REVIEW ARTICLE

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Crystallopathies

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Deposits of crystals, misfolded proteins, or airborne particulate matter of nanoparticle or microparticle size (all of which are hereafter referred to as crystal deposits) (see the Glossary) cause diverse medical disorders (Table 1) that can manifest as either acute or chronic organ injuries (Fig. 1). Recent discoveries in the study of crystal biology suggest that unifying pathophysiological mechanisms underlie these disorders and may identify molecular targets for innovative therapies.

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General Features of Crystal-Associated Diseases

In nature, organisms catalyze the aggregation of atoms and ions into amorphous crystals, which are built into complex structures such as corals, shells, bones, and teeth; these structures provide structural stiffness and durability. In the wrong place, the same process can be injurious — for example, calcifications of vascular walls or tendons. The aggregation of atoms or ions in a periodic manner leads to self-perpetuating growth of regular-shaped crystals (Fig. 2). Single crystals sticking together or glued together by cement-like amorphous crystals can form polycrystalline masses, such as calculi.

Intrinsic and Extrinsic Sources of Crystals

Local supersaturation with minerals, dietary metabolites, or drug metabolites occurs most often in excretory organs, such as the biliary and urinary tracts, where concentration and supersaturation are thought to be common initiators of the crystallization process and stone formation. Endogenous proteins or paraproteins can also undergo self-aggregation to form polycrystalline-like microparticles (Table 1). For example, the process of beta-sheet fibrils self-perpetuating fibrillation to form plaquelike amyloid deposits in amyloidosis or Alzheimer's disease resembles mineral crystallization.

Crystals and particulate matter from outside the body usually enter the lungs from airborne occupational or environmental sources, including dust from cigarette smoke (Table 1).[1] Other sources of extrinsic particles include cosmetics, nanoparticle carriers for drugs, and metallic, plastic, or silicone implants.

Particle Size

Particle size is a critical determinant of the tissue response (Fig. 2). Macrophages and other phagocytes are usually the first cells to engulf particles for phagocytosis; this process is possible for nanoparticles and microparticles of a few micrometers in diameter.[2] Phagosomes fuse with lysosomes that contain lytic proteases. The inability to digest crystalline nanoparticle or microparticle cargo destabilizes lysosomes and induces cell stress, autophagy, and leakage of lysosomal proteases into the cytosol. Massive loads of particles may give macrophages

Glossary

Apoptosis-associated speck-like (ASC) protein speck complex: ASC protein is a central component of cytosolic inflammasomes. On inflammasome activation, ASC assembles into a single large protein complex up to 1 μm in size, which is termed a "speck." On release from dying macrophages, ASC speck microparticles activate inflammasomes in other cells.

Autophagy: Catabolism-like adaptive mechanism of stressed or starving cells that reduces energy expenditure by breaking down cell organelles.

Calciphylaxis: Severe form of medial calcific (Mönckeberg's) sclerosis leading to vascular obstruction, thrombosis, or both. The most frequent clinical presentation is painful skin necrosis.

Crystal: Solid particle with a geometric shape because its atoms, ions, or molecules are arranged in a regular ordered structure. Polycrystals are crystals irregularly fused together — for example, in a stone. Amorphous solids also form crystal-like microparticles but have no periodic arrangement.

Crystallopathy: Disease that involves crystals of crystal-like particulate matter in the pathogenesis of tissue injury.

Damage-associated molecular pattern (DAMP): Intracellular elements that are released into the extracellular space by plasma membrane rupture during cell necrosis and act as danger signals by eliciting proinflammatory effects through the activation of receptors of the innate immune system on other cells.

Ferroptosis: An iron-dependent form of regulated necrosis involving impaired glutathione peroxidase 4 generation, which promotes lipid peroxidation–driven cell death.

Hydroxyapatite: An amorphous calcium phosphate crystal that occurs in bone, dental enamel, dentin, kidney stones, and vascular or soft-tissue calcifications.

Inflammasome: A multiprotein oligomer complex that integrates numerous danger signals inside the intracellular cytosol to activate the inflammatory caspase 1 and sometimes caspase 5 (caspase 11 in mice). The consequences are release of mature interleukin-1β and eventually pyroptosis.

Mixed lineage kinase domain–like (MLKL): A pseudokinase that, when phosphorylated by RIPK3, contributes to the execution of necroptosis.

NACHT, LRR, and PYD domains–containing protein 3 (NLRP3): A central component of the inflammasome.

Necroinflammation: Necrotic tissue injury associated with an intense inflammatory response. This occurs because cell necrosis triggers inflammation and vice versa. Examples include the crescendo phase of acute gouty arthritis and crystal-induced acute kidney injury.

Necroptosis: Receptor-interacting protein kinase 3 (RIPK3)–dependent form of regulated necrosis, which is induced by extracellular signals through tumor necrosis factor receptor 1 (TNFR1), toll-like receptor 4 (TLR4)–TRIF, toll-like receptor 3 (TLR3)–TRIF, or CD95.

Nephrocalcinosis: Deposition of calcium salts such as calcium phosphate or calcium oxalate in the parenchyma of the kidney.

NETosis: A form of neutrophil death associated with the formation of a neutrophil extracellular net (NET).

Particulate matter: Microscopic solid or liquid matter — for example, suspended in the air. Such atmospheric particulate matter can be atmospheric dust, biologic particles (e.g., viruses, bacteria, allergens, and pollen), or particulate contaminants (e.g., tobacco smoke, smog, fly ash, and occupational dusts).

Pyroptosis: A form of regulated necrosis dependent on caspase 4, caspase 5, or caspase 11, described mainly as occurring on recognition of bacterial endotoxin inside the cytoplasm of infected macrophages.

Receptor-interacting protein kinase 1 (RIPK1): One of the possible upstream triggers of the necrosome formation.

Receptor-interacting protein kinase 3 (RIPK3): The key molecule in necroptosis.

Sialolithiasis: Salivary stones, usually in the duct of the salivary gland.

Urolithiasis: Calculi or stones in the kidney (nephrolithiasis), ureter (ureterolithiasis), or urinary bladder (cystolithiasis).

a "foam cell" appearance.[3] Crystal needles and other larger particles that exceed the size of macrophages may induce the formation of giant cells, which are able to internalize even larger particles.[4] The presence of calculi or implants can result in a state called "frustrated phagocytosis," which involves the formation of giant cells and a persistent release of phagocytic enzymes attempting extracellular digestion (Fig. 2).[2]

MECHANICAL OBSTRUCTION

Nonaggregating crystal masses remain in liquid form — for example, monosodium urate crystals in bird droppings or tophi in persons with

gout. In contrast, polycrystalline aggregates may solidify and grow into cavity-filling calculi that can cause tissue injury through mechanical obstruction that leads to colic and organ failure or vascular obstruction. The concentration of minerals in excretory fluids promotes supersaturation and crystallization, and therefore the biliary and urogenital tracts are susceptible to stone formation.

Bile is rich in electrolytes, bile acids, cholesterol, phospholipids, and conjugated bilirubin and frequently forms stones inside the gallbladder. Mobilization of calculi into extrahepatic bile ducts causes biliary colic. In the urinary tract, crystallization usually starts in the renal tubules, where supersaturation results from a stepwise concentration of the glomerular filtrate and from the active secretion of calcium, uric acid, oxalate, phosphate, or drug metabolites (Table 1).[5] After single crystals adhere to the luminal membrane of renal tubules by attaching to annexin II, CD44, or osteopontin, they serve as a nucleus for the building of larger polycrystalline plugs that obstruct the tubules.[6] Diffuse crystal-plug formation can cause acute kidney injury — for example, in acute oxalate nephropathy induced by polyethylene glycol intoxication or in myeloma cast nephropathy (Table 1).[7] In genetic forms of hyperoxaluria or hypercalciuria, persistent crystallization leads to progressive nephrocalcinosis and chronic kidney disease (Table 1).[8] Such "stony kidneys" have a white appearance on ultrasonographic or radiographic images.[8] Larger crystal aggregates may form in the renal pelvis, where there is more space and where calcified Randall's plaques (mineral concentrations on renal papillae) are sites of stone formation.[5] Dislocation of such calculi causes transient or persistent obstruction of urine flow, which manifests clinically as renal colic. In addition, drug-related or diet-related crystalluria can lead to unilateral or bilateral renal colic and even to acute renal failure.[9,10] Gallstones blocking the pancreatic outflow cause acute pancreatitis. Hydroxyapatite calculi in the ducts of salivary glands (sialoliths) cause sialdenitis (Table 1).

Crystal masses may also cause vascular obstruction through a number of different mechanisms. Atherosclerosis is caused by accumulations of cholesterol crystals in the intima of the arterial wall, or atheromas.[11] Atheromatous plaques eventually obstruct the vascular lumen and lead to tissue ischemia, as well as to plaque cap rupture and subsequent thrombotic vascular occlusion and tissue infarction.[3] Plaque rupture in the aorta or its major branches can cause cholesterol emboli or ischemic necrosis (Fig. 2).[12] Cholesterol crystals appear as spindle-shaped luminal clefts in tissue biopsy specimens or on funduscopic inspection of retinal arteries.[12] Finally, vascular calcifications — calcium phosphate deposits in the medial layer of muscular arteries — are common among the elderly, patients with uremia, and patients with primary hyperparathyroidism.[13] This medial calcific sclerosis confers a loss of vascular compliance and eventually causes peripheral artery disease or calciphylaxis, as well as possible ischemic tissue necrosis in association with high mortality. Together, obstructions caused by crystals can result in colic, inflammation, and tissue necrosis and can sometimes lead to fatal organ failure.

NECROINFLAMMATION

Crystals elicit direct cytotoxic effects, inflammation, and inflammation-driven cell necrosis (Fig. 3), in an autoamplifying loop that is referred to as "necroinflammation."[14]

CRYSTAL CYTOTOXIC EFFECTS

Crystals kill cells in various ways (Fig. 3). Phagocytosis of indigestible nanocrystals overloads phagolysosomes. As is the case in metabolic storage diseases, such cells undergo substantial stress, actin depolymerization, production of reactive oxygen species, and enhanced autophagy, but the mode of cell death can vary.[15] Amorphous calcium phosphates in acidic lysosomes release large amounts of calcium into the cytosol, driving cell necrosis.[16] In addition, crystals of calcium oxalate, calcium pyrophosphate, cystine, or monosodium urate induce a type of epithelial and mesenchymal cell necrosis through receptor-interacting protein kinase 3 (RIPK3)–mediated phosphorylation of the pseudokinase mixed lineage kinase domain–like (MLKL).[17] This form of regulated necrosis is referred to as necroptosis.[18] *Ripk3*-deficient or *Mlkl*-deficient mice are protected from acute kidney injury related to oxalate crystal–induced tubular necrosis.[17] Ferroptosis, another form of regulated necrosis, contributes to the same disease; whether this happens in parallel with necroptosis remains unclear.[19]

Table 1. Diseases Related to Crystals and Other Microparticles.*

Crystal or Particle and Disorder	Major Disease Manifestations
Intrinsic inorganic crystals	
Brushite: nephrolithiasis or urolithiasis	Renal colic
Calcium carbonate	
Cholecystolithiasis or choledocholithiasis	Biliary colic
Nephrolithiasis or urolithiasis	Renal colic
Calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite)	
Nephrolithiasis or urolithiasis	Renal colic
Acute oxalate nephropathy	Acute kidney injury
Polyethylene glycol poisoning	Acute kidney injury
Dietary oxalosis: black tea, starfruit, rhubarb, vitamin C, nuts	Acute kidney injury, renal colic
Bariatric surgery–related or short bowel–related	Acute kidney injury, renal colic
Chronic oxalate nephropathy (e.g., primary hyperoxaluria)	Chronic kidney disease, organ oxalosis
Calcium pyrophosphate or calcium phosphate	
Pseudogout, chondrocalcinosis, hemochromatosis, hyperparathyroidism	Acute monarthritis, periarthritis, bursitis, osteoarthritis
Hyperphosphatemic familial tumoral calcinosis	Soft-tissue calcification, tissue ischemia, ischemic necrosis
Vascular calcification, calciphylaxis, warfarin calcification	Tissue ischemia, ischemic necrosis, chronic kidney disease
Dent's disease	Nephrocalcinosis
Hydroxyapatite	
Vascular calcification, calciphylaxis	Tissue ischemia, ischemic necrosis
Atherosclerosis	Tissue ischemia, ischemic necrosis
Acute phosphate nephropathy	Acute kidney injury
Nephrolithiasis or urolithiasis	Renal colic
Sialolithiasis	Painful swelling of the salivary gland
Breast microcalcifications	—
Struvite: nephrolithiasis or urolithiasis	Renal colic
Intrinsic organic crystals or microparticles	
Adenine: adenine phosphoribosyltransferase deficiency	Nephrolithiasis or urolithiasis, renal colic, chronic kidney disease
Amyloid	
Amyloid-β in Alzheimer's disease	Dementia
Amylin in diabetes	Hyperglycemia
Transthyretin amyloidosis	Polyneuropathy, cardiomyopathy
Bile pigment	
Cholecystolithiasis or docholithiasis	Biliary colic, pancreatitis
Bile cast nephropathy	Acute kidney injury
Cholesterol	
Atherosclerosis	Tissue ischemia, ischemic necrosis
Cholesterol embolism	Ischemic necrosis
Nonalcoholic steatohepatitis	Acute lipotoxic liver disease
Cholesteryl ester storage disease	Chronic lipotoxic liver disease
Cholesterol granuloma	Bone lesions
Cholecystolithiasis or docholithiasis	Biliary colic

Table 1. (Continued.)

Crystal or Particle and Disorder	Major Disease Manifestations
Cystine: cystinosis	Chronic kidney disease, urolithiasis, extrarenal manifestations
Light chains	
Myeloma cast nephropathy	Acute kidney injury
Crystalloglobulinemia	Thrombotic microangiopathy
Light-chain Fanconi's syndrome	Renal tubulopathy, chronic kidney disease
Crystal-storing histiocytosis	Renal tubulopathy, chronic kidney disease
Fibrillary glomerulonephritis	Proteinuria, chronic kidney disease
Immunotactoid glomerulopathy	Proteinuria, chronic kidney disease
Monosodium urate	
Gout	Acute monarthritis, bursitis; chronic tophous gout
Nephrolithiasis or urolithiasis	Renal colic
Urate nephropathy	Acute kidney injury
Myoglobin or heme: myoglobin cast nephropathy	Acute kidney injury
Fibrillar α-synuclein: Parkinson's disease	Motor symptoms (parkinsonism)
Prion peptide: spongiform encephalopathy diseases	Variable encephalopathies
Uromodulin: cast nephropathies	Acute kidney injury
Extrinsic crystals or particulates	
Asbestos: lung asbestosis, cancer	Pulmonary fibrosis, mesothelioma
Drugs (acyclovir, methotrexate, indinavir, sulfadiazine) causing drug-related kidney injury	Acute kidney injury, renal colic
Hemozoin: malaria	Hemolysis, systemic inflammatory response syndrome
Implants, implant debris particles: implant-related injury	Monarthritis, aseptic osteolysis, foreign-body reactions
Occupational dusts: silica, asbestos, cotton, charcoal	
Acute dust-induced lung injury	Dust-induced respiratory failure
Pneumoconiosis (silicosis, asbestosis, anthracosis)	Lung fibrosis
Tobacco smoke particulates: smoking-related COPD, emphysema	Chronic respiratory distress
Air pollutants: smog-related asthma, pneumonitis, COPD	Acute and chronic respiratory distress

* COPD denotes chronic obstructive pulmonary disease.

RIPK1 in its unstimulated polyubiquitinated form and drugs that maintain this state, such as necrostatin-1, suppress necroptosis[20] — for example, in crystalline acute kidney injury.[17] Necrostatin-1 also suppresses heme-related cytotoxicity,[21] which may contribute to rhabdomyolysis-related muscle or tubular epithelial-cell necrosis. In contrast, malaria parasites convert heme into hemozoin crystals to reduce the toxic effects of heme.[22]

The RIPK3–MLKL–dependent signaling pathway also mediates monosodium urate crystal–induced neutrophil necrosis — that is, neutrophil necroptosis.[23] Neutrophil necrosis is a central process in acute gouty arthritis: it releases alarmins (molecules produced by damaged tissue that activate inflammation) such as interleukin-1α and neutrophil extracellular traps (NETs), the latter of which consist of chromatin that has become "decorated" with (i.e., that has become secondarily attached to) cytoplasmic components such as neutrophil elastase, cathepsins, and myeloperoxidase.[24] Crystal-induced necroptosis of parenchymal cells and neutrophils promotes the release of large amounts of histones into the extracellular space that elicit further direct cytotoxic effects on surrounding cells.[25] In this manner, crystals induce autoamplification of necroinflammation involving numerous processes of cell necrosis (Fig. 3). Silica crystals, however, promote caspase-dependent apoptosis of bronchial

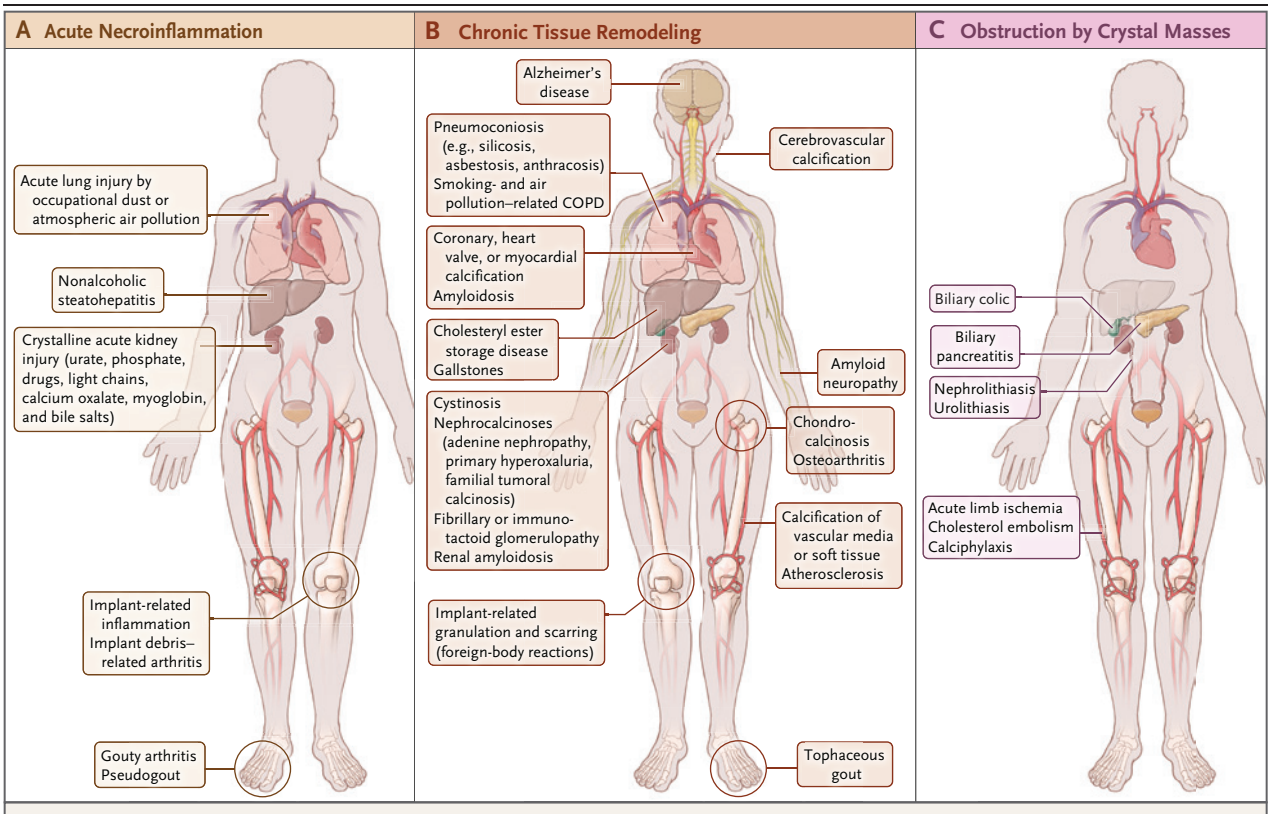

Figure 1. Crystallopathies Grouped According to Predominant Pathologic Mechanism.

Crystal-related or microparticle-related disorders can be grouped in three categories, according to their predominant pathologic mechanism: necroinflammation, chronic tissue remodeling leading to tissue atrophy and scarring, and obstruction of ducts, cavities, or vessels by large crystal masses, calculi, or stones. COPD denotes chronic obstructive pulmonary disease.

epithelial cells, which leads to epithelial barrier damage.[26] Whether the variety of the observed forms of regulated cell death depends on the crystal type, crystal size, or the affected cell type remains to be further characterized in detail.

CRYSTAL-INDUCED INFLAMMATION

By killing cells, crystals trigger inflammation through the release of proinflammatory elements, such as alarmins, proteases, and so-called damage-associated molecular patterns (DAMPs), by necrotic cells. These elements have the capacity to activate toll-like receptors or inflammasomes (Fig. 3). DAMPs include nucleoprotein HMGB1, histones, mitochondrial DNA, demethylated DNA or RNA, ATP, uric acid, and double-stranded DNA.[27] Through the activation of toll-like receptors or inflammasomes, dying cells induce the expression and secretion of cytokines, kinins, and lipid mediators, leading to a local inflammatory response — vasodilation (redness), pain, endothelial dysfunction with increased vascular permeability (swelling), and leukocyte adhesion (leukocyte influx). Local complement activation can also be involved.[28] These processes contribute to the typical clinical presentation of acute crystallopathies — for example, in gouty arthritis, dust-related respiratory distress, and crystalline acute kidney injury.

However, crystals also trigger inflammation directly.[29] In fact, Martinon, Tschopp, and colleagues made a seminal discovery when they found that monosodium urate and calcium pyrophosphate dihydrate crystals activate the NLRP3 inflammasome to trigger the release of mature interleukin-1β from macrophages.[30] Subsequently, research teams all over the world confirmed this finding and reported that many types of inorganic crystals and organic or synthetic microparticles have the same effect.[29,31] These include crystals or particulates of cholesterol,[32] calcium oxalate,[33] calcium phosphate,[34] calcium pyrophos-

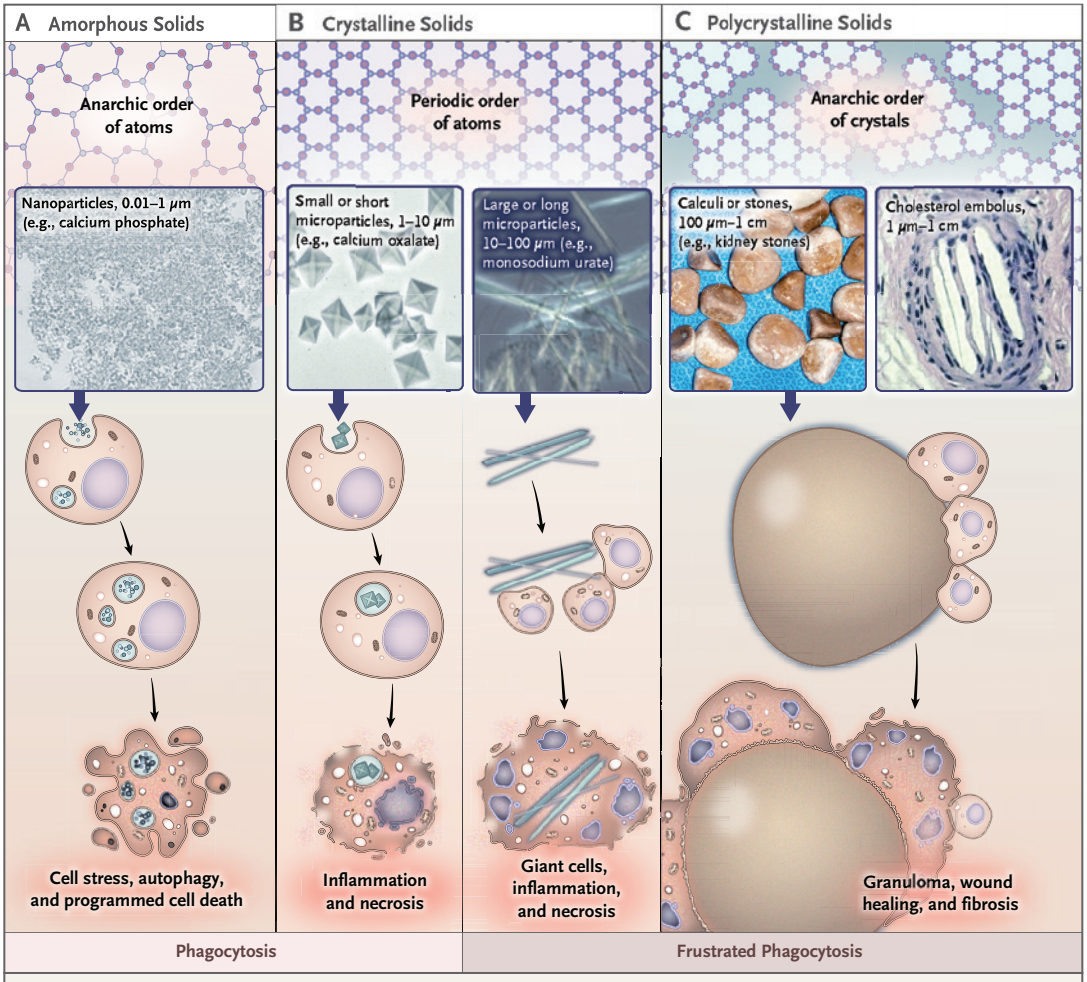

Figure 2. Handling of Crystalline Solids of Different Sizes.

Mineral solids form in the human body during homeostasis (bone formation and turnover) and during excretion of organic metabolites (urates and oxalates), but sometimes they cause disease (e.g., cholesterol crystals in atherosclerosis, urate crystals in gout, and oxalate crystals in kidney-stone disease). Extrinsic solids can also enter the body (as a result of air pollution, exposure to occupational dusts, and implants). Particle size is a critical determinant of crystal handling by phagocytes. Extracellular nanoparticle deposits increase tissue stiffness and reduce tissue compliance — for example, in vascular calcifications. The inability to digest microparticles inside lysosomes promotes lysosomal instability and leakage, which is an intracellular danger signal that triggers inflammation and cell death. "Frustrated phagocytosis" of larger particles (the inability of phagocytes to engulf the particles) fosters the formation of giant cells, a polyploid version of proinflammatory macrophages. Calculi, stones, or implants that are too large to be internalized even by giant cells are segregated from the parenchymal tissue by phagocytes in a granuloma-like lesion. The persistent attack of the particle by enzyme release is also associated with injury and scar formation in the surrounding tissue. The result is a fibrotic destruction of organs — for example, progressive lung fibrosis in silicosis, asbestosis, and chronic lung diseases related to occupational dusts.

phate,[35] cystine,[36] silica,[37,38] asbestos,[38] aluminum salts,[37,39] malarial hemozoin,[40] uromodulin glycoprotein,[41] ASC speck complexes,[42] myoglobin,[43] misfolded protein aggregates or amyloid fibrils (such as Alzheimer's-related amyloid-β),[44] Parkinson's-related fibrillar α-synuclein,[45] neurotoxin prion peptide,[46] diabetes-related amyloid polypeptide,[47] cigarette smoke–related microparticles,[48] and nanoparticles[49] (e.g., formed by titanium dioxide, carbon, polysterene, or liposomes). These findings suggested the concept of a unifying pathophysiological mechanism underlying otherwise apparently diverse disorders.[29] The NLRP3 inflammasome is a multiprotein oligomer com-

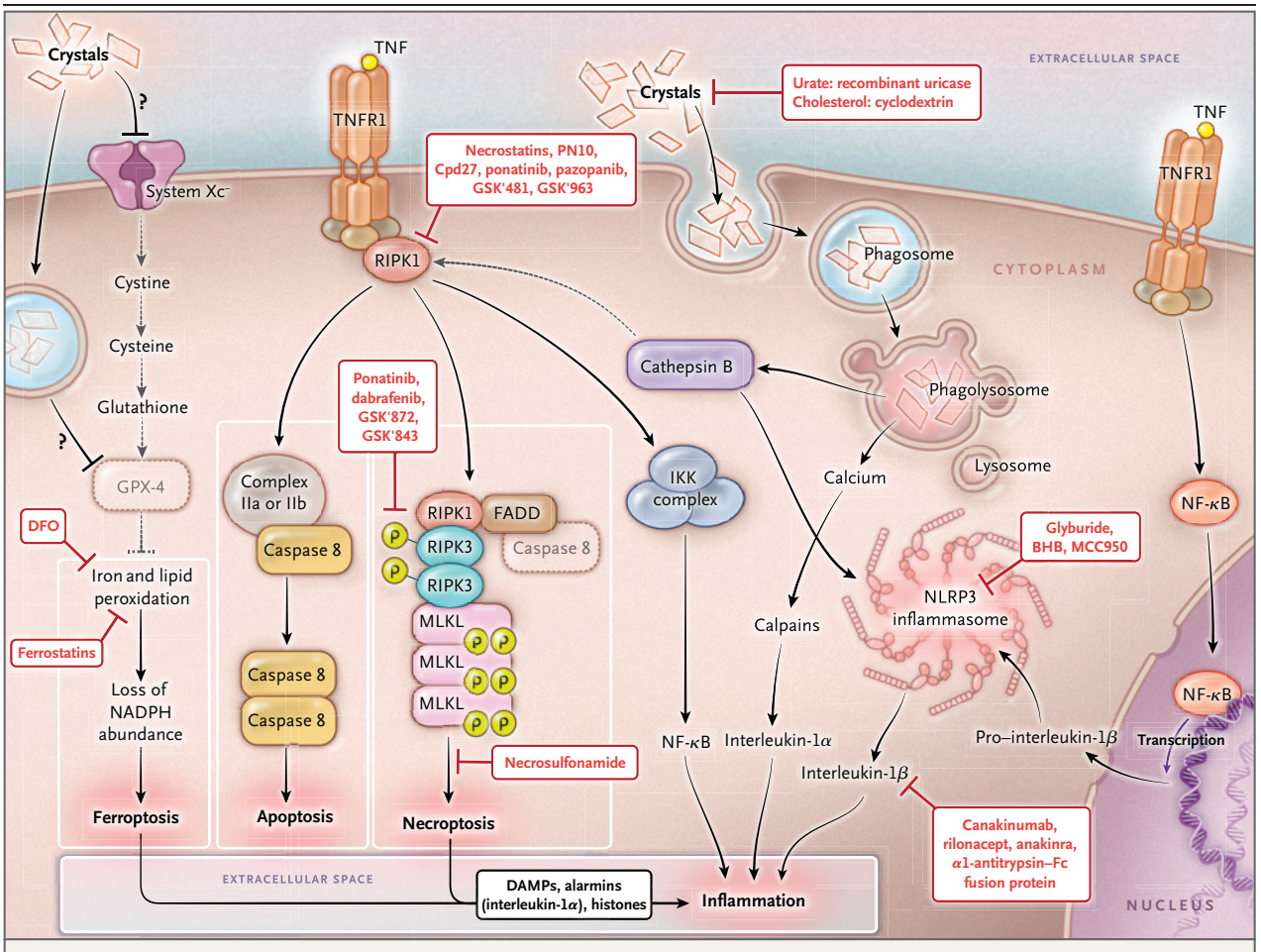

Figure 3. Molecular Mechanisms of Crystal-Related Necroinflammation.

Crystal-induced necroinflammation involves numerous signaling pathways that lead to cell death (apoptosis, necroptosis, and ferroptosis), inflammation, or both. Crystals may suppress glutathione peroxidase-4 (GPX-4) directly or by suppressing system Xc^- to induce loss of NADPH abundance and ferroptosis. The activation of death receptors involves receptor-interacting protein kinase 1 (RIPK1) for triggering either caspase 8–dependent apoptosis or receptor-interacting protein kinase 3 (RIPK3)–mixed lineage kinase domain–like (MLKL)–dependent necroptosis. Any cytokine or toll-like receptor induces the transcription of pro–interleukin-1β. After crystal phagocytosis leads to lysosomal destabilization, the leakage of cathepsin B, among other signals, triggers the activation of the NACHT, LRR, and PYD domains–containing protein 3 (NLRP3) inflammasome, which is a central primary and secondary driver of crystal-related inflammation. This mechanism induces the enzymatic cleavage of pro–interleukin-1β into its mature form. Mature interleukin-1β is either secreted or passively released together with interleukin-1α on inflammatory necrosis of the cell. New therapeutic options are shown in red boxes. BHB denotes β-hydroxybutyrate, DAMP danger-associated molecular pattern, DFO desferoxamine, FADD Fas-associated protein with death domain, GSH glutathione, IKK IκB kinase, NF-κB nuclear factor κB, P phosphorylated, ROS reactive oxygen species, and TNFR tumor necrosis factor (TNF) receptor.

plex formed by the cytosolic proteins NLRP3, ASC, and caspase 1.[31] In this complex, NLRP3 serves as a sensor that integrates intracellular danger signals, such as mitochondrial release of reactive oxygen species, potassium efflux, or protease leakage from lysosomal compartments, into the formation of inflammasome complexes.[31] In this manner, NLRP3 translates danger recognition into danger response by activating caspase 1, which subsequently cleaves pro–interleukin-1β into its bioactive and secreted form, promotes macrophage polarization into the proinflammatory M1 phenotype, and leads to cell necrosis. However, the precise crystal-induced and NLRP3-mediated mode of necrosis is still unclear (Fig. 3).[50] The NLRP3 inflammasome is specifically active in macrophages and dendritic cells that act as danger sentinels in all tissues

(Fig. 3). The released interleukin-1β activates the interleukin-1 receptor (interleukin-1R) on various cells in tissues.[51] Cells activated by interleukin-1R signaling promote inflammation by secreting cytokines and chemokines.[51] Systemic release of interleukin-1β induces fever and a systemic inflammatory response syndrome, as occurs during acute gout and other crystalline acute organ injuries.

The molecular mechanism whereby crystals trigger NLRP3 activation can vary. For example, extracellular cholesterol crystals trigger cytokine release on binding to the human macrophage-inducible C-type lectin (hMincle).[52] Silica and monosodium urate crystals can trigger NLRP3 activation by attaching to the outer plasma membrane.[53] Monosodium urate crystal uptake into acidic lysosomes causes massive release of sodium into the cell, which increases tonicity and leads to secondary water influx. This process dilutes the intracellular potassium concentration, which is an intracellular danger signal that activates the NLRP3 inflammasome.[54] When amorphous calcium phosphate crystals reach the acidic environment of phagolysosomes, they release large amounts of calcium. Calcium release activates not only NLRP3 but also the calcium-dependent protease calpain,[55] which processes interleukin-1α into its mature form (Fig. 3). However, most phagocytosed crystals and microparticles probably activate the NLRP3 inflammasome by destabilizing phagolysosomes, which prompts the release of lytic proteases into the cytosol (Fig. 3).[37,44,56] In malaria, plasmodium-infected red cells trigger the release of interleukin-1β by host immune cells through a dual mechanism. While hemozoin crystals induce lysosomal destabilization and uric acid release and activate the NLRP3 inflammasome, plasmodium-derived DNA attached to hemozoin activates the AIM2 inflammasome in the cytosol.[57] In summary, crystals induce interleukin-1–dependent inflammation in dendritic cells and macrophages and possibly other immune and nonimmune cells. In addition, crystal-induced cell necrosis triggers inflammation by releasing numerous elements that induce proinflammatory mediators. In turn, some cytokines trigger necroptosis (Fig. 3). This autoamplifying necroinflammation leads to the crescendo of painful swelling or organ dysfunction during the first hours after exposure to crystals.[14]

IMMUNE ANERGY

Not all pathologic crystal deposits are associated with acute necroinflammation. Gallstones and kidney stones, chondrocalcinosis, heart-valve calcifications, tophi in chronic gout, and silica-related lung fibrosis are instead associated with chronic tissue remodeling and scarring. One explanation is compartmentalization — for example, of gallstones in the gallbladder or kidney stones in the renal pelvis. In addition, crystals and calculi are covered with proteins that mask their cytotoxic and immunostimulatory potential. For example, the glycoprotein uromodulin is selectively secreted in the kidney in the ascending loop of Henle. Within the tubular lumen, uromodulin is immunologically inert and binds crystals together with other urinary proteins, a process that masks their cytotoxic potential and that enhances their clearance from the urinary tract. However, uromodulin itself has a tendency to form immunostimulatory microparticles that activate toll-like receptor 4 (TLR4) and the NLRP3 inflammasome, but this occurs only when tubular epithelial-cell damage exposes uromodulin particles to interstitial dendritic cells.[41,58] Granuloma formation is another form of compartmentalization. For example, silica and asbestos particles in the lung cannot be cleared by alveolar and infiltrating macrophages and remain within granulomata, and the impaired wound-healing process leads to pulmonary scarring. This process involves numerous immune-cell subsets with antiinflammatory and wound-healing phenotypes.[59]

The mystery of why monosodium urate crystals cause necroinflammation in an acute gout attack but not in a chronic gout tophus was recently solved. Tophus formation involves large numbers of neutrophils undergoing "NETosis" (a form of neutrophil death associated with NET formation) but surprisingly without any release of proinflammatory cytokines.[24] The process of massive NETosis results in the release of large amounts of proteases that digest all cytokines and chemokines; this process now appears to be a central element of immune anergy within tophi in chronic gout. The same mechanism may contribute to the spontaneous resolution of an acute gout attack.

Finally, crystals can interact directly with cell-surface receptors that down-regulate immuno-

stimulatory pathways. For example, monosodium urate crystals bind specifically to C-type lectin receptor Clec12a (also called myeloid inhibitory C-type lectin–like receptor), which counterbalances sterile inflammation in macrophages, dendritic cells, and neutrophils.[60,61] Whether this mechanism also contributes to the absence of inflammation in association with calcium phosphate crystals in soft tissues or vascular walls is currently unclear, but this process resembles proactive ossification. Thus, crystals and other microparticles do not always cause acute necroinflammation. Despite the irritating nature of microparticles, several molecular mechanisms counterbalance persistent inflammation and promote chronic wound healing processes that eventually destroy tissues through granuloma formation associated with scarring.

MOLECULAR TARGETS FOR INNOVATIVE THERAPIES

The shared pathologic mechanisms of crystal-related and microparticle-related diseases may provide new therapeutic targets, as illustrated in Figure 3. Inflammasome-mediated release of interleukin-1β was validated as a pathway common to most of the diseases discussed here, through demonstration of protection from inflammation and injury in *Nlrp3*-deficient mice[32,33,38,44,47,56,62] and of amelioration of inflammation and injury by interleukin-1 antagonist therapy in mice and humans.[33-35,40,63] Drugs that can suppress interleukin-1–related inflammation include the interleukin-1β neutralizing antibody canakinumab, the fusion protein of the interleukin-1R and human IgG rilonacept, and the recombinant human interleukin-1R antagonist anakinra. Canakinumab was approved in Europe for the treatment of recurrent gouty arthritis on the basis of its capacity to rapidly suppress inflammatory pain in gouty arthritis and to prevent further gout attacks.[64] In addition, α1-antitrypsin–Fc fusion protein can abrogate interleukin-1–driven inflammation in gouty arthritis.[65] These data indicate that similar effects might be achieved in the treatment of other acute crystallopathies. Canakinumab was tested for the treatment of early type 1 diabetes in two clinical trials, but no major effect was shown.[66] Studies involving type 2 diabetes and atherosclerosis are still under way.[67] Several small-molecule–based NLRP3 antagonists have been validated in preclinical studies,[68-70] but it remains unclear whether they offer benefits beyond interleukin-1 blockade in acute and chronic diseases related to crystals or particulate matter.

The discovery of crystal-related cytotoxicity involving the pathways of necroptosis and ferroptosis offers a new set of molecular targets for the treatment of crystallopathies. Small-molecule inhibitors are available to block necroptosis and ferroptosis (Fig. 3).[71] There is a concern that long-term therapy with cell-death inhibitors may increase the risk of cancer, but short-term therapy with these agents might abrogate necroinflammation at an early stage of acute crystal-induced tissue injuries — for example, in the lung immediately after dust exposure.

Disease related to misfolded proteins may be targeted by pharmacologic chaperones or aromatic small molecules that specifically refold or stabilize misfolded proteins.[72,73] However, the most intriguing concept is the dissolution of crystals as a cure for chronic crystallopathies. Many crystals easily dissolve at a certain pH. Cyclodextrin can dissolve cholesterol crystals in vitro and in animals with atherosclerosis.[74] Remedies that were claimed to dissolve gallbladder or kidney stones proved largely inefficient and have been replaced by surgical or shockwave interventions. However, gout tophi can be resolved effectively with recombinant uricase, an enzyme that breaks down urates, including monosodium urate crystals. Unfortunately, repeated use of rasburicase is associated with anaphylaxis in up to 6.2% of patients.[75] However, recombinant uricase remains in use for prophylaxis of the tumor lysis syndrome, although its potential to reduce the rate of death or of acute kidney injury has been questioned in a large meta-analysis.[76]

Finally, the immunostimulatory potential of microparticles has been implemented in vaccination strategies. Aluminum salts exert their vaccine-adjuvant effects by activating the NLRP3 inflammasome,[37,39] and nanoparticles constructed from biocompatible polyesters can have the same effect.[77]

CONCLUSIONS

Clinically diverse crystal-related and particulate matter–related disorders are now known to share molecular pathologic mechanisms, such as necro-

inflammation driven by NLRP3, caspase 1, caspase 11, and interleukin-1 or by RIPK1, RIPK3, and MLKL. Since therapeutic blockade of interleukin-1 has reached the clinic for the treatment of gout, there is increasing hope that some of the other evolving drug molecular targets will also eventually become innovative cures for patients with diseases related to crystals, particulate matter, or misfolded proteins.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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