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Innate immunity to inhaled particles: A new paradigm of collective recognition

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Abstract

Major progress has been achieved in recent years to elucidate mechanisms driving the early response of pulmonary innate immune cells to inhaled micrometric and nanometric particles. Mononuclear phagocytes promptly categorize particles, alert immune network and engage crescendo responses for particle clearance and homeostasis restoration. Negatively charged particles directly interact with scavenger receptors A and B (SR-A and SR-B) and consequently activate specific signaling pathways, resulting in the production of TNF and IL-1 family members, which coordinate effective innate immune responses. Cytokine secretion also arises after a simple contact between particle-associated radicals and cell membranes. Reactive particles engage the passive release of constitutive alarmins, ensuing particle- or TNF-α-induced cell death and membranolysis. Finally, the inflammasome machinery represents the decisive intracellular platform that finely tune immune pathways engaged after SR activation, alarmin release, TNF-α production and cell homeostasis perturbations. Disturbance of these collective recognition processes prolongs particle persistence and innate immune responses that generate long-lasting adaptive immunity and cause chronic lung diseases.

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Keywords

Sensing, Particles, Nanoparticles, Silica, Innate immunity, PRR, DAMP, HAMP, Cytokines, Alarmins and inflammasome.

Abbreviation

PRRs, Pattern Recognition Receptors; DAMPs, Damage-Associated Molecular Patterns; HAMPs, Homeostasis-Altering Molecular Processes; LMP, Lysosomal Membrane Permeabilization; CDE, Clathrin-Dependent Endocytosis; NLRP, NOD-like Receptor Rroteins.

1. Introduction

Interest in clarifying the immuno-pathophysiology of lung disorders induced by inorganic particles was initiated almost 30 years ago with the first description of a marked accumulation of neutrophils and activated macrophages (or mononuclear phagocytes) in the lungs of dust-exposed individuals with respiratory impairments [1]. Although additional immune cells and pathways have been identified that refine our understanding of the immune mechanisms leading to particle-induced chronic diseases [2], it remains to elucidate how innate immunity senses particles (inert or reactive) and elicits early tissue responses that have an essential role in eliminating particles or driving diseases such as fibrosis and cancer.

The innate immune system integrates a distinct set of receptors on phagocytes designated pattern recognition receptors (PRRs) and serving as sensors for monitoring the extracellular and intracellular compartments for signs of infection or tissue injury [3]. These sentinel receptors rely on sensing common structural and functional features associated with different classes of microorganisms termed pathogen-associated molecular patterns (PAMPs). The PRR system also detects debris from dying cells, known as danger-associated molecular patterns (DAMPs that comprise alarmins) and perturbations in cytoplasmic homeostasis, recently defined as homeostasis-altering molecular processes (HAMPs) [4]. The engagement of PRRs by PAMPs, DAMPs or HAMPs results in the production of master cytokines such as IL-1 and TNF family members that orchestrate effective immune responses [5].

A similar PRR-mediated sensing system for inhaled particles did initially not appear plausible because particles are different from biological structures such as microorganism cell-wall components or viral nucleic acids, which are avidly and specifically recognized by PRRs. The discovery that scavenger receptors (SR), a subfamily of PRRs, are dedicated to sense endogen low-density lipoprotein (LDL) particles and asbestos [6] changed the opinion of researchers in particle toxicology and suggested that innate immunity can specifically recognize particles and initiate responses against particles. In 1998, three distinct reports [7–9] concurrently revealed a new PRR-related intracellular sensing axis comprising nod-like receptors (NLRP), termed

inflammasome, that is pivotal in particle recognition and immune system activation (reviewed in Ref. [10]). Altogether, these unforeseen aspects of particle-sensing processes by PRRs have shaken up our knowledge of early host defense responses against particles.

Available evidence supports the view that the innate immune system senses particles such as silica, asbestos or titanium dioxide to promote their clearance and to prevent tissue injury. However, the inability of phagocytes to eliminate particles can result in inappropriate and prolonged activation of innate immunity responses [11]. The progressive development of fibrosis, cancer or autoimmune diseases after particle exposure appears when particles are refractory to clearance process, constantly activate PRR-mediated particle recognition, induce cytokine release and promote long-lasting adaptive immune responses and drive chronic diseases [12]. Thus, the fine regulation of innate immunity after its activation by particles is essential to restore homeostasis (Fig. 1).

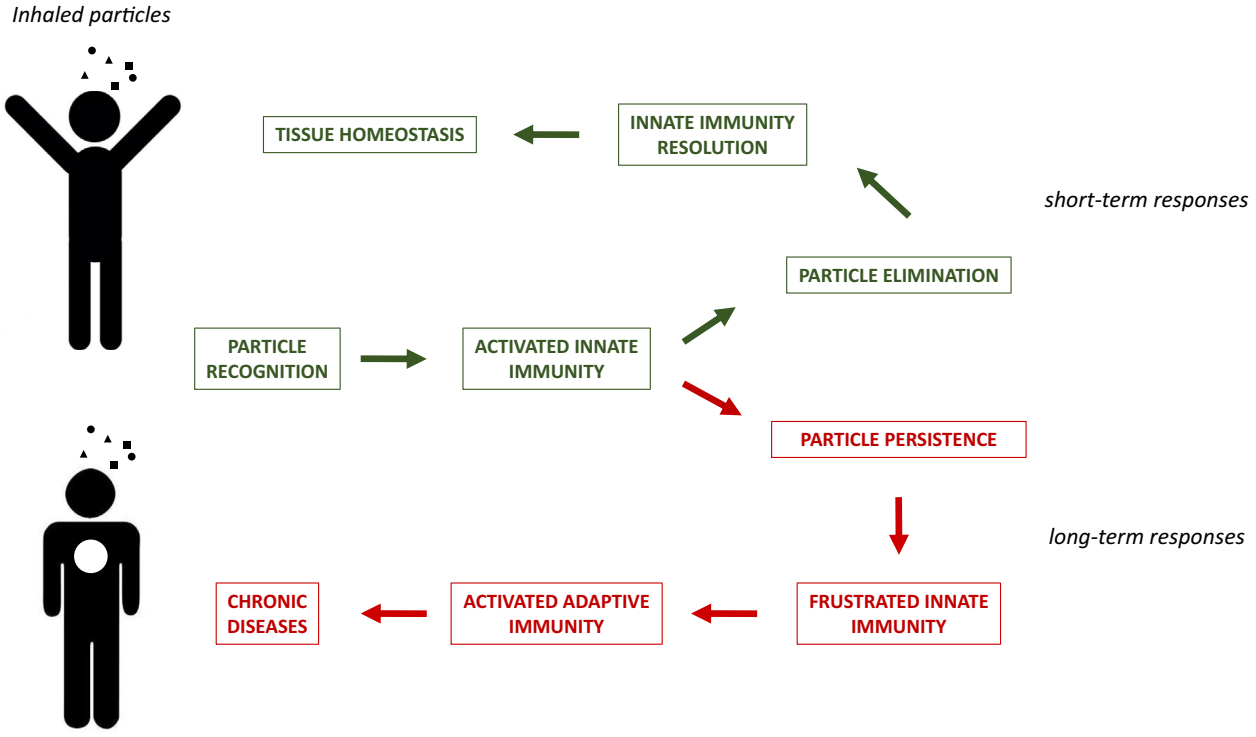
Here, we discuss some of the recent developments in particle sensing and describe the emerging concepts of micro- and nanoparticle-recognition systems that include different classes of PRRs (scavenger receptors and inflammasome machinery), DAMPs (alarmins) and HAMPs (membrane destabilization). These recognition systems survey the extracellular or cytosolic spaces for detecting particles themselves or particle-related cell signatures and operate in a complementary manner to promote effective responses to particles. Exploring the collective actions of the PRR pathways sensing particles represents a new frontier in particle toxicity, and is the focus of this review.

2. Initial pattern recognition receptor activation by particles

Scavenger receptor (SR) are integral membrane proteins that contribute to the recognition and elimination of foreign or altered-self targets. The SR subfamily abundantly present on mononuclear phagocytes comprises a diverse array of functional innate receptors sharing the ability to recognize polyionic ligands such as oxidized LDL particles [13]. SR-mediated sensing also represents the main PRR-related system to detect inhaled particles and initiate early tissue responses [14].

Among SR members, compelling studies support SR-A6 (MARCO) as critical in particle recognition. Expression

Fig. 1

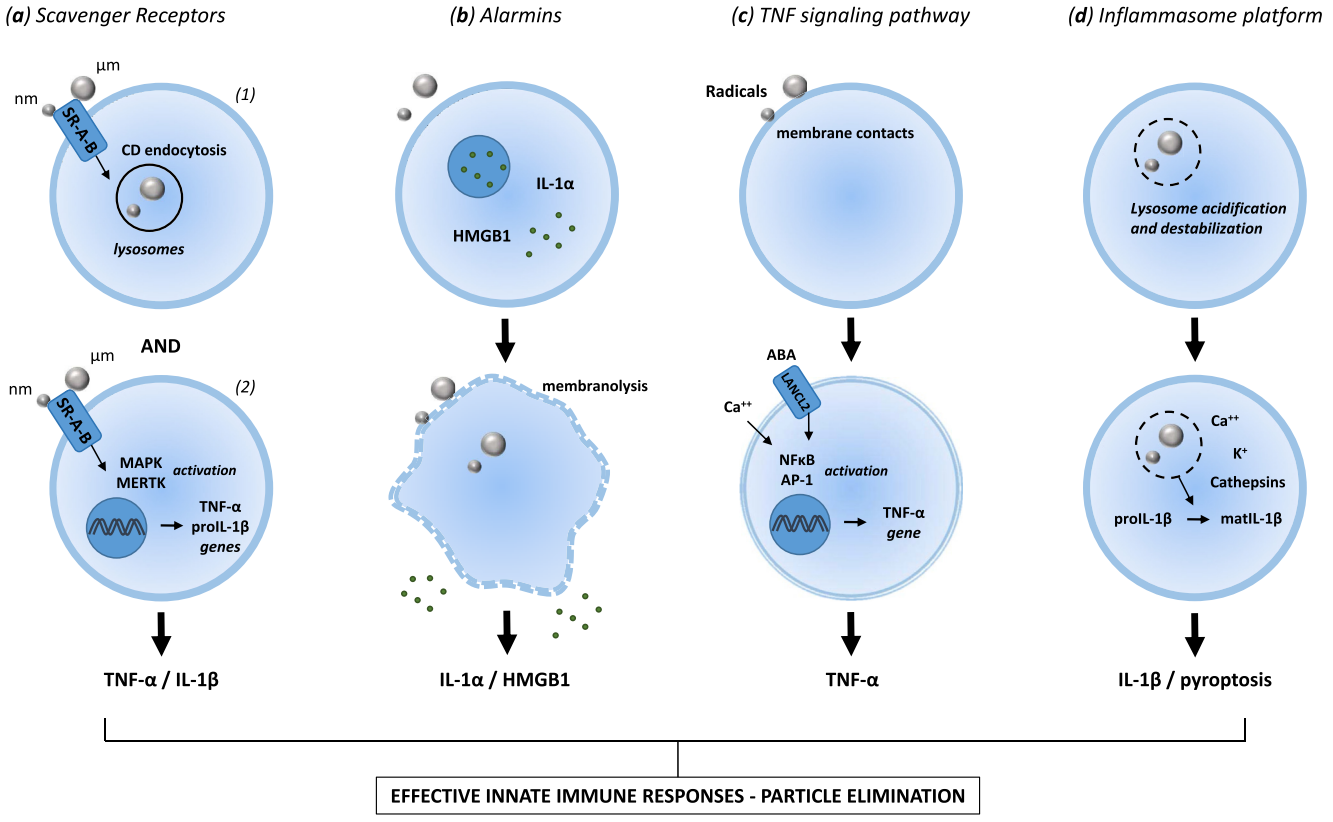


Inappropriate activation of sensing processes leads to persistent particle accumulation, uncontrolled innate immune responses and chronic disease development. Phagocytes possess a sensing arsenal of pattern recognition receptors (PRRs) capable of recognizing and taking up inhaled particles. Engagement of this recognition system results in the deployment of innate immune responses accountable to clear particles from tissue to avoid tissue injury. After particle elimination, innate immune responses are controlled and tissue homeostasis is restored. The inability of the innate immune system to degrade and clear particles results into frustrated innate immune responses that lead to the establishment of long-lasting adaptive immunity and cause chronic diseases for which no specific therapy is available.

of SR-A6 in macrophages mediates silica particle binding and endocytosis with cell death [15] (Fig. 2a1). Pulmonary macrophage SR-A6 also mediates clearance of silica from lung tissue [16]. The role of SR-A1 (CD204) is currently less clear but probably comparable to MARCO when considering particle endocytosis [14]. SR-A1 and silica particles can undergo internalization from the plasma membrane via clathrin-dependent endocytosis (CDE) [17]. SR-A6 and -A1 recognize various particles (silica, metallic particles and latex beads), irrespective of their primary size (from micrometric to nanometric) [18–20]. Importantly, SR-A-mediated particle endocytosis can result in lysosomal membrane permeabilization (LMP) and cell death, two crucial events leading to inflammasome engagement and alarmin release, respectively (see below, points 5 and 3). Recently, another SR of the B subfamily (SR-B1) has been identified to specifically sense amorphous and crystalline nano- and micro-sized silica particles. Consistent with previous characterization of SR-B1 as a non-endocytic receptor, this PRR is, however, not required for silica internalization as the SR-A members [21].

Intracellular signaling pathways are also directly activated after SR-A/B and particle interaction (Fig. 2a2). Activation of SR-A6 and SR-A1 by silica is implicated in free radical and TNF-α production in bone-marrow-derived mast cells and macrophages [22,23]. SR-A1 and SR-B1 also mediate IL-1β release [20,21]. These data strongly indicate that SR-A/B directly induces mitogen-activated protein kinase (MAPK) phosphorylation and NF-κB and/or AP-1 nuclear translocation after

Fig. 2



Early sensing and alerting processes are combined and mutually linked in response to inhaled particles. (**a1**) Micrometric (µm) and nanometric (nm) particles are internalized by phagocytes through the scavenger receptors (SR) A and B and clathrin-dependent (CD) endocytosis. (**a2**) Particle sensing by these subclasses of pattern recognition receptors (PRRs) also results in the activation of MAPK and MerTK signal transduction leading to TNF-α and IL-1β secretion, which instruct innate immune responses and inflammasome platform (see *d*). (**b**) Endocytosis of particles can result in cell death and membranolysis, permitting the passive release of alarmins (subclass of danger-associated molecular patterns, DAMPs) in the tissue environment. Beside their direct activity on innate immune cell recruitment and stimulation, alarmins are also powerful stimulators of immature proIL-1β production and mature (mat) IL-1β secretion (*d*). (**c**) Radical groups on particle surface induce plasma membrane peroxidation, calcium flux perturbation, abscisic acid (ABA) release and LANCL2 receptor activation that consequently result in TNF-α release. In addition to its own innate immune activity, TNF-α is known to actively increase the pool of proIL-1β available for the inflammasome machinery (*d*) and to induce cell death and membranolysis (*b*). (**d**) Reactive particles which are taken up by phagocytes (*see a1*) induce perturbations in cytoplasmic homeostasis (homeostasis-altering molecular processes, HAMPs such as ion concentration modifications and lysosomal leakage of cathepsin K and S) that are sensed by the intracellular PRR-related inflammasome complex (NLRP) and cause NLRP engagement and mature IL-1β release from inactive proIL-1β. Inflammasome engagement results in a cell death termed pyroptosis that can contribute to alarmin release (*b*). The stepwise engagement of PRRs with the progressively increase of serial cytokine secretion coordinates effective immune responses and promotes particle elimination.

particle exposure [13,24]. The Mer receptor tyrosine kinase (MerTK) signal cascade is also activated after silica sensing by SR-A1 and mediates IL-1β release by macrophages [20]. However, SR-A6- and SR-A1-deficient mice display exacerbated cytokine production and neutrophilic accumulation after administration of silica particles [16,25]. Exacerbated LMP, cholesterol recycling dysfunction and increased IL-1β release are also observed in macrophages deficient for SR-A6 [26]. In contrast, SR-B1 depleted mice treated with silica particles showed, as expected, reduced IL-1β levels and neutrophilia [21]. Thus, the SR-A subfamily signals through different intracellular pathways with distinct effects on immune responses to inhaled particles.

The mechanisms underlying the direct recognition of particles by SR-A and -B have been elegantly clarified in recent studies. The positively charged amino acids of SR are involved in the sensing processes of particles with negative surface charges. A conserved R-X-R motif of domain V (also termed C-terminal SR cysteine-rich domain or SRCR) of SR-A6 containing positively charged residues is required to initiate cell signaling after macrophage exposure to silica or asbestos particles. Crystalline or amorphous silica and TiO_2 particles bind at different sites in domain V, which may elicit specific and different downstream effects and tissue responses [18,23]. SR-B1 binding to silica is also linked to the particle surface charge and a basic amino acid cluster (α4 to α5) at the apex position of the receptor [21].

3. Membranolysis and alarmin release

Dying cells and cell death pathways have an important role in the initiation of host defense against bacterial and viral infection or during tissue injury [27]. The release of necrotic cell or apoptotic body contents after membrane rupture (membranolysis) acts as a signal to initiate rapid immune responses. Molecules generated by dying cells include DAMPs that account for the upstream immunological cues regulating innate immunity and initiating adaptive immune responses [28].

Membranolysis during cell death induced by silica in phagocytes [29] is initiated by the peroxidation of membrane lipids caused by reactive surface silanol patches present on particle surface [30–33]. Alarmins are the main endogenous danger signals among DAMPs released to the extracellular milieu after particle exposure [10] (Fig. 2b). Unlike most cytokines, which are up regulated upon stimulation, alarmins are constitutively present in resting cells and play important intracellular functions as transcription factors under homeostatic conditions. Alarmins possess extracellular functions when passively released by dying macrophages after membranolysis and bind membrane receptors in adjacent phagocytes not yet affected or newly recruited [28]. IL-1α, along with other constitutive alarmins such as High Mobility Group Box 1 (HMGB1) is abundantly secreted after particle exposure and promotes IL-1β gene transcription by activating NFκB or AP-1 translocation (see point 5 and reference [10]).

IL-1α is released from cellular stocks in pulmonary macrophages after exposure to silica or carbon nanotubes (CNT). This effect is size-dependent since nanoparticles where more efficient to induce IL-1α release than the corresponding micrometric particles. IL-1α release by macrophages was predictive of the acute inflammogenic potential (active IL-1β levels and neutrophil accumulation) of micro- and nano-silica [34]. Passive release of HMGB1 is documented in cultures of macrophages exposed to silica or CNT [35]. The presence of HMGB1 in the extracellular environment increases IL-1β secretion by CNT-treated macrophages and inhibition of extracellular HMGB1 reduces CNT-induced IL-1β secretion and inflammation *in vivo* [35].

4. Particle recognition and TNF-α signaling

There is very little doubt that TNF-α is a key cytokine in lung responses to inhaled particles [36]. Release of TNF-α by phagocytes is typically observed after *in vitro* or *in vivo* exposure to diverse particles [37]. TNF-α is a powerful activator of NFκB and AP-1 transcription factors and is known to orchestrate cytokine expression during the early inflammatory responses to particles [38].

Recent elements emphasize TNF-α as the first innate immune signal immediately released after reactive particle exposure. While its early production dependents on SR or Fas ligand (see the above point 2 and reference [39]), evidence also suggests that particle-generated radicals and TNF-α expression are functionally linked. A simple contact between the macrophage plasma membrane and particles is sufficient to trigger TNF-α production in the absence of phagocytosis [40–42]. Radicals generated at the surface of particles cause membrane lipid peroxidation, extracellular Ca^{2+} influx, ABA/LANCL2 interaction and TNF-α release, which occurs within the first minutes of cell exposure to particles such as silica [40,41] (Fig. 2c). Simple particle contacts with the cell membrane are also evoked as triggers for IL-1β secretion [43].

Under resting conditions, TNF-α translation is repressed in most cells but rapidly restored under stress conditions [44]. In addition, a membrane-bound precursor of TNF-α is processed by a TNF-α converting enzyme (TACE) to generate secreted mature TNF-α [45], confirming that TNF-α is very promptly secreted, independently of transcriptional induction or other early immune mediators.

The capacity of TNF-α to transduce particle detection to alert the immune network is not limited to its direct

effects. In response to particles, TNF-α also emerges as one of the major cytokines mediating proIL-1β expression and initiating inflammasome activation (point 5 and reference [38]). Finally, this early cytokine is involved in cell death of phagocytes and subsequent alarmin release after particle treatment (point 3 and [46]).

5. The inflammasome platform: the ultimate regulator of innate immune responses to particles

Beside SR expressed on their surface (point 2), phagocytes can elicit discriminating responses to inhaled particles by deploying a sophisticated PRR-related intracellular machinery termed inflammasome (or NLRP) [9] (Fig. 2d). This key component of cytosolic surveillance is a multiprotein signaling platform that controls activation of the proteolytic enzyme caspase-1 which, in turn, regulates the secretion of mature IL-1β and IL-18, coordinates host defenses and induces pyroptotic cell death [47]. The inflammasome components NLRP3 (NOD-, LRR- and pyrin domain-containing 3) and pyrin do not directly detect microorganism or particle patterns, but instead act as signal integrators detecting perturbations in cytoplasmic homeostasis. These cell perturbations now termed HAMPs underlie inflammasome assembly and biologically active IL-1β release that play a crucial role in the establishment of innate and adaptive immune responses [4].

Intensive investigations exploring HAMPs have led to propose different cell processes accounting for inflammasome activation and IL-1β maturation in response to inhaled particles. The main perturbations of cell homeostasis detected by NLRP3 are LMP and subsequent lysosome content release after particle internalization. Following particle uptake by the phagolysosomal pathway, LMP elicits the leakage of cathepsin B and S that, in turn, regulates inflammasome assembly and activation [48,49]. Lysosome acidification probably represents a prerequisite and an upstream inflammasome activator after particle endocytosis [50]. Early modifications of cell volume, intracellular ionic concentrations (mainly K^+ and Ca^{++}) and redox balance are also sensed by NLRP3 axis as major cellular events requiring IL-1β activation [43,51]. Additional cell perturbations crucial to NLRP3 activation comprise mitochondrial damage and autophagy dysfunction [52]. Physicochemical characteristics of particles such as size and shape are decisive for particle internalization and lysosomal alteration. The smallest and fiber- or needle-like particles are particularly active to induce inflammasome activation. Surface area properties and reactivity also govern cell function, lysosomal damage, and subsequent inflammasome/IL-1β processing [10].

Surprisingly, other activating stimuli are required for phagocytes to express the immature precursor form of IL-1β (proIL-1β), a prerequisite for active IL-1β release. The endogenous mediators that prime the transcription of pro-IL-1β through the NFκB/AP-1 signal transduction are the alarmins IL-1α and HMGB1 released after particle-induced cell membranolysis (see point 3 and [10]). Besides alarmins, TNF-α is another crucial priming factor promptly released by phagocytes after radical sensing (point 4) and scavenger receptor activation (point 2 and [38]). Finally, pyroptosis is the cell death pathway mediated by caspase-1 activation. Thus, pyroptosis can amplify innate immune responses to particles through the release of dying cell contents, including alarmins.

Interestingly, the secretion of mature IL-1β strikingly requires collective and concomitant signals i.e. HAMPs (which activate inflammasome) and DAMPs (which induce proIL-1β production). It is thus probable that the detection of crescendo signals after PRR system engagement allows the host to gauge particle reactivity, particle deposition or tissue damages and ultimately adjust effective innate immune responses to particles.

6. General scenario and predictive tests

In conclusion, this review highlights that the innate immune system integrates SR on phagocytes that sense inhaled particles, activate signal transduction pathways and initiate particle endocytosis. The PRR system associated to inhaled particles also detects components from dying phagocytes after particle endocytosis that comprise DAMPs (alarmins and abscisic acid) and HAMPs (ion movements and cathepsin release). The engagement of PRRs by the particles themselves, DAMPs or HAMPs results in the production of potent pro-inflammatory cytokines (mainly IL-1 and TNF) that orchestrate early immune responses against particles.

Simple and quantitative models or assays based on PRR engagement may serve in (nano) toxicology to predict the *in vivo* toxicity of new materials and particles and to reduce animal uses. Alarmin release in macrophage cultures has been proposed to serve as a basis for evaluating the inflammogenic activity of nano- and micrometric particles [34]. The binding of particles at different sites of SR may also be used to distinguish reactive and inert particles [18,23]. Finally, the activation of inflammasome machinery appears highly predictive of the potential capacity of micro- and nanoparticles to activate innate immune responses [10]. Indeed, these intracellular PRRs can be activated by a large panel of cell perturbations induced by diverse reactive particles.

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Conflict of interest

The author has no conflicting financial interest.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Rom WN, Travis WD, Brody AR: **Cellular and molecular basis of the asbestos-related diseases**. *Am Rev Respir Dis* 1991, **143**:408–422.
2. Pollard KM: **Silica, silicosis, and autoimmunity**. *Front Immunol* 2016, **7**:97.
3. Kieser KJ, Kagan JC: **Multi-receptor detection of individual bacterial products by the innate immune system**. *Nat Rev Immunol* 2017, **17**:376–390.
4. Liston A, Masters SL: **Homeostasis-altering molecular processes as mechanisms of inflammasome activation**. *Nat Rev Immunol* 2017, **17**:208–214.
5. Afonina IS, Zhong Z, Karin M, Beyaert R: **Limiting inflammation-the negative regulation of NF-kappaB and the NLRP3 inflammasome**. *Nat Immunol* 2017, **18**:861–869.
6. Resnick D, Freedman NJ, Xu S, Krieger M: **Secreted extracellular domains of macrophage scavenger receptors form elongated trimers which specifically bind crocidolite asbestos**. *J Biol Chem* 1993, **268**:3538–3545.
7. Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J: **Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica**. *Science* 2008, **320**: 674–677.
8. Cassel SL, Eisenbarth SC, Iyer SS, Sadler JJ, Colegio OR, Tephly LA, Carter AB, Rothman PB, Flavell RA, Sutterwala FS: **The Nalp3 inflammasome is essential for the development of silicosis**. *Proc Natl Acad Sci USA* 2008, **105**:9035–9040.
9. •• Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E: **Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization**. *Nat Immunol* 2008, **9**:847–856.

In investigating how silica particles activate NLRP3 inflammasome, this paper was the first and perhaps the most convincing to propose that lysosomal destabilization after particle endocytosis results in inflammasome assembly, IL-1β secretion and subsequent innate immune responses to particles.

10. Rabolli V, Lison D, Huaux F: **The complex cascade of cellular events governing inflammasome activation and IL-1beta processing in response to inhaled particles**. *Part Fibre Toxicol* 2016, **13**:40.
11. Iwasaki A, Medzhitov R: **Control of adaptive immunity by the innate immune system**. *Nat Immunol* 2015, **16**:343–353.
12. Cao X: **Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease**. *Nat Rev Immunol* 2016, **16**:35–50.
13. Zani IA, Stephen SL, Mughal NA, Russell D, Homer-Vanniasinkam S, Wheatcroft SB, Ponnambalam S: **Scavenger receptor structure and function in health and disease**. *Cells* 2015, **4**:178–201.
14. Thakur SA, Hamilton Jr RF, Holian A: **Role of scavenger receptor a family in lung inflammation from exposure to environmental particles**. *J Immunotoxicol* 2008, **5**:151–157.
15. Hamilton Jr RF, Thakur SA, Mayfair JK, Holian A: **MARCO mediates silica uptake and toxicity in alveolar macrophages from C57BL/6 mice**. *J Biol Chem* 2006, **281**:34218–34226.
16. Thakur SA, Beamer CA, Migliaccio CT, Holian A: **Critical role of MARCO in crystalline silica-induced pulmonary inflammation**. *Toxicol Sci* 2009, **108**:462–471.
17. Orr GA, Chrisler WB, Cassens KJ, Tan R, Tarasevich BJ, Markillie LM, Zangar RC, Thrall BD: **Cellular recognition and trafficking of amorphous silica nanoparticles by macrophage scavenger receptor A**. *Nanotoxicology* 2011, **5**:296–311.
18. Thakur SA, Hamilton Jr R, Pikkarainen T, Holian A: **Differential binding of inorganic particles to MARCO**. *Toxicol Sci* 2009, **107**:238–246.
19. Palecanda A, Paulauskis J, Al-Mutairi E, Imrich A, Qin G, Suzuki H, Kodama T, Tryggvason K, Koziel H, Kobzik L: **Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles**. *J Exp Med* 1999, **189**:1497–1506.
20. Nishijima N, Hirai T, Misato K, Aoyama M, Kuroda E, Ishii KJ, Higashisaka K, Yoshioka Y, Tsutsumi Y: **Human scavenger receptor A1-mediated inflammatory response to silica particle exposure is size specific**. *Front Immunol* 2017, **8**:379.
21. Tsugita M, Morimoto N, Tashiro M, Kinoshita K, Nakayama M: **SR-B1 is a silica receptor that mediates canonical inflammasome activation**. *Cell Rep* 2017, **18**:1298–1311.
22. Brown JM, Swindle EJ, Kushnir-Sukhov NM, Holian A, Metcalfe DD: **Silica-directed mast cell activation is enhanced by scavenger receptors**. *Am J Respir Cell Mol Biol* 2007, **36**: 43–52.
23. •• Murthy S, Larson-Casey JL, Ryan AJ, He C, Kobzik L, Carter AB: **Alternative activation of macrophages and pulmonary fibrosis are modulated by scavenger receptor, macrophage receptor with collagenous structure**. *FASEB J* 2015, **29**: 3527–3536.

A comprehensive study showing how particles can directly bind and activate scavenger receptors.

24. Hamilton Jr RF, Thakur SA, Holian A: **Silica binding and toxicity in alveolar macrophages**. *Free Radic Biol Med* 2008, **44**: 1246–1258.
25. Beamer CA, Holian A: **Scavenger receptor class A type I/II (CD204) null mice fail to develop fibrosis following silica exposure**. *Am J Physiol Lung Cell Mol Physiol* 2005, **289**: L186–L195.
26. Biswas R, Hamilton Jr RF, Holian A: **Role of lysosomes in silica-induced inflammasome activation and inflammation in absence of MARCO**. *J Immunol Res* 2014, **2014**:304180.
27. Jorgensen I, Rayamajhi M, Miao EA: **Programmed cell death as a defence against infection**. *Nat Rev Immunol* 2017, **17**: 151–164.
28. Nefla M, Holzinger D, Berenbaum F, Jacques C: **The danger from within: alarmins in arthritis**. *Nat Rev Rheumatol* 2016, **12**: 669–683.
29. Joshi GN, Knecht DA: **Silica phagocytosis causes apoptosis and necrosis by different temporal and molecular pathways in alveolar macrophages**. *Apoptosis* 2013, **18**:271–285.
30. Pavan C, Turci F, Tomatis M, Ghiazza M, Lison D, Fubini B: **Zeta potential evidences silanol heterogeneity induced by metal contaminants at the quartz surface: implications in membrane damage**. *Colloids Surf B Biointerfaces* 2017, **157**: 449–455.
31. Pavan C, Fubini B: **Unveiling the variability of "Quartz Hazard" in light of recent toxicological findings**. *Chem Res Toxicol* 2017, **30**:469–485.
32. Pavan C, Rabolli V, Tomatis M, Fubini B, Lison D: **Why does the hemolytic activity of silica predict its pro-inflammatory activity?** *Part Fibre Toxicol* 2014, **11**:76.
33. •• Turci F, Pavan C, Leinardi R, Tomatis M, Pastero L, Garry D, Anguissola S, Lison D, Fubini B: **Revisiting the paradigm of silica pathogenicity with synthetic quartz crystals: the role of crystallinity and surface disorder**. *Part Fibre Toxicol* 2016, **13**:32.

A seminal paper demonstrating the ability of irregular surfaces and radical patches on particles interacting with membranes to induce lysis and cytokine release.

34. Rabolli V, Badissi AA, Devosse R, Uwambayinema F, Yakoub Y, Palmai-Pallag M, Lebrun A, De Gussem V, Couillin I, Ryffel B, *et al.*: **The alarmin IL-1alpha is a master cytokine in acute lung inflammation induced by silica micro- and nanoparticles**. *Part Fibre Toxicol* 2014, **11**:69.

35. • Jessop F, Holian A: **Extracellular HMGB1 regulates multi-walled carbon nanotube-induced inflammation in vivo**. *Nanotoxicology* 2015, **9**:365–372.

This and the paper from Rabolli were the first to suggest that alarmins passively released after particle-induced cell death play a major role in proIL-1β expression and the establishment of innate immune responses.

36. Fazzi F, Njah J, Di Giuseppe M, Winnica DE, Go K, Sala E, St Croix CM, Watkins SC, Tyurin VA, Phinney DG, *et al.*: **TNFR1/phox interaction and TNFR1 mitochondrial translocation Thwart silica-induced pulmonary fibrosis**. *J Immunol* 2014, **192**:3837–3846.

37. Shvedova AA, Kisin ER, Mercer R, Murray AR, Johnson VJ, Potapovich AI, Tyurina YY, Gorelik O, Arepalli S, Schwegler-Berry D, *et al.*: **Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice**. *Am J Physiol Lung Cell Mol Physiol* 2005, **289**:L698–L708.

38. Franchi L, Eigenbrod T, Nunez G: **Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation**. *J Immunol* 2009, **183**:792–796.

39. Borges VM, Falcao H, Leite-Junior JH, Alvim L, Teixeira GP, Russo M, Nobrega AF, Lopes MF, Rocco PM, Davidson WF, *et al.*: **Fas ligand triggers pulmonary silicosis**. *J Exp Med* 2001, **194**:155–164.

40. •• Magnone M, Sturla L, Jacchetti E, Scarfi S, Bruzzone S, Usai C, Guida L, Salis A, Damonte G, De Flora A, *et al.*: **Autocrine abscisic acid plays a key role in quartz-induced macrophage activation**. *FASEB J* 2012, **26**:1261–1271.

This manuscript (with others such as reference #43) elegantly demonstrated that a simple particle and cell membrane contact results in cytokine secretion, hence reinforcing the idea that the sensing mechanism of particles is prompt and varied.

41. Scarfi S, Magnone M, Ferraris C, Pozzolini M, Benvenuto F, Benatti U, Giovine M: **Ascorbic acid pre-treated quartz stimulates TNF-alpha release in RAW 264.7 murine macrophages through ROS production and membrane lipid peroxidation**. *Respir Res* 2009, **10**:25.

42. Haberzettl P, Duffin R, Kramer U, Hohr D, Schins RP, Borm PJ, Albrecht C: **Actin plays a crucial role in the phagocytosis and biological response to respirable quartz particles in macrophages**. *Arch Toxicol* 2007, **81**:459–470.

43. Hari A, Zhang Y, Tu Z, Detampel P, Stenner M, Ganguly A, Shi Y: **Activation of NLRP3 inflammasome by crystalline structures via cell surface contact**. *Sci Rep* 2014, **4**:7281.

44. Han J, Brown T, Beutler B: **Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level**. *J Exp Med* 1990, **171**:465–475.

45. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, *et al.*: **A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells**. *Nature* 1997, **385**:729–733.

46. Gozal E, Ortiz LA, Zou X, Burow ME, Lasky JA, Friedman M: **Silica-induced apoptosis in murine macrophage: involvement of tumor necrosis factor-alpha and nuclear factor-kappaB activation**. *Am J Respir Cell Mol Biol* 2002, **27**:91–98.

47. Broz P, Dixit VM: **Inflammasomes: mechanism of assembly, regulation and signalling**. *Nat Rev Immunol* 2016, **16**:407–420.

48. Hughes CS, Colhoun LM, Bains BK, Kilgour JD, Burden RE, Burrows JF, Lavelle EC, Gilmore BF, Scott CJ: **Extracellular cathepsin S and intracellular caspase 1 activation are surrogate biomarkers of particulate-induced lysosomal disruption in macrophages**. *Part Fibre Toxicol* 2016, **13**:19.

49. Bunderson-Schelvan M, Holian A, Hamilton Jr RF: **Engineered nanomaterial-induced lysosomal membrane permeabilization and anti-cathepsin agents**. *J Toxicol Environ Health B Crit Rev* 2017, **20**:230–248.

50. Jessop F, Hamilton Jr RF, Rhoderick JF, Fletcher P, Holian A: **Phagolysosome acidification is required for silica and engineered nanoparticle-induced lysosome membrane permeabilization and resultant NLRP3 inflammasome activity**. *Toxicol Appl Pharmacol* 2017, **318**:58–68.

51. Rabolli V, Wallemme L, Lo Re S, Uwambayinema F, Palmai-Pallag M, Thomassen L, Tyteca D, Octave JN, Marbaix E, Lison D, *et al.*: **Critical role of aquaporins in interleukin 1beta (IL-1beta)-induced inflammation**. *J Biol Chem* 2014, **289**:13937–13947.

52. Jessop F, Hamilton RF, Rhoderick JF, Shaw PK, Holian A: **Autophagy deficiency in macrophages enhances NLRP3 inflammasome activity and chronic lung disease following silica exposure**. *Toxicol Appl Pharmacol* 2016, **309**:101–110.