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MINIREVIEWS

Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage

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Abstract

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The inflammasome is a large, multiprotein complex that drives proinflammatory cytokine production in response to infection and tissue injury; the best-characterized inflammasome is the nod-like receptor protein-3 (NLRP3). Once activated, inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of interleukin-1beta. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes were recently reported, *via* the activation of the inflammasome that consists of NLRP3, apoptosis associated speck-like protein and caspase-1. Several manuscripts seem to identify NLRP3 inflammasome as a possible therapeutic target in the treatment of progressive chronic kidney disease. Serum amyloid A (SAA), as acute-phase protein with also proinflammatory properties, has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. SAA is a well recognised potent activator of the NLRP3. Here we will address our description on the involvement of the kidney in autoinflammatory diseases driven mainly by secondary, or reactive, AA amyloidosis with a particular attention on novel therapeutic approach which has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration.

Key words: Inflammation; Autoinflammatory disease; Chronic kidney disease; Interleukin-1; Dialysis; Caspase; Proteinuria; Amyloidosis; Nod-like receptor protein-3

Core tip: Inflammation may also negatively produce elevation of proinflammatory cytokines. Recently, attention was addressed to the formation of the intracellular inflammasome nod-like receptor protein-3 (NLRP-3) activating caspase-1, the enzyme required for the maturation of interleukin-1. IL1, in turn, regulate serum amyloid A, a major acute-phase with also proinflammatory properties. An interesting new scenario on the pathogenesis of renal diseases (namely ANCA-

associated glomerulonephritis vasculitis, urate-crystal nephropathy, contrast nephropathy, acute kidney injury, reactive systemic amyloidosis) and reactive systemic amyloidosis was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of chronic kidney disease.

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INTRODUCTION

Inflammation is a protective process, an attempt of the organism to respond to the harmful stimuli and at the same time to initiate the healing process for the tissue. Although the release of proinflammatory cytokines may have acute beneficial effects, chronic systemic elevation is likely to produce detrimental effects[1].

Inflammation is central to the pathogenesis of many renal diseases: The innate immune system, a first line defense against pathogens, is usually involved in the initiation and propagation of inflammation and moreover, chronic inflammation may contribute to progression of acute or chronic kidney disease (CKD).

NLRP3 mediated inflammation

Recently, several authors[2,3] seem to indicate additional mechanisms that may orchestrate renal inflammatory and fibrotic processes by the formation and activation of the intracellular inflammasome that consists of nod-like receptor protein-3 (NLRP-3), apoptosis associated speck-like protein (ASC) and caspase-1.

In the last few years several authors[2-9] underlined the importance of the NLRP3 inflammasome activation, the currently most fully characterized inflammasome, as an important player in renal injury. An interesting new scenario on the pathogenesis of renal diseases beyond the acquired knowledge in the rheumatologic field was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of progressive CKD.

Inflammasome: The inflammasome is a large, multi-protein complex that drives proinflammatory cytokine production in response to infection and tissue injury.

The best-characterized inflammasome is the NLRP3 inflammasome. On assembly of the NLRP3 inflammasome, post-translational processing and secretion of pro-inflammatory cytokines IL-1β and IL-18 occurs; in addition, cell death may be mediated *via* caspase-1[10].

Interleukin-1 (IL-1), previously known as endogenous pyrogen, osteoclast activating factor, catabolin, hemopoietin-1, lymphocyte activating factor, or epidermal-derived thymocyte activating factor, is produced as an inactive precursor form upon cell activation. Its release requires the activation of different molecules gathered under the name of "inflammasome".

The activation of inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of IL-1. The release of IL-1 requires the activation of the cell by ATP through its P2X7 receptor that involvement of K^+ and Ca^+ channels and the action of a phosphatidylcholine-specific phospholipase. Necrotic cells produced by pressure disruption, but also hypoxic injury, uric acid crystals, bacterial toxins[11] or complement-mediated damage were capable of activating the NLRP3 inflammasome, triggered in part through ATP produced by mitochondria released by damaged cells (Table 1).

Some authors[12] indicate that the activation of the NLRP3 inflammasome requires two separate signals (Figure 1). The first signal, which can derive from Toll-like receptors, Tumor Necrosis Factor Receptors or IL-1R signaling, needs to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) for the transcription and translation of the immature pro-forms of IL-1β and IL-18. As a second step, enzymatic cleavage is needed to secrete these pro-inflammatory ILs into the extracellular space.

The non-immune renal parenchymal cells do not seem to release IL-1β, as they do not express pro-IL-1β upon NF-κB activation[8], however, several reports document the expression and release of IL-18 from tubular epithelial cells (TECs)[13-15]. This would seem to indicate that the NLRP3 inflammasome and caspase-1 axis may also be in renal non-immune cells. Moreover, Zhang *et al*[16] using a confocal microscopy, documented NLRP3 and ASC to be expressed by glomerular podocytes.

Intrinsic renal cells express components of the inflammasome pathway: This is mostly prominent in TECs and, to a lower degree, in glomeruli. Several primary renal diseases and systemic diseases affecting the kidneys are associated with NLRP3 inflammasome/IL-1β /IL-18 axis activation. Most of the disorders studied have been acute inflammatory diseases: The disease spectrum includes ureteric obstruction, ischaemia reperfusion injury, glomerulonephritis, sepsis, hypoxia, glycerol-induced renal failure, and crystal nephropathy[17].

The German group from Munich recently described[7] the role of the NLRP3 inflammasome in oxalate nephropathy and found that calcium oxalate crystals kill TECs, which leads to the release of ATP and potentially other NLRP3-agonistic DAMPs that trigger IL-1β secretion by renal dendritic cells.

In addition, renal dendritic cells ingest oxalate crystals by phagocytosis and subsequent lysosomal leakage activates NLRP3. Acute oxalate nephropathy was significantly attenuated in NLRP3-, ASC- and caspase-1-deficient mice. Finally, acute oxalate nephropathy had been shown to be prevented by therapeutic IL-1 blockade with anakinra, a IL-1 receptor antagonist approved by the United States Food and Drug Administration for the treatment of rheumatoid arthritis. The results of this study suggest a potentially similar pathogenic role

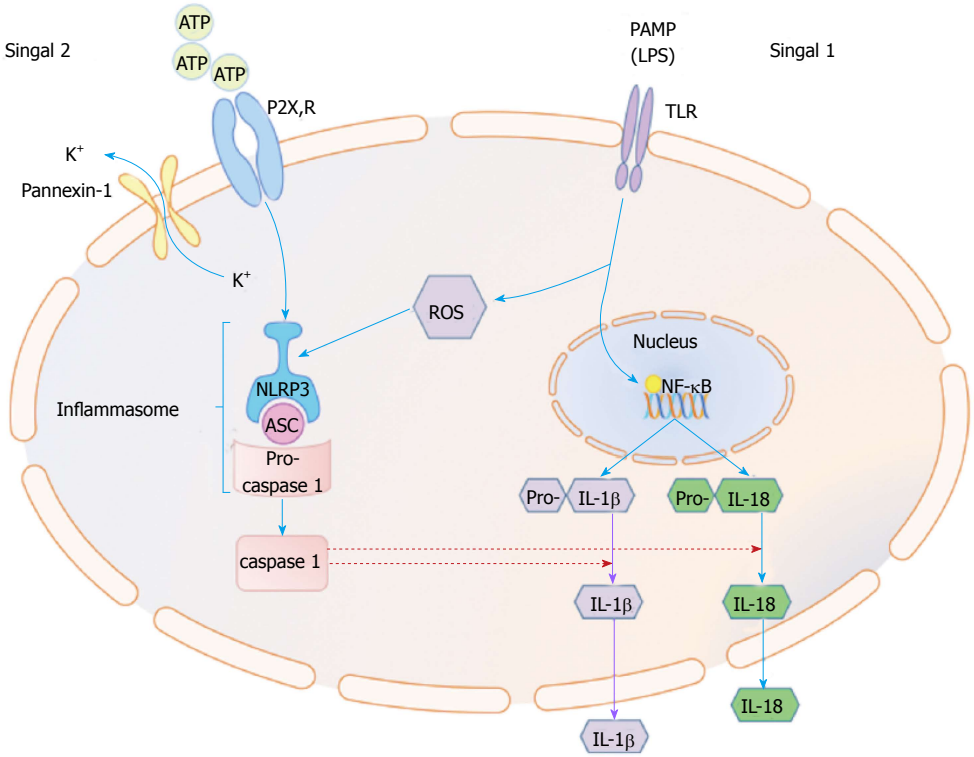

Figure 1 Model of nod-like receptor protein-3 inflammasome activation and the role of the nod-like receptor protein-3 inflammasome in the two-step activation of interleukin-1β and interleukin-18[10,12]. Activation of the NLPR3 inflammasome requires two signals. Signal 1: Activation of TLRs, IL-1Rs and TNFRs induces the transcription and translation of NF-κB to produce pro-forms of IL-1β and IL-18; Signal 2: Enzymatic cleavage by (caspase-11-driven) caspase-1 to secrete mature cytokines, IL-1β and IL-18. ROS: Reactive oxygen species; TLR: Toll-like receptor.

of the NLRP3 inflammasome in other crystal-related nephropathies such as cast nephropathy, contrast nephropathy, acute kidney injury (AKI) in rhabdomyolysis or urate nephropathy[17].

The role of the NLRP3 inflammasome arthritis in urate crystal-induced is well described[18] and the block of IL-1 may be considered a good therapeutic option in patients with gouty arthritis and renal failure[19].

Moreover, other authors reported that the upcoming data on the NLRP3 inflammasome support the evolving danger signaling concept of renal inflammation[20]. More recently Schreiber *et al*[21] described their experience in antineutrophil cytoplasmic antibodies (ANCA)-activated phagocytes that cause vasculitis and necrotizing crescentic glomerulonephritis (NCGN). The authors supposed that ANCA-induced phagocyte NADPH oxidase generated tissue-damaging reactive oxygen species that restrains inflammation, downregulated caspase-1, thereby keeping the inflammasome in check, reducing IL-1 generation and limiting ANCA-induced inflammation. The authors concluded that IL-1 receptor blockade by anakinra might provide a promising strategy in NCGN. More than 25 years ago, even in patients on hemodialysis, it was shown that the involvement of monocyte activation brings to the release of IL-1 and related cytokines, as already reported in 1988 by Dinarello[22].

Mulay *et al*[7] experimentally showed in mice that renal CaOx crystal deposition was associated with diffuse neutrophil infiltrates and tubular necrosis mainly at the inner stripe of the outer medulla, as demonstrated by the disintegration of TECs and granular casts in tubular lumen. The structural alterations of oxalate nephropathy were associated with renal failure[4]. Clodronate liposome was used in WT mice or diphtheria toxin in CD11c DTRg mice to demonstrate that CaOx-induced intrarenal IL-1 secretion originated from the intrarenal network of interstitial mononuclear phagocytes.

On hypothesizing that therapeutic blockade of IL-1 might be able to interfere with this pathomechanism and protect against renal failure, the authors[2] used anakinra: Intraperitoneal injection of anakinra dose-dependently reduced tubular injury and neutrophil recruitment and improved renal excretory function during oxalate nephropathy in mice. The authors concluded that IL-1 mediated inflammation and tissue damage in kidney injury induced by CaOx crystals and thus IL-1 blockade protected from renal failure in oxalate nephropathy in mice.

Both experimental and human studies show a detrimental role for NLRP3 in the development of acute and chronic tubule-interstitial disease[6]. To confirm this Duewell *et al*[23], using a novel microscopic technique, a combination of laser reflection and fluorescence confocal microscopy to identify in mice crystalline materials and immune cells, recently reported that minute cholesterol crystals were present in early diet-induced atherosclerotic lesions and that their appearance coincided with

Table 1 Pathogen associated molecular pattern and damage associated molecular pattern that trigger nod-like receptor protein-3 activation[12]

Type	Molecule/molecular pattern
PAMP	Leptospiral interrogans/glycolipoprotein
	Influenza
	Streptococcus pyogenes/streptolysin O
	Staphylococcus aureus/alpha hemolysin
DAMP	ATP
	Nigericin
	Histones
	U1snRNP ribonucleoprotein
	dsDNA/nucleosomes
	MSU crystals
	Uromodulin
	Biglycan
	Silica
	Alum
	Calcium oxalate
	Asbestos
	Amyloid-β
	Hemazoin
	Hyaluronan

PAMP: Pathogen associated molecular pattern; DAMP: Damage associated molecular pattern; ROS: Reactive oxygen species/oxidative stress; PAMP: Pathogen-associated molecular pattern; DAMP: Damage-associated molecular pattern; ATP: Adenosine tri phosphate; MSU: Mono sodium urate.

the first appearance of inflammatory cells.

To test whether cholesterol crystals could activate the release of IL-1β, the authors incubated lipopolysaccharides-primed human peripheral blood mononuclear cells with cholesterol crystals: Cholesterol crystals induced a robust, dose-responsive release of cleaved IL-1β in a caspase-1 dependent manner. The authors also demonstrated that cholesterol crystals also activated the NLRP3 inflammasome in phagocytes *in vitro* in a process that involved phago-lysosomal damage. Crystalline cholesterol acts as an endogenous danger signal and its deposition in arteries or elsewhere was an early cause rather than a late consequence of inflammation.

Most importantly, mice whose bone marrow-derived cells lacked NLRP3 inflammasome components, or IL-1 cytokines, were markedly resistant to developing atherosclerosis. The lesional area in the aorta of these mice was reduced on average by 69%, compared to chimeric LDLR-deficient mice that had wild-type bone marrow.

Amyloidosis: Amyloidosis is a disorder of protein folding in which normally whole or fragments of normally soluble proteins are deposited as abnormal, insoluble fibrils that disrupt tissue structure, so causing disease. In systemic amyloidosis the deposits may be present in the parenchyma of the viscera and tissues, causing progressive organ dysfunction leading patients to death. Systemic amyloidosis, fatal within 6 mo of diagnosis in up to 20% of patients, causes about one per thousand deaths in developed countries and remains an important unmet medical need. There are about 30 different types of amyloid in humans, characterized by the particular specific protein that forms the fibrils[24] (Figure 2).

Table 2 Over 30 proteins capable of amyloid formation have been identified

Immunoglobulin light chains in primary systemic amyloidosis
Ig heavy chain
Beta2-microglobulin in dialysis-associated arthropathy
Amyloid beta protein in alzheimer disease and down syndrome
Hereditary forms (including transthyretin, apolipoprotein A-Ⅰ and A-Ⅱ, gelsolin, lysozyme, fibrinogen a-alpha chain
Amyloid a in secondary amyloidosis

The core structure of all amyloid fibrils consists of antiparallel β-pleated sheets arranged with their long axes perpendicular to the long axis of the fibril. This structure specifically binds the histochemical dye, Congo-red, from alkaline alcoholic solutions, in an ordered molecular array which gives pathognomonic red-green birefringence when viewed in strong cross-polarized light. This is the gold standard for histological diagnosis of amyloid[24] (Table 2).

There are therefore both acquired and hereditary forms of amyloidosis. The most common form of systemic amyloidosis is the AL type. The international nomenclature comprises A for amyloidosis and the second and other letters identify the amyloid fibril protein, in this case L for monoclonal immunoglobulin light chains[24].

AL amyloidosis, formerly known as primary amyloidosis, is thus a complication of monoclonal gammopathy of any type ranging from myeloma through monoclonal gammopathy of uncertain significance, to the whole variety of B/plasma cell dyscrasias. It accounts for about 60% of all cases.

AA amyloidosis, formerly known as secondary or reactive systemic amyloidosis, is a complication of chronic inflammatory and infective diseases in which there is a sustained acute-phase response with overproduction of serum amyloid A (SAA) protein, a very sensitive and dynamic major acute-phase protein. Although becoming rare in the developed world due to greatly improved treatments for inflammatory arthritides, Crohn's disease, chronic infection, *etc.* AA amyloidosis is still a fairly common disease in medicine department and nephrologist's counseling may be required for the detection of proteinuria or renal failure[25].

In the past ten years, thanks to more aggressive treatment schedules and to the increasing availability of anti-TNF treatments, some authors[26,27] report that the incidence of AA amyloidosis in chronic arthritides has slowly decreased.

This has led to a relative increase in the rate of other conditions that are well-recognized to significantly associate with AA, such as Crohn's disease, hereditary periodic fevers, malignancies, systemic vasculitides and diseases predisposing to recurrent infections, including

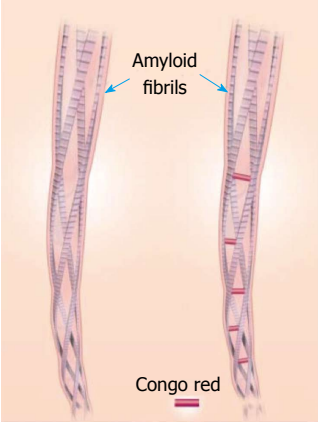

Figure 2 Structural features of amyloid[25].

cystic fibrosis, bronchiectasis, epidermolysis bullosa, cyclic neutropenia, acquired or inherited immunodeficiencies, injection-drug use and acne conglobata.

SAA is a major acute-phase protein present in serum but also shown to possess proinflammatory properties, as meaning that it can induce the release of cytokines from different cell types, including THP-1 monocytes, human neutrophils, and mast cells.

SAA is mainly produced in the liver under the regulation of IL-1, IL-6, and TNF-, but its expression has also been demonstrated in other cell types, including macrophages, endothelial cells, and smooth muscle cells.

Moreover, SAA has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. As processing of SAA by cathepsin B may result in production of amyloidogenic SAA fragments: Experimentally, SAA has demonstrated to induce a strong expression of IL1β and TNFα in human macrophages[28].

SAA mediates its effect through activation of NLRP3 inflammasome: SAA is a potent activator of the NLRP3 inflammasome *via* a cathepsin B- and P2X7-dependent manner and is the first physiological proinflammatory mediator that can provide signals needed for expression of pro-IL-1 (as shown in Figure 1) and activation of the inflammasome cascade, resulting in activation of caspase-1 and secretion of mature IL-1β so resulting in formation of amyloidogenic fragments[28]. The conversion of the circulating soluble protein SAA into stable, highly ordered, amyloid fibrils that accumulate extracellularly causing organ damage is a multi-step process.

As an acute phase reactant secreted by the liver under the transcriptional control of IL-1 and IL-6, SAA increases up to 1000 fold following an inflammatory stimulation. If such stimuli persist, as occurs in several chronic diseases, SAA concentration may reach a critical threshold over which it becomes prone to aggregation. Moreover, the estimated ten years' survival was reported to be much higher in the patients with lower SAA levels, below 10 mg/dL[29].

A β-2m amyloidosis, so-called DIALYSIS-RELATED AMYLOIDOSIS, is a serious complication of long-term dialysis for end-stage renal failure in which β2-microglobulin, normally catabolized by the kidneys, is not adequately cleared and accumulates in the plasma, rising in concentration from its normal value of 1-2 mg/L to up to 70 mg/L[30].

All amyloid deposits have the feature to be largely ignored by the usually very efficient physiological mechanisms by which abnormal protein debris is cleared from the interstitial space in the tissues. Dead cells, effete matrix and structural proteins, blood cells and plasma proteins extravasated in injury, are normally rapidly cleared with no local or systemic clinical consequences. In contrast, although macrophages and giant cells are occasionally seen, especially around local rather than deposited as amyloid fibrils in and around bones and joints, causing pain, bone cysts and pathological fractures.

Here we will address our description on the involvement of the kidney in autoinflammatory disease driven mainly by secondary, or reactive, AA amyloidosis.

AA AMYLOIDOSIS

A clear example of renal involvement in autoinflammatory disease with amyloid A deposition may be considered the Muckle-Wells (MWS) disease associated with AA-amyloidosis. MWS is inherited as an autosomal dominant condition, meaning each child of a sufferer has a 50% chance of developing the syndrome.

MWS is a rare genetic autoinflammatory syndrome and the intermediate-severity form of cryopyrin-associated periodic syndrome (CAPS). As with other forms of this syndrome, it presents with recurrent episodes of fever, skin rash, joint pain, abdominal pain and conjunctivitis, but in addition sufferers typically develop a progressive sensorineural deafness and amyloidosis[9].

The protein affected in MWS is cryopyrin, produced by the NLRP3 gene located on chromosome 1. The gene is expressed in white blood cells (mainly neutrophils) and chondrocytes (cartilage cells). Cryopyrin is an essential component of the inflammasome, an intracellular protein complex involved in the innate immune system. The abnormal inflammasome in MWS allows unrestricted activation of the enzyme caspase-1, which in turn causes overproduction of active IL-1, switching on the inflammatory cascade in an uncontrolled manner[31].

MWS can have severe consequences due to chronic high levels of inflammation in the body. This can be life-threatening if generalized amyloidosis of the AA type develops, due to long-term buildup of amyloid protein products from the chronic inflammation in MWS. Organ damage results from the extracellular deposition of proteolytic fragments of the acute-phase reactant SAA as amyloid fibrils[9]. A sustained high concentration of SAA is the prerequisite for developing AA amyloidosis.

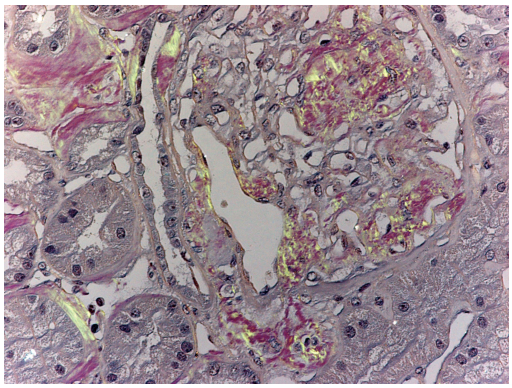

Figure 3 Renal biopsy: Amyloid fibrils bind congo red stain, yielding the pathognomonic apple-green birefringence under cross-polarized light microscopy[9].

The kidneys, liver and spleen are the main target organs of AA amyloid deposits (Figure 3).

In more than 90% of the patients proteinuria, nephrotic syndrome and/or renal dysfunction dominate the clinical picture at onset[9,24,26,27]. If not effectively treated, this disease invariably leads to end stage kidney disease[9] and renal replacement therapy, that are still associated with a poor outcome[27].

Over 25% of MWS patients have elevated serum amyloid, and at least 25% have amyloidosis. Serum AA testing is essential to follow, along with C-Reactive Protein (C-RP), Erythrocyte Sedimentation Rate (ESR) and other laboratory tests. Amyloidosis is also a risk to some patients affected by different types of CAPS, a group of autoinflammatory disorders characterized by recurrent episodes of systemic inflammation marked by fever, tissue inflammation, particularly of the joints and skin, and other constitutional symptoms, clinically defined by a spectrum of varying severity. Amyloidosis may be associated in familial cold autoinflammatory syndrome and in neonatal-onset multisystem inflammatory disease/chronic infantile neurological cutaneous and articular syndrome, but not so much as in MWS. Generalized amyloidosis is due to a permanent buildup of amyloid in the kidneys, liver and elsewhere, that can be fatal[32].

Clinical AA amyloidosis is typically preceded by many years of active inflammation before presenting, most commonly with renal involvement[33].

In AA amyloidosis renal dysfunction is reported to be the predominant disease manifestation. Mortality, amyloid burden, and renal prognosis all significantly correlated with the SAA concentration during follow-up. The risk of death was reported to be 17.7 times as high among patients with highest SAA concentrations. In the previously reported[25] largest study on AA amyloidosis involving 374 patients, the most frequent underlying disorder was inflammatory arthritis and only rare causes of AA amyloidosis included vasculitis, sickle cell anemia, malignant disease, epidermolysis bullosa, and cyclic neutropenia. Renal involvement was reported to be frequent: In 97% of patients, more than 500 mg of proteinuria per day were present or the serum creatinine concentration was more than 1.5 mg/dL. The relative risk of progression to end-stage renal failure was also increased among patients whose renal function was relatively worse at baseline, with an increase by a factor of 5 for each doubling of the baseline serum creatinine concentration ($P < 0.001$).

Fortunately, the cardiac involvement is not so frequent in AA amyloidosis and it is reported to be present in only 1 patient, and findings consistent with cardiac infiltration were present in only 2 among 224 patients who underwent echocardiography[34].

A worse renal outcome in patients with chronic sepsis or Crohn's disease was reported[28], possibly related to the high frequency of surgical intervention and administration of immunosuppressive drugs, probably due to greater severity of disease associated or not at increased risk of infection.

Therapy

Further studies need to elucidate whether persistent inflammation serves as a catalyst by sensing and converting the endothelium into a proinflammatory surface that makes the vasculature more vulnerable to the effects of other circulating risk factors. Such a scenario is supported by the strong documented association between inflammatory markers and endothelial dysfunction in patients with CKD.

Similarly, effective anti-inflammatory treatment, or whatever is needed to control the acute-phase response and maintain circulating SAA serum levels in the normal range, is life saving in AA amyloidosis[9,25,27]. Rigorous compliance with colchicine therapy for Familiar Mediterranean Fever (FMF) prevents and ameliorates AA amyloidosis even in patients who do not experience complete relief of symptoms. The key is to control SAA production, closely monitoring SAA serum levels in all patients with AA amyloidosis, and tailoring their treatment to keep these as low as possible. However, many patients are already in severe or end-stage organ failure when diagnosed with amyloidosis and new approaches are desperately needed to save them.

Treatment of AA amyloidosis has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration as much as possible. If not effectively treated, this disease invariably leads to end stage kidney disease and renal replacement therapy, that are still associated with high mortality rate[25].

In a unpublished experience we observed in a female patient aged 40, affected by Chron disease with nephrotic proteinuria of 14 g/daily and CKD stage 3 secondary to renal AA amyloidosis histologically proven, the control of the baseline chronic bowel inflammatory disease with the monoclonal antibody adalimumab, a TNF-alfa inhibitor, significantly reduced the proteinuria levels up to 6 g/daily, while still remaining in the nephrotic range. The TNF-inhibitor therapy also reduced SAA levels from more than 4 mg/dL (normal values <

0.5) up to quite normal values (0.61 mg/dL).

Among AA amyloidosis therapy, some years ago interest was pointed on eprodisate, structurally similar to heparin sulfate, a glycosaminoglycan that is known to promote fibril assembly, inducing amyloid formation. Eprodisate, negatively charged, sulfonated molecule that is structurally similar to heparin sulfate and works by competitively inhibiting the interaction between SAA and glycosaminoglycans.

A RCT was conducted[35] enrolling 180 patients with AA amyloidosis-associated nephropathy; patients were treated with eprodisate or placebo for 24 mo: The authors reported that the treatment was associated with a 42% reduction in the risk of worsening renal disease (as measured by creatinine clearance) or death (CI: 0.37-0.93; P = 0.02), compared with placebo. Surprisingly, there was no significant difference in terms of the overall changes in proteinuria: A second phase Ⅲ trial is now ongoing.

Higher levels of aspecific laboratory inflammatory markers such as C-RP and sTNF are independently associated with faster rates of kidney function loss in CKD[36]. Pravastatin, a HMG-CoA reductase inhibitor, was reported to prevent loss of kidney function to a greater extent in CKD individuals with coronary artery disease (CAD) with greater evidence of inflammation, although this was of borderline significance. These data suggest that inflammation may mediate the loss of kidney function among subjects with CKD and concomitant CAD[37].

Some years ago, other authors experimentally found that inhibition of the isoprenoid pathway by another statin, lovastatin, resulted in a dose-dependent reduction of amyloid formed in mouse recombinant SAA produced in Escherichia coli, hypothesizing the isoprenoid metabolism as a potential target for prevention and treatment of AA amyloidosis[38].

More recently, Luo *et al*[39] studied the effects of another statin, rosuvastatin (RSV), and observed that, compared with controls, diabetic Sprague-Dawley rats showed severe metabolic disorder, cardiac dysfunction, fibrosis, disorganized ultrastructure, and excessive activation NLRP3 inflammasome, ASC, IL-1β and mitogen-activated protein kinases. The NLRP3 inflammasome was found activated in response to high levels of glucose. RSV was added and continued for 8 wk. The effect and underlying mechanisms of action of RSV in diabetic cardiomyopathy (DCM) and whether NLRP3 was a target for RSV in DCM, was studied. The authors concluded that, compared with diabetics rats alone, RSV experimentally ameliorated the overexpression of NLRP3 inflammasome and silencing NLRP3, ameliorated cardiac remodeling and dysfunction, so identifying RSV as a significant potential therapy *via* inhibition of NLRP3 inflammasome.

Due to the strong association between proinflammatory cytokines and complications common in ESRD, such as vascular calcification and wasting, the potential role of both general and targeted anticytokine treatment strategies in ESRD patients needs further evaluation[40]. Inflammation has to be considered an important target for pathogenetic interventions both in AKI and in progression of CKD, as recently suggested[41].

Therapeutic interventions that suppress inflammation and oxidative stress may address both short-term (dynamic) and long-term (structural) contributors to a decline in the GFR in patients with CKD and could possibly stabilize or even improve kidney function[9,42].

However, despite major technologic improvements in dialysis techniques, a lot of haemodialysis and peritoneal dialysis patients show serological evidence of an activated inflammatory response, as clearly indicated by increased circulating levels of non-specific markers of inflammation and proinflammatory cytokines such as IL-6.

Dialysis treatment save the lives of patients with ESRD but it does not cure the burden of clinical consequences related to uremic state, *i.e.,* the marked risk for atherosclerotic cardiovascular disease and inflammation. Renal transplantation (TPX) can be considered in selected patients progressing to ESRD, but unfortunately, it is a choice not offered to all ESRD patients due to the low number of transplants performed in some countries.

Novel treatments to control inflammation processes, and also to prevent progression of renal damage, are under development and anti-cytokine agents are becoming the mainstay of therapy to prevent and treat AA, including patients with FMF that do not respond or do not tolerate adequate colchicine dosages and targeting key molecular events in the fibrillogenesis process[43]; also the role of other drugs are in progress[44].

Unfortunately, control of fibril-protein production is not possible in some forms of amyloidosis and in others it is often slow. There is no therapy that directly targets amyloid deposits for enhanced clearance. However, all amyloid deposits contain the normal, non-fibrillar plasma glycoprotein, serum amyloid P component (SAP).

Other authors[45] showed that administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, macrophage-derived giant cell reaction that swiftly removes massive visceral amyloid deposits without adverse effects. Interestingly, the authors found that a combination of a drug that depletes circulating SAP and an antibody that targets residual SAP within the deposits results in clearance of amyloid deposits. A humanized version of the anti-SAP antibody has been developed with a view to clinical evaluation of this dual approach, hypothesizing this combined therapy to eliminate amyloid deposits.

IL-1 blockade

Clinical observations to date suggest that although IL-1 plays a key role in activation of the innate immune system, blockade of this cytokine appears to have few adverse effects. Anti-IL-1 therapy appears to

increase the risk of infection only marginally, and there is no clear evidence for increased risk of malignancy, despite lymphoma and other types of cancer have been reported in children treated with TNF blockers, often when along with certain other drugs (such as azathioprine or 6-mercaptopurine). Safety block of IL-1 after 12 mo after renal TPX was reported[46] also in a renal transplanted patients affected by MWS with systemic amyloidosis treated with triple immunosuppressive drug regimen and at the same time canakinumab: No flares of MWS was observed during this period.

In our experience we did not observe increased hospitalization rate due to infections or malignancy in two patients affected by Muckle Wells syndrome treated with IL-1 blockers who had been followed for over three years[9].

CONCLUSION

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The best-characterized inflammasome is the NLRP3 that, once activated, leads to the active form of caspase-1, the enzyme required for the maturation of IL-1β. SAA, as acute-phase protein with also proinflammatory properties, is a well recognized potent activator of the NLRP3. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes, resulting in kidney insufficiency were recently reported, *via* the activation of the inflammasome.

Currently, treatment options in amyloidosis rely on reducing the supply of the precursor protein and thus depend absolutely upon accurate typing of the amyloid. Intercalating agents able to induce physical disruption of the fibrillar structure of the native fibrils, once mature fibrils have been deposited, are under study in some types of non-AA amyloidosis, hence producing an intermediate: It so resulting to be more readily available for enzymatic degradation.

The administration of anti-human SAP antibodies[47] to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, reaction that swiftly removes massive visceral amyloid deposits without adverse effects. These promising results achieved in mouse models based on intermediary metabolism may not be extended to humans, so specifical trials are needed to test this hypothesis also in humans.

The role of statins is a new aspect targeted towards NLRP3 and not only in ameliorating dyslipidemic profile: Treatment with statins may represent a promising further test for this well-known class of drugs beyond the CV risk reduction, mediated by the reduction of lipidic profile[39].

Recently, great interest is growing on the role of NLRP3 inflammasome that incorporates several signals of tissue injury, infectious or non-infectious, and consequently brings, *via* the activation of caspase-1, to the secretion of the pro-inflammatory cytokines IL-1β and IL-18. Block of the IL-1 system seems to be a fascinating option to counteract caspase activation and reducing IL-1 levels and consequently also SAA levels, that appear to be dramatically reduced within normal values even in patients with border line levels up to thousands of times. The mainway is to control the primary cause of inflammation and IL-1 blockers have demonstrated in rheumatologic field to be really effective and safe, even when associated to important immunosuppressant therapy, such as in kidney transplant patients. Moreover, a possible targeted intervention of IL-1 receptor blockade, even in active vasculitis, was recently suggested[22].

Several question points remain open, such as whether it is right to consider IL-1 block as target for treating CKD. And also if it is really the NLRP3 inflammasome a gauge of kidney injury damage or if we can specifically target the NLRP3 inflammasome for therapeutic intervention, as recently postulated by other authors[12].

The direct involvement of NLRP3 in kidney disease has not been demonstrated yet, despite recently several manuscripts address a reasonable suspicion about it. Moreover, a deeper knowledge on the role of NLRP3 inflammasome and of reactive AA amyloidosis in renal diseases is requested. Whether blocking IL-1 is really effective in delaying the progression of renal damage has yet to be demonstrated by large trials, despite, at the moment, the high costs severely limit the use of such drugs.

REFERENCES

1 **Carrero JJ**, Stenvinkel P. Persistent inflammation as a catalyst for other risk factors in chronic kidney disease: a hypothesis proposal. *Clin J Am Soc Nephrol* 2009; **4** Suppl 1: S49-S55 [PMID: 19996005 DOI: 10.2215/CJN.02720409]

2 **Pulskens WP**, Butter LM, Teske GJ, Claessen N, Dessing MC, Flavell RA, Sutterwala FS, Florquin S, Leemans JC. Nlrp3 prevents early renal interstitial edema and vascular permeability in unilateral ureteral obstruction. *PLoS One* 2014; **9**: e85775 [PMID: 24454932 DOI: 10.1371/journal.pone.0085775]

3 **Iyer SS**, Pulskens WP, Sadler JJ, Butter LM, Teske GJ, Ulland TK, Eisenbarth SC, Florquin S, Flavell RA, Leemans JC, Sutterwala FS. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc Natl Acad Sci USA* 2009; **106**: 20388-20393 [PMID: 19918053 DOI: 10.1073/pnas.0908698106]

4 **Vilaysane A**, Chun J, Seamone ME, Wang W, Chin R, Hirota S, Li Y, Clark SA, Tschopp J, Trpkov K, Hemmelgarn BR, Beck PL, Muruve DA. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. *J Am Soc Nephrol* 2010; **21**: 1732-1744 [PMID: 20688930 DOI: 10.1681/ASN.2010020143]

5 **Vesey DA**, Cheung C, Cuttle L, Endre Z, Gobe G, Johnson DW. Interleukin-1beta stimulates human renal fibroblast proliferation and matrix protein production by means of a transforming growth factor-beta-dependent mechanism. *J Lab Clin Med* 2002; **140**: 342-350 [PMID: 12434136]

6 **Anders HJ**, Muruve DA. The inflammasomes in kidney disease. *J Am Soc Nephrol* 2011; **22**: 1007-1018 [PMID: 21566058 DOI: 10.1681/ASN.2010080798]

7 **Mulay SR**, Kulkarni OP, Rupanagudi KV, Migliorini A, Darisipudi

MN, Vilaysane A, Muruve D, Shi Y, Munro F, Liapis H, Anders HJ. Calcium oxalate crystals induce renal inflammation by NLRP3-mediated IL-1β secretion. *J Clin Invest* 2013; **123**: 236-246 [PMID: 23221343 DOI: 10.1172/JCI63679]

8 **Lichtnekert J**, Kulkarni OP, Mulay SR, Rupanagudi KV, Ryu M, Allam R, Vielhauer V, Muruve D, Lindenmeyer MT, Cohen CD, Anders HJ. Anti-GBM glomerulonephritis involves IL-1 but is independent of NLRP3/ASC inflammasome-mediated activation of caspase-1. *PLoS One* 2011; **6**: e26778 [PMID: 22046355 DOI: 10.1371/journal.pone.0026778]

9 **Scarpioni R**, Rigante D, Cantarini L, Ricardi M, Albertazzi V, Melfa L, Lazzaro A. Renal involvement in secondary amyloidosis of Muckle-Wells syndrome: marked improvement of renal function and reduction of proteinuria after therapy with human anti-interleukin-1β monoclonal antibody canakinumab. *Clin Rheumatol* 2015; **34**: 1311-1316 [PMID: 24510061 DOI: 10.1007/s10067-013-2481-2]

10 **Turner CM**, Arulkumaran N, Singer M, Unwin RJ, Tam FW. Is the inflammasome a potential therapeutic target in renal disease? *BMC Nephrol* 2014; **15**: 21 [PMID: 24450291 DOI: 10.1186/1471-2369-15-21]

11 **Church LD**, Savic S, McDermott MF. Long term management of patients with cryopyrin-associated periodic syndromes (CAPS): focus on rilonacept (IL-1 Trap). *Biologics* 2008; **2**: 733-742 [PMID: 19707454]

12 **Lorenz G**, Darisipudi MN, Anders HJ. Canonical and non-canonical effects of the NLRP3 inflammasome in kidney inflammation and fibrosis. *Nephrol Dial Transplant* 2014; **29**: 41-48 [PMID: 24026244 DOI: 10.1093/ndt/gft332]

13 **Faust J**, Menke J, Kriegsmann J, Kelley VR, Mayet WJ, Galle PR, Schwarting A. Correlation of renal tubular epithelial cell-derived interleukin-18 up-regulation with disease activity in MRL-Faslpr mice with autoimmune lupus nephritis. *Arthritis Rheum* 2002; **46**: 3083-3095 [PMID: 12428253 DOI: 10.1002/art.10563]

14 **VanderBrink BA**, Asanuma H, Hile K, Zhang H, Rink RC, Meldrum KK. Interleukin-18 stimulates a positive feedback loop during renal obstruction via interleukin-18 receptor. *J Urol* 2011; **186**: 1502-1508 [PMID: 21855933 DOI: 10.1016/j.juro.2011.05.046]

15 **Franke EI**, Vanderbrink BA, Hile KL, Zhang H, Cain A, Matsui F, Meldrum KK. Renal IL-18 production is macrophage independent during obstructive injury. *PLoS One* 2012; **7**: e47417 [PMID: 23077611 DOI: 10.1371/journal.pone.0047417]

16 **Zhang C**, Boini KM, Xia M, Abais JM, Li X, Liu Q, Li PL. Activation of Nod-like receptor protein 3 inflammasomes turns on podocyte injury and glomerular sclerosis in hyperhomocysteinemia. *Hypertension* 2012; **60**: 154-162 [PMID: 22647887 DOI: 10.1161/HYPERTENSIONAHA.111.189688]

17 **Mulay SR**, Evan A, Anders HJ. Molecular mechanisms of crystal-related kidney inflammation and injury. Implications for cholesterol embolism, crystalline nephropathies and kidney stone disease. *Nephrol Dial Transplant* 2014; **29**: 507-514 [PMID: 24163269 DOI: 10.1093/ndt/gft248]

18 **Martinon F**, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006; **440**: 237-241 [PMID: 16407889 DOI: 10.1038/nature04516]

19 **Scarpioni R**. An Innovative Approach to Managing a Patient With Complicated Gouty Arthritis and Chronic Kidney Disease. *Joint Spine Bone* 2015; In press

20 **Schroder K**, Tschopp J. The inflammasomes. *Cell* 2010; **140**: 821-832 [PMID: 20303873 DOI: 10.1016/j.cell.2010.01.040]

21 **Schreiber A**, Luft FC, Kettritz R. Phagocyte NADPH oxidase restrains the inflammasome in ANCA-induced GN. *J Am Soc Nephrol* 2015; **26**: 411-424 [PMID: 25012177 DOI: 10.1681/ASN.2013111177]

22 **Dinarello CA**. Interleukin-1--its multiple biological effects and its association with hemodialysis. *Blood Purif* 1988; **6**: 164-172 [PMID: 3293622]

23 **Duewell P**, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nuñez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010; **464**: 1357-1361 [PMID: 20428172 DOI: 10.1038/nature08938]

24 **Merlini G**, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003; **349**: 583-596 [PMID: 12904524 DOI: 10.1056/NEJMra023144]

25 **Lachmann HJ**, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, Hawkins PN. Natural history and outcome in systemic AA amyloidosis. *N Engl J Med* 2007; **356**: 2361-2371 [PMID: 17554117 DOI: 10.1056/NEJMoa070265]

26 **Immonen K**, Finne P, Grönhagen-Riska C, Pettersson T, Klaukka T, Kautiainen H, Hakala M. A marked decline in the incidence of renal replacement therapy for amyloidosis associated with inflammatory rheumatic diseases - data from nationwide registries in Finland. *Amyloid* 2011; **18**: 25-28 [PMID: 21284495 DOI: 10.3109/13506129.2010.549252]

27 **Obici L**, Merlini G. AA amyloidosis: basic knowledge, unmet needs and future treatments. *Swiss Med Wkly* 2012; **142**: w13580 [PMID: 22653707 DOI: 10.4414/smw.2012.13580]

28 **Niemi K**, Teirilä L, Lappalainen J, Rajamäki K, Baumann MH, Öörni K, Wolff H, Kovanen PT, Matikainen S, Eklund KK. Serum amyloid A activates the NLRP3 inflammasome via P2X7 receptor and a cathepsin B-sensitive pathway. *J Immunol* 2011; **186**: 6119-6128 [PMID: 21508263 DOI: 10.4049/jimmunol.1002843]

29 **Gillmore JD**, Lovat LB, Persey MR, Pepys MB, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet* 2001; **358**: 24-29 [PMID: 11454373 DOI: 10.1016/S0140-6736(00)05252-1]

30 **Bataille S**, Fernandez C, Zink JV, Brunet P, Berland Y, Burtey S. The Case | A hip fracture in a hemodialysis patient. Pathologic right-hip fracture from β2-microglobulin amyloidosis. *Kidney Int* 2013; **83**: 1211-1212 [PMID: 23728017 DOI: 10.1038/ki.2012.436]

31 **Kümmerle-Deschner JB**, Tyrrell PN, Reess F, Kötter I, Lohse P, Girschick H, Huemer C, Horneff G, Haas JP, Koitschev A, Deuter C, Benseler SM. Risk factors for severe Muckle-Wells syndrome. *Arthritis Rheum* 2010; **62**: 3783-3791 [PMID: 20722029 DOI: 10.1002/art.27696]

32 **Cantarini L**, Rigante D, Brizi MG, Lucherini OM, Sebastiani GD, Vitale A, Gianneramo V, Galeazzi M. Clinical and biochemical landmarks in systemic autoinflammatory diseases. *Ann Med* 2012; **44**: 664-673 [PMID: 21972825 DOI: 10.3109/07853890.2011.598546]

33 **Simons JP**, Al-Shawi R, Ellmerich S, Speck I, Aslam S, Hutchinson WL, Mangione PP, Disterer P, Gilbertson JA, Hunt T, Millar DJ, Minogue S, Bodin K, Pepys MB, Hawkins PN. Pathogenetic mechanisms of amyloid A amyloidosis. *Proc Natl Acad Sci USA* 2013; **110**: 16115-16120 [PMID: 23959890 DOI: 10.1073/pnas.1306621110]

34 **Dubrey SW**, Cha K, Simms RW, Skinner M, Falk RH. Electrocardiography and Doppler echocardiography in secondary (AA) amyloidosis. *Am J Cardiol* 1996; **77**: 313-315 [PMID: 8607418 DOI: 10.1016/S0002-9149(97)89403-9]

35 **Dember LM**, Hawkins PN, Hazenberg BP, Gorevic PD, Merlini G, Butrimiene I, Livneh A, Lesnyak O, Puéchal X, Lachmann HJ, Obici L, Balshaw R, Garceau D, Hauck W, Skinner M. Eprodisate for the treatment of renal disease in AA amyloidosis. *N Engl J Med* 2007; **356**: 2349-2360 [PMID: 17554116 DOI: 10.1056/NEJMoa065644]

36 **Scarpioni R**, Ricardi M, Albertazzi V, Melfa L. Treatment of dyslipidemia in chronic kidney disease: Effectiveness and safety of statins. *World J Nephrol* 2012; **1**: 184-194 [PMID: 24175258 DOI: 10.5527/wjn.v1.i6.184]

37 **Tonelli M**, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int* 2005; **68**: 237-245 [PMID: 15954913 DOI: 10.1111/j.1523-1755.2005.00398.x]

38 **van der Hilst JC**, Kluve-Beckerman B, Bodar EJ, van der Meer JW, Drenth JP, Simon A. Lovastatin inhibits formation of AA amyloid. *J Leukoc Biol* 2008; **83**: 1295-1299 [PMID: 18285405

DOI: 10.1189/jlb.1107723]
39 **Luo B**, Li B, Wang W, Liu X, Liu X, Xia Y, Zhang C, Zhang Y, Zhang M, An F. Rosuvastatin alleviates diabetic cardiomyopathy by inhibiting NLRP3 inflammasome and MAPK pathways in a type 2 diabetes rat model. *Cardiovasc Drugs Ther* 2014; **28**: 33-43 [PMID: 24254031 DOI: 10.1007/s10557-013-6498-1]
40 **Stenvinkel P**, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimbürger O, Cederholm T, Girndt M. IL-10, IL-6, and TNF-alpha: central factors in the altered cytokine network of uremia--the good, the bad, and the ugly. *Kidney Int* 2005; **67**: 1216-1233 [PMID: 15780075 DOI: 10.1111/j.1523-1755.2005.00200.x]
41 **Cantaluppi V**, Quercia AD, Dellepiane S, Ferrario S, Camussi G, Biancone L. Interaction between systemic inflammation and renal tubular epithelial cells. *Nephrol Dial Transplant* 2014; **29**: 2004-2011 [PMID: 24589723 DOI: 10.1093/ndt/gfu046]
42 **Cachofeiro V**, Goicochea M, de Vinuesa SG, Oubiña P, Lahera V, Luño J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int Suppl* 2008; **(111)**: S4-S9 [PMID: 19034325 DOI: 10.1038/ki.2008.516]
43 **Obici L**, Merlini G. Amyloidosis in autoinflammatory syndromes. *Autoimmun Rev* 2012; **12**: 14-17 [PMID: 22878269 DOI: 10.1016/j.autrev.2012.07.016]
44 **Lachmann HJ**, Quartier P, So A, Hawkins PN. The emerging role of interleukin-1β in autoinflammatory diseases. *Arthritis Rheum* 2011; **63**: 314-324 [PMID: 20967858]
45 **Sayed RH**, Hawkins PN, Lachmann HJ. Emerging treatments for amyloidosis. *Kidney Int* 2015; **87**: 516-526 [PMID: 25469850 DOI: 10.1038/ki.2014.368]
46 **Bodin K**, Ellmerich S, Kahan MC, Tennent GA, Loesch A, Gilbertson JA, Hutchinson WL, Mangione PP, Gallimore JR, Millar DJ, Minogue S, Dhillon AP, Taylor GW, Bradwell AR, Petrie A, Gillmore JD, Bellotti V, Botto M, Hawkins PN, Pepys MB. Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. *Nature* 2010; **468**: 93-97 [PMID: 20962779 DOI: 10.1038/nature09494]
47 **Kortus-Götze B**, Hoyer J. Successful renal transplantation in Muckle-Wells syndrome treated with anti-IL-1β-monoclonal antibody. *NDT Plus* 2011; **4**: 404-405 [PMID: 25984207 DOI: 10.1093/ndtplus/sfr118]

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