidney injury and inflammation

| (i) Do the various types of crystals and particles share identical molecular mechanisms in inducing inflammation and does the NLRP3 inflammasome also mediate light chain-, myoglobin- or other CN-related particle-mediated renal inflammation? |
| (ii) Is crystal size an important aspect in CN-associated inflammation, e.g. because a certain crystal size is needed for phagocytic uptake? |
| (iii) What is the role of IL-18 in NLRP3-mediated CN? |
| (iv) Does crystal-induced NLRP3 activation cause pyroptotic cell death? |
| (v) Does the inflammatory response affect the physicochemical conditions that contribute to intrarenal crystal formation? |
| (vi) What factors regulate crystal clearance by intrarenal mononuclear phagocytes? |
| (vii) Which other molecular and cellular mechanisms contribute to the resolution of AKI in CN? |
| (viii) Do renal manifestations of CN differ due to the different site of crystallization and does CN-related renal inflammation largely relates to crystals that arrive in the interstitial compartment where they activate dendritic cells? |
| (ix) To what extent does crystal-induced inflammation contribute to the chronic kidney remodeling in CN? |
| (x) To what extent can the experimental data be validated in human CN? |

SUMMARY AND PERSPECTIVE

A group of pathophysiological diverse kidney disorders shares intrarenal crystal deposition as a common pathway to trigger kidney inflammation and injury [48]. The molecular mechanisms of crystal-induced inflammation involve crystal uptake into intracellular lysosomes and eventually lysosomal leakage, which activates the NLRP3 inflammasome independent from the type of crystal. NLRP3 activation triggers caspase-1-dependent secretion of IL-1β and IL-18 secretion, which induces a general inflammatory response including the recruitment of neutrophils and macrophages to the site of crystal formation. While these enhance local inflammation, macrophages may also contribute to crystal clearance or to progressive scarring, respectively. However, we have just started to understand the pathophysiology of crystal-induced injury and tissue remodeling. In the future, it will be essential to validate the mouse data in human CN. This requires careful immunohistochemical and transcriptome analyses of different CN entities to see if they share common immune pathways or if crystal-specific patterns exist. There are several other questions that deserve to be addressed (Table 2). The translational mission of research in this area, however, may also focus on those cytokine signals.
that drive the recruitment of cells that support crystal clearance as a potential therapeutic target to minimize crystal-related tissue remodeling. Moving beyond the traditional view of obstruction-related renal dysfunction in CN may offer the chance for novel discoveries and even therapeutic perspective of CNs.

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CONFLICT OF INTEREST STATEMENT

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

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