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Molecular Activation of NLRP3 Inflammasome by Particles and Crystals: A Continuing Challenge of Immunology and Toxicology

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NLRP3, inflammasome, particle, crystal, inflammation, chronic disease

Abstract

Particles and crystals constitute a unique class of toxic agents that humans are constantly exposed to both endogenously and from the environment. Deposition of particulates in the body is associated with a range of diseases and toxicity. The mechanism by which particulates cause disease remains poorly understood due to the lack of mechanistic insights into particle-biological interactions. Recent research has revealed that many particles and crystals activate the NLRP3 inflammasome, an intracellular pattern-recognition receptor. Activated NLRP3 forms a supramolecular complex with an adaptor protein to activate caspase 1, which in turn activates IL-1β and IL-18 to instigate inflammation. Genetic ablation and pharmacological inhibition of the NLRP3 inflammasome dampen inflammatory responses to particulates. Nonetheless, how particulates activate NLRP3 remains a challenging question. From this perspective, we discuss our current understanding of and progress on revealing the function and mode of action of the NLRP3 inflammasome in mediating adaptive and pathologic responses to particulates in health and disease.

1. PARTICLES AND CRYSTALS IN HEALTH AND DISEASE

Particles and crystals are part of a large group of particulate materials and substances that have unique toxicological relevance to human health and disease (1–5). Biologically relevant particles and crystals are commonly in the range of micro- to nanometers in size, which allows for the deposition, phagocytosis, and migration of particulates in the body. Nevertheless, these particulates differ substantially in their size, shape, composition, surface chemistry, and agglomeration state, which in turn determine their interaction with biological systems. The health effects of particulates encompass a broad spectrum of toxicity and disease conditions, ranging from chronic diseases associated with the accumulation of endogenous particulates, such as atherosclerosis, gout and pseudogout, and Alzheimer's disease, to diseases of the lung and pleura caused by the inhalation of mineral particles and fibers, exemplified by pneumoconiosis and mesothelioma. Moreover, the list of toxic particulates from endogenous and exogenous sources has expanded substantially and their role in disease pathogenesis has been increasingly recognized over the past two decades, which has propelled a rapid increase in research on the biology and health effects of particles and crystals (**Figure 1**).

Particles and crystals are formed endogenously or are encountered in the environment (6). Endogenous particulates derive from excess metabolites, cholesterol, misfolded proteins, and immune complexes that deposit and accumulate in target tissue, causing inflammation and injury.

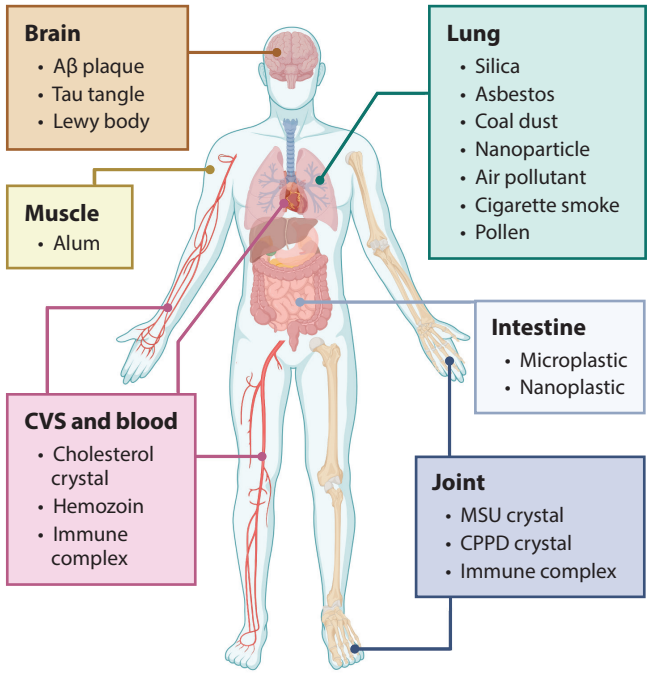

Figure 1

Deposition of particles and crystals in the body. Respiration and ingestion are major routes of exposure to exogenous particles. Internal organs succumb to endogenously produced particulates from excess metabolites, cholesterol, and protein aggregates and fibrils. Exceptions include alum, which is injected into muscles as a vaccination adjuvant, and hemozoin, a crystalline heme waste from the digestion of hemoglobin by blood-feeding parasites. Abbreviations: Aβ, amyloid-β; CPPD, calcium pyrophosphate dihydrate; CVS, cardiovascular system; MSU, monosodium urate. Figure adapted from images created with BioRender.com.

Disease-associated endogenous crystals and protein aggregates include monosodium urate (MSU) crystals and calcium pyrophosphate dihydrate (CPPD) crystals that deposit in joints to cause gout and pseudogout (2); cholesterol crystals that accumulate in artery walls to stimulate the development of atherosclerotic lesions, leading to cardiovascular disease and stroke (3, 7); amyloid plaques that derive from the oligomerization of amyloid-beta (Aβ) peptides and cause the degeneration and death of neurons in the brains of aging individuals and patients with Alzheimer's disease (8); Lewy bodies that comprise fibrillar α-synuclein (α-Syn) aggregates and result in central nervous system (CNS) pathology, called synucleinopathy, which causes Parkinson's disease when they are formed in the dopaminergic neurons of the substantia nigra pars compacta of the brain (9); and immune complexes that cause serum sickness and other immune complex–mediated hypersensitivity diseases (10). Given the high prevalence and medical and social burdens of chronic disease, the role of endogenous particles and crystals in the pathogenesis of some common chronic diseases has received particular attention. Indeed, the research in this direction has provided major insights into the formation and mechanisms of action of endogenous particulates under varied physiological and disease conditions in recent years.

Environmental particulates are exemplified by mineral particles and fibers such as crystalline silica, asbestos fiber, and coal dust. Mineral particles and fibers exist in the earth's crust. Humans are exposed to mineral particulates mainly through mining and industrial and commercial usage of the materials. Inhalation of mineral particulates is the direct cause of silicosis, asbestosis, and coal miner's lung disease, collectively known as pneumoconiosis. Pulmonary exposure to mineral dust is known to increase the incidence of lung cancer, while mesothelioma is considered synonymous with inhalation of asbestos fibers (5, 11–13). Additionally, tuberculosis commonly accompanies silicosis, whereas a condition termed massive progressive fibrosis has been noted in patients with coal dust exposure in recent years (14). Historically, lung disease caused by exposure to silica, asbestos, and coal dust was a major cause of mortality, morbidity, and disability in the Western world during industrialization. Although exposure to these classical mineral particles is significantly reduced in developed countries, substantial exposures continue to exist and cause disease in the lung and pleura worldwide, including in some professions in developed countries (15). Environmental exposure to particulates from cigarette smoke, combustion exhaust, and air pollution is widespread and is associated with increased incidences of lung cancer, asthma, chronic obstructive pulmonary disease, and cardiovascular disease (16–20). Particles and nanoparticles are also created for beneficial health effects. Alum refers to trivalent inorganic aluminum salts, such as potassium aluminum sulfate, and is used as an effective adjuvant to boost vaccination in humans and animals (21). Nanoparticles are being increasingly exploited as diagnostics and therapeutics that collectively comprise nanomedicine (22). Overall, the rapid advancement of nanotechnology in the last two decades has led to the creation and use of numerous nanomaterials with a broad range of applications and, at the same time, raised new concerns over the possible health effects of nanoparticle exposure among workers and consumers (23, 24).

A key question for understanding the biology of particles and crystals is how particulates are sensed by mammalian cells to elicit specific responses that may lead to disease development. In this regard, major progress has been made in recent years to uncover a critical role of the nucleotide-binding, oligomerization domain NOD-like receptor family (NLR) pyrin domain–containing 3 (NLRP3) inflammasome in particle sensing and integration of effector responses to particles and crystals in health and disease (2, 25–27). NLRP3 is a multidomain protein that oligomerizes and recruits partner proteins through evolutionarily conserved domains to form an inflammasome to control inflammation. NLRP3 was initially identified as NALP3, named after its three domains characteristic of NALP proteins: the domain present in NLP family apoptosis inhibitor protein (NAIP), MHC class II transcription factor (CIITA), incompatibility locus

protein from *Podospora anserina* (HET-E), and telomerase-associated protein (TP1) (NACHT); the leucine-rich repeat domain (LRR); and the pyrin domain (PYD) (28). As NACHT is a common domain in the NLR family mediating nucleotide-binding and oligomerization, it was renamed NLRP3 (25). NLRP3 is also known as cryopyrin as certain mutations of NLRP3 are the direct cause of human autoinflammatory disease (25, 29).

2. NLRP3 INFLAMMASOME: WHERE PARTICLES MEET IMMUNITY

The innate immune system protects the body against pathogens, injury, and other harmful stimuli by detecting specific signals and enabling effector responses that are inflammatory and adaptive in nature (30). Innate immune sensing is mediated through germline-encoded pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) derived from invading microbes and danger/damage-associated molecular patterns (DAMPs) released from damaged tissue (31). Membrane PRRs consist of Toll-like receptors (TLRs) that scan extracellular PAMPs and activate signaling pathways, such as nuclear factor κB (NF-κB), to control gene regulation and other responses to modulate inflammation (32). Intracellular PRRs are inflammasomes that detect intracellular PAMPs and DAMPs and integrate inflammatory responses by controlling the maturation and secretion of proinflammatory cytokines and other mediators (25).

The term inflammasome was initially coined to describe intracellular supramolecular complexes formed in response to microbial and sterile stimuli to mediate caspase 1 (Casp1)-dependent processing of interleukin (IL)-1β (33). Upon activation, the NLRP3 protein oligomerizes and recruits associated speck-like protein containing a caspase-recruitment and activation domain (ASC). Activated ASC has prion-like properties and assembles into filamentous bundles to form microscopically visible specks. ASC specks recruit multiple pro-Casp1s through their caspase-recruitment and activation domains (CARDs). Pro-Casp1 undergoes proximity-induced autoactivation and mediates the cleavage and maturation of IL-1β and IL-18. Mature IL-1β and IL-18 are released into the extracellular space to function as autocrine and paracrine agents, respectively, to initiate and amplify inflammation in tissue and systemically (26).

In recent years, the list of inflammasome-activated mediators has expanded substantially beyond IL-1β and IL-18. Casp1 cleaves gasdermin D (GSDMD), and the resulting N-terminal domain (N-GSDMD) inserts into the cytoplasmic membrane to form membrane pores, causing cell swelling and pyroptosis, a form of inflammatory cell death. Pyroptotic cells release proinflammatory mediators and concurrently kill and eliminate intracellular pathogens (34). IL-1α and the high-mobility group box 1 protein (HMGB1) are among the mediators released from pyroptotic cells. Recent studies also revealed a large group of proteins having Casp1 cleavage sites, 20 of which were validated as Casp1 substrates, including Casp7 (35). Cleavage of poly(ADP-ribose) polymerase 1 (PARP1) by Casp3 and Casp7 is a hallmark of apoptosis. Activation of Casp7 and PARP1 through the NLRP3 inflammasome implicates the proteins in pyroptosis independently of apoptosis (36). Alternatively, the finding suggests a cross-interaction between pyroptosis and apoptosis through the Casp7-PARP1 axis, as has been suggested in the concept of PANoptosis (37). These new target proteins of NLRP3 and Casp1 likely provide new mechanisms to explain the expanding functions of the NLRP3 inflammasome in physiology and disease beyond those initially identified and mediated by IL-1β.

Most inflammasomes detect a small number of signals, whereas the NLRP3 inflammasome is activated by a wide range of activators with diverse structures and properties (25). The PAMPs known to activate NLRP3 include nigericin, muramyl dipeptide, double-strand RNA, and single-strand RNA, while NLRP3-activating DAMPs are exemplified by extracellular ATP and reactive oxygen species (ROS) (25). Uniquely, NLRP3 is activated by a variety of particulates of both

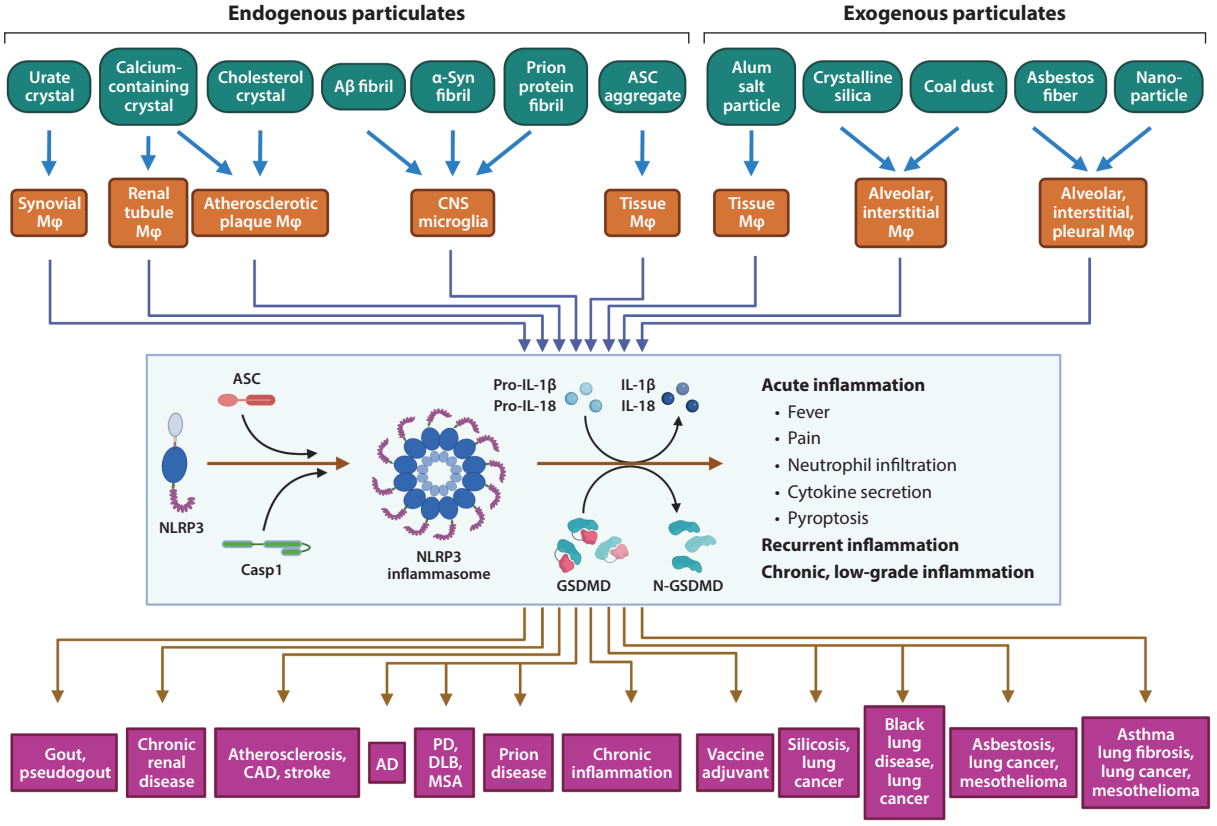

Figure 2

Activation of the NLRP3 inflammasome by particles and crystals in health and disease. Many biologically relevant particles and crystals produced endogenously or obtained from the environment are taken up by tissue macrophages and monocytes. The NLRP3 inflammasome serves as an innate immune sensor and effector to detect DAMPs and elicit inflammatory responses to maintain tissue homeostasis. Prolonged activation of NLRP3 causes persistent tissue damage and chronic inflammation, leading to disease and toxicity. Abbreviations: α-Syn, α-synuclein; Aβ, amyloid-β; AD, Alzheimer's disease; ASC, associated speck-like protein containing a caspase-recruitment and activation domain; CAD, coronary artery disease; Casp1, caspase 1; CNS, central nervous system; DAMP, danger/damage-associated molecular pattern; DLB, dementia with Lewy bodies; GSDMD, gasdermin D; IL, interleukin; N-GSDMD, GSDMD N-terminal domain; Mφ, macrophage; MSA, multiple system atrophy; NLRP3, nucleotide-binding, oligomerization domain NOD-like receptor family pyrin domain–containing 3; PD; Parkinson's disease. Figure adapted from images created with BioRender.com.

endogenous and environmental origins (27). In these cases, activation of NLRP3 augments the production of IL-1β and enhances proinflammatory responses that are implicated in the pathogenesis of disease and toxicity associated with particles and crystals. In this connection, the NLRP3 inflammasome serves as a supramolecular platform that bridges particles and crystals to innate immune functions and disease pathogenesis (**Figure 2**). The research in this direction has progressed rapidly to reveal new mechanisms by which particles and crystals activate innate immune sensing and signaling and induce adaptive and pathologic responses that lead to disease and toxicity.

3. MOLECULAR ACTIVATION OF NLRP3 INFLAMMASOME

The NLRP3 inflammasome is composed of three major protein components: the sensor protein NLRP3, adaptor ASC, and effector Casp1 (25). Each protein consists of several distinctive

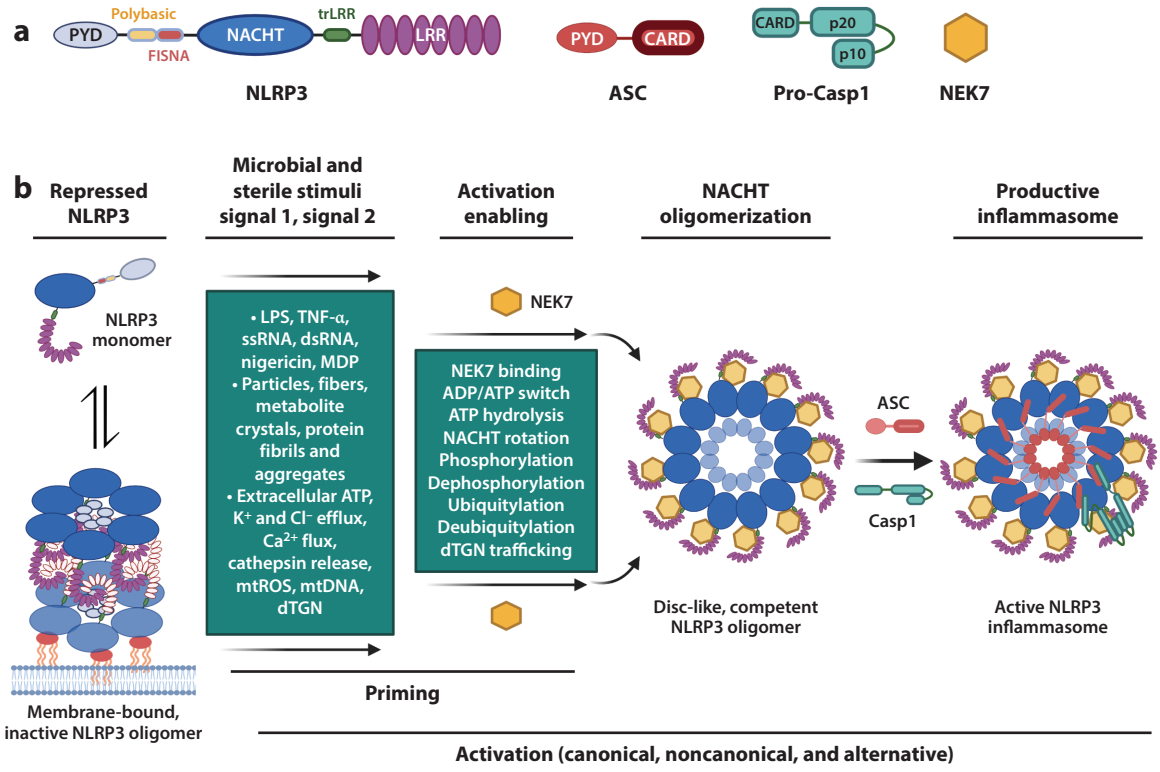

Figure 3

Integration of NLRP3 inflammasome activation. (*a*) Domain structures of NLRP3 and inflammasomal components. (*b*) Multiple steps of NLRP3 activation. Quiescent NLRP3 exists in an equilibrium between cytosolic monomer and membrane-associated oligomer. Microbial and sterile stimuli activate NLRP3 through primming and activation via inducer-specific pathways. The association of NLRP3 oligomers with dTGN vesicles facilitates their translocation into the nucleus for binding to nuclear NEK7, which enables NLRP3 to become competent for activation, resulting in inflammasome assembly and activation. Abbreviations: ASC, associated speck-like protein containing a CARD; CARD, caspase-recruitment and activation domain; Casp1, caspase 1; dsRNA, double-strand RNA; dTGN, dispersed *trans*-Golgi network; FISNA, fish-specific NACHT-associated domain; LPS, lipopolysaccharide; LRR, leucine-rich repeat; MDP, muramyl dipeptide; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; NACHT, domain present in NAIP, CIITA, HET-E, and TP1; NEK7, never in mitosis gene A–related kinase 7; NLRP3, nucleotide-binding, oligomerization domain NOD-like receptor family pyrin domain–containing 3; PYD, pyrin domain; ssRNA, single-strand RNA; TNF-α, tumor necrosis factor alpha; trLRR, transition LRR. Figure adapted from images created with BioRender.com.

domains (**Figure 3*a***). Assembly of the inflammasome is largely mediated through homotypic domain interactions (26). NLRP3 is a tripartite protein consisting of an N-terminal PYD, a central nucleotide triphosphatase NACHT domain, and a C-terminal LRR domain (27). ASC contains a PYD and a CARD, whereas Casp1 comprises a CARD and a p20-p10 catalytic domain that is cleaved into p20 and p10 in active Casp1. Upon activation, NLRP3 oligomerizes and recruits ACSs through homotypic PYD-PYD binding. ASC in turn recruits multiple pro-Casp1s through CARD-CARD interactions. Pro-Casp1 is cleaved via proximity-induced autoactivation. Active Casp1 contains two dimers of p20 and p10 that cleave and activate IL-1β, IL-18, and GSDMD.

NLRP3 is a member of the signal transduction ATPases with numerous domains (STAND) family of proteins that typically integrate sensing, regulation, and scaffolding functions through a single multidomain protein within a large regulatory network (38). NLRP3 senses diverse PAMP and DAMP signals, including signals associated with particles and crystals, to activate

inflammasomal assembly and signaling to regulate inflammation (27). The mechanism by which NLRP3 is activated by PAMPs and DAMPs remains incompletely understood, but several major steps and pathways in the activation, assembly, and signaling of the NLRP3 inflammasome have been implicated (**Figure 3*b***).

3.1. Priming

In quiescent cells, NLRP3 exists in a latent form at a low level. Activation of the NLRP3 inflammasome often involves two separable processes. Some NLRP3-activating signals (signal 1) induce the transcription of genes encoding NLRP3, other inflammasomal components, and Casp1 substrates to increase their protein levels. This process, termed priming, increases NLRP3 and makes it competent for activation (39). Priming is mediated through membrane-associated TLRs or cytoplasmic nucleotide-binding oligomerization domain–containing 2 (NOD2). Activation of NF-κB is important for induction of NLRP3 and IL-1β gene expression, but other transcription factors may also contribute to the regulation of transcription of the genes such as the nuclear receptor subfamily 1 group D member 1 mediating the circadian control of *Nlrp3* transcription (40, 41).

Posttranscriptional modification and protein-protein interaction modulate NLRP3 activities in both priming and activation steps (26). As an example, phosphorylation at Ser5 of human NLRP3 inhibits PYD-mediated assembly due to increased charge repulsion between PYDs, whereas dephosphorylation of the residue by protein phosphatase 2A promotes NLRP3 and ASC binding via their PYDs (42). Resting NLRP3 is ubiquitylated, while deubiquitylation of NLRP3 facilitates its priming and activation (43). Never in mitosis gene A–related kinase 7 (NEK7) is a mitotic kinase that binds NLRP3 at the interphase of the cell cycle, which licenses NLRP3 for activation (44).

3.2. Canonical Activation

In primed cells, activation of the NLRP3 inflammasome is triggered by PAMPs and DAMPs (signal 2) via three discrete but interrelated pathways (26). Activation of the NLRP3 inflammasome by many signals involves a multistep process known as the canonical pathway. Canonical activation consists of the release of autoinhibition of NLRP3, oligomerization of the NLRP3 NACHT domain, formation of ASC specks, recruitment and autoactivation of Casp1, and cleavage and secretion of IL-1β (26, 27). Several events are commonly observed in the canonical activation of NLRP3, though there remains no single consensus mechanism to account for activation of NLRP3 by all canonical activators.

3.2.1. Ion flux. Potassium (K^+) efflux is among the first events required for activation of NLRP3 by many signals, such as the microbial ionophore toxins nigericin and gramicidin, extracellular ATP, and particles and crystals (45–47). Calcium (Ca^{2+}) influx and release of Ca^{2+} from the endoplasmic reticulum to the cytoplasm and efflux of chloride ion (Cl^-) are also involved in NLRP3 activation. Flux of each ion, that is, K^+ efflux, Ca^{2+} flux, and Cl^- efflux, as well as ATP signaling, is mediated through specific and interrelated membrane channels in an inducer- and context-dependent manner.

3.2.2. Lysosomal damage. Pathogens, cell debris, and particles and crystals are commonly cleared from tissue by phagocytes. Upon phagocytosis, microbial and tissue debris are digested by lysosomal enzymes in the acidic environment of lysosomes. But inorganic particles and crystals are resistant to lysosomal degradation, resulting in the accumulation of particulates and consequently the damage and destabilization of lysosomes. Lysosomal damage and rupture release the particulates and lysosomal contents, such as cathepsins, into the cytoplasm. Lysosomal destabilization

and cathepsin release have been implicated in NLRP3 activation by several crystals and particles, as well as some soluble lysosomotropic agents such as the dipeptide Leu-Leu-OMe (48, 49).

3.2.3. Release of mitochondrial ROS and DNA. A common feature in the activation of NLRP3 by diverse signals is the production of ROS, including superoxide anion radical ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) (50). Mitochondria consume about 90% of the oxygen in mammalian cells to produce ATP through the mitochondrial respiratory chain. However, electrons may leak from the chain at complexes I and III to reduce O_2 to $O_2^{-\bullet}$, giving rise to mitochondrial reactive oxygen species (mtROS) (51, 52). Production of mtROS is increased upon stimulation with NLRP3 activators, including particles and crystals (53). The NLRP3 activator may also induce ROS production through membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), such as NOX2, that oxidize NADPH to reduce O_2 to $O_2^{-\bullet}$. How ROS activate NLRP3 remains uncertain, though several possibilities have been proposed. ROS may release thioredoxin-interacting protein (TXNIP), which binds thioredoxin in resting cells. Released TXNIP binds to quiescent NLRP3 to relieve NLRP3 from autoinhibition (54). The mitochondria contain circular DNA that can be oxidized and released into the cytoplasm and the extracellular space, such as blood plasma, by NLRP3 activators (55). Oxidized mitochondrial DNA serves as a DAMP that preferentially activates NLRP3 compared to other inflammasomes such as the absent in melanoma 2 inflammasome (56).

3.2.4. Membrane association and trafficking. NLRP3 exists in the cytoplasm or in association with a membrane structure. Upon activation, NLRP3 associates with the mitochondrial membrane by binding to cardiolipin, which is exposed to the mitochondrial outer membrane upon membrane damage by ROS (57). In the presence of viral infection, NLRP3 becomes associated with mitochondria by binding to mitofusin 2 (58). The dispersed *trans*-Golgi network (dTGN) serves as a scaffold to recruit NLRP3 for aggregation and trafficking in which NLRP3 binds to phosphatidylinositol 4-phosphate in the membrane (59). NLRP3 may also associate with the Golgi by binding to the complex between the sterol regulatory element-binding protein 2 (SREBP2) and the SREBP cleavage-activating protein (SCAP) to form a signaling hub that integrates cholesterol synthesis and inflammasome activation during inflammation (60). A recent study revealed that inactive NLRP3 forms double-ring and cage-like structures that are associated with Golgi membranes and dTGN. Moreover, association of NLRP3 cage structures with dTGN is necessary for transporting NLRP3 into the nucleus to reach and bind NEK7, which makes it competent for activation (61).

3.3. Noncanonical Activation

Phagocytosis of gram-negative bacteria results in the release of lipopolysaccharides (LPSs) inside phagocytes, which activate Casp4/5 in humans and Casp11 in mice (62). Activated Casp4/5/11 cleave GSDMD, release ATP, and induce K^+ efflux, all of which activate NLRP3 inflammasome formation (63). This noncanonical pathway of NLRP3 activation does not depend on priming as Casp4 levels are high in resting macrophages.

3.4. Alternative Activation

LPS can stimulate human monocytes to activate Casp8, which is commonly associated with apoptosis. In this scenario, activated Casp8 serves as an alternative to Casp1 to cleave IL-1β and IL-18, resulting in the alternative activation of the NLRP3 inflammasome (64). This alternative pathway is mediated through the signaling cascade consisting of TLR4, the Toll/IL-1 receptor domain-containing adaptor protein inducing interferon β (TRIF), the receptor-interacting

serine/threonine-protein kinase 1 (RIPK1), the Fas-associated death domain protein (FADD), and CASP8. This alternative pathway does not depend on Casp1. In mouse dendritic cells, prolonged exposure to LPSs may also activate alternative NLRP3 activation independently of the purinergic receptor $P2X_7$ involved in K^+ efflux (65).

3.5. Inflammasomal Assembly

Recent structural studies provided new insights into the structures of inactive NLRP3 and the assembly of the NLRP3 inflammasome. Both murine and human NLRP3 proteins exist in an equilibrium between monomeric and oligomeric structures and are localized between the cytoplasm and intracellular membranes in unstimulated cells (61, 66, 67). Mouse NLRP3 forms 12- to 16-mer double-ring cage-like structures that are predominantly associated with intracellular membranes. The multimer cages of NLRP3 are held together by binding between LRR domains, with PYDs buried within the complex to avoid premature activation. Association of double-ring cages with dTGN is required for activation of NLRP3 by activators such as nigericin, as double-ring-defective NLRP3 fails to be activated (61). dTGN may facilitate the transport of NLRP3 oligomers to reach NEK7 in the nucleus for binding and becoming competent for activation (68). Formation of the NLRP3 inflammasome is a complex process and is not well understood. The homotypic PYD-PYD interactions are critical for NLRP3 oligomerization, recruitment of ASC, and assembly of ASC into fibrils and specks. A recent study revealed that ASC filament elongation is unidirectional, originates from the B end of the NLRP3 filament, and is directed toward the formation of prion-like filament bundles and functional inflammasome specks (69).

4. ACTIVATION OF NLRP3 BY PARTICULATES: A CONTINUING CHALLENGE

The findings discussed above suggest a multistep process for the activation of NLRP3 by major activators (**Figure 3*b***). In this working model, priming and activation of NLRP3 occur at multiple steps in an activator- and context-dependent manner. These events are integrated to increase the protein levels of NLRP3, other inflammasomal components, and Casp1 substrates; induce NLRP3 oligomerization and its association with membranes both in the inactive state and during activation; and ultimately, result in the formation of a functional NLRP3 inflammasome. Nonetheless, how NLRP3 is activated by PAMPs and DAMPs with diverse structures at the molecular level under varied physiological and disease conditions remains largely unclear. This is particularly the case for particles and crystals.

Ongoing attempts to understand the activation of the NLRP3 inflammasome by particulates have focused on three mechanisms: K^+ efflux, ROS sensing, and release of lysosomal contents. Ion flux, particularly, K^+ efflux, was shown to be involved in the action of particles and crystals activating NLRP3, including silica, CPPD crystals, and asbestos (70, 71). Increased production of ROS is commonly observed in cells exposed to particles and crystals, and ROS have been proposed to serve as a common signal to activate NLRP3 by many activators, including particles and crystals such as silica, asbestos, MSU, and CPPD; nanotubes and nanoparticles; alum; and protein aggregates such as α-Syn fibrils (71–75). The molecular target of ROS in NLRP3 activation remains controversial and awaits further clarification. Upon deposition in tissue, most particulates are phagocytosed by macrophages. However, many particles are resistant to lysosomal degradation. As such, engulfed particulates would cause the destabilization of lysosomes, resulting in lysosomal leakage and rupture to release their contents, such as cathepsin B, into the cytoplasm. Lysosomal destabilization and leakage have been demonstrated for silica, alum, nanoparticles, crystalline cholesterol, and protein aggregates such as Aβ fibrils and α-Syn fibrils (7, 48, 73, 75, 76). How lysosomal molecules,

such as cathepsin B, activate NLRP3 and whether other cathepsins besides cathepsin B contribute to NLRP3 activation remain unclear.

5. NLRP3 INFLAMMASOME IN PARTICLE-DRIVEN DISEASE AND TOXICITY

The NLRP3 inflammasome has been notably involved in the development of several common chronic diseases and toxicity driven by particles and crystals.

5.1. Atherosclerosis

Atherosclerotic cardiovascular disease, such as coronary artery disease and stroke, underlies a leading cause of morbidity and mortality worldwide. Atherosclerosis is the progressive narrowing and hardening of arteries due to the accumulation of fatty materials and cholesterol, which form atheromatous plaques and cause chronic inflammation in artery walls. In this regard, atherosclerosis reflects the maladaptation of the innate immune system to excess deposition of fat and cholesterol in the artery wall. Recent studies reveal that excess cholesterol forms crystals early in the development of atherosclerosis; moreover, microscopic crystalline cholesterol acts as a DAMP signal to instigate inflammatory responses by activating the NLRP3 inflammasome in artery walls (7, 77, 78). Inhibition of NLRP3 activation by MCC950 suppresses atherosclerotic development in blood vessel walls in a mouse model of atherosclerosis with apolipoprotein E deficiency (79).

5.2. Gout and Pseudogout

Gout is an arthritis characterized by an elevated level of blood urea and deposition of MSU crystals in joint synovial fluid. Three stages are commonly noted: asymptomatic hyperuricemia, periodic acute gout attacks, and chronic tophaceous gout in which large deposits of MSU crystals are formed in joints and tissues (2). The pathologic role of MSU crystals was established when MSU crystals were injected into human knees and caused acute gouty attacks (80). MSU was shown to act as a danger signal from injured tissue (81, 82), whereas MSU crystals were among the first particulates identified as an endogenous NLRP3 inflammasome activator (70). The inflammatory activity of MSU crystals depends on the activation of the NLRP3 inflammasome and IL-1β production but not the functions of TLRs (70, 83). Deficiency of NLRP3 or ASC reduced MSU crystal–induced production of IL-1β in macrophages and dampened peritonitis and neutrophil infiltration in an MSU crystal–induced mouse model of peritonitis (70, 84). Deposition of CPPD crystals in the cartilage and synovial fluid causes CPPD crystal deposition disease or pseudogout arthritis. The etiology that causes CPPD crystal formation and deposition is unknown. Similar to MSU crystals in gout, CPPD crystals activate the NLRP3 inflammasome and instigate inflammation by elevating IL-1β production in affected joints (70).

Clinically, gout and pseudogout resemble autoinflammatory disease in that both have spontaneous episodes of acute inflammation in the absence of high titers of autoantibodies or antigen-specific T cells as in autoimmune disease (85, 86). The autoinflammatory nature of gout and pseudogout stems from the recurrent activation of the NLRP3 inflammasome by MSU and CPPD crystals caused by underlying metabolic abnormalities of unknown etiology, which is different from autoinflammation caused by autoactivation of NLRP3 through mutations of NLRP3 that disrupt the autoinhibition mechanisms in nascent NLRP3 (27).

5.3. Neurodegeneration

Neurodegeneration is the progressive atrophy and loss of function of neurons and is both the direct cause and a prominent feature of aging and neurodegenerative disease. In many cases,

neurodegeneration is associated with the toxicity of misfolded proteins that form aggregates and fibrils, exemplified by Aβ fibrils and tau tangles in Alzheimer's disease and α-Syn fibrils in Parkinson's disease.

5.3.1. Alzheimer's disease. A critical role of the NLRP3 inflammasome in Alzheimer's pathological development in association with neuronal protein aggregates was suggested when Aβ fibrils were shown to activate the NLRP3 inflammasome to produce IL-1β from microglia in vitro and to activate and recruit microglia to senile plaques in mouse brain when injected into the striatum by microinjection (76). Indeed, recent studies have revealed intricate interplays among Aβ fibrils, tau tangles, NLRP3 activation, and IL-1β-associated chronic inflammation in aging and Alzheimer's pathology (**Figure 4**). Microglia are the principal innate immune cells in the CNS. Microglia exist near neurons, and both cells maintain a controlled homeostasis by mutually modulating their activities. In aging and neurodegenerative brains, damaged and dying neurons release Aβ to form fibrils. Aβ fibrils serve as DAMPs to activate the NLRP3 inflammasome in microglia. Chronic activation of NLRP3 leads to persistently elevated inflammatory cytokines and chronic inflammation in the brain, resulting in the propagation of neurodegeneration (87). Activation of NLRP3 also induces the hyperphosphorylation of tau tangles by upregulating protein kinases, such as the glycogen synthase kinase 3β (GSK-3β), thereby contributing to tauopathies in aging and Alzheimer's brains (88). Additionally, pyroptotic microglia release ASC specks that bind Aβ

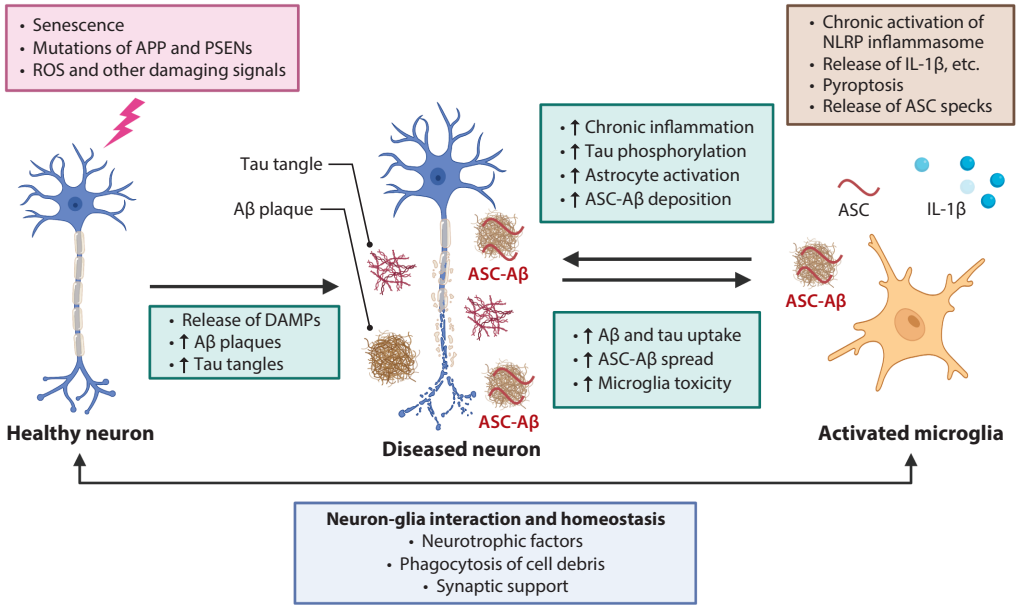

Figure 4

Multiple roles of the NLRP3 inflammasome in neurodegeneration associated with aging and Alzheimer's disease. Healthy neurons and microglia exist in proximity and in controlled homeostasis. Damaged neurons release Aβ fibrils that serve as DAMPs to activate the NLRP3 inflammasome in microglia, resulting in elevated inflammation in the brain. Activation of NLRP3 causes hyperphosphorylation of the tau tangles that boost tauopathy. Pyroptotic microglia release ASCs that bind Aβ fibrils to form ASC-Aβ composites, which further activate microglia through a vicious feedforward cycle, propagating chronic inflammation and neurodegeneration. Abbreviations: Aβ, amyloid-β; APP, amyloid precursor protein; ASC, associated speck-like protein containing a caspase-recruitment and activation domain; ASC-Aβ, composites of ASC and Aβ proteins; DAMP, danger/damage-associated molecular pattern; IL, interleukin; NLRP3, nucleotide-binding, oligomerization domain NOD-like receptor family pyrin domain–containing 3; PSEN, presenilin; ROS, reactive oxygen species; tau, tubulin associated unit. Figure adapted from images created with BioRender.com.

aggregates to form ASC-Aβ composites. ASC-Aβ composites can damage neurons and are taken up by neighboring microglia to amplify NLRP3-mediated inflammatory responses, which sets in motion a vicious feedforward cycle resulting in elevated neuroinflammation and degeneration (89). The mechanism by which Aβ filaments and tau tangles activate NLRP3 remains unclear, but lysosomal destabilization and cathepsin B release have been implicated as molecular links between the protein aggregates and NLRP3 activation (76).

5.3.2. Parkinson's disease and other central nervous system disorders. Parkinson's disease is characterized by the formation of Lewy bodies in the dopaminergic neurons of the substantia nigra pars compacta. Lewy bodies are mainly composed of α-Syn fibrils and are associated with the death of dopaminergic neurons. Released α-Syn fibrils are taken up by microglia through phagocytosis. Phagocytosed α-Syn acts as a DAMP to activate the NLRP3 inflammasome in the microglial cell by causing lysosomal leakage and release of cathepsin B into the cytoplasm (75). Dementia with Lewy bodies and multiple system atrophy are also synucleinopathies whose development involves the activation of NLRP3 by α-Syn aggregates (**Figure 2**). Activation of the NLRP3 inflammasome is also implicated in the neuroinflammation and pathogenesis of several other CNS diseases, including amyotrophic lateral sclerosis associated with superoxide dismutase 1 (SOD1) mutation and protein aggregation, prion disease, and traumatic brain injury (90–92). Whether and how the protein aggregates and fibrils play a role in the activation of the NLRP3 inflammasome in these disease conditions are unknown and await future investigation.

5.4. Pneumoconiosis and Nanotoxicity

Pneumoconiosis is a group of interstitial lung diseases resulting from the inhalation and deposition of dust particles in the lung. Pneumoconiosis is characterized by chronic inflammation, interstitial fibrosis, and restrictive impairment of the lung. Exposure to silica, asbestos, and coal dust causes silicosis, asbestosis, and coal miner's lung disease, respectively, which are the three most common types of pneumoconiosis. The pathogenesis of pneumoconiosis disease shares a common theme: Chronic or recurrent inhalation of dust particles and fibers leads to their persistent deposition in the lung, causing chronic inflammation and the development of fibrosis and cancer in the lung and the pleura. Acute inhalation of a large quantity of respirable dusts causes acute and rapidly progressing conditions. In both acute and chronic exposures, inflammation is commonly the first tissue response to the deposition of inhaled particles, which aids in particle clearance and tissue repair and the maintenance of homeostasis in the lung. However, airborne mineral dusts are resistant to phagocytic digestion and would persist in the tissue, resulting in a microenvironment that favors the chronic progression of inflammation and fibrosis. Therefore, pneumoconiosis is the result of maladaptation of innate immunity and tissue repair to persistent stimulation by particles and fibers. Like MSU and cholesterol crystals, mineral dusts, such as silica particles and asbestos fibers, activate the NLRP3 inflammasome to elevate the production of IL-1β and IL-18 in macrophages in vitro and in vivo (48, 71, 72). Deficiency of NLRP3 and associated inflammasome components in mice significantly and consistently reduced pulmonary inflammation upon inhalation of asbestos fibers or intratracheal instillation of silica particles (71, 72).

The rapid advancement in nanotechnology has led to the creation and utilization of numerous nanomaterials in industry and commercial products, including nanomedicines. Some nanomaterials exhibit properties of toxic particles and fibers, such as resistance to degradation, large surface area, and large aspect ratio, raising safety concerns on the inhalation of nanomaterials. Animal studies revealed asbestos-like and silica-like health effects of some engineered nanoparticles and nanotubes in the lung, which typically manifests a prominent inflammatory response with a propensity for progression to chronic inflammation, fibrosis, and malignancy. In one study, long

and needle-like carbon nanotubes (CNTs) and asbestos induced the secretion of IL-1β in LPS-primed macrophages by activating the NLRP3 inflammasome (73). Induction required functional NLRP3 and involved the production of ROS, release of cathepsin B, and activities of the $P2X_7$ receptor and Src and Syk kinases. In a separate study, nano-TiO_2 and nano-SiO_2, but not nano-ZnO, were shown to activate the NLRP3 inflammasome to release IL-1β, as well as IL-1α, from macrophages (93). It was also shown that, like silica, long and slim or short and rigid multiwalled CNTs stimulate macrophages to secrete IL-1β in vitro by activating the NLRP3 inflammasome (94). These findings suggest a prominent role of the NLRP3 inflammasome in nanotoxicity, which warrants further investigation to aid in the safety evaluation of engineered nanomaterials and nanomedicines.

6. CONCLUSION

Mammalian species protect against endogenous and environmental particles and crystals by way of phagocytotic clearance and inflammation. At the core of innate immune reactions is the recognition of signals derived from the deposition of particulates in tissues. The uncovering of the NLRP3 inflammasome as a key PRR for sensing PAMPs and DAMPs, including those associated with particles and crystals, has provided a new platform to elucidate the mechanism underlying innate immune sensing of particulates and how the adaptive inflammatory responses to particulates are propagated and go astray to cause chronic progression and disease. Still, considerable challenges exist and await further investigation. First, the mechanism by which the NLRP3 inflammasome is activated by particles and crystals of innumerable forms at the molecular level remains largely unclear. Second, how the varied physical and chemical properties of particulates influence the interaction between particles and innate immunity and the pathological and toxicological outcomes of such interaction are unknown yet critical for the safety evaluation of many man-made and naturally occurring particulate materials. Third, the functions of the NLRP3 inflammasome that may account for diverse pathological and toxicological effects of particulates beyond those associated with IL-1β production are not well understood. Fourth, given the persistent nature of particles and crystals and their associated health effects, a role of resolution or failed resolution of inflammation is expected in the pathogenesis of chronic inflammation and fibrosis caused by particles and crystals, which is largely unaddressed. Last but not least, how the rich body of knowledge generated from the study of the NLRP3 inflammasome and its role in particle-driven disease and toxicity can be harnessed to provide new insights into potential targets for the control and treatment of disease and toxicity in humans is a promising, albeit challenging, question to pursue in the near future.

DISCLOSURE STATEMENT

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