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Cells under stress: The mechanical environment shapes inflammasome responses to danger signals

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Abstract

Many intracellular signals, such as host danger associated molecules and bacterial toxins during infection, elicit inflammasome activation. However, the mechanical environment in tissues may also influence the sensitivity of various inflammasomes activation. The cellular mechanical environment is determined by the extracellular tissue stiffness, or its inverse, tissue compliance. Tissue stiffness is sensed by the intracellular cytoskeleton through a process termed mechanotransduction. Thus, extracellular compliance and the intracellular cytoskeleton may regulate the sensitivity of inflammasome activation. Control of pro-inflammatory signaling by tissue compliance may contribute to the pathogenesis of diseases such as ventilator-induced lung injury during bacterial pneumonia and tissue fibrosis in inflammatory disorders. The responsible signaling cascades in inflammasome activation pathways and mechanotransduction cross-talk are not yet fully understood. This rather different immunomodulatory perspective will be reviewed and open questions discussed here.

Summary sentence:

This review summarizes available evidence showing the internal cytoskeleton and extracellular compliance modulates inflammasome activation.

Graphical Abstract

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Keywords

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INTRODUCTION:

The innate immune system senses invading pathogens and clears infections by mounting inflammatory responses. A major pro-inflammatory signal is IL-1 β , which is produced following inflammasome activation. The inflammasome is a high molecular weight (~700kDa) multimeric complex that activates caspase-1 to cleave pro-IL-1 β and pro-IL-18 into the bioactive, mature forms of IL-1 β and IL-18 [1]. Inflammasome activation has been conceptualized as a two-step process. First, priming of the inflammasome occurs through pathogen mediated TLR signaling. Priming requires NF- κ B-mediated transcription and upregulates synthesis of inflammasome components and pro-IL-1 β . In the second step, inflammasome assembly results in the cleavage and production of mature IL-1 β . Assembly is initiated by binding of danger-associated molecules, such as uric acid [2] ATP [3], dsDNA [4], or bacterial toxins [5] to inflammasome receptors. Some inflammasome receptors include “nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing receptors,” such as NLRP3, NLRC4, and NLRP1, “absent in melanoma-2” (AIM2), and Pyrin inflammasomes [6]. Recruitment of pro-caspase-1 during inflammasome assembly is facilitated by an adaptor protein termed “apoptosis-associated speck-like protein containing a CARD” (ASC; see Fig. 1) [1, 7, 8]. Signals that induce inflammasome priming are often called “signal 1,” while those inducing inflammasome assembly can be referred to as “signal 2.” The amount of mature IL-1 β produced after inflammasome activation can be modulated by affecting signal 1, signal 2, or both.

In inflammatory disorders, enhanced inflammasome activation often correlates with reduced tissue compliance and subsequent compromised organ function. Tissue compliance is a measure of the degree to which a hollow organ can expand in volume in response to a

pressure increase [9]. Healthy lungs exhibit a high degree of tissue compliance, expanding in volume easily when airway pressure increases. However, in disease states, lung compliance decreases (stiffness increases), and in consequence volume expansion does not occur as easily with increased airway pressure. In ventilator-induced lung injury (VILI), a model of acute lung injury (ALI), sudden decreases in lung compliance (increased stiffness) is associated with increased inflammation. Reduced lung compliance in pulmonary fibrosis is also correlated with enhanced inflammation [10, 11]. Remodeling of tissue during inflammation can drastically increase the stiffness of soft tissues. For instance, the elastic modulus of lung increases from ~2 kPa in healthy conditions to ~20 – 50 kPa in fibrotic conditions [9, 12]. Tissue stiffening exerts increased resistance or mechanical stress on adherent cells. Translation of extracellular mechanical forces, such as those exerted by changes in tissue compliance, into intracellular signaling is termed mechanotransduction. Mechanical signaling in cells is propagated by the cytoskeleton network of actin microfilaments, intermediate filaments and microtubules after cells engage other cells or extracellular matrix (ECM). Substrate stiffness has been shown to alter the cellular responsiveness to inflammatory factors, such as IL-1 β , through modulation of the cytoskeleton. Mechanotransduction changes cellular outcomes such as proliferation and collagen secretion [13]. Thus, understanding the molecular mechanisms by which inflammation and the mechanical environment may create reinforcing feedback loops could illuminate the pathophysiology of many inflammatory disorders.

Cell adhesion is mediated by the extracellular region of integrins at contact areas, which in some cells are called focal adhesions (FAs; see Fig. 1). Integrin engagement induces clustering, which then recruits an array of adaptor and signaling proteins to transduce mechanical signals. One such adaptor protein is talin, which links the cytoplasmic end of the integrin- β -subunit to the actin cytoskeleton. Other adaptor proteins include paxillin and vinculin. Vinculin is essential for cellular mechanical force sensing [14, 15]. Integrin signaling through talin, paxillin and vinculin recruits additional actin-binding proteins to promote cell adhesion and migration [16–20]. Additionally, integrin-mediated adhesion sites incorporate the actin-based motor protein myosin. Cells use myosin to “pull” against adhesion sites, thus sensing mechanical stress when tension is exerted by integrin-mediated attachments on ECM [9]. Thus, adhesion sites not only sense mechanical tension but can also exert mechanical force through myosin [20, 21].

External forces are exerted upon cells by adhesion to ECM. Cells also experience physical resistance from specific extracellular environments, as when leukocytes experience shear stress induced by fluid flow while in the circulation, or by the mechanical stretch experienced by tissue-resident cardiac macrophages. These mechanical stimuli are also transduced from the cytoskeleton to cellular adhesion sites via integrin alignment mediated by F-actin retrograde flow [22]. Importantly, extracellular mechanical forces alter cell biology. For example, increasing the substrate stiffness of dendritic cells stimulated mechanotransduction, increased β 2-integrin expression, increased cellular adherence and slowed migration [23].

Increased tissue stiffness is a hallmark of various inflammatory diseases. For instance, arterial stiffness is increased in cardiovascular diseases [24, 25], lung compliance is

decreased in chronic obstructive pulmonary fibrosis and ALI [26], airway walls stiffen in asthma [27], and intestinal walls stiffen in ulcerative colitis [28]. Increased tissue stiffness has been shown, in some instances, to enhance inflammatory responses. For instance, increased lung tissue stiffness enhanced LPS-induced inflammation [29]. Additionally, mechanosensitive inflammasome activation has been implicated in acute respiratory distress syndrome (ARDS) pathophysiology. In a model of ventilator-induced lung injury (VILI), mechanical stress, induced by mechanical ventilation, activated the NLRP3 inflammasome in macrophages and endothelial cells, contributing to lung damage [30, 31]. Similarly, actin depolymerization induced by the mechanical stimulus of cyclic stretch also enhanced NLRP3 activation in tenocytes and consequently promoted tendinitis progression [32]. Similarly, blockade of integrin signaling in intestinal epithelial cells results in diminished NLRP3 activation and reduced IL-18 production [33]. However, the molecular mechanisms linking mechanotransduction to inflammasome activation remain unclear.

Due to its macromolecular nature, inflammasome assembly has been hypothesized to be facilitated by cytoskeleton re-organization (see Fig. 1) [34–37]. Understanding the molecular mechanisms by which mechanotransduction modulates inflammasome activation will help in developing therapies to ameliorate the above-mentioned inflammatory diseases. In this review, we highlight the cross-talk of mechanosensitive cytoskeletal elements with inflammasome signaling to provide a unique perspective on the pathophysiology of inflammatory disorders. Understanding how mechanotransduction may impact inflammation could support new targeted therapies for inflammatory diseases.

Cytoskeleton-mediated inflammasome regulation:

Cytoskeleton substructure formation occurs in response to mechanical stimulus and provides scaffolds for cell adhesion, migration and signaling [38]. Cell adhesion sites created after integrin-based attachment recruit different arrays of proteins and have different half-lives in different cell types, and therefore are variously called focal adhesions (in fibroblasts), focal complexes, invadopodia (in cancer cells), or podosomes (in hematopoietic cells, such as osteoclasts, dendritic cells, monocytes and macrophages; [39, 40]). Signaling through integrin-mediated cell adhesions employs dynamic F-actin microfilaments. F-actin polymerization is required for integrin-initiated mechanotransduction, to maintain cell membrane tension and to stabilize cell adhesions. Actin depolymerization drugs such as Cytochalasin D and Latrunculin destabilize F-actin, reducing F-actin filament length and crosslinking. Destabilizing F-actin reduces the elastic and viscous modulus of cells, thus inducing a more fluid-like membrane, reducing adhesion stability and impairing mechanosensitivity [41]. Thus, directly modifying F-actin through actin-modifying agents can be used to test the requirement of mechanotransduction for inflammasome activation.

One recent report directly linked F-actin polymerization to NLRP3 inflammasome regulation. F-actin interacts with NLRP3 and ASC, and actin polymerization inhibits NLRP3 activation via Flightless-1 (Fli-1) and leucine-rich repeat Fli-1-interaction protein 2 (LRRFIP2) [42]. In the same report, direct inhibition of actin polymerization by treating cells with Latrunculin B or Cytochalasin D enhanced NLRP3 activation induced by LPS and ATP, and subsequently increased maturation of IL-1 β and IL-18 [42]. Fli-1 is a member of

the actin-remodeling gelsolin family, and was previously described as a regulator of IL-1 β induced by NLRP3 through inhibition of caspase-1. Other work revealed that Flil-1 acts as pseudosubstrate for caspase-1, and reduced pro-IL-1 β processing and subsequent release of mature IL-1 β [43]. Actin network stabilization by Jasplakinolide, which binds to F-actin and prevents depolymerization, also exacerbated LPS-induced IL-18 and IL-1 β production, although the exact mechanism is not clear [39]. While Jasplakinolide can increase the cellular content of F-actin, it is important to remember that this mechanism of action does not necessarily produce the “opposite” effect of the depolymerizing agent Cytochalasin D. Filament stabilization also prevents turnover and remodeling of filaments, and it is often F-actin remodeling—not the static amount of polymerized or depolymerized actin—that supports adhesion, motility and signaling. Thus, both Cytochalasin D and Jasplakinolide can produce similar cellular outcomes because both prevent actin remodeling.

In contrast, F-actin depolymerization via Cytochalasin-D did attenuate asbestos-induced NLRP3 activation and decreased maturation of IL-1 β and IL-18 in mesothelial cells [44]. F-actin de-polymerization limits phagocytosis of asbestos and the subcellular localization of inflammasome assembly [44]. Actin polymerization is also required for NLRC4 activation in macrophages, as shown by reduced ASC speck formation and IL-1 β maturation following Cytochalasin-D treatment [34]. While the molecular mechanism behind these discrepant observations is not yet clear, one explanation may be a requirement for F-actin in the phagocytosis of the inflammasome stimulant (asbestos) which is dispensable when the inflammasome stimulant (LPS/ATP in above mentioned report [42]) is not phagocytosed. Additionally, it is possible that the subcellular localization of inflammasome assembly varies with different inflammasome stimuli, and therefore differentially requires F-actin.

Vimentin, a component of the intermediate filament cytoskeleton, also enhances inflammasome activation through direct interaction with NLRP3 and Caspase-1. Binding of vimentin to the NLRP3 inflammasome induces IL-1 β maturation in macrophages. Interestingly, Vimentin-deficient mice are resistant to lung fibrosis induced by Bleomycin, LPS, and Asbestos [12]. Resistance to lung fibrosis corresponds with a reduction in IL-1 β -mediated inflammation in the lungs [12]. Intermediate filaments are essential to subcellular trafficking and the formation of mechanosensitive adhesive structures, such as podosomes [39, 44–46]. Thus, loss of vimentin can impair cellular adhesiveness and mechanosensitivity. Reduced inflammasome activation in Vimentin deficient macrophages, indicated by significantly diminished IL-1 β and Caspase-1 maturation, may therefore arise from decreased mechanosensitivity. If so, the reduction in lung fibrosis in bleomycin-treated, vimentin-deficient mice may result from diminished IL-1 β -stimulated collagen production. Reduced collagen deposition would decrease lung stiffness and thus reduce mechanical stress on macrophages, possibly further dampening NLRP3 activation. Reduced fibrosis in vimentin-deficient animals provides a clinically-relevant example of how a better understanding of the molecular links between mechanotransduction and inflammasome activation may illuminate the pathophysiology of poorly understood diseases, such as idiopathic pulmonary fibrosis.

Mechanotransduction-associated kinases in inflammasome signaling

While anchoring cells by linking ECM to the cytoskeleton, FAs and podosomes also initiate intracellular signaling through the recruitment of kinases. Spleen tyrosine kinase (Syk), a Src-homology 2 (SH-2) containing non-receptor kinase, binds to integrins in a Src-kinase dependent manner [47]. At adhesion sites, Syk recruits and phosphorylates SH2-domain containing non-receptor tyrosine kinases such as focal adhesion kinase (FAK) or the homologous FAK-related proline-rich tyrosine kinase 2 (Pyk2; [48]). FAK and Pyk2 also bind to actin-binding proteins, like Talin and Paxillin, through the focal adhesion targeting (FAT) domain [49]. In macrophages, Pyk2 is recruited to podosomes. Integrin clustering activates Pyk2 kinase activity [50, 51]. Pyk2 is required for podosome formation via FAT domain-dependent actin and microtubule stabilization [52].

Syk and Pyk2 are highly expressed in hematopoietic cells, while FAK functions have mostly been described in non-hematopoietic cells [47, 53]. Syk and Pyk2 also transduce mechanical signals. For instance, macrophages adhering to stiff implants generate a foreign body response (FBR) that is mediated by Syk. Syk activation results in ASC and caspase-1 activation and subsequent IL-1 β production [54]. Activation of the downstream kinase Pyk2 also escalates lung inflammation in LPS-induced lung injury [55]. Pyk2 can increase the oligomerization and cross-linking of ASC via phosphorylation at Tyr146 in the CARD domain. Phosphorylation of ASC increased NLRP3 inflammasome assembly and enhanced IL-1 β production. FAK, however, does not directly phosphorylate ASC, but enhances NLRP3 and AIM2 inflammasome activation via interactions with ASC through unknown mechanisms [56] (Fig. 1). Because Pyk2 connects cytoskeletal components via the FAT domain and also interacts directly with the inflammasome adaptor ASC, Pyk2 may be a common mediator of cross-talk between mechanotransduction and inflammasome activation.

Additionally, integrin induction of FAK signaling activates small GTPases, such as RhoA. RhoA activation in turn stimulates Rho associated protein kinase (ROCK). Activated ROCK induces F-actin polymerization [57]. RhoA also activates PKN1/2 kinase, which phosphorylates the Pyrin inflammasome at S205 and S241. Phosphorylation of the pyrin inflammasome enables binding of the inhibitory protein 14-3-3. 14-3-3 is a phosphoserine binding protein, and binding of 14-3-3 to pyrin blocks the pyrin domain of the Pyrin inflammasome, inhibiting its assembly with ASC and caspase-1 [58]. When RhoA is inactivated through inhibitory toxins (e.g. IbpA (*Histophilus somni*)/VopS (*Vibrio parahaemolyticus*), TcdA (*C. difficile*) [59] or TecA (*Burkholderia cenocepacia*) [60], non-phosphorylated Pyrin inflammasome is released from 14-3-3 inhibition and becomes activated, subsequently inducing IL-1 β and IL-18 maturation. Similarly, microtubule destabilization via treatment with the tubulin depolymerizer Colchicine enhances Pyrin inflammasome-mediated IL-1 β release [58]. However, the mechanism by which tubulin depolymerization causes inflammasome activation is currently unknown [61]. RhoA is also implicated in LPS-mediated ALI and increased lung stiffness induced by LPS-mediated inflammation increased expression of guanine exchange factor-H1 (GEF-H1) [62]. GEF-H1 increases activity of Rho, further enhancing lung inflammation [29], probably by Pyrin inflammasome activation via binding 14-3-3 [63]. Regulation of the Pyrin inflammasome via mechanosensitive integrin-FAK signaling and the microtubule network strengthens the

mechanosensitive inflammasome response. The feedback loops created between mechanotransduction and almost any inflammasome assembly may assist in explaining the pathophysiology of many disorders in which inflammatory pathways become dysregulated.

Concluding remarks:

In this review, we have discussed cytoskeleton-mediated mechanotransduction and the link to inflammasome pathways (Fig. 1). This link is exemplified in inflammatory lung injury diseases like VILI where mechanical ventilation exerts mechanical stress. Ventilator-induced cyclic stretch of alveolar macrophages activates the macrophage NLRP3 inflammasome [64], and also in lung endothelial cells [30, 31], to produce IL-1 β . Mechanotransduction modulates the macrophage inflammatory response in other systems as well [65]. For instance, macrophages cultured on softer substrates downregulated inflammatory cytokine expression [66]. As discussed previously, the kinase Pyk2 plays a crucial role in both mechanotransduction and inflammation. Pyk2 can prime inflammasomes after LPS stimulation, and Pyk2 initiates F-actin re-organization during cell adhesion [67]. Additionally, the requirement for Pyk2 in macrophage podosome formation [52] and in NLRP3 inflammasome activation [56] may explain reduced lung injury observed in Pyk2 deficient mice following LPS-induced ALI [55]. Finally, a causal link between mechanotransduction and inflammasome activation was suggested by the observation that lipoxin treatment both increased tissue compliance and consequently reduced lung inflammation in a model of LPS-induced ALI [68].

Cytoskeleton destabilization by actin depolymerizers (*e.g.* Cytochalasin-D, and Latrunculin) enhanced inflammasome activation. We speculate on two possible explanations for how reduced F-actin polymerization may enhance inflammation that warrant further investigation. First, destabilized F-actin reduces cell adhesion, thus reducing ECM-integrin signaling and “relaxing” the cell [41]. In less stretched (or “relaxed”) cells, shorter F-actin filaments increase retrograde flow, augmenting cytoskeleton-integrin signaling and thus promoting inflammasome activation [22]. Secondly, shortened F-actin filaments may enhance inflammasome assembly simply because the physical proximity of inflammasome components is reduced.

Some inconsistencies in the data thus far reported on the cytoskeleton and mechanoregulation of inflammasome activation reveal the complex regulatory mechanisms involved. For instance, Cytochalasin-D induced actin depolymerization has also been reported to reduce inflammasome activation [34, 44] and cyclic stretch reduced IL-1 β secretion via adenosine monophosphate kinase (AMPK) mediated caspase-1 inhibition [69]. While the differences may be due to differences in cell type and inflammasome activator, much work remains to be done to clarify how F-actin and other cytoskeletal components modify inflammatory outcomes [12].

Other open questions revolve around the function of FAK and Pyk2, which may also regulate inflammasome assembly in multidimensional ways. The future evaluation of actin dynamics and an increased focus on actin-modulatory proteins will greatly enhance our understanding of the mechanosensitivity of inflammasome activation. While no published study yet definitively consolidates mechanotransduction with inflammasome activation, we

suggest that the correlative evidence available in the literature offers substantial opportunities for significant and innovative research. Defining the molecular links between mechanotransduction and inflammasome activity may illuminate the pathophysiology of inflammatory diseases such as ALI, arteriosclerosis and ulcerative colitis, where tissue fibrosis directly correlates with inflammation and disease severity.

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Abbreviations:

AIM2	absent in melanoma-2
ALI	acute lung injury
ARDS	acute respiratory distress syndrome
ASC	apoptosis associated speck like protein containing a CARD
AMPK	adenosine monophosphate kinase
ATP	adenosine triphosphate
CARD	caspase activation and recruitment domain
COPD	chronic obstructive pulmonary disease
DAMPs	danger associated molecular patterns
ECM	extracellular matrix
FA	focal adhesions
FAK	focal adhesion kinase
FAT	focal adhesion targeting domain
FBR	foreign body response
Fil	flightless-1
GEF-H1	Guanine exchange factor -H1
IL-1β	interleukin 1 β
IL-18	interleukin 18
LPS	Lipopolysachharide
LRRFIP2	leucine-rich repeat FliI-interaction protein 2
NLRP	Nod like receptor protein or nucleotide-binding domain, leucine-rich-containing family (NLR), pyrin domain-containing

NLRC4	NLR family CARD containing protein-4
PAMPs	pathogen associated molecular patterns
PKN-1/2	(Serine/threonine)-protein kinase –1/2
Pyk2	proline-rich tyrosine kinase 2
ROCK	Rho associated protein kinase
Syk	spleen tyrosine kinase
TLR	toll like receptors
VILI	ventilator induce lung injury

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Figure 1. Schematic presentation of mechanotransduction and inflammasome signaling.

The inflammasome is activated through pattern recognition receptors that provide signal 1, inducing expression of pro-IL-1 β , pro-IL-18 and inflammasome components. Danger-associated molecules provide signal 2, initiating inflammasome receptor oligomerization, which activates caspase-1 through the adaptor protein ASC. Active caspase-1 cleaves pro-IL-1 β and pro-IL-18, releasing the mature, bio-active forms of IL-1 β and IL-18.

The inflammasome pathway is regulated by cytoskeleton-mediated mechanotransduction.

Cell adhesion based mechanotransduction activates ASC by Pyk2 and FAK to enhance NLRP3, NLRC4 and AIM2 activity, whereas cell adhesion can inhibit the pyrin inflammasome through activation of the GTPase RhoA via FAK. The NLRP3 inflammasome is also regulated by direct interactions with F-actin and the intermediate filament protein vimentin. Mechanical forces, including those exerted by changes in tissue stiffness and ECM, can thus be translated through cell adhesion sites to modulate inflammasome activity in multiple cells types.