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Crystal Formation in Inflammation

Bernardo S. Franklin,[1] Matthew S. Mangan,[1,2] and Eicke Latz[1,2,3,4]

[1]Institute of Innate Immunity, University Hospitals, University of Bonn, Bonn 53127, Germany; email: franklin@uni-bonn.de, matthew.mangan@dzne.de, eicke.latz@uni-bonn.de

[2]German Center for Neurodegenerative Diseases, Bonn 53175, Germany

[3]Department of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

[4]Centre of Molecular Inflammation Research, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim 7491, Norway

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Abstract

The formation and accumulation of crystalline material in tissues is a hallmark of many metabolic and inflammatory conditions. The discovery that the phase transition of physiologically soluble substances to their crystalline forms can be detected by the immune system and activate innate immune pathways has revolutionized our understanding of how crystals cause inflammation. It is now appreciated that crystals are part of the pathogenesis of numerous diseases, including gout, silicosis, asbestosis, and atherosclerosis. In this review we discuss current knowledge of the complex mechanisms of crystal formation in diseased tissues and their interplay with the nutrients, metabolites, and immune cells that account for crystal-induced inflammation.

1. INTRODUCTION

The formation of crystalline materials is a common phenomenon in biological systems, and its precise regulation is important for physical, chemical, and biological processes. Examples include the crystallization of inorganic minerals that mollusks use to form their shells, and the formation of bones and teeth in vertebrates. Crystal formation is essential in many multicellular organisms for the development of (exo)skeletons and for orientation, navigation, and homing, whereas other organisms use crystals to detoxify metabolically harmful heavy metals.

Crystallization usually occurs through nucleation, a stochastic process in which a preaggregated seed serves as a template for a self-perpetuating aggregation within a biological system. In nucleation, each molecular layer serves as a matrix for the deposition of the next one. Nucleation plays a pivotal role in the determination of crystal structure, size, and distribution, and its precise regulation allows the formation of structures that are essential to life, such as the exoskeleton in invertebrates. To form such vital crystalline structures with specific sizes, shapes, and layering, the precipitation of soluble material must be tightly regulated. Crystals can break and replicate as they grow, disseminate, and seed new crystals. They can also catalyze chemical reactions by binding to organic molecules. Tiny changes in their structures, shape, and distribution can lead to altered adhesiveness, dissolution, and diffusion through tissues, as well as uncontrolled crystal growth, and such changes can also influence their potential immunogenicity (1).

Organic molecules such as proteins, lipids, and organic minerals can also form crystals, and this process often involves the heterogeneous precipitation of several components, such as minerals and proteins, minerals and lipids, or lipids and proteins (2). Nucleation can also occur in proteinaceous material, which can form crystal-like amyloids or glass-like amorphous aggregates (3). Indeed, several proteins and peptide hormones are stably stored as amyloids to develop their physiological functions (4). Amyloid fibrillation resembles crystallization in two ways (5, 6): Firstly, both amyloid fibrils and crystals form by nucleation and similar growth mechanisms; secondly, both amyloid fibrils and crystals grow instantly from preaggregated seeds. The amorphous aggregation of proteins is similar to the glass transition, in which heterogeneous conformations are fixed by attractive forces from various sites of interaction. Glass-like states of proteins can be produced by adding high concentrations of salt, such as ammonium sulfate or sodium chloride (a procedure known as salting-out).

Aging, dietary changes, exposure to environmental insults, infections, inflammatory reactions, and various disease states can disturb the balance of mineral/protein precipitation, causing pathological mineralization. Aberrant crystallization can result in the formation of pathological crystals within the human body, leading to diverse disease states (**Figure 1**). Likewise, peptides or proteins can aggregate erroneously, giving rise to pathological conditions ranging from neurodegenerative disorders to systemic amyloidosis. Several studies highlight the formation and pathogenesis of protein aggregates (7, 8), and this topic will not be discussed further here. In this review we summarize our current knowledge about crystal formation and how crystal deposition can progress to disease. We focus on inflammation-related crystals and on how inflammation can contribute to pathological crystallization.

2. PHYSIOLOGICAL CRYSTALS

Every living organism consists of organic and inorganic materials that can combine to fulfill various purposes, such as defense against predators, maintenance of body structure, orientation and balance, storage of certain elements, enzymatic reactions, and detoxification of heavy metals. Examples of the uses of these biominerals include the exoskeletons of crustaceans, the gastroliths of crayfishes, the otoliths in the inner ear of vertebrates, fish scales, mollusk shells, and coral

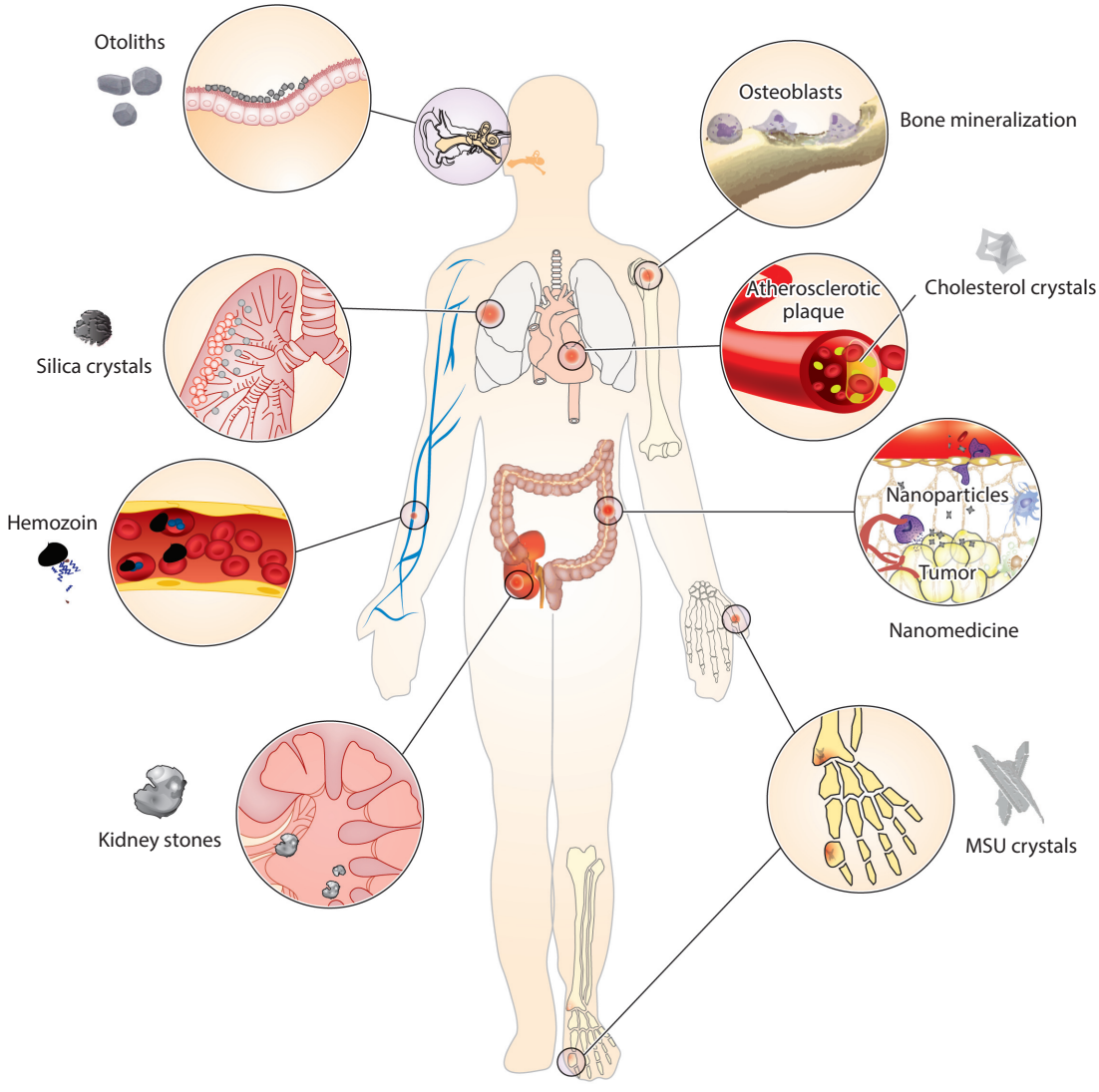


Figure 1

Physiological and pathological crystals. Crystals form within the human body and have a role in many cellular and physiological processes; the otolith crystals in the vestibular apparatus, for example, provide a sense of balance. Osteoblasts produce and deposit a calcium- and phosphate-based mineral into a collagen organic matrix in a precise regulated manner to form and repair bones. Aberrant crystallization, however, often occurs and can lead to several disease states. Free cholesterol in the blood can crystalize and accumulate in arterial walls, forming atherosclerotic plaques that limit the flow of oxygen-rich blood to organs; excess uric acid forms needle-shaped crystals that accumulate in the joints, causing gout. Perturbations in calcium mineralization can give rise to pathological conditions, including kidney stones. *Plasmodium falciparum*, the main cause of human malaria, digests the contents of red blood cells to produce free heme, which crystallizes within the parasites' digestive vacuoles into hemozoin, known as the malaria toxin because of its inflammatory potential. Exposure to environmental pollutants, such as silica, results in accumulation of crystals in the lungs and elsewhere and, in turn, inflammation. Finally, vaccine adjuvants and nanomedicine rely on the diffusion and inflammatory activity of crystals for efficient drug delivery and to elicit a protective immune response. Abbreviation: MSU, monosodium urate.

skeletons, among others. Hence, the crystallization of biomaterial is a physiological process that does not cause immune activation under homeostatic conditions. For example, the hardness of an exoskeleton is maintained by the deposition of calcium carbonate supplied by the epithelium, which is in close contact with the exoskeleton. Thus, the homeostasis of epithelial cells should not be disturbed by crystal deposition. Another example of the physiological interplay between immune competent cells and crystalline material is the interface between bone structures and cells involved in their remodeling, such as osteoclasts and osteoblasts. Under physiological conditions the interaction between these cells and crystalline material does not cause an inflammatory reaction. Furthermore, orientation, navigation, and homing are critical for the survival of many organisms, ranging from bacteria to vertebrates. In some species, these traits rely on sensitivity to magnetic fields, which is provided by a highly fine-tuned sensory system based on minute crystals of single-domain magnetite (Fe_3O_4) or greigite (Fe_3S_4) (9). This system is essential for the orientation of some magnetotactic organisms and was among the first sensory systems to evolve.

The precise, controlled nucleation of crystals is also important in our inner ear organ, where the sense of balance and orientation is driven by an intricate system in the vestibular labyrinth known as the otolith organs (from the Greek *oto*, for ear, and *lithus*, for stone). This system is composed of semicircular canals; the utricle, which gives us the sense of horizontal movement; and the sacs (saccule), a bed of sensory cells that gives information about vertical acceleration. The otolith organs, present in all vertebrates, use microscopic stones and viscous fluid to stimulate hair cells to detect motion and orientation, monitoring balance, movement, and direction. These stones are small particles, composed of a combination of a gelatinous matrix and calcium carbonate, and they are found in the viscous fluid of the saccule and utricle. The movement of these particles causes them to stimulate hair cells connected to sensory nerves when the head moves, giving the perception of motion. Hence, crystalline materials fulfill a range of functions, and under physiological conditions and in specialized areas of our bodies, they are tolerated by the immune system.

3. PATHOLOGICAL CRYSTALS

Although numerous examples exist in which physiological crystals do not provoke the immune system, aberrant crystallization of organic materials within the body, or exposure to external crystalline material, can cause an inflammatory response that is associated with pathogenesis in various diseases. The phase transition of normally soluble material into crystalline substances can act as a danger signal that triggers an immune response. Recent studies have demonstrated that material from environmental sources, dietary sources, and infectious organisms, or material introduced in the course of therapy, can cause inflammation through immune recognition of crystals (**Figure 1**). These pathological crystalline materials are recognized by the immune system as danger signals, leading to an inflammatory reaction that, if it persists, leads to pathologies in various diseases.

4. MECHANISMS OF CRYSTAL-INDUCED INFLAMMATION

Frustrated Phagocytosis and Lysosomal Damage

Although crystal contact with cellular membranes can, in some instances, be sufficient to trigger inflammation (10), a great part of the inflammation caused by pathological crystals depends on their phagocytosis by immune cells. Phagocytosis is an actin-based process involving adhesion, receptor clustering, and tyrosine phosphorylation. Receptor clustering at the attachment site leads to local polymerization of actin filaments and particle internalization. This usually results in trafficking of the phagocytosed material to phagolysosomes and the breakdown of the engulfed target, resulting

in little or no inflammation. Nevertheless, phagocytosis can go awry depending on the physical parameters of the particle being phagocytosed. This process is termed frustrated phagocytosis and can dictate the extent of the immune reaction (**Figure 2**).

Phagocytosis of larger particles can damage lysosomal membranes, causing lysosomal damage (LD) (**Figure 2**). LD is a common outcome of the phagocytosis of many physicochemically different crystals, such as silica (11, 12), asbestos (12), cholesterol (13), alum (11), and monosodium urate (MSU; 14) crystals (**Figure 2*b***). Lysosomes store high concentrations of hydrolytic enzymes, which makes them potentially harmful to the cell. Crystalline material, among other substances, can cause LD, resulting in leakage of lysosomal proteases (e.g., cathepsins) into the cell cytosol, where they digest vital proteins and affect other organelles, such as the mitochondria.

Immune cells have intricate mechanisms that detect alterations in both actin rearrangement and the stability of lysosomes and mitochondria. Hence, either the direct crystal engagement of cellular membranes in the absence of phagocytosis or the phagocytosis of crystals can result in inflammation.

Cathepsins released following LD can damage mitochondria and cause mitochondrial outer membrane permeabilization–dependent cell death. This results in proapoptotic molecules such as cytochrome *c* being released into the cytosol and activates caspases, the main drivers of cell death (15; **Figure 3**). Thus, the extent of LD can determine whether cells undergo necrosis or enact a caspase activation cascade (15, 16).

Depending on their type and morphology, crystals can mediate the release of a myriad of proinflammatory mediators. This includes cytokines, but also inflammatory lipid mediators (17, 18). Despite the large number of proinflammatory molecules released by crystal-induced LD, the release of the proinflammatory cytokines of the interleukin-1 family (IL-1β, IL-18, and IL-1α) is a central mechanism connecting all crystal-induced pathologies. IL-1 cytokines exert a host of immunological activities and have a well-established role in autoinflammatory diseases (19). As will be discussed further, IL-1 cytokines are, in several instances, the main drivers of crystal-induced pathology (20–25). Expression of IL-1 cytokines is tightly regulated, and most IL-1 family members lack a signal peptide and require proteolytic cleavage to exhibit biological effects (19). Three major pathways have been identified that result in the release of IL-1 cytokines in crystal diseases.

LD: lysosomal damage

MSU: monosodium urate

NLRP3: NACHT, LRR, and PYD domains–containing protein 3

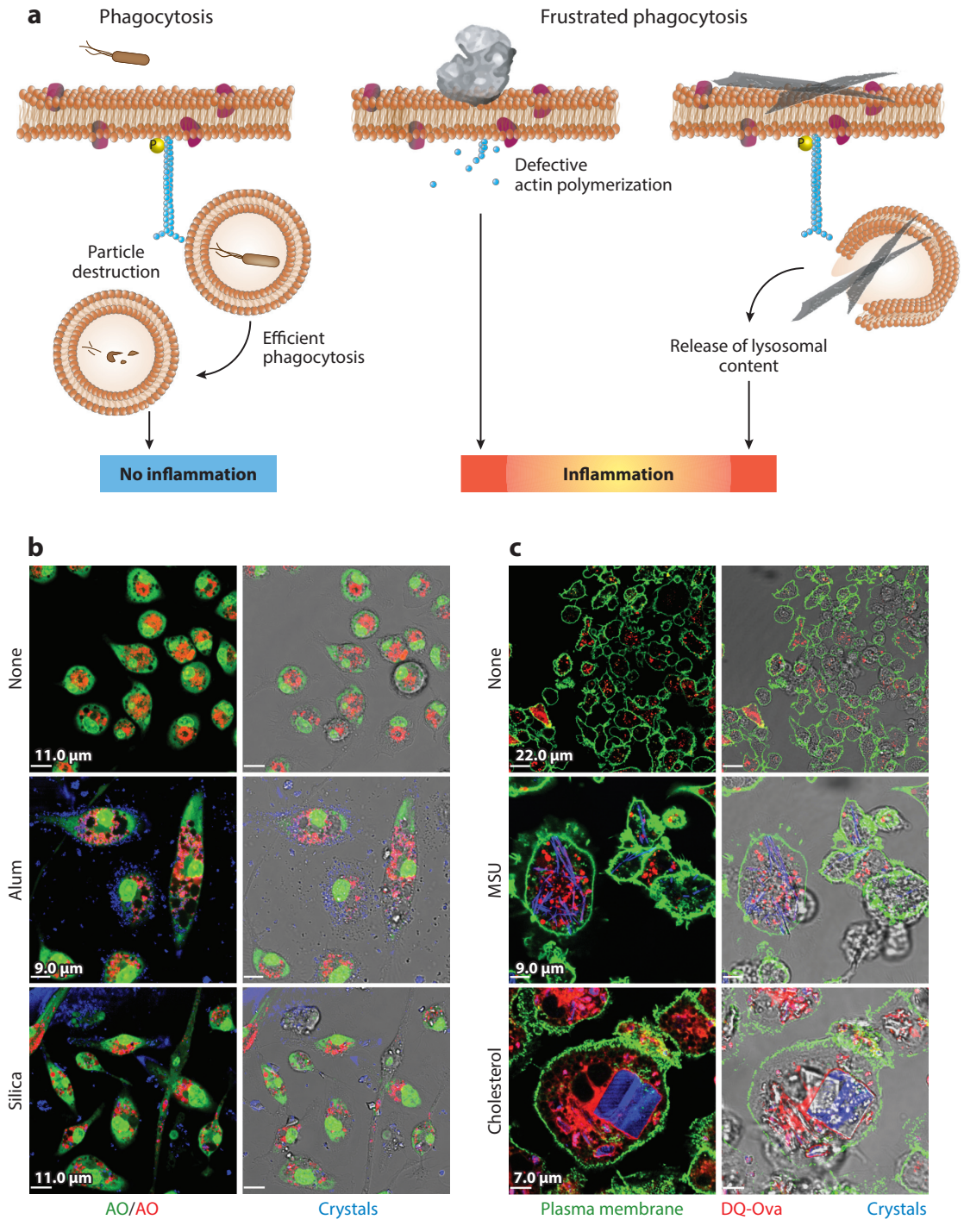
NLR: NOD-like receptor

CARD: caspase recruitment domain

Inflammasome Activation by Crystals

One of the most studied mechanisms of crystal-induced inflammation is crystals' ability to activate the cytosolic receptor complex called the NACHT, LRR, and PYD domains–containing protein 3 (NLRP3) inflammasome (26; **Figure 3**). NLRP3, a member of the NOD-like receptor (NLR) family, is the best-characterized NLR family member capable of forming an inflammasome. Following activation of NLRP3, the adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD) (ASC) is recruited to NLRP3, which induces the formation of ASC filaments (27, 28). ASC provides a molecular platform for the recruitment of procaspase-1 via CARD/CARD interactions. Recruitment of procaspase-1 to ASC filaments results in autoproteolytic caspase-1 activation. Active caspase-1, in turn, cleaves the precursors of IL-1β and IL-18 cytokines and mediates their release from cells.

Many triggers, including bacterial pore-forming toxins, extracellular ATP acting on P2X7 receptors, and a range of crystalline or protein aggregates can activate NLRP3 (29). These signals may also act in concert, as crystal-induced LD causes mass release of danger-associated molecular patterns (DAMPs) through necrosis and plasma membrane rupture, which can potentiate crystal-induced inflammation (30). ATP, which is among the triggers that can activate NLRP3 and is


a
Phagocytosis
Frustrated phagocytosis
Defective
actin polymerization
Particle
destruction
Efficient
phagocytosis
Release of lysosomal
content
No inflammation
Inflammation
b
None
Alum
Silica
11.0 µm
9.0 µm
11.0 µm
AO/AO
Crystals
c
None
MSU
Cholesterol
22.0 µm
9.0 µm
7.0 µm
Plasma membrane
DQ-Ova
Crystals

presumably released from cells that have phagocytosed crystals, contributes to crystal-induced inflammation (31).

The precise mechanism of crystal-induced NLRP3 activation is debated. One accepted model however, involves crystal-mediated LD (11, 32), although it remains unclear how exactly this process initiates NLRP3 activation. Lysosomal calcium release, proteolysis by cathepsin B, and mitochondrial reactive oxygen species (ROSs) have all been implicated in the process, but the individual contributions of each of these mechanisms remain unknown (11, 12, 33–36; **Figure 3**). A recent report suggested that the stress-responsive MAP kinase JNK is activated after LD and that inhibition or knockdown of JNK blocks NLRP3 activation. It was further demonstrated that LD induces a calcium-dependent pathway upstream of NLRP3 (37).

ROS: reactive oxygen species

LD is a common NLRP3 trigger induced by many different crystals (11–14, 34, 38). Thus, it seems that the NLRP3 inflammasome monitors the integrity of the lysosomal compartment and thereby detects LD caused by crystals, protein aggregates, invading microbes, and, potentially, imbalances in osmotic pressure. Crystals and other aggregates that directly damage lysosomes can cause NLRP3 activation. For example, depletion of zinc in macrophages causes LD, activates NLRP3, and induces IL-1β secretion (39). Hence, it will be interesting to test whether noncrystalline substances that destabilize lysosomes are also NLRP3 activators, and whether factors that prevent LD can reduce levels of NLRP3 activation. Interestingly, a recent study suggests that probenecid, used in the treatment of gout, can reduce levels of LD following reperfusion injury of the brain (40).

Another mechanism suggested to be involved in crystal-induced NLRP3 activation is the crystals' ability to induce ROSs, which are generated on exposure of macrophages to crystals (12, 38, 41). The source of ROSs remains controversial. Although both the knockdown of NADPH oxidase, an essential enzyme for ROS production, and the use of antioxidants inhibit different crystals from activating NLRP3 (12), genetic NADPH deficiency does not prevent NLRP3-dependent caspase-1/IL-1β activation (42–45). NLRP3 activation by crystals or other triggers in macrophages typically requires cell priming by a proinflammatory stimulus, such as a Toll-like receptor agonist, cytokines, or other NF-κB activators (46, 47). A model has been proposed by which the inflammasome requires at least two signals: Signal 1 provides the priming signal for cells, whereas signal 2 leads to inflammasome assembly (**Figure 4**). Recent work has demonstrated that crystals can provide both signal 1 and signal 2. Crystals can be recognized by the complement system, which results in the production of the proinflammatory mediator C5a. C5a is sufficient to provide the priming signal for subsequent inflammasome activation by the crystals (48–50; **Figure 4**). In other settings, such as atherosclerosis, oxidized low-density lipoprotein is recognized at the cell surface by TLR4/6 and the scavenger receptor CD36 (51), leading to macrophage priming. Hence, several innate immune signaling pathways cooperate in the recognition of certain crystals and provoke an inflammatory response.

Figure 2

Frustrated phagocytosis and lysosomal damage (LD). (*a*) Physiological phagocytosis requires protein phosphorylation, actin polymerization, and efficient internalization and destruction of the targeted particle. This usually results in a localized and controlled immune response with little or no inflammation. Phagocytosis of crystals can go awry and cause aberrant actin polymerization or LD, such that the lysosome's contents leak into the cell cytosol. (*b*,*c*) LD is a common outcome of the phagocytosis of alum, silica, monosodium urate (MSU), and cholesterol crystals. LD can be assessed with lysosomotropic probes such as (*b*) acridine orange (AO), a molecular probe used to measure the functional state of lysosomes, and (*c*) fluorescent ovalbumin (DQ-Ova), a quenched conjugate consisting of a pH-insensitive dye and ovalbumin.

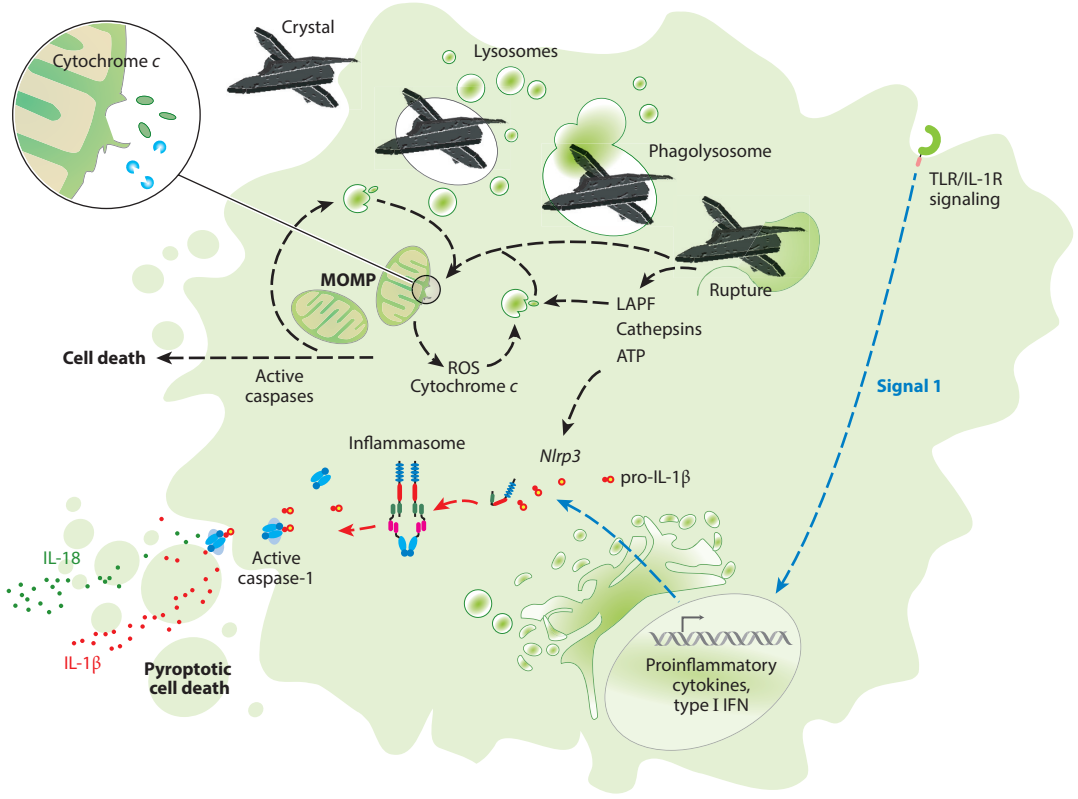


Figure 3

Inflammasome activation by crystals. Interleukin-1 (IL-1) cytokines are central to most, if not all, crystal-induced inflammation. Expression of IL-1 cytokines requires signaling through the Toll-like receptor (TLR) or IL-1R (signal 1; *blue arrow*). This results in production of immature procytokines that need further activation by caspase-1. NACHT, LRR, and PYD domains–containing protein 3 (NLRP3) patrols the cytosol and senses disturbances in lysosomal membranes and the mitochondria. Activated NLRP3 oligomerizes with apoptosis-associated speck-like protein containing a CARD (ASC) to form a caspase-1–activating scaffold. Active caspase-1 subsequently cleaves IL-1β and IL-18 into their bioactive forms and causes pyroptosis, an inflammatory type of cell death. The precise molecular mechanism of crystal-induced NLRP3 activation remains unknown. Release of the lysosomal contents, including cathepsins, LAPF, and ATP, into the cytosol has been proposed. Furthermore, lysosomal damage (LD) can cause mitochondrial outer membrane permeabilization (MOMP), which results in release of proapoptotic molecules such as cytochrome *c* into the cytosol, and further contributes to the activation of cell death–mediator caspases. MOMP also boosts reactive oxygen species (ROS) generation and contributes to NLRP3 activation directly and indirectly by inducing further LD.

NLRP3/Caspase-1–Independent, Crystal-Induced IL-1 Release

Increasing evidence suggests that inflammasomes and caspase-1 are not the only mechanism for processing IL-1 cytokines (52). Pore-forming toxins and extracellular ATP, both of which are NLRP3 activators, mediate IL-1β activation and release, as well as pyroptotic cell death through caspase-1. By contrast, lysosome-disrupting agents require caspase-1 for IL-1β activation but can induce cell death in a caspase-1–independent manner (16, 53). Many enzymes, including proteases, lipases, and nucleases, normally reside in the lysosomal compartment and could contribute to cellular demise following LD. Additionally, calpain-like proteases are activated by calcium released from damaged lysosomes (54) and specifically induce IL-1α release independently of NLRP3 and

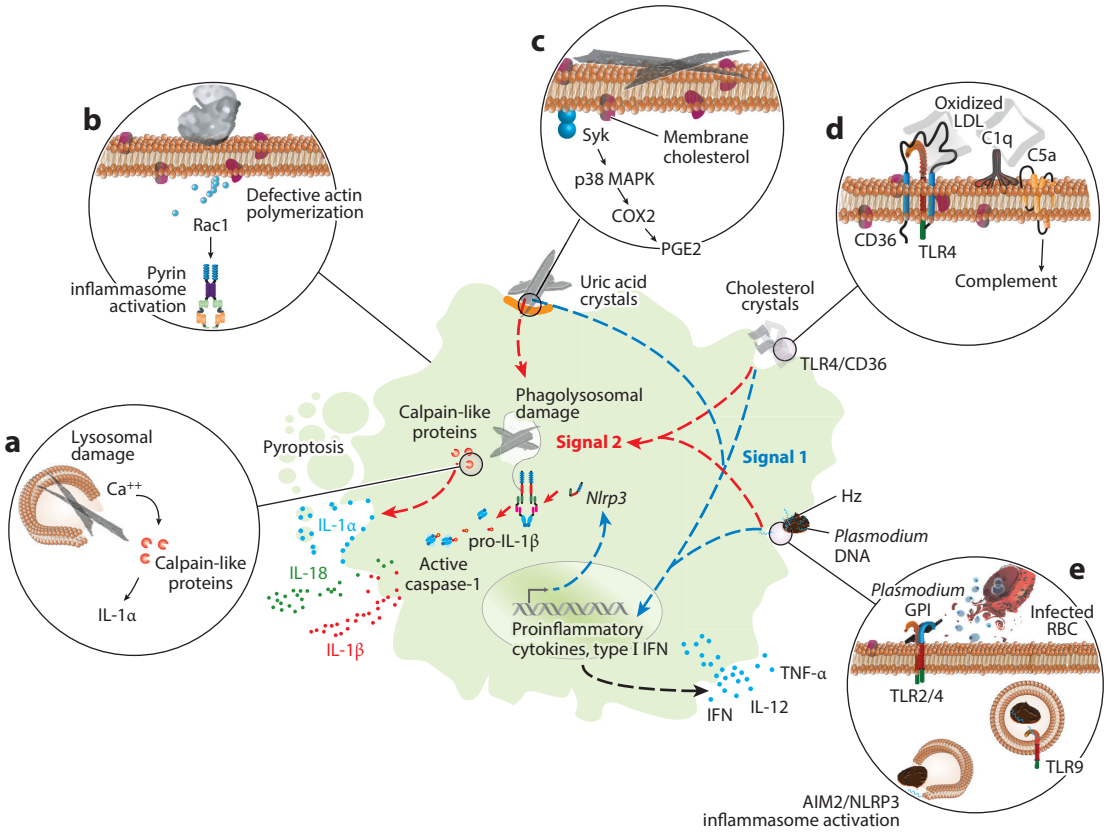


Figure 4

NACHT, LRR, and PYD domains–containing protein 3 (NLRP3)-dependent and -independent crystal induced inflammation. In addition to causing the lysosomal damage (LD) that activates NLRP3, crystals provide signal 1 for NLRP3 and interleukin-1 (IL-1) expression. Furthermore, they activate NLRP3-independent proinflammatory pathways. (*a*) Calcium released from LD can activate calpain-like proteases and result in IL-1α production independently of NLRP3. (*b*) The pyrin inflammasome directly senses disturbances in actin polymerization during frustrated phagocytosis. (*c*) Uric acid crystals can directly engage cellular membranes, without the involvement of any known cellular receptor, and induce proinflammatory cytokine production via the activation of SYK kinases in dendritic cells. (*d*) Oxidized low-density lipoprotein (LDL) can be recognized at the cell surface by Toll-like receptor 4 (TLR4) and the scavenger receptor CD36. Furthermore, members of the complement system C5a and C1q potentiate uric acid or cholesterol crystals in IL-1β production. (*e*) Glycophosphatidylinositol (GPI) anchored to the plasma membrane of *Plasmodium* parasites activates TLR4/2, and the malaria pigment hemozoin (Hz) carries *Plasmodium* DNA into the phagolysosomal compartment, where it activates TLR9, which primes NLRP3 and pro-IL-1 expression. Furthermore, phagocytosis of Hz induces LD and NLRP3 activation and releases parasite DNA into the cytosol, where it activates the AIM2 inflammasome. Abbreviations: IFN, interferon; RBC, red blood cell.

caspase-1. This demonstrates that calpain-like proteases can be involved in NLRP3-independent activation of IL-1R signaling downstream of crystal recognition.

Phagocytosis-Independent, Crystal-Mediated Inflammation

Crystal contact with membranes in the absence of phagocytosis can be sufficient to activate NLRP3 in a manner that depends on ion influx (55). The size, shape, and charge of the crystal may therefore dictate which particular pathway is triggered to activate NLRP3. Pyrin, another member

of the NLR family that forms an inflammasome with ASC and caspase-1, can detect alterations in actin rearrangement and induce IL-1 secretion (56). Pyrin directly binds to, and colocalizes with, polymerizing actin (57–59).

The guanosine triphosphatases (GTPases) of the Rho family are master regulators of cytoskeletal organization and phagocytosis, and they are frequent targets of a broad range of mucosal bacterial pathogens (60, 61). The Rho GTPase Rac1 can control inflammasome assembly and caspase-1 activation (60). Importantly, Rac1 is directly involved in mechanosensing of phagocytosed targets (62). Thus, it is possible that phagocytosis of larger crystalline material results in inflammasome activation through disruption of actin polymerization and/or GTPase activity. This hypothesis, however, needs to be experimentally investigated.

5. SOURCES OF PATHOLOGICAL CRYSTALS

The incidence of chronic inflammatory diseases is increasing, both in developed and developing countries, concomitant with a rise in living standards and the adoption of a western lifestyle. Economic development has led to an improved food supply and the gradual elimination of dietary deficiencies, improving overall nutritional status in many countries. This is especially true in low- and middle-income countries. However, the rise in living standards and urbanization that accompanies economic development has had consequences for the lifestyles and dietary patterns of individuals, not all of which are positive. Modern populations not only consume more energy-dense food (with a greater ratio of fats and added sugars) than former generations but also have significantly different patterns of work, leisure, and physical activity. Strong evidence exists that changes in the nutritional status of western populations contribute to the causal factors underlying noncommunicable diseases. Moreover, the pace of these changes seems to be accelerating, suggesting that more damage is to come. Many endogenous crystals form in our body as a result of the lifestyle adopted in modern societies.

Endogenous Pathological Crystals

Erroneous crystallization of endogenous proteins, lipids, or minerals can lead to the formation and accumulation of pathological crystals in the body and drive a wide spectrum of inflammatory diseases.

Cholesterol crystals and associated diseases. Cholesterol is an essential structural component of cell membranes. It is required to maintain both membrane integrity and fluidity, and it is also a precursor for the biosynthesis of hormones, bile acids, and vitamin D. Cholesterol is produced by cells and acquired through the consumption of food containing animal fat. Although cholesterol is essential to many cellular processes, increased serum cholesterol levels are strongly associated with the development and progression of atherosclerosis. Cholesterol molecules are incorporated in cellular membranes, and excess free cholesterol becomes esterified.

Cholesterol esters are insoluble in water at 37°C and form lipid droplets, which are abundant in macrophage foam cells. Furthermore, free cholesterol can form cholesterol crystals, and the deposition of these crystals in the subintimal space of arteries is recognized as a hallmark of atherosclerotic lesions (63). Macrophages within the arterial wall take up excessive amounts of cholesterol and transform into foam cells, a process that can impair their function (64). Furthermore, cholesterol crystals can cause inflammation after they are phagocytosed by macrophages, which results in secretion of IL-1 cytokines (13).

Cholesterol crystals have also been implicated in diseases other than atherosclerosis, such as cholecystolithiasis. In this disorder, a biochemical imbalance of lipids and salts in the bile can mediate precipitation of cholesterol, leading to the formation of gallstones and inflammation in the gallbladder. Indeed, cholesterol monohydrate crystals form in supersaturated bile and are regarded as a prerequisite for the development of cholesterol gallstones (65).

MAC: medial artery calcification

Phosphate crystal diseases. In addition to cholesterol, phosphate-containing crystals can deposit in the vascular wall within the extracellular matrix of vascular smooth muscle cells. This leads to Mönckeberg's sclerosis, a special form of arteriosclerosis characterized by calcification and ossification of the media of medium-size arteries. Calcium pyrophosphate dihydrate, hydroxyapatite, and other crystals are found in the amputated lower legs of patients with Mönckeberg's sclerosis (66). Such crystals may cause fibrosis and intimal proliferation, which may contribute to the progressive occlusion of blood vessels, resulting in ischemic symptoms.

Calcium crystals and associated diseases. Calcium is an essential component in the cellular physiology of living organisms and the most abundant element by mass in many animals. Calcium is also a major component of several physiological and pathological mineralized deposits present in the human body. The main calcium mineralized products (enamel, dentin, and bone) are formed by well-controlled processes of mineralization. Deregulated calcium mineralization can occur, giving rise to pathological conditions including dental calculus, urinary stones, arthritic cartilage, heart valve calcification, and placental calcification, among other disorders. With advanced age, vascular inflammation, hypertension, and certain metabolic disorders such as type 2 diabetes (T2D), calcium crystals can accumulate in the arterial microvasculature. Such mineralization can occur at various sites (cardiac valves, arterial intima or media, capillaries), involve localized or diffuse widespread calcification, and result from numerous causes that provoke active inflammatory and osteogenic processes.

Vascular calcification is a form of ectopic calcification that commonly occurs in atherosclerosis. Atherosclerosis and vascular calcification are closely related and often coexist in patients with chronic kidney disease (CKD). Atherosclerosis is considered the main cause of the elevated cardiovascular risk associated with CKD (67). Vascular calcification, part of the atherosclerotic process, occurs via the deposition in the arterial intima of basic calcium phosphate crystals, primarily consisting of calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], similar to the crystals that mineralize bones. These calcium phosphate crystals are phagocytosed by macrophages, resulting in secretion of proinflammatory cytokines, including TNF-α, IL-1, and IL-8 (68).

Deposits of two types of calcium crystals can cause sudden attacks of painful inflammation in and around joints: (*a*) calcium pyrophosphate crystals, which deposit within the joints and can cause acute crystal arthritis (also known as pseudogout), and (*b*) apatite crystals, which can occur inside joints but are also deposited in tendons and cause acute calcific tendinitis. Calcium pyrophosphate crystals induce strong IL-1β responses (14), and patients with pseudogout respond well to IL-1–blocking therapies (69).

Placental calcification is a common pathological condition in obstetrics. A study by Lu and colleagues (70) indicates that infection with a species of nanobacterium is related to placental calcification. The authors identified the 16S ribosomal DNA of the nanobacterium strain X98418 in nanobacteria-like particles from 16 cases of placental calcification.

Medial artery calcification (MAC) can be identified in patients with CKD and T2D (71). MAC is a characteristic feature of diabetes and a strong predictor of cardiovascular disease in patients with diabetes (71, 72). In T2D, MAC significantly increases the risk of mortality and amputation. Multiple factors contribute to the induction and progression of diabetic MAC, including

inflammation, oxidative stress, adiposity, insulin resistance, advanced glycation end products, and hyperphosphatemia (73). Diabetes and dyslipidemia induce oxidative stress, low-grade inflammation, and angiogenesis in the adventitia of diabetic arteries.

Hydroxyapatite crystal disease. Hydroxyapatite, an essential mineral component of normal bones and teeth, can also cause disease by forming microscopic crystals that accumulate in or around joints, tendons, and ligaments, resulting in inflammation and pain. Hydroxyapatite crystals have been described as a cause of inflammation in rotator cuff disease, which is caused by damage to any of the four tendons that stabilize the shoulder joint. Current treatments for hydroxyapatite crystal disease rely on anti-inflammatory and pain relief measures. Macrophages may promote a vicious cycle of inflammation and calcification in the vessel wall by ingesting neointimal calcific deposits (predominantly hydroxyapatite) and secreting TNF-α (68). This creates a degenerative cycle, as TNF-α itself is a vascular calcifying agent and further aggravates the vascular calcification in diseases such as atherosclerosis (74).

Kidney stones. Renal calculi are mineral deposits present in the urine that accumulate in the kidneys. The renal tubular fluid in the distal nephron of the kidney is supersaturated with calcium oxalate (CaOx), which crystallizes in the tubules as either calcium oxalate monohydrate (COM), or calcium oxalate dehydrate (COD). These aggregates are known as kidney stones, and their primary inorganic constituent is most often COM microcrystals. They also contain small amounts of embedded proteins, which are thought to serve as adhesive bridges between crystals (75).

Kidney stones usually form when urine becomes supersaturated. This occurs when the balance of solute and solvent in urine is disturbed, either by pH- or diet-related changes in mineral composition. Supersaturation, often expressed as the ratio of urinary CaOx, or calcium phosphate, concentration to solubility, is the driving force in kidney stone formation. At levels of supersaturation below 1, crystals dissolve, whereas at supersaturation levels above 1, crystals nucleate and grow, promoting stone formation. Approximately 80% of stones are composed of CaOx with variable amounts of calcium phosphate. Kidney stones typically leave the body via passage in the urine stream, and many stones form and pass without causing symptoms. However, when stones reach a size that obstructs the ureter or renal pelvis, they cause excruciating, intermittent pain, known as renal colic, which radiates from the flank to the groin or inner thigh. Pain control is usually the first measure taken, followed by anti-inflammatory medication, suggesting that inflammation also plays a role. Indeed, supersaturation of the urine is a necessary, but not sufficient, condition for the development of a urinary calculus, and patients with inflammatory bowel disease (Crohn's disease, ulcerative colitis) have a tendency to form stones (76).

Uric acid crystals. Gout, a chronic inflammatory disease characterized by recurrent attacks of acute joint inflammation, is regarded as the prototypical crystal-induced arthropathy. It is perhaps the oldest known type of arthritis, first described and identified by Hippocrates and the Egyptians in 2046 BCE. The causative agent of gout, however, was only identified in the 1960s, when, for the first time, the presence of urate crystals in the synovial fluid of gout patients was visualized with polarized light microscopy. Indeed, the diagnostic standard remains identification of negatively birefringent MSU crystals in synovial fluid or tophus aspirate using polarizing microscopy (77). An estimated 6.1 million adults in the United States have had gout. The prevalence increases with age and is higher among men than women, with an overall ratio of 3 or 4 to 1.

Uric acid is a danger signal released from injured cells that acts as an adjuvant signal to the immune system (78). Uric acid stimulates dendritic cell (DC) maturation and, when coinjected with antigen in vivo, significantly enhances responses from $CD8^+$ T cells. When uric acid reaches

an abnormally high concentration in the blood, needlelike MSU crystals form. They form in collecting ducts and in the surrounding interstitium of the renal medulla, where urine osmolarity reaches its maximum. Uric acid crystals can also deposit in other tissues in the body, especially in joints, giving rise to intense inflammation accompanied by pain and disability.

Uric acid crystals can also form deposits in the kidney or urinary tract, leading to kidney stone formation and occasionally impairing kidney function. Deposition of uric acid crystals can occur asymptomatically for months or years. However, despite the continuous deposition of uric acid crystals in the joints, sudden and severely painful attacks of gout are sporadic. Triggers for recurrent flares include recent diuretic use, alcohol intake, hospitalization, and surgery. For many years the molecular cause of inflammation in patients with gout remained unknown. Developments in the pathophysiology of gout have revealed a role for neutrophils and IL-1 in disease and shed light on possible mechanisms. Neutrophils ingress into the joint cavity, where they can be activated by direct contact with crystals; because of their ability to stimulate the release of numerous inflammatory mediators, they are thought to create an arthritic condition. Without long-term treatment, this can lead to joint damage and significant functional impairment. Induction of the release of proinflammatory cytokines and chemokines by MSU crystals has also been suggested to play an important role (79). IL-1β release, for example, is a central event in the inflammatory reaction of a gout attack (14, 23). Thus, IL-1–blockage therapies, using either recombinant IL-1 receptor antagonist (IL-1Ra; anakinra) or anti–IL-1β antibodies, are effective in gout (24, 25, 77).

Options for managing acute attacks include the use of nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, glucocorticoids, and possibly corticotropin (80). NSAIDs and colchicine are first-line agents for acute attacks (80). Oral colchicine has long been used, although it was only recently (in 2009) approved by the US Food and Drug Administration (FDA) for use in patients with acute gout. It prevents uric acid–induced IL-1β release from macrophages (14, 81, 82).

Crystalline retinopathy. Crystalline retinopathy is a rare complication of chronic retinal detachment. The observation of crystalline retinal deposits should prompt the search for chronic retinal detachment. The composition of these crystals is unknown (83).

Environmental Pathological Crystals

Economic development occurs at the cost of environmental changes, such as air and water pollution, which expose populations to harmful byproducts of modern human activity.

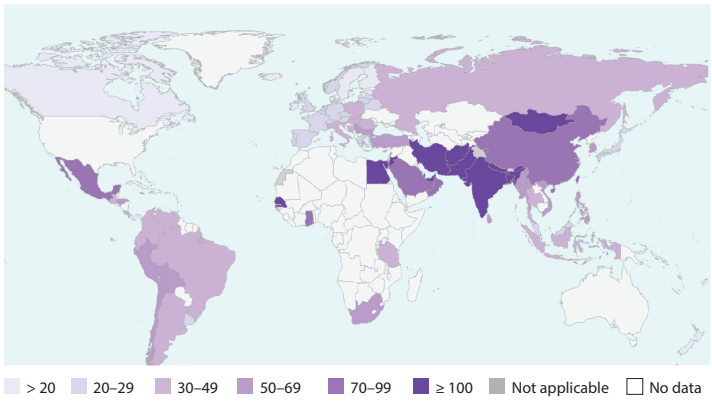
Air pollution. The World Health Organization (WHO) estimates that 3.7 million deaths could be attributed to ambient (outdoor) air pollution in 2012 (84). Approximately 88% of those deaths occurred in low- and middle-income countries, with the greatest burden falling on the Western Pacific and South-East Asian WHO regions (**Figure 5**). In 2012, 4.3 million deaths were triggered by household (indoor) air pollution. Almost all of these deaths occurred in low- and middle-income countries. Altogether, air pollution is responsible for approximately 1 in 8 deaths worldwide. The primary pollutants produced by human activity come from the combustion of carbon sources, including coal and petroleum, or from the sulfur dioxide released from factories. These carbon-based pollutants contain significant amounts of particulate matter (PM), known as carbon black nanoparticles (CBNPs) or fine particles.

Exposure to ambient particulate pollutants causes adverse health effects and can aggravate existing respiratory conditions, such as asthma and chronic obstructive pulmonary disease (COPD). Experimental evidence exists that inhalation of ambient PM promotes alveolar and systemic inflammatory responses in rats (85, 86). Furthermore, PM can combine with atmospheric oxidants,

PM: particulate matter

CBNPs: carbon black nanoparticles

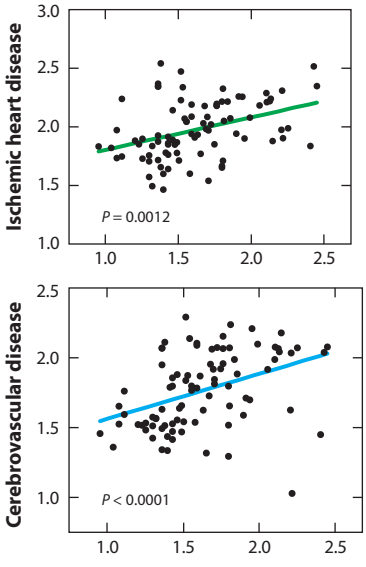
a Exposure to PM10 (μg/m³) in urban areas, 2008–2013



b

Country	PM10 (μg/m³) (per 100,000)	Ischemic heart disease (per 100,000)	Cerebrovascular disease (per 100,000)
Afghanistan	268.0	328.6	109.5
Andorra	24.0	45.4	32.7
Argentina	30.0	70.6	43.5
Austria	27.0	72.7	26.0
Bahrain	254.0	68.8	28.2
Bangladesh	163.0	203.7	108.3
Belarus	24.0	348.1	129.3
Belgium	27.0	58.0	29.7
Bhutan	23.0	221.7	117.7
Bolivia	51.0	93.5	70.1
Bosnia & Herz.	49.0	96.1	115.5
Brazil	41.0	81.2	74.0
Brunei Daruss.	12.0	54.3	45.0
Bulgaria	58.0	151.4	142.5
Canada	11.0	66.2	22.9
Chile	64.0	51.5	45.3
China	90.0	79.7	161.9
Colombia	43.0	85.8	42.2
Costa Rica	31.0	74.0	29.5
Cuba	36.0	111.3	58.5
Cyprus	29.0	77.4	35.9
Denmark	13.0	55.9	39.3
Ecuador	38.0	39.9	34.5
Egypt	136.0	174.0	110.4
Estonia	13.0	173.3	57.8
Finland	12.0	93.8	33.5
France	25.0	29.2	21.7
Germany	23.0	75.0	31.2
Ghana	98.0	120.1	125.5
Greece	26.0	60.3	61.6
Guatemala	45.0	58.9	20.8
Honduras	58.0	152.4	90.8
Hungary	33.0	169.0	68.8
Iceland	9.0	68.1	28.7
India	134.0	165.8	116.4
Indonesia	48.0	150.8	90.0
Iran (Islamic Republic of)	127.0	194.5	97.4
Ireland	18.0	79.2	30.4
Israel	63.0	46.4	19.7
Italy	32.0	51.7	34.9
Jamaica	36.0	110.7	62.9
Japan	21.0	31.2	36.7
Jordan	128.0	162.5	119.4
Latvia	23.0	220.8	117.1
Lebanon	63.0	164.4	62.7
Lithuania	23.0	233.7	89.2
Luxembourg	18.0	53.6	34.1
Malaysia	29.0	138.7	75.8
Maldives	20.0	50.9	37.6
Mauritius	72.0	118.4	76.7
Mexico	79.0	87.7	38.8
Monaco	20.0	37.4	26.6
Mongolia	140.0	75.7	150.9
Myanmar	69.0	164.7	97.4
Nepal	114.0	152.6	82.6
Netherlands	25.0	39.8	27.3
New Zealand	16.0	76.5	33.3
Norway	20.0	57.2	32.6
Oman	82.0	181.9	51.5
Pakistan	282.0	222.9	119.6
Peru	63.0	45.0	33.0
Philippines	50.0	121.6	82.8
Poland	39.0	122.4	60.8
Portugal	27.0	43.8	62.7
Qatar	165.0	80.1	10.7
Republic of Korea	51.0	34.7	65.1
Romania	39.0	155.0	127.1
Russian Federation	33.0	296.7	195.8
Saudi Arabia	87.0	180.6	50.0
Senegal	179.0	97.3	118.0
Serbia	53.0	105.1	118.6
Singapore	27.0	82.4	33.7
Slovakia	34.0	217.7	74.7
Slovenia	31.0	60.0	45.5
South Africa	56.0	71.0	83.4
Sri Lanka	23.0	84.5	42.8
Sudan	64.0	212.0	118.0
Sweden	17.0	71.0	32.9
Switzerland	23.0	52.2	22.0
Thailand	39.0	87.1	123.0
Turkey	58.0	157.1	116.5
United Arab Emirates	161.0	94.5	42.3
United Kingdom	21.0	68.8	36.9
United Republic of Tanzania	35.0	117.6	137.7
Uruguay	27.0	70.8	71.9
Venezuela (Bolivarian Republic of)	47.0	107.3	53.4
Vietnam	65.0	112.5	173.0

c Age-standardized death rates per 100,000 habitants of both sexes, by cause and member state, 2008



Exposure to particulate matter PM10 (μg/m³) in urban areas, 2008–2013

which can potentiate negative health effects. In experiments with rats, greater lung injury and inflammation was observed in animals exposed to diesel exhaust particles oxidized by ozone (O_3) than those exposed to nonoxidized diesel particles (87). Such findings hold true for other combustion particles exposed to O_3 (88, 89).

CS: cigarette smoke

In **Figure 5**, we use publicly available data from the report *Global Health Observatory Data: Exposure to Ambient Air Pollution* (84) to analyze the correlation between PM concentrations measured in urban areas during the years 2008–2012 and mortality rates for cardiovascular diseases, available in the report *Global Atlas on Cardiovascular Disease Prevention and Control* (90). The data encompass annual mean PM concentrations from a total of 1,600 cities within 87 countries; many of the concentrations exceed the WHO's Air Quality Guideline values. Annual mean concentrations of PM with an aerodynamic diameter of 10 μm or less (PM10) were significantly correlated with mortality from ischemic heart disease and cerebrovascular disease (**Figure 5*b*,*c***).

Data from the United States were not available in the WHO report. However, one pioneer study revealed a striking association between cardiovascular disease and reported air pollution in Los Angeles, California, in 2005 (91). Specifically, the authors found a significant correlation between long-term exposure to ambient air pollution and the development of cardiovascular disease. Importantly, a study published during the preparation of this manuscript reported air pollution as the main cause of premature mortality in the West (92).

Many recent studies have focused on the impact of PM on human health. It is now agreed that exposure to CBNPs constitutes a significant environmental risk factor for humans, particularly in susceptible populations with predisposing lung conditions. Strong epidemiological associations also exist between exposure to PM in air pollution and the incidence of COPD, ischemic heart disease, stroke, and lung cancer in human populations. Experiments in mice have demonstrated that inhalation of CBNPs in aerosols exacerbates lung inflammation, as evidenced by histopathological analysis and by the expression levels of IL-6, fibronectin, and interferon-γ (IFN-γ) mRNAs in lung tissue (93). In humans, exposure of asthmatic children to CBNPs and carbon monoxide from traffic pollution is associated with asthma exacerbation (94). One of the main toxicological effects of atmospheric PM inhalation is oxidative stress, which results in the generation of ROSs by affected cells (95, 96). CBNPs can directly affect the endothelium by generating ROSs, leading to cytotoxic injury and an inflammatory response (97).

Particulate matter from cigarette smoke. Cigarette smoke (CS) contains two major components, the vapor or smoke phase and the particulate phase (98). CS is composed of dense tar, which, like CBNPs, is carbon based and so has a similar effect as PM from air pollution (98).

Figure 5

World exposure to ambient air pollution and cardiovascular diseases. (*a*) World map showing the annual mean concentrations of particulate matter with an aerodynamic diameter of 10 μm or less (PM10) in urban areas, estimated on the basis of measurements from a total of 1,600 cities within different countries (measurements range from 9 to more than 100 $\mu g/m^3$). The reported country averages rely on measurements made during the years 2008–2012, with the great majority made during the years 2011 and 2012. Adapted from a map by the World Health Organization (**http://www.who.int/gho/phe/outdoor_air_pollution/exposure/en/**). (*b*) Mean PM10, as in panel *a*, and age-standardized death rates per 100,000 for both sexes, by cause and member state, 2008. Source: World Health Organization, *Global Atlas on Cardiovascular Disease Prevention and Control* (**http://whqlibdoc.who.int**). (*c*) Correlation between the reported average PM10 in a country's urban areas and death rates from cardio- and cerebrovascular disease. Parametric correlation (Pearson's $r = 0.3424$, $P = 0.0012$, PM10 versus ischemic heart disease) was performed when data fit a normal distribution. Nonparametric correlation (Spearman's $r = 0.4703$, $P < 0.0001$, PM10 versus cerebrovascular disease) was performed when data did not fit a normal distribution. All data are available at **http://www.who.int/gho/phe/outdoor_air_pollution/exposure/en/** and **http://www.who.int/cardiovascular_diseases/publications/atlas_cvd/en/**.

When inhaled, the particulate phase is deposited in the lungs, where it initiates an inflammatory response by generating ROSs. This subsequently leads to the synthesis and release of inflammatory cytokines from lung endothelial cells (99). This results in chronic inflammation, which manifests as COPD (100).

IL-1R signaling is crucial for CS-induced airway inflammation (101), and it is believed that IL-1 cytokines are the main drivers of COPD in smokers (102, 103). IL-1α, IL-1β, and IL-18 levels are elevated in bronchoalveolar lavage fluid and sputum from COPD patients (104–106), and mice overexpressing IL-1β or IL-18 display a COPD-like phenotype (102, 103). Although pulmonary inflammation is significantly attenuated by neutralization of IL-1α or IL-1β (104), CS-mediated lung neutrophilia seems to be exclusively driven by IL-1α (107).

Evidence exists that the NLRP3 inflammasome plays a role in CS-induced pathology (104, 108, 109). Nevertheless, NLRP3 activation has not yet been specifically associated with the particulate phase of CS. Thus, it would be interesting to investigate whether NLRP3 is activated by CS using mechanisms similar to those used by other crystals.

Silica crystals and silicosis. Silicosis, a disease marked by chronic inflammation and fibrosis of the lung, is a preventable condition caused by inhalation of crystalline silica particles. Silicosis is considered an occupational disease, as exposure to crystalline silica particles occurs primarily on building or mining sites (110). Silica particles stimulate an inflammatory response in the lungs through interactions with both alveolar macrophages and endothelial cells (11, 111). As silica particles are unable to be degraded, tissue damage persists and chronic inflammation develops. Persistent inflammation drives T cell responses, which can result in lung fibrosis and the development of autoimmune reactions elsewhere in the body (112).

IL-1 appears to be the most important cytokine involved in silicosis, as blockage of IL-1 greatly reduces expression of other inflammatory molecules, including IL-6, IL-8, and TNF-α (20). This suggests that IL-1 acts upstream of an inflammatory cascade activated in silicosis. This concept is supported by the findings that the release of IL-1α precedes the release of other DAMPs and that IL-1α-blockage largely prevents silica-induced inflammation (21). Similarly, systemic inhibition of NF-κB, the primary proinflammatory transcription factor activated by IL-1R signaling, but not epithelial cell–specific NF-κB, reduces inflammation in human silicosis (113), and blockage of IL-1 reduces the fibrotic response (114).

Silicosis is also associated with increased risk of autoimmune disorders and fibrosis, particularly in the heart and kidney (115). This occurs when chronic inflammation, driven by the innate immune response, deregulates T cells. Silica-driven inflammation decreases the proportion of regulatory T cells and increases the proportion of proinflammatory T helper (IL-)17 cells, which contributes to the ongoing pathogenesis of silicosis (116). The balance between these two T cell subtypes is also regulated by IL-1β generated in response to silica, as inhibition of IL-1β both locally and systemically dramatically reduces fibrosis and restores balance in the T cell compartment (117). Thus, currently available IL-1R antagonists may represent a useful therapeutic strategy to counter the chronic inflammatory condition associated with silicosis.

Asbestos-induced inflammation and asbestosis. Asbestosis is caused by inhalation of asbestos, a structured, fibrous silicate mineral used as a heat-resistant building material and insulator. Although asbestosis is caused by crystals that are structurally distinct from those that cause silicosis, the two diseases share fundamental properties, including crystal-mediated generation of acute and chronic inflammatory responses and ongoing fiber deposition in the lungs. In addition, inflammation is primarily driven by IL-1 in both diseases (118–120). Despite these similarities, the inflammatory signaling pathways instigated by these crystals differ. This is evident

in the differential effects of asbestos- and silica-driven inflammation. A recent study examining transcriptional responses toward these two different crystals found that silica induces a more proinflammatory response, whereas asbestos favors aberrant cell proliferation (121). This difference is manifested in the propensity of asbestos, but not silica, to instigate tumorigenesis.

Hz: hemozoin

Asbestos is the primary cause of mesothelioma, and 3,000 people in America are diagnosed with asbestos-induced mesothelioma each year. Although it is strongly linked to inflammation, asbestos-mediated mesothelioma occurs independent of the inflammatory response initiated by NLRP3, as NLRP3-deficient mice exhibit a similar incidence of asbestos-induced mesothelioma as wild-type mice, although they have an attenuated inflammatory response (122). Similarly, the inflammatory reaction induced by asbestos is greatly reduced in IL-1R–deficient mice, but the incidence of mesothelioma does not change (122). Rather, these mice display an increased Th2 response, with an increase in IL-13 and PGE_2, suggesting that the cancerous phenotype is more closely associated with a Th2 immune response. Recently, inhibition of the alarmin HMGB-1, which is released from mesothelioma cells, has been demonstrated to be sufficient to attenuate the cancerous phenotype of these cells (123). Collectively, these results suggest that the carcinogenic effect of asbestos is not IL-1–driven. This illustrates that even minor structural differences in crystals can alter the inflammatory pathways initiated and their pathological outcomes.

Infection-Related Crystalline Material

Infectious agents, such as bacteria, viruses, or parasites, can also cause the accumulation of crystalline material inside cells or in the intercellular space.

Hemozoin and malaria. Parasites of the genus *Plasmodium* cause malaria, one of the most devastating diseases in the world. The WHO estimates that 3.3 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk (>1 in 1,000 chance of getting malaria in a year) (124). Malaria remains a significant cause of illness and death in children and adults in the countries in which it is endemic.

Plasmodium parasites replicate inside red blood cells, where they feed on hemoglobin as a source of amino acids. Digestion of hemoglobin by *Plasmodium* results in the accumulation of heme, which is toxic for the parasite. To remove the heme from their environment, plasmodial species detoxify it inside their food vacuole. This is accomplished by an enzyme expressed by the parasite that polymerizes heme into a crystal byproduct called hemozoin (Hz). Hz is released from *Plasmodium*-infected erythrocytes during the schizont burst (also known as the burst of infected erythrocytes) and is rapidly phagocytosed by immune cells, such as macrophages, neutrophils, and DCs.

Plasmodium infection can provide both signals required for inflammasome activation: firstly, Glycophosphatidylinositol, anchored to the plasma membrane of *Plasmodium* parasites, activates TLR4/2 (125) and induces NF-κB signaling; furthermore, Hz carries *Plasmodium* DNA into the phagolysosomal compartment, where it activates the TLR9/NF-κB signaling pathway, thus providing signal 1 for NLRP3 and IL-1 expression (126). Subsequently, phagocytosis of Hz induces LD and NLRP3 activation (34) and releases parasite DNA into the cytosol, where it activates the AIM2 inflammasome (127).

Charcot-Leyden crystals. Charcot-Leyden crystals (CLCs) were described more than 160 years ago (128) as a hallmark of eosinophilic leukocyte infiltration into tissues. They are primarily associated with allergic reactions, infection with helminths, and hematologic malignancies with eosinophilia (129, 130). CLCs are characteristically long (up to 50 μm in length), thin, and dipyramidal. Eosinophils have been established as the unique cellular source of this crystalline material,

NANOTECHNOLOGY—THE NEXT FRONTIER OR AN INFLAMMATORY TIME BOMB?

Nanotechnology is an emerging area of research involving the use of nanoparticles (NPs), which are highly reactive nano-sized particles with a diameter of 100 nm or less. They have been integrated into many applications, including the delivery of medical treatments (169).

Despite their already widespread use, our understanding of how these crystalline particles interact with the immune system is still developing. It is becoming increasingly clear, however, that NPs can activate multiple inflammatory pathways. Both silica and titanium NPs can activate the NLRP3 inflammasome (170), inducing IL-1–mediated lung inflammation and neutrophilia. Other NPs, including carbon nanotubes, which have a similar morphology to asbestos microfibers, can cause asbestos-like inflammation and pathology (171). In addition, NPs are transported or can penetrate through tissues, enabling them to cause inflammation distant from their entry point (172).

Given both the benefits NPs offer and their inflammatory potential, improving their use is imperative to ensure their safe and continued development. This improvement should include rational NP design based on an increased understanding of how they mediate inflammation. For example, the surface of the NP plays an important role in triggering inflammation. An alternative coating can be applied to the surface of silica NPs with amine or phospho groups, greatly reducing their inflammatory potential (173). Increased understanding of how NPs trigger inflammation is still required, however, and until this understanding is reached, caution must prevail.

which consists of lysophospholipase, a protein synthesized by eosinophils and stored in the large granules of these cells (131).

Since the protein composing these crystals was discovered, no biochemical activity or biological function has been established for it. Nevertheless, CLCs persist in the sputum, feces, and tissues of patients with eosinophil inflammatory processes and are resistant to proteolytic digestion. Hence, their persistence in tissues, even after the disintegration of eosinophils at the inflammatory site, may have an important impact on tissue inflammation and homeostasis. Although necrosis is a prominent cytological feature in several diseases in which CLCs form (130, 132), the inflammatory potential of such residual crystalline artifacts in tissues remains to be explored.

Pharmacologically Induced Crystal Formation

Many therapeutic compounds currently used to treat diseases are able to form crystals (see sidebar, Nanotechnology—The Next Frontier or an Inflammatory Time Bomb?). The kidneys are often a major site of crystal accumulation. Crystal formation of pharmacological agents frequently occurs in the distal tubule of the kidney, as this is the main site of proton secretion. Indeed, intratubular acidosis can promote the precipitation of certain solutes and drugs, and inefficient acidification promotes the precipitation of other pharmacological compounds.

Atazanavir. Atazanavir is a protease inhibitor commonly used to treat patients infected with human immunodeficiency virus (HIV). It is one of the key drugs used in combination antiretroviral therapy against HIV. One year after its approval by the FDA in 2003, the first report of acute interstitial nephritis associated with atazanavir was published (133). After that case, several other studies reported renal insufficiency in HIV patients as a result of interstitial nephritis. This type of interstitial nephritis is characterized by the presence of crystalline deposits, 60% of which include atazanavir metabolites (134–138). From these findings, it was concluded that atazanavir can form needle-shaped crystals in urine and renal interstitial tissues, leading to crystalluria,

urolithiasis, acute kidney injury, and CKD. In epidemiological studies, atazanavir/ritonavir alone or in combination with tenofovir has been associated with increased risk of progression to CKD.

Sevelamer crystals. Sevelamer (also known as Renagel) is a phosphate-binding drug used to treat hyperphosphatemia in patients with CKD. It is prescribed for the management of hyperphosphatemia in adult patients with stage 4 or 5 CKD who are on hemodialysis. When taken with meals, it binds to dietary phosphate and prevents its absorption. Sevelamer forms crystals that deposit in the gastrointestinal tract and injure the mucosa, leading to inflammation (139). In general, sevelamer crystals display broad, curved, and irregularly spaced fish scales with a variable eosinophilic-to-rusty-brown color on hematoxylin and eosin (H&E) staining and a violet color on alcian blue–periodic acid–Schiff (PAS) staining with diastase. Interestingly, histological processing of crushed sevelamer tablets reveals crystals identical to those found in patient samples.

Particulate immune adjuvants. Vaccines are composed of two main components, the antigenic material and the adjuvant. Adjuvants possess specific immune-stimulatory properties, leading to an adaptive immune response and immunity. Given the immune-stimulatory properties of particulate materials, their extensive use as adjuvants is not surprising.

The foremost example of particulate adjuvants is aluminum salts, or alum. Alum has been the most widely used adjuvant for the past 80 years, since the observation that coinjection with alum leads to robust, antigen-specific T and B cell responses (140). It initiates a Th2 response when administered in a vaccine, resulting in an IgG antibody response to the antigen and establishing humoral immunity. However, how alum exerts this effect is still unclear. Alum induces IL-1β release from macrophages (11). However, whether IL-1 is required to establish humoral immunity is controversial (38, 141–143). As different types of alum have different immune effects, the controversy might be a result of differences in the formulations of alum used (144). However, given that the humoral response to alum is MyD88-independent (145), it seems unlikely that IL-1 plays an important role in the formation of immunity. Subsequent studies have also established that PGE_2 is released in response to alum by a mechanism related to crystal phagocytosis (17) or upon triggering abortive phagocytosis, leading to increased antigen uptake (146). Inhibition of this response decreases the humoral response to antigen.

The properties of a crystal that enhance or suppress its immunoreactivity are currently being investigated to design superior immune adjuvants. This is particularly challenging because the exact mechanism of action, as well as the crystalline properties controlling it, remains unidentified. However, much insight has been gained recently.

6. CRYSTAL MORPHOLOGY AND INFLAMMATION

Crystals can assume infinite, disparate morphologies depending on the relative growth rates of their various surfaces and the availability of soluble counterparts in the environment. Most of an inflammatory reaction caused by pathological crystals is dependent on the phagocytosis of the crystals by immune cells. Therefore, the cues that immune cells use to determine whether a target should be ingested play a crucial role in crystal-induced inflammation. Although cellular receptors are clearly important for the phagocytosis of crystalline particles, other physical characteristics of crystals play an important role in the activation of immune cells in a manner that does not involve cellular receptors. Features including size, rigidity, texture, and surface charge not only play a role in determining how phagocytic cells select crystals as targets for phagocytosis, they also influence inflammatory output from these cells (147–149; **Figure 6**).

Polymeric microparticles, a new generation adjuvant, have also been tested for immunogenicity, and, compared with alum, they promote enhanced antigen transport and more effective immune

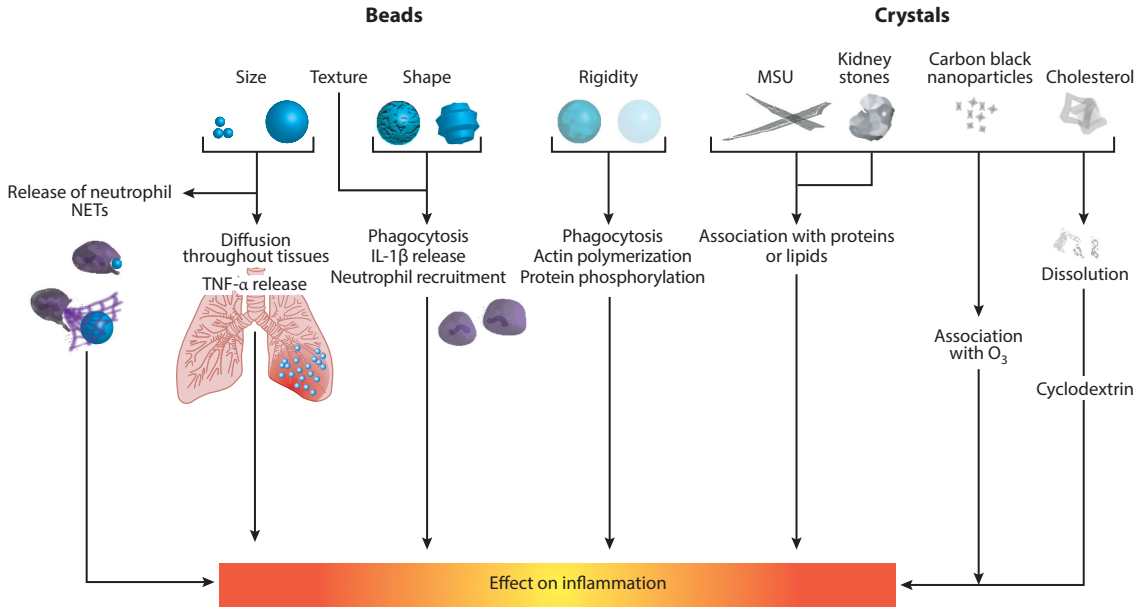


Figure 6

Crystal morphology and inflammation. Crystals can assume infinite shapes, combine with organic elements, and take on electrical charges; all of these factors have effects on their immunogenicity. Cells of the immune system are able to distinguish and differentially respond to tiny variations in crystal morphology, such as size; texture; rigidity; and association with proteins, lipids, or inorganic elements. All of these crystal features influence phagocytosis by immune cells and the ensuing inflammatory response. Abbreviations: MSU, monosodium urate; NET, neutrophil extracellular trap.

cell activation (150). To further understand the properties essential for the immunogenicity of these particles, Williams and colleagues (151) have investigated layered double hydroxide crystals of varying size, ion composition, and charge. The charge of the particle, as well as the separation of the layers of ions, directly controls the immunogenicity of the crystal, enabling researchers to tune the immune response. Such crystalline adjuvants show promise in eliciting a controlled immune response after vaccination.

In a study in which microparticles of similar composition but varying texture were examined for immunogenicity in a mouse peritonitis model, particles with rough surfaces were not only more readily phagocytosed in vitro, but they also induced more rapid neutrophil recruitment than round particles. Higher levels of IL-1β were secreted from macrophages that had phagocytosed rough particles (149). In agreement with these findings, another study employing cobalt-chromium-molybdenum alloy particles reported that larger, irregular particles caused greater LD and induced significantly more IL-1β release from macrophages than equal doses of smaller, perfectly spherical particles (152).

It is noteworthy that crystal size and texture have opposite effects on TNF-α secretion and IL-1β release. TNF-α secretion by macrophages is inversely related to hydroxyapatite crystal size, suggesting that the microscopic calcific deposits found in the early stages of atherosclerosis pose a greater inflammatory risk than the macroscopic deposits in more advanced lesions (153). An explanation for these findings may rely on the potential of smaller particles to translocate through tissues and cause inflammation. In inhalation experiments with rats using anatase particles of different sizes, smaller particles elicit a persistently high inflammatory reaction in the lungs compared to larger particles. Although no difference in mass deposition exists between smaller

and larger particles, in the lower respiratory tract smaller particles display increased translocation to the pulmonary interstitium, followed by increased inflammation and fibrosis (154). Smaller silica particles are more immunogenic than larger particles (155).

The size of particles can also affect the function of neutrophils in immunity. Neutrophils selectively release neutrophil extracellular traps (NETs) depending on microbe size. NETs are released in response to large pathogen formations, such as *Candida albicans* hyphae and extracellular aggregates of *Mycobacterium bovis*, but not in response to small yeast or single bacteria that can be phagocytosed. Similarly, MSU, a large crystal, causes release of NETs upon its phagocytosis by neutrophils. This response has been suggested to immobilize MSU crystals and prevent them from being further phagocytosed, thus limiting their immune-stimulating potential (156). In an additional effort to restrain MSU crystal–driven inflammation, NETs aggregate and degrade MSU-driven cytokines and chemokines (157). It would be interesting to determine whether smaller particles reduce NET formation, and whether this is associated with lower or higher levels of inflammation.

NETs: neutrophil extracellular traps

CD: cyclodextrin

Other mechanical features of crystals can modulate the efficiency of phagocytosis. Macrophages appear to display a strong preference for rigid objects. When microbeads with identical chemical properties, size, and surfaces, but different stiffness, are fed to macrophages, soft beads are unable to stimulate the assembly of the actin filaments required to form and close phagosomes. Phagocytosis, cytoskeleton rearrangement, and protein phosphorylation are sensitive to rigidity signals (62). Other features of crystals that interfere with their proinflammatory potential are their clearance and dissolution propensity. For example, macrophages can dissolve MSU crystals, but they are not able to efficiently eliminate silica or asbestos crystals.

Cyclodextrins (CDs) are FDA-approved cyclic oligosaccharides that dissolve lipids in their hydrophobic core (158). They are used to solubilize and entrap various lipophilic pharmaceutical agents for delivery into human cells. β-CDs mediate cholesterol efflux from cells more efficiently than high-density lipoproteins (159). CDs increase cholesterol solubility, promote the removal of cholesterol from foam cells in vitro, and initiate anti-inflammatory mechanisms (160–162).

Our group has found that CD is able to dissolve cholesterol crystals in vitro and clear them from atherosclerotic plaques, thus reducing systemic inflammation in an in vivo model of atherosclerosis (A. Grebe & E. Latz, unpublished data). Treatment of hypercholesterolemic *ApoE*$^{-/-}$ mice with CD after 12 weeks of high fat diet–induced atherosclerosis results in a significant reduction in atherosclerotic plaque size. Furthermore, CD reduces the amount of cholesterol crystals in atherosclerotic plaques in mice fed a continuous high-cholesterol diet. CD also increases the cholesterol efflux capacity of macrophages that have phagocytosed cholesterol crystals in vitro (A. Grebe & E. Latz, unpublished data). Thus, the dissolution propensity of some crystals can be explored for therapeutic purposes.

Finally, the propensity of crystals to form complexes with proteins and lipids affects their immunogenicity and inflammatory potential. The difference in the pathological behaviors of COM and COD crystals can be explained by the poor adhesion of COD crystals and their reduced capacity to form stable aggregates with proteins (163). The adjuvanticity of alum is greatly affected by its interaction with membrane lipids in DCs (146). Furthermore, initial studies performed on different compositions of alum have identified both the crystallinity and the exposure of hydroxyl groups on the surface of the crystal as important factors in activating an immune response (164).

7. HOW INFLAMMATION DRIVES CRYSTALLIZATION

As discussed, the formation of bones in vertebrates and their remodeling after fracture are examples of the highly controlled crystallization of minerals in physiological functions. The process of

bone formation involves complex interactions between osteoblasts and osteoclasts, the primary bone-building and bone-resorbing cells, and other cells in the microenvironment. The activity of osteoclasts is regulated by the action of systemic cytokines and hormones such as parathyroid hormone, estrogen, and calcitonin. TNF-α and IL-1 induce a marked increase in osteoclast activity and play an important role in postmenopausal osteoporosis. TGF-α is produced by several solid tumors associated with hypercalcemia of malignancy and can stimulate osteoclastic bone resorption in cultures of mouse organs.

Inflammation and proinflammatory cytokines can also affect the deposition of calcium crystals in different organs, giving rise to calcifying disorders. Vascular calcification is an ectopic calcification that commonly occurs in atherosclerosis, diabetes mellitus, and end-stage renal disease, as well as with advancing age. Oxysterol 25-hydroxycholesterol and TGF-β are both present in atherosclerotic lesions and induce calcification of vascular cells (165). The monocyte- and macrophage-derived proinflammatory cytokines TNF-α and IL-1β activate tissue nonspecific alkaline phosphatases, which cause mineralization of cultured vascular smooth muscle cells (71, 74), and influence cardiovascular calcification (74). Indeed, vascular calcification is associated with increased levels of circulating TNF-α and other proinflammatory markers in patients with Type 1 and Type 2 diabetes (166, 167), and TNF-α neutralization using antibodies slows osteogenesis and vascular calcium deposition in diabetic mice (168). Thus, vascular calcification is actively related to the inflammatory process of atherosclerosis.

8. CONCLUSIONS

Crystal-related diseases are prevalent and increasing in incidence worldwide. Diseases such as atherosclerosis, stroke, COPD, and gout cause immense morbidity and together surpass infectious diseases as a cause of death. We have seen major advances in the understanding of the pathophysiological origins of crystal-mediated diseases in recent years, and this knowledge has spurred the development of new therapies. The production of proinflammatory cytokines of the IL-1 family seems to be at the core of crystal-induced inflammation. Therefore, therapies aimed at blocking IL-1 signaling are being tested and have proved to be efficient in many instances. However, therapeutic approaches aimed at the prevention of crystallization or the dissolution of crystallized materials should also be considered.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Poloni LN, Ward MD. 2014. The materials science of pathological crystals. *Chem. Mater.* 26(1):477–95
2. Khan SR, Glenton PA, Backov R, Talham DR. 2002. Presence of lipids in urine, crystals and stones: implications for the formation of kidney stones. *Kidney Int.* 62(6):2062–72

3. Yoshimura Y, Lin Y, Yagi H, Lee Y-H, Kitayama H, et al. 2012. Distinguishing crystal-like amyloid fibrils and glass-like amorphous aggregates from their kinetics of formation. *PNAS* 109(36):14446–51
4. Maji SK, Perrin MH, Sawaya MR, Jessberger S, Vadodaria K, et al. 2009. Functional amyloids as natural storage of peptide hormones in pituitary secretory granules. *Science* 325(5938):328–32
5. Jarrett JT, Lansbury PT. 1993. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* 73(6):1055–58
6. Come JH, Fraser PE, Lansbury PT. 1993. A kinetic model for amyloid formation in the prion diseases: importance of seeding. *PNAS* 90(13):5959–63
7. Chiti F, Dobson CM. 2006. Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* 75:333–66
8. Blancas-Mejía LM, Ramirez-Alvarado M. 2013. Systemic amyloidoses. *Annu. Rev. Biochem.* 82:745–74
9. Kirschvink JL, Walker MM, Diebel CE. 2001. Magnetite-based magnetoreception. *Curr. Opin. Neurobiol.* 11(4):462–67
10. Ng G, Sharma K, Ward SM, Desrosiers MD, Stephens LA, et al. 2008. Receptor-independent, direct membrane binding leads to cell-surface lipid sorting and Syk kinase activation in dendritic cells. *Immunity* 29(5):807–18
11. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, et al. 2008. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* 9(8):847–56
12. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. 2008. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320(5876):674–77
13. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, et al. 2010. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464(7293):1357–61
14. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nat. Cell Biol.* 440(7081):237–41
15. Boya P, Kroemer G. 2008. Lysosomal membrane permeabilization in cell death. *Oncogene* 27(50):6434–51
16. Lima H, Jacobson LS, Goldberg MF, Chandran K, Diaz-Griffero F, et al. 2013. Role of lysosome rupture in controlling Nlrp3 signaling and necrotic cell death. *Cell Cycle* 12(12):1868–78
17. Kuroda E, Ishii KJ, Uematsu S, Ohata K, Coban C, et al. 2011. Silica crystals and aluminum salts regulate the production of prostaglandin in macrophages via NALP3 inflammasome–independent mechanisms. *Immunity* 34(4):514–26
18. Satpathy SR, Jala VR, Bodduluri SR, Krishnan E, Hegde B, et al. 2015. Crystalline silica-induced leukotriene B4-dependent inflammation promotes lung tumour growth. *Nat. Commun.* 6:7064
19. Dinarello CA. 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27(1):519–50
20. Herseth J, Refsnes M, Låg M, Hetland G, Schwarze P. 2008. IL-1β as a determinant in silica-induced cytokine responses in monocyte–endothelial cell co-cultures. *Hum. Exp. Toxicol.* 27(5):387–99
21. Rabolli V, Badissi AA, Devosse R, Uwambayinema F, Yakoub Y, et al. 2014. The alarmin IL-1α is a master cytokine in acute lung inflammation induced by silica micro- and nanoparticles. *Part. Fibre Toxicol.* 11(1):69
22. Bäck M, Hansson GK. 2015. Anti-inflammatory therapies for atherosclerosis. *Nat. Rev. Cardiol.* 12(4):199–211
23. di Giovine FS, Malawista SE, Nuki G, Duff GW. 1987. Interleukin 1 (IL 1) as a mediator of crystal arthritis. Stimulation of T cell and synovial fibroblast mitogenesis by urate crystal–induced IL 1. *J. Immunol.* 138(10):3213–18
24. So A, De Smedt T, Revaz S, Tschopp J. 2007. A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res. Ther.* 9(2):R28
25. Terkeltaub R, Sundy JS, Schumacher HR, Murphy F, Bookbinder S, et al. 2009. The interleukin 1 inhibitor rilonacept in treatment of chronic gouty arthritis: results of a placebo-controlled, monosequence crossover, non-randomised, single-blind pilot study. *Ann. Rheum. Dis.* 68(10):1613–17
26. Martinon F, Mayor A, Tschopp J. 2009. The inflammasomes: guardians of the body. *Annu. Rev. Immunol.* 27(1):229–65

27. Cai X, Chen J, Xu H, Liu S, Jiang Q-X, et al. 2014. Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell* 156(6):1207–22
28. Lu A, Magupalli VG, Ruan J, Yin Q, Atianand MK, et al. 2014. Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell* 156(6):1193–1206
29. Latz E, Xiao TS, Stutz A. 2013. Activation and regulation of the inflammasomes. *Nat. Rev. Immunol.* 13(6):397–411
30. Schaefer L. 2014. Complexity of danger: the diverse nature of damage-associated molecular patterns. *J. Biol. Chem.* 289(51):35237–45
31. Monção-Ribeiro LC, Faffe DS, Santana PT, Vieira FS, da Graça CLAL, et al. 2014. P2X7 receptor modulates inflammatory and functional pulmonary changes induced by silica. *PLOS ONE* 9(10):e110185
32. Hornung V, Latz E. 2010. Critical functions of priming and lysosomal damage for NLRP3 activation. *Eur. J. Immunol.* 40(3):620–23
33. Murakami T, Ockinger J, Yu J, Byles V, McColl A, et al. 2012. Critical role for calcium mobilization in activation of the NLRP3 inflammasome. *PNAS* 109(28):11282–87
34. Dostert C, Guarda G, Romero JF, Menu P, Gross O, et al. 2009. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLOS ONE* 4(8):e6510
35. Petrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J. 2007. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell Death Differ.* 14(9):1583–89
36. Zhou R, Yazdi AS, Menu P, Tschopp J. 2011. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469(7329):221–25
37. Okada M, Matsuzawa A, Yoshimura A, Ichijo H. 2014. The lysosome rupture-activated TAK1-JNK pathway regulates NLRP3 inflammasome activation. *J. Biol. Chem.* 289(47):32926–36
38. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. 2008. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 453(7198):1122–26
39. Summersgill H, England H, Lopez-Castejon G, Lawrence CB, Luheshi NM, et al. 2014. Zinc depletion regulates the processing and secretion of IL-1. *Cell Death Dis.* 5(1):e1040
40. Wei R, Wang J, Xu Y, Yin B, He F, et al. 2015. Probenecid protects against cerebral ischemia/reperfusion injury by inhibiting lysosomal and inflammatory damage in rats. *Neuroscience* 301:168–77
41. Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, et al. 2008. The Nalp3 inflammasome is essential for the development of silicosis. *PNAS* 105(26):9035–40
42. Meissner F, Seger RA, Moshous D, Fischer A, Reichenbach J, Zychlinsky A. 2010. Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. *Blood* 116(9):1570–73
43. Rada B, Park JJ, Sil P, Geiszt M, Leto TL. 2014. NLRP3 inflammasome activation and interleukin-1β release in macrophages require calcium but are independent of calcium-activated NADPH oxidases. *Inflamm. Res.* 63(10):821–30
44. van de Veerdonk FL, Smeekens SP, Joosten LAB, Kullberg B-J, Dinarello CA, et al. 2010. Reactive oxygen species–independent activation of the IL-1β inflammasome in cells from patients with chronic granulomatous disease. *PNAS* 107(7):3030–33
45. van Bruggen R, Köker MY, Jansen M, van Houdt M, Roos D, et al. 2010. Human NLRP3 inflammasome activation is Nox1-4 independent. *Blood* 115(26):5398–400
46. Franchi L, Eigenbrod T, Nuñez G. 2009. Cutting edge: TNF-α mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. *J. Immunol.* 183(2):792–96
47. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, et al. 2009. Cutting edge: NF-κB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183(2):787–91
48. An LL, Mehta P, Xu L, Turman S, Reimer T, et al. 2014. Complement C5a potentiates uric acid crystal–induced IL-1β production. *Eur. J. Immunol.* 44(12):3669–79
49. Nymo S, Niyonzima N, Espevik T, Mollnes TE. 2014. Cholesterol crystal–induced endothelial cell activation is complement-dependent and mediated by TNF. *Immunobiology* 219(10):786–92
50. Samstad EO, Niyonzima N, Nymo S, Aune MH, Ryan L, et al. 2014. Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. *J. Immunol.* 192(6):2837–45

51. Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, et al. 2009. CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat. Immunol.* 11:155–61
52. Netea MG, van de Veerdonk FL, van der Meer JWM, Dinarello CA, Joosten LAB. 2015. Inflammasome-independent regulation of IL-1–family cytokines. *Annu. Rev. Immunol.* 33(1):49–77
53. Jacobson LS, Lima H, Goldberg MF, Gocheva V, Tsiperson V, et al. 2013. Cathepsin-mediated necrosis controls the adaptive immune response by Th2 (T helper type 2)-associated adjuvants. *J. Biol. Chem.* 288(11):7481–91
54. Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, et al. 2012. Inflammasome activators induce interleukin-1α secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 36(3):388–400
55. Hari A, Zhang Y, Tu Z, Detampel P, Stenner M, et al. 2014. Activation of NLRP3 inflammasome by crystalline structures via cell surface contact. *Sci. Rep.* 4:7281
56. Kim ML, Chae JJ, Park YH, De Nardo D, Stirzaker RA, et al. 2015. Aberrant actin depolymerization triggers the pyrin inflammasome and autoinflammatory disease that is dependent on IL-18, not IL-1. *J. Exp. Med.* 212(6):927–38
57. Mansfield E, Chae JJ, Komarow HD, Brotz TM, Frucht DM, et al. 2001. The familial Mediterranean fever protein, pyrin, associates with microtubules and colocalizes with actin filaments. *Blood* 98(3):851–59
58. Richards N, Schaner P, Diaz A, Stuckey J, Shelden E, et al. 2001. Interaction between pyrin and the apoptotic speck protein (ASC) modulates ASC-induced apoptosis. *J. Biol. Chem.* 276(42):39320–29
59. Waite AL, Schaner P, Hu C, Richards N, Balci-Peynircioglu B, et al. 2009. Pyrin and ASC co-localize to cellular sites that are rich in polymerizing actin. *Exp. Biol. Med.* 234(1):40–52
60. Schotte P, Denecker G, Van Den Broeke A, Vandenabeele P, Cornelis GR, Beyaert R. 2004. Targeting Rac1 by the *Yersinia* effector protein YopE inhibits caspase-1–mediated maturation and release of interleukin-1β. *J. Biol. Chem.* 279(24):25134–42
61. Aktories K. 2011. Bacterial protein toxins that modify host regulatory GTPases. *Nat. Rev. Microbiol.* 9:487–98
62. Beningo KA, Wang Y-L. 2002. Fc-receptor–mediated phagocytosis is regulated by mechanical properties of the target. *J. Cell Sci.* 115(Pt. 4):849–56
63. Small DM. 1988. George Lyman Duff memorial lecture. Progression and regression of atherosclerotic lesions: insights from lipid physical biochemistry. *Arteriosclerosis* 8(2):103–29
64. Tabas I. 2002. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *J. Clin. Investig.* 110(7):905–11
65. Sedaghat A, Grundy SM. 1980. Cholesterol crystals and the formation of cholesterol gallstones. *N. Engl. J. Med.* 302(23):1274–77
66. Bély M, Apáthy A. 2013. Mönckeberg's sclerosis—crystal induced angiopathy. *Orv. Hetil.* 154(23):908–13
67. Tsirpanlis G. 2007. Is inflammation the link between atherosclerosis and vascular calcification in chronic kidney disease? *Blood Purif.* 25(2):179–82
68. Nadra I, Mason JC, Philippidis P, Florey O, Smythe CDW, et al. 2005. Proinflammatory activation of macrophages by basic calcium phosphate crystals via protein kinase C and MAP kinase pathways: a vicious cycle of inflammation and arterial calcification? *Circ. Res.* 96(12):1248–56
69. McGonagle D, Tan AL, Madden J, Emery P, McDermott MF. 2008. Successful treatment of resistant pseudogout with anakinra. *Arthritis Rheum.* 58(2):631–33
70. Lu H, Guo Y-N, Liu S-N, Zhang D-C. 2012. Nanobacteria may be linked to calcification in placenta. *Ultrastruct. Pathol.* 36(3):160–65
71. Chistiakov DA, Sobenin IA, Orekhov AN, Bobryshev YV. 2014. Mechanisms of medial arterial calcification in diabetes. *Curr. Pharm. Des.* 20(37):5870–83
72. Reaven PD, Sacks J, Investigators for the VADT. 2005. Coronary artery and abdominal aortic calcification are associated with cardiovascular disease in type 2 diabetes. *Diabetologia* 48(2):379–85
73. Towler DA, Bidder M, Latifi T, Coleman T, Semenkovich CF. 1998. Diet-induced diabetes activates an osteogenic gene regulatory program in the aortas of low density lipoprotein receptor–deficient mice. *J. Biol. Chem.* 273(46):30427–34
74. Yin TT, Patel J, Parhami F, Demer LL. 2000. Tumor necrosis factor-α promotes in vitro calcification of vascular cells via the cAMP pathway. *Circulation* 102(21):2636–42

75. Govindaraj A, Selvam R. 2001. Increased calcium oxalate crystal nucleation and aggregation by peroxidized protein of human kidney stone matrix and renal cells. *Urol. Res.* 29(3):194–98
76. Worcester EM. 2002. Stones from bowel disease. *Endocrinol. Metab. Clin. N. Am.* 31(4):979–99
77. Neogi T. 2011. Clinical practice: gout. *N. Engl. J. Med.* 364(5):443–52
78. Shi Y, Evans JE, Rock KL. 2003. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 425(6957):516–21
79. di Giovine FS, Malawista SE, Thornton E, Duff GW. 1991. Urate crystals stimulate production of tumor necrosis factor α from human blood monocytes and synovial cells. Cytokine mRNA and protein kinetics, and cellular distribution. *J. Clin. Investig.* 87(4):1375–81
80. Zhang W, Doherty M, Bardin T, Pascual E, Barskova V, et al. 2006. EULAR evidence based recommendations for gout. Part II: management. Report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). *Ann. Rheum. Dis.* 65:1312–24
81. Taskiran EZ, Cetinkaya A, Balci-Peynircioglu B, Akkaya YZ, Yilmaz E. 2012. The effect of colchicine on pyrin and pyrin interacting proteins. *J. Cell Biochem.* 113(11):3536–46
82. Balci-Peynircioglu B, Waite AL, Schaner P, Taskiran ZE, Richards N, et al. 2008. Expression of ASC in renal tissues of familial Mediterranean fever patients with amyloidosis: postulating a role for ASC in AA type amyloid deposition. *Exp. Biol. Med.* 233(11):1324–33
83. Finck S, Lepièce G, Bonnet S. 2014. Diagnosis of chronic retinal detachment following the discovery of a crystalline retinopathy. *Rev. Med. Liege* 69(11):586–89
84. World Health Organ. 2014. *Global Health Observatory Data: Exposure to Ambient Air Pollution*. Geneva: World Health Organ. **http://www.who.int/gho/phe/outdoor_air_pollution/exposure/en**
85. Becker S, Soukup JM, Gilmour MI, Devlin RB. 1996. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. *Toxicol. Appl. Pharmacol.* 141(2):637–48
86. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, et al. 2001. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM_{10}). *Am. J. Respir. Crit. Care Med.* 164(5):826–30
87. Madden MC, Richards JH, Dailey LA, Hatch GE, Ghio AJ. 2000. Effect of ozone on diesel exhaust particle toxicity in rat lung. *Toxicol. Appl. Pharmacol.* 168(2):140–48
88. McWhinney RD, Gao SS, Zhou S, Abbatt JPD. 2011. Evaluation of the effects of ozone oxidation on redox-cycling activity of two-stroke engine exhaust particles. *Environ. Sci. Technol.* 45(6):2131–36
89. Rattanavaraha W, Rosen E, Zhang H, Li Q, Pantong K, Kamens RM. 2011. The reactive oxidant potential of different types of aged atmospheric particles: an outdoor chamber study. *Atmos. Environ.* 45(23):3848–55
90. Mendis S, Puska P, Norrving B. 2011. *Global Atlas on Cardiovascular Disease Prevention and Control*: *Policies, Strategies and Interventions.* Geneva: World Health Organ.
91. Künzli N, Jerrett M, Mack WJ, Beckerman B, LaBree L, et al. 2005. Ambient air pollution and atherosclerosis in Los Angeles. *Environ. Health Perspect.* 113(2):201–6
92. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. 2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 525(7569):367–71
93. Saputra D, Yoon J-H, Park H, Heo Y, Yang H, et al. 2014. Inhalation of carbon black nanoparticles aggravates pulmonary inflammation in mice. *Toxicol. Res.* 30(2):83–90
94. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. 2014. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. *Environ. Res.* 129:11–19
95. Gurgueira SA, Lawrence J, Coull B, Murthy GGK, González-Flecha B. 2002. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. *Environ. Health Perspect.* 110(8):749–55
96. González-Flecha B. 2004. Oxidant mechanisms in response to ambient air particles. *Mol. Aspects Med.* 25(1–2):169–82
97. Yamawaki H, Iwai N. 2006. Mechanisms underlying nano-sized air-pollution–mediated progression of atherosclerosis: Carbon black causes cytotoxic injury/inflammation and inhibits cell growth in vascular endothelial cells. *Circ. J.* 70(1):129–40

98. Valavanidis A, Vlachogianni T, Fiotakis K. 2009. Tobacco smoke: involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. *Int. J. Environ. Res. Public Health* 6(2):445–62
99. Churg A, Dai J, Tai H, Xie C, Wright JL. 2002. Tumor necrosis factor-α is central to acute cigarette smoke–induced inflammation and connective tissue breakdown. *Am. J. Respir. Crit. Care Med.* 166(6):849–54
100. Angelis N, Porpodis K, Zarogoulidis P, Spyratos D, Kioumis I, et al. 2014. Airway inflammation in chronic obstructive pulmonary disease. *J. Thorac. Dis.* 6(Suppl. 1):S167–72
101. Doz E, Noulin N, Boichot E, Guénon I, Fick L, et al. 2008. Cigarette smoke–induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. *J. Immunol.* 180(2):1169–78
102. Lappalainen U, Whitsett JA, Wert SE, Tichelaar JW, Bry K. 2005. Interleukin-1β causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. *Am. J. Respir. Cell Mol. Biol.* 32(4):311–18
103. Hoshino T, Kato S, Oka N, Imaoka H, Kinoshita T, et al. 2007. Pulmonary inflammation and emphysema: role of the cytokines IL-18 and IL-13. *Am. J. Respir. Crit. Care Med.* 176(1):49–62
104. Pauwels NS, Bracke KR, Dupont LL, Van Pottelberge GR, Provoost S, et al. 2011. Role of IL-1α and the Nlrp3/caspase-1/IL-1β axis in cigarette smoke–induced pulmonary inflammation and COPD. *Eur. Respir. J.* 38(5):1019–28
105. Petersen AMW, Penkowa M, Iversen M, Frydelund-Larsen L, Andersen JL, et al. 2007. Elevated levels of IL-18 in plasma and skeletal muscle in chronic obstructive pulmonary disease. *Lung* 185(3):161–71
106. Kang M-J, Homer RJ, Gallo A, Lee CG, Crothers KA, et al. 2007. IL-18 is induced and IL-18 receptor α plays a critical role in the pathogenesis of cigarette smoke–induced pulmonary emphysema and inflammation. *J. Immunol.* 178(3):1948–59
107. Botelho FM, Bauer CMT, Finch D, Nikota JK, Zavitz CCJ, et al. 2011. IL-1α/IL-1R1 expression in chronic obstructive pulmonary disease and mechanistic relevance to smoke-induced neutrophilia in mice. *PLOS ONE* 6(12):e28457
108. Eltom S, Belvisi MG, Stevenson CS, Maher SA, Dubuis E, et al. 2014. Role of the inflammasome–caspase1/11-IL-1/18 axis in cigarette smoke driven airway inflammation: an insight into the pathogenesis of COPD. *PLOS ONE* 9(11):e112829
109. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, et al. 2014. The adaptor ASC has extracellular and "prionoid" activities that propagate inflammation. *Nat. Immunol.* 15(8):727–37
110. Leung CC, Yu ITS, Chen W. 2012. Silicosis. *Lancet* 379(9830):2008–18
111. Peeters PM, Perkins TN, Wouters EFM, Mossman BT, Reynaert NL. 2013. Silica induces NLRP3 inflammasome activation in human lung epithelial cells. *Part. Fibre Toxicol.* 10:3
112. Lee S, Matsuzaki H, Kumagai-Takei N, Yoshitome K, Maeda M, et al. 2014. Silica exposure and altered regulation of autoimmunity. *Environ. Health Prev. Med.* 19(5):322–29
113. Di Giuseppe M, Gambelli F, Hoyle GW, Lungarella G, Studer SM, et al. 2009. Systemic inhibition of NF-κB activation protects from silicosis. *PLOS ONE* 4(5):e5689
114. Guo J, Gu N, Chen J, Shi T, Zhou Y, et al. 2013. Neutralization of interleukin-1 β attenuates silica-induced lung inflammation and fibrosis in C57BL/6 mice. *Arch. Toxicol.* 87(11):1963–73
115. Maeda M, Nishimura Y, Kumagai N, Hayashi H, Hatayama T, et al. 2010. Dysregulation of the immune system caused by silica and asbestos. *J. Immunotoxicol.* 7(4):268–78
116. Song L, Weng D, Dai W, Tang W, Chen S, et al. 2014. Th17 can regulate silica-induced lung inflammation through an IL-1β–dependent mechanism. *J. Cell Mol. Med.* 18(9):1773–84
117. Guo J, Shi T, Cui X, Rong Y, Zhou T, et al. 2015. Effects of silica exposure on the cardiac and renal inflammatory and fibrotic response and the antagonistic role of interleukin-1 β in C57BL/6 mice. *Arch. Toxicol.* 2015:1–12
118. Hillegass JM, Miller JM, MacPherson MB, Westbom CM, Sayan M, et al. 2013. Asbestos and erionite prime and activate the NLRP3 inflammasome that stimulates autocrine cytokine release in human mesothelial cells. *Part. Fibre Toxicol.* 10:39
119. Thompson JK, Westbom CM, MacPherson MB, Mossman BT, Heintz NH, et al. 2014. Asbestos modulates thioredoxin–thioredoxin interacting protein interaction to regulate inflammasome activation. *Part. Fibre Toxicol.* 11:24

120. Murthy S, Adamcakova-Dodd A, Perry SS, Tephly LA, Keller RM, et al. 2009. Modulation of reactive oxygen species by Rac1 or catalase prevents asbestos-induced pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* 297(5):L846–55
121. Perkins TN, Peeters PM, Shukla A, Arijs I, Dragon J, et al. 2015. Indications for distinct pathogenic mechanisms of asbestos and silica through gene expression profiling of the response of lung epithelial cells. *Hum. Mol. Genet.* 24(5):1374–89
122. Chow MT, Tschopp J, Möller A, Smyth MJ. 2012. NLRP3 promotes inflammation-induced skin cancer but is dispensable for asbestos-induced mesothelioma. *Immunol. Cell Biol.* 90(10):983–86
123. Yang H, Pellegrini L, Napolitano A, Giorgi C, Jube S, et al. 2015. Aspirin delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression. *Cell Death Dis.* 6:e1786
124. World Health Organ. 2014. *Global Health Observatory Data: World Health Statistics 2014*. Geneva: World Health Organ. **http://www.who.int/gho/publications/world_health_statistics/2014/en/**
125. Krishnegowda G, Hajjar AM, Zhu J, Douglass EJ, Uematsu S, et al. 2005. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J. Biol. Chem.* 280(9):8606–16
126. Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, et al. 2007. Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *PNAS* 104(6):1919–24
127. Kalantari P, Deoliveira RB, Chan J, Corbett Y, Rathinam V, et al. 2013. Dual engagement of the NLRP3 and AIM2 inflammasomes by *Plasmodium*-derived hemozoin and DNA during malaria. *Cell Rep.* 16:1–15
128. Clarke CA, Lee CM, Furbert-Harris PM. 2015. The enigmatic Charcot-Leyden crystal protein (Galectin-10): speculative role(s) in the eosinophil biology and function. *J. Clin. Cell Immunol.* 6:323
129. Weller PF, Goetzl EJ. 1979. The regulatory and effector roles of eosinophils. *Adv. Immunol.* 27:339–71
130. Manny JS, Ellis LR. 2012. Acute myeloid leukemia with Charcot-Leyden crystals. *Blood* 120(3):503
131. Weller PF, Goetzl EJ, Austen KF. 1980. Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. *PNAS* 77(12):7440–43
132. Arora VK, Singh N, Bhatia A. 1997. Charcot-Leyden crystals in fine needle aspiration cytology. *Acta Cytol.* 41(2):409–12
133. Brewster UC, Perazella MA. 2004. Acute interstitial nephritis associated with atazanavir, a new protease inhibitor. *Am. J. Kidney Dis.* 44(5):e81–84
134. Izzedine H, M'rad MB, Bardier A, Daudon M, Salmon D. 2007. Atazanavir crystal nephropathy. *AIDS* 21(17):2357–58
135. Hara M, Suganuma A, Yanagisawa N, Imamura A, Hishima T, Ando M. 2015. Atazanavir nephrotoxicity. *Clin. Kidney J.* 8(2):137–42
136. Viglietti D, Verine J, De Castro N, Scemla A, Daudon M, et al. 2011. Chronic interstitial nephritis in an HIV type-1–infected patient receiving ritonavir-boosted atazanavir. *Antivir. Ther.* 16(1):119–21
137. Kanzaki G, Tsuboi N, Miyazaki Y, Yokoo T, Utsunomiya Y, Hosoya T. 2012. Diffuse tubulointerstitial nephritis accompanied by renal crystal formation in an HIV-infected patient undergoing highly active antiretroviral therapy. *Intern. Med.* 51(12):1543–48
138. Coelho S, Aparício SR, Manso R, Soto K. 2012. Kidney failure in an HIV-positive patient. *Am. J. Kidney Dis.* 59(6):A27–30
139. Swanson BJ, Limketkai BN, Liu T-C, Montgomery E, Nazari K, et al. 2013. Sevelamer crystals in the gastrointestinal tract (GIT): a new entity associated with mucosal injury. *Am. J. Surg. Pathol.* 37(11):1686–93
140. Lindblad EB. 2004. Aluminium compounds for use in vaccines. *Immunol. Cell Biol.* 82(5):497–505
141. Kool M, Pétrilli V, De Smedt T, Rolaz A, Hammad H, et al. 2008. Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *J. Immunol.* 181(6):3755–59
142. McKee AS, Munks MW, MacLeod MKL, Fleenor CJ, Van Rooijen N, et al. 2009. Alum induces innate immune responses through macrophage and mast cell sensors, but these sensors are not required for alum to act as an adjuvant for specific immunity. *J. Immunol.* 183(7):4403–14

143. Franchi L, Nuñez G. 2008. The Nlrp3 inflammasome is critical for aluminium hydroxide–mediated IL-1β secretion but dispensable for adjuvant activity. *Eur. J. Immunol.* 38(8):2085–89
144. Cain DW, Sanders SE, Cunningham MM, Kelsoe G. 2013. Disparate adjuvant properties among three formulations of "alum." *Vaccine* 31(4):653–60
145. Gavin AL, Hoebe K, Duong B, Ota T, Martin C, et al. 2006. Adjuvant-enhanced antibody responses in the absence of Toll-like receptor signaling. *Science* 314(5807):1936–38
146. Flach TL, Ng G, Hari A, Desrosiers MD, Zhang P, et al. 2011. Alum interaction with dendritic cell membrane lipids is essential for its adjuvanticity. *Nat. Med.* 17(4):479–87
147. Cannon GJ, Swanson JA. 1992. The macrophage capacity for phagocytosis. *J. Cell Sci.* 101(Pt. 4):907–13
148. Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. 1993. Macrophage/particle interactions: effect of size, composition and surface area. *J. Biomed. Mater. Res.* 28(1):81–90
149. Vaine CA, Patel MK, Zhu J, Lee E, Finberg RW, et al. 2013. Tuning innate immune activation by surface texturing of polymer microparticles: the role of shape in inflammasome activation. *J. Immunol.* 190(7):3525–32
150. Zhang W, Wang L, Liu Y, Chen X, Li J, et al. 2014. Comparison of PLA microparticles and alum as adjuvants for H5N1 influenza split vaccine: adjuvanticity evaluation and preliminary action mode analysis. *Pharm. Res.* 31(4):1015–31
151. Williams GR, Fierens K, Preston SG, Lunn D, Rysnik O, et al. 2014. Immunity induced by a broad class of inorganic crystalline materials is directly controlled by their chemistry. *J. Exp. Med.* 211(6):1019–25
152. Caicedo MS, Samelko L, McAllister K, Jacobs JJ, Hallab NJ. 2013. Increasing both CoCrMo-alloy particle size and surface irregularity induces increased macrophage inflammasome activation in vitro potentially through lysosomal destabilization mechanisms. *J. Orthop. Res.* 31(10):1633–42
153. Nadra I, Boccaccini AR, Philippidis P, Whelan LC, McCarthy GM, et al. 2008. Effect of particle size on hydroxyapatite crystal–induced tumor necrosis factor α secretion by macrophages. *Atherosclerosis* 196(1):98–105
154. Oberdörster G, Ferin J, Lehnert BE. 1994. Correlation between particle size, in vivo particle persistence, and lung injury. *Environ. Health Perspect.* 102(Suppl. 5):173–79
155. Kusaka T, Nakayama M, Nakamura K, Ishimiya M, Furusawa E, Ogasawara K. 2014. Effect of silica particle size on macrophage inflammatory responses. *PLOS ONE* 9(3):e92634
156. Schorn C, Janko C, Krenn V, Zhao Y, Munoz LE, et al. 2012. Bonding the foe—NETting neutrophils immobilize the pro-inflammatory monosodium urate crystals. *Front. Immunol.* 3:376
157. Schauer C, Janko C, Munoz LE, Zhao Y, Kienhöfer D, et al. 2014. Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. *Nat. Med.* 20(5):511–17
158. Gould S, Scott RC. 2005. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. *Food Chem. Toxicol.* 43(10):1451–59
159. Kilsdonk E, Yancey PG, Stoudt GW, Bangerter FW, Johnson WJ, et al. 1995. Cellular cholesterol efflux mediated by cyclodextrins. *J. Biol. Chem.* 270(29):17250–56
160. Liu SM, Cogny A, Kockx M, Dean RT, Gaus K, et al. 2003. Cyclodextrins differentially mobilize free and esterified cholesterol from primary human foam cell macrophages. *J. Lipid Res.* 44(6):1156–66
161. Kritharides L, Kus M, Brown AJ, Jessup W, Dean RT. 1996. Hydroxypropyl-β-cyclodextrin–mediated efflux of 7-ketocholesterol from macrophage foam cells. *J. Biol. Chem.* 271(44):27450–55
162. Atger VM, de la Llera Moya M, Stoudt GW, Rodrigueza WV, Phillips MC, Rothblat GH. 1997. Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells. *J. Clin. Investig.* 99(4):773–80
163. Sheng X. 2005. Crystal surface adhesion explains the pathological activity of calcium oxalate hydrates in kidney stone formation. *J. Am. Soc. Nephrol.* 16(7):1904–8
164. Sun B, Ji Z, Liao Y-P, Wang M, Wang X, et al. 2013. Engineering an effective immune adjuvant by designed control of shape and crystallinity of aluminum oxyhydroxide nanoparticles. *ACS Nano* 7(12):10834–49
165. Watson KE, Boström K, Ravindranath R, Lam T, Norton B, Demer LL. 1994. TGF-β 1 and 25-hydroxycholesterol stimulate osteoblast-like vascular cells to calcify. *J. Clin. Investig.* 93(5):2106–13

166. Alman AC, Kinney GL, Tracy RP, Maahs DM, Hokanson JE, et al. 2013. Prospective association between inflammatory markers and progression of coronary artery calcification in adults with and without type 1 diabetes. *Diabetes Care* 36(7):1967–73
167. Thomsen SB, Rathcke CN, Zerahn B, Vestergaard H. 2010. Increased levels of the calcification marker matrix Gla protein and the inflammatory markers YKL-40 and CRP in patients with type 2 diabetes and ischemic heart disease. *Cardiovasc. Diabetol.* 9:86
168. Al-Aly Z, Shao J-S, Lai C-F, Huang E, Cai J, et al. 2007. Aortic *Msx2-Wnt* calcification cascade is regulated by TNF-α–dependent signals in diabetic $Ldlr^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* 27(12):2589–96
169. Mulder WJM, Jaffer FA, Fayad ZA, Nahrendorf M. 2014. Imaging and nanomedicine in inflammatory atherosclerosis. *Sci. Transl. Med.* 6(239):239sr1
170. Yazdi AS, Guarda G, Riteau N, Drexler SK, Tardivel A, et al. 2010. Nanoparticles activate the NLR pyrin domain containing 3 (Nlrp3) inflammasome and cause pulmonary inflammation through release of IL-1α and IL-1β. *PNAS* 107(45):19449–54
171. Dong J, Porter DW, Batteli LA, Wolfarth MG, Richardson DL, Ma Q. 2015. Pathologic and molecular profiling of rapid-onset fibrosis and inflammation induced by multi-walled carbon nanotubes. *Arch. Toxicol.* 89(4):621–33
172. Husain M, Wu D, Saber AT, Decan N, Jacobsen NR, et al. 2015. Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. *Nanotoxicology* 9:1013–22
173. Marzaioli V, Aguilar-Pimentel JA, Weichenmeier I, Luxenhofer G, Wiemann M, et al. 2014. Surface modifications of silica nanoparticles are crucial for their inert versus proinflammatory and immunomodulatory properties. *Int. J. Nanomed.* 9:2815–32

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