Biochimica et Biophysica Acta 1840 (2014) 1433–1440

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Ambiguities in NLRP3 inflammasome regulation: Is there a role for mitochondria?☆

Kate E. Lawlor, James E. Vince *

The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
Department of Medical Biology, the University of Melbourne, Parkville, Victoria 3050, Australia

ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form 10 August 2013
Accepted 19 August 2013
Available online 27 August 2013

Keywords:
Inflammasome
NLRP3
Mitochondria
Reactive oxygen species
Caspase-1
IL-1β

ABSTRACT

Background: The NLRP3 inflammasome is a sensor of specific pathogen, host and environmental danger molecules. Upon activation NLRP3 recruits caspase-1, which cleaves and thereby activates precursor interleukin-1β (IL-1β) and IL-18 to initiate immune responses. Several recent studies have posited that the mitochondria are a central regulator of NLRP3 function.
Scope of review: Mitochondrial reactive oxygen species (mtROS) production, mitochondrial apoptosis, mitochondrial DNA (mtDNA) release, mitophagy, calcium induced mitochondrial damage and mitochondrial co-ordination of NLRP3 localization have all been implicated in regulating NLRP3 activity. In this article we review the literature both for and against these models of NLRP3 inflammasome activation, and highlight other recent contentious issues concerning NLRP3 functioning.
Major conclusions: Although many mechanisms have been proposed for activating NLRP3, no unified model has yet to gain acceptance. Further research is required to clarify how the mitochondria might influence NLRP3 activity.
General significance: While the NLRP3 inflammasome is important for host protection against microbial infection, rare genetic mutations in NLRP3 also cause severe auto-inflammatory diseases. More recent research has implicated NLRP3 activity in pathologies such as atherosclerosis, cancer, type 2 diabetes and Alzheimer's disease. Understanding the mechanisms of NLRP3 inflammasome formation and regulation therefore has the potential to uncover new inflammasome and disease specific therapeutic targets. This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

1. Introduction

Inflammasomes are multiprotein complexes that form upon exposure to pathogenic microbes and host danger signals to activate caspase-1 and cause IL-1β and IL-18 maturation. Caspase-1 activation can also result in an inflammatory form of cell death known as pyroptosis, which can play an important physiological role in response to chemotherapy and microbial infection [1,2]. Several cytosolic pattern recognition receptors (PRRs) act as sensors of pathogen associated molecular patterns (PAMPS) and damage associated molecular patterns (DAMPS) to initiate formation of inflammasome complexes, including 6 members of the NOD-like receptor family (NLRP1, NLRP3, NLRP6, NLRP7, NLRP12, NLRC4) and the HIN-200 family member, AIM2, which uniquely recognizes and directly binds cytosolic DNA [3]. Many of these inflammasomes, including NLRP3 and AIM2, utilize the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) to recruit caspase-1 via CARD–CARD homotypic interactions (Fig. 1).

NLR family pyrin domain containing 3 (NLRP3; also referred to as NALP3 and cryopyrin) has been the most widely studied inflammasome. Amongst known inflammasome sensors, what appears unique to NLRP3 is its ability to not just sense DAMPs and PAMPs, but environmental irritants and hazardous human made chemicals, such as asbestos particles, biomaterials, nanoparticles and chemotherapeutic drugs. Genetic deletion of NLRP3 in mice has implicated it in host protection to pathogenic viruses, bacteria and fungi [3]. Gain of function mutations in NLRP3 also occur in a spectrum of auto-inflammatory diseases termed cryopyrin-associated periodic syndromes [4]. However, it is NLRP3's inappropriate activation by host DAMPs, such as cholesterol crystals, saturated fatty acids, islet amyloid polypeptide and amyloid-β that has implicated it in widespread human conditions, including atherosclerosis, type 2 diabetes and Alzheimer's disease [5].

The pathways and mechanisms that regulate NLRP3 function have attracted considerable attention [6] (Fig. 2). A priming signal (or signal 1) is required for transcriptional induction of inactive precursor IL-1β (pro-IL-1β) and NLRP3, and experimentally, this is often performed by Toll-like receptor (TLR) stimulation. Signal 1 stimuli are usually separable from formation of a functional NLRP3 inflammasome, which is induced by a second trigger, the NLRP3 activator.

Junying Yuan and colleagues originally demonstrated a critical role for caspase-11 in caspase-1 activation following endotoxic shock [7]. The non-canonical caspase-11 inflammasome has more recently been shown to be activated by many gram negative bacteria and some

☆ This article is part of a Special Issue entitled Frontiers of Mitochondrial Research.

* Corresponding author at: The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia. Tel.: +61 3 93452524.
E-mail address: vince@wehi.edu.au (J.E. Vince).

0304-4165/$ – see front matter.
http://dx.doi.org/10.1016/j.bbagen.2013.08.014

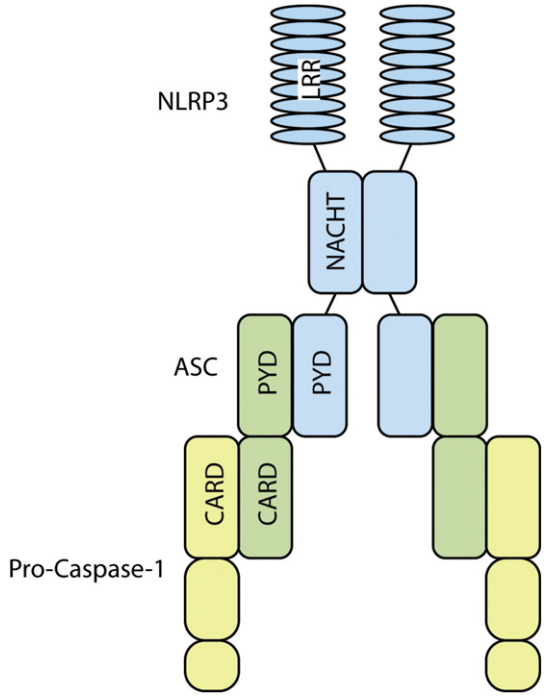

Fig. 1. NLRP3 inflammasome components. NLRP3 is comprised of a series of leucine-rich repeats (LRR) that may function as the sensor domain; a NACHT domain, common to all NLR family members, which enables ATP-dependent oligomerization and activation of the complex; a PYD (Pyrin) domain that facilitates its interaction with the adaptor protein ASC (apoptosis-associated speck-like protein) that recruits caspase-1 via homotypic interactions between CARD (Caspase recruitment domain) domains.

bacterial toxins (Fig. 2) and can induce lytic cell death in the absence of caspase-1. Unlike the canonical NLRP3 inflammasome, this pathway requires both NLRP3 and caspase-11 to cause caspase-1 activation and IL-1β maturation [8]. Although the mechanism by which caspase-11 is activated remains unclear, it has been proposed that gram negative bacteria induce signaling via the LPS-TLR4 adaptor proteins TRIF and Myd88, and downstream type I interferon production, to induce caspase-11 expression and activation [9–11]. However recent research indicates that caspase-11 activation is caused by bacteria or LPS entry into the host cytosol independent of TLR4, and that TLR4 and type I interferon's may simply act to induce the expression of caspase-11 [12–14]. It is therefore likely that the activation of the caspase-11 inflammasome relies upon interactions between specific bacterial molecules, such as LPS, and an unidentified cytosolic host receptor.

Unlike soluble NLRP3 triggers, particulate or crystalline stimuli have been proposed to activate NLRP3 through phagolysosomal destabilization and the release of proteolytic enzymes (Fig. 2). Many studies have also linked the efflux of potassium (K^+) with NLRP3 stimuli, including crystalline and pore-forming toxins [15], and the exposure of cells to physiological concentrations of K^+ or chemical inhibition of K^+ efflux blocks NLRP3 inflammasome activation [16–18]. Pannexin-1 was suggested to associate with the P2X7 receptor upon ATP binding to form a non-selective hemi-channel allowing DAMPs and PAMPs access to NLRP3, ion exchange and the release of IL-1β [19,20]. However, pannexin-1 deficient macrophages respond normally to P2X7R and NLRP3 activation to activate caspase-1 and cause IL-1β release [21]. More recently, work by the laboratory of Gabriel Nunez has proposed that K^+ efflux is perhaps the common denominator essential for NLRP3 activation by all NLRP3 stimuli [22].

Increased intracellular calcium (Ca^{2+}) levels have also been suggested as a pre-requisite for NLRP3 activation. NLRP3 stimuli are proposed to cause the influx of calcium (Ca^{2+}) via the cell surface transient receptor potential cation channel, subfamily M, member 2 (TRPM2), and also activate the calcium sensing receptor (CASR) to promote the release of Ca^{2+} stores from the endoplasmic reticulum (ER) [23–26]. The mechanism by which increased cytosolic Ca^{2+} facilitates NLRP3 activation requires further research, although it has recently been highlighted that NLRP3 activation caused by excess Ca^{2+} may result from the precipitation of calcium crystals (in high phosphate ion media), and as such, presumably acts as a particulate NLRP3 activator to cause phagolysosomal disruption [22]. However, decreased NLRP3 functioning upon genetic deletion of the calcium sensing gene GPRC6A or channel TRPM2 [23,24] implies a role for Ca^{2+} mediated NLRP3 activation beyond calcium crystal mediated lysosomal damage.

1.1. A role for mitochondria in NLRP3 function?

Mitochondria play a central role in energy generation and are essential for Bax and Bak dependent, or "mitochondrial," apoptosis. Mitochondria have also been directly implicated in innate immune signal transduction, as the outer mitochondrial membrane protein MAVS (mitochondrial anti-viral signaling) is an essential adaptor protein for signaling anti-viral responses following stimulation of the dsRNA receptors RIG-I and Mda5 [27]. More recently, evidence has been accumulating that links NLRP3 inflammasome activation to mitochondrial metabolic activity, mitochondrial apoptosis, and MAVS itself (Fig. 3). However, as outlined below, further work is clearly required to unify these models and establish their existence beyond reasonable doubt.

1.2. Bcl-2 and inflammasome activation

The first link to inflammasome activation and the mitochondria came from studies conducted in the laboratory of John Reed in 2007, which suggested that the mitochondrial anti-apoptotic proteins Bcl-2 and Bcl-xL bind to and suppress NLRP1 inflammasome activity [28]. Reconstitution of the NLRP1 inflammasome in 293 and HeLa cells demonstrated that Bcl-2 or Bcl-xL expression limited NLRP1 activation of caspase-1. Consistent with this idea, it was recently shown that a vaccinia virus Bcl-2 homologue, FL1, also prevents NLRP1 activation [29]. Notably, key experiments implicating Bcl-2 in NLRP1 regulation were performed in ATP stimulated macrophages [28], and it is now known that ATP is a potent NLRP3 activator. Cells deficient in Bcl-2 secreted more IL-1β in response to ATP when compared to wildtype macrophages, while those overexpressing Bcl-2, displayed decreased IL-1β secretion. Therefore one interpretation of this data is that Bcl-2 can limit NLRP3 activation. While two recent studies using Bcl-2 overexpressing cells also implied that Bcl-2 expression limits NLRP3 activity [30,31], other research has found no influence of increased Bcl-2 expression on NLRP3 function [32].

1.3. Mitophagy, ROS and NLRP3 activation

In 2008 the laboratory of Shizuo Akira demonstrated that deletion of a key mammalian autophagic gene, Atg16L1, caused IL-1β secretion in response to LPS alone, and also increased IL-1β secretion in response to the canonical NLRP3 stimuli ATP or monosodium urate crystals (MSU) [33]. In contrast, LPS induced secretion of cytokines such as TNF, is unaffected by the loss of Atg16L1. Similarly, it has been shown that genetic deletion of other genes required for autophagic functioning, such as LC3B (microtubule-associated protein 1 light chain 3B) and Beclin1, also increases NLRP3 activation [34]. Therefore autophagy clearly plays a role in limiting inflammasome activity.

One mechanism by which autophagy may limit NLRP3 activity is by sequestering and facilitating the degradation of inflammasome components, such as pro-IL-1β, NLRP3, caspase-1 or ASC. Despite showing increased NLRP3 function, $LC3B^{-/-}$ and $Beclin1^{+/-}$ macrophages did not show significant differences in pro-IL-1β, ASC or caspase-1 expression before or after inflammasome activation [34]. Similarly,

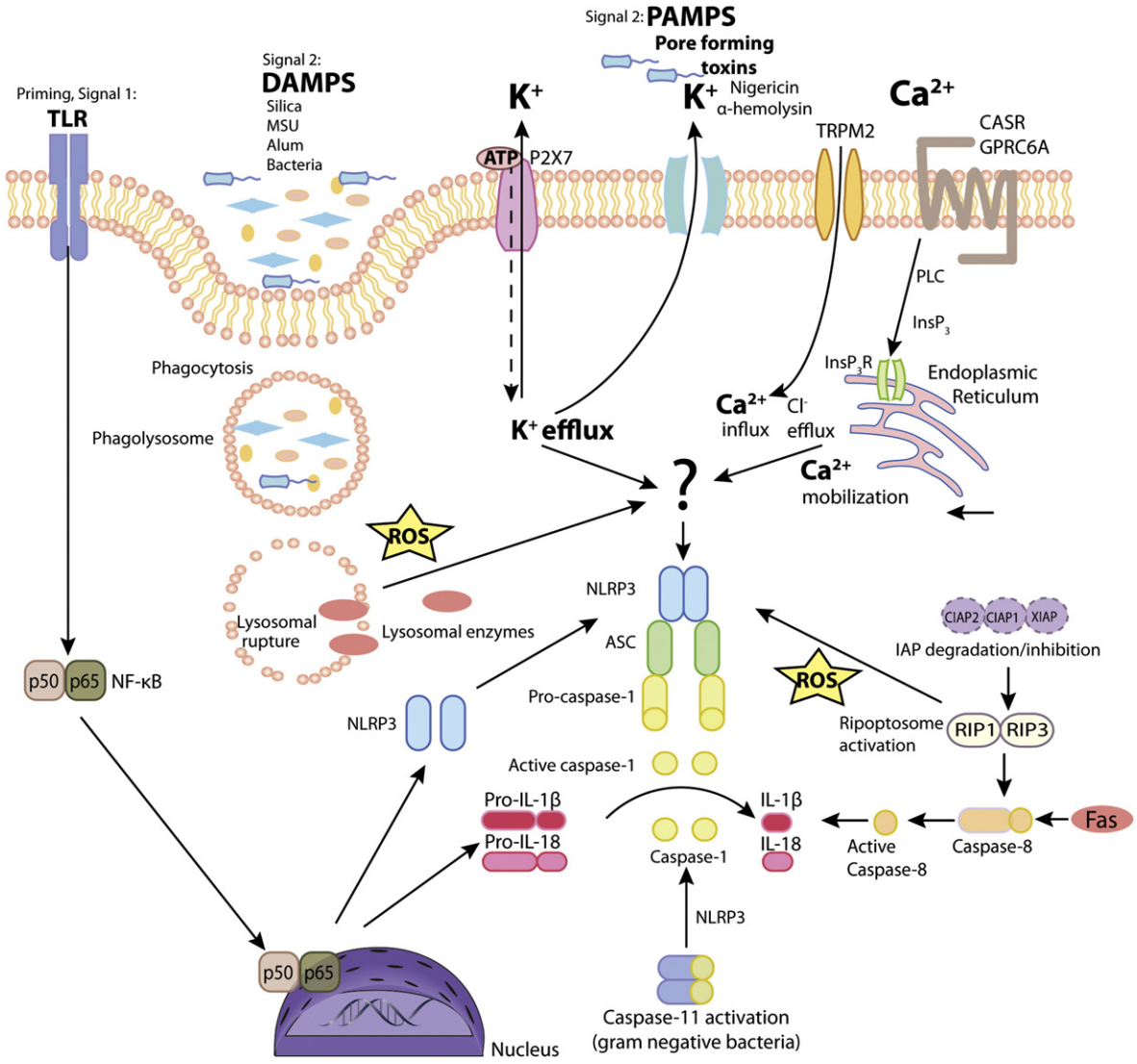

Fig. 2. Mechanisms proposed for NLRP3 inflammasome activation. A TLR priming signal (signal 1) is required for transcription of NLRP3 and pro-IL-1β, while a variety of PAMPS and DAMPS (signal 2) activate NLRP3 by initiating formation of a complex comprising of NLRP3, the adaptor protein ASC and pro-caspase-1. Auto-catalytically processed caspase-1 subsequently cleaves pro-IL-1β to mature bioactive IL-1β. The exact mechanism for NLRP3 activation is unclear but may include pore formation and K^+ efflux/Ca^{2+} influx, lysosomal rupture and ROS production. In contrast to canonical NLRP3 activators, cytosolic LPS derived from gram-negative bacteria is likely to bind an unknown receptor to activate a non-canonical caspase-11 containing inflammasome that can also activate caspase-1. Inhibition of IAPs using a mimetic of the natural IAP antagonist mitochondrial protein SMAC/DIABLO has been shown to activate the NLRP3 inflammasome via ripoptosome formation and RIP3 signaling. IAP inhibition or Fas signaling can also activate IL-1β via caspase-8 (CASR: calcium-sensing receptor; DAMPS: damage-associated molecular patterns; InsP3: inositol triphosphate; PAMPs: pathogen-associated molecular patterns; PLC: phospholipase C; ROS: reactive oxygen species; TLR: toll like receptor; TRP: transient receptor potential channel).

Atg16L1$^{-/-}$ cells also displayed normal caspase-1 levels [33]. These findings suggest that the NLRP3-caspase-1 inflammasome complex is not dramatically regulated by degradation within autophagosomes. In contrast, it was proposed that following AIM2 activation, ubiquitylation of ASC causes it to associate with the autophagic adaptor, p62, and thereby targets the AIM2 inflammasome for autophagic destruction [35]. Using chemical inhibitors and activators of autophagy, it has also recently been suggested that pro-IL-1β may be regulated by autophagy mediated degradation [36].

LPS priming and NLRP3 activation has often been observed to correlate with oxidative stress [37]. While initial shRNA gene knockdown studies in immortalized Thp1 cells suggested NADPH oxidases as the source of ROS [38], subsequent studies using primary cells lacking NADPH oxidases failed to confirm these findings [39]. In 2011 the laboratories of Jurg Tschopp and Augustine Choi independently proposed that mitochondria are the source of ROS that are either required for, or facilitate, NLRP3 activation [30,34]. Autophagic clearance of damaged mitochondria (mitophagy) was required for preventing mtROS production and excessive NLRP3 signaling (Fig. 3) [30,34], and recently, RIP2 dependent mitophagy and mtROS suppression was proposed as key to limiting pathologic NLRP3 activation of IL-18 following influenza A virus infection [40]. Although few studies have examined the role of mitochondrial mediated NLRP3 regulation in human disease, mitochondrial ROS has been linked to cytokine secretion, including IL-1β, in TNF-receptor-associated periodic syndrome (TRAPS) patient monocytes [41], as well as increased NLRP3 signaling in fibromyalgia patients [42].

In line with ROS being important for NLRP3 function, anti-oxidants have been extensively used to efficiently inhibit NLRP3 activity. Nevertheless, it has been suggested that anti-oxidants interfere with the LPS priming step and that they prevent LPS induced expression of NLRP3, and not NLRP3 activity per se [43]. However, it has also been reported that anti-oxidant treatment after LPS stimulation can still inhibit NLRP3 activity [24,44,45], and interestingly, Kate Schroder's laboratory has shown that even a short LPS stimulation

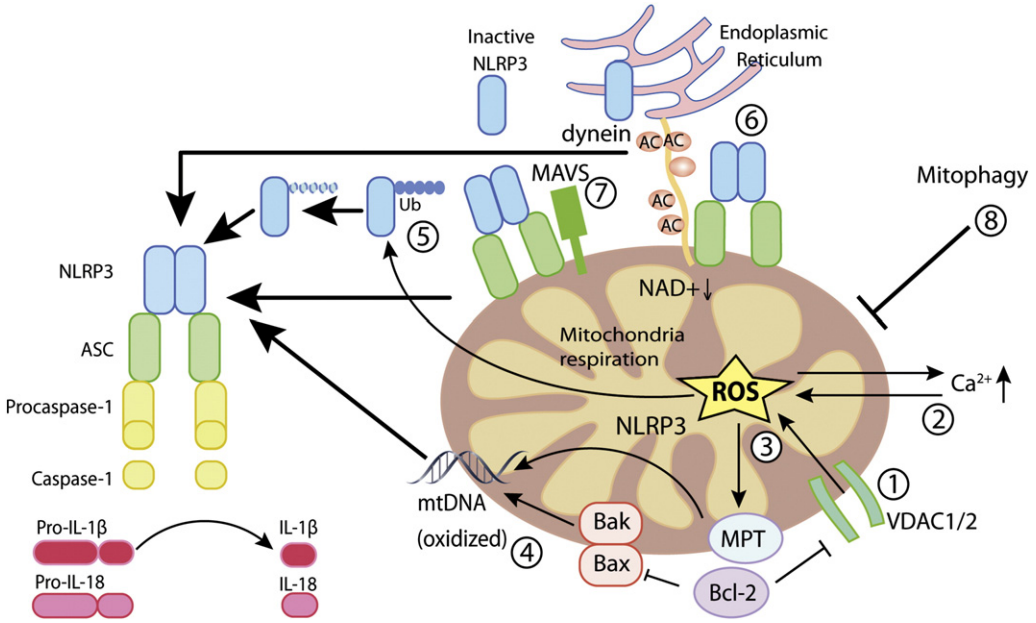

Fig. 3. Models for mitochondrial regulation of the NLRP3 inflammasome. LPS priming and NLRP3 activating agents stimulate mtROS production. This may occur by 1. VDAC activation, which is reportedly repressed by Bcl-2. 2. Increased cytosolic calcium levels may trigger mitochondrial damage and mtROS production, and/or mtROS may also increase cytosolic calcium. The excess production of mitochondrial ROS has a number of proposed consequences. 3. Increased mtROS production causes mitochondrial permeability transition (MPT) pore opening and subsequently mtDNA release. 4. Alternatively, mtROS causes mtDNA oxidation, while at the same time NLRP3 stimuli induce Bax/Bak mediated mitochondrial membrane damage allowing mtDNA release. As a consequence of 3 or 4, mtDNA binds and directly activates the NLRP3 inflammasome. 5. NLRP3 activation is repressed by ubiquitylation. Mitochondrial ROS promotes NLRP3 de-ubiquitylation to facilitate its activation. Other proposed mechanisms by which the mitochondria may regulate the formation and activation of the NLRP3 inflammasome, include 6, microtubule-driven spatial rearrangement. Diminished NAD^+ leads to increased acetylation (AC) of α-tubulin to facilitate dynein-mediated transport of the mitochondria to the endoplasmic reticulum, which promotes NLRP3-ASC interactions. 7. Alternatively, or in addition to 6, the mitochondrial-associated adaptor protein MAVS (mitochondrial antiviral signaling) recruits cytosolic NLRP3 to the mitochondria to facilitate optimal NLRP3 activation. 8. As a consequence of 1 to 7, the clearance of damaged ROS producing mitochondria through autophagy (mitophagy) is important for limiting excess NLRP3 signaling. Please refer to the main text for the limitations associated with these proposed mechanisms, and the appropriate references.

facilitates NLRP3-caspase-1 activation independent of NLRP3 induction [46]. Recent studies by several groups suggest that LPS priming or NLRP3 activating molecules prompt the de-ubiquitylation of NLRP3, possibly in a mitochondrial ROS dependent manner, and that the de-ubiquitylation of NLRP3 is critical for NLRP3 activation (Fig. 3) [47–49]. This research implies that LPS induced mitochondrial ROS may alert the cell to potential danger by calling NLRP3 to attention, but does not send NLRP3 into battle.

The role of ROS induced by NLRP3 triggers, or signal 2, remains less clear, but may involve the promotion of Ca^{2+} influx. However, there are conflicting reports on whether Ca^{2+} influx occurs downstream or upstream of mitochondrial damage and ROS production [24,25]. Using anti-oxidants combined with liposomes, silica or alum particles to activate NLRP3, the data of Liang Quo and colleagues suggested that Ca^{2+} influx was dependent on NLRP3 mediated ROS production [24]. In contrast, the laboratory of Tiffany Horng proposed that Ca^{2+} influx induced by the soluble NLRP3 stimulus, ATP, was the cause of mitochondrial dysfunction and ROS production (Fig. 3) [25]. Similarly, while Jae Jin Chae and colleagues proposed that cyclic AMP (cAMP) binds and inhibits NLRP3, and that Ca^{2+}-CASR stimulation reduces cellular cAMP to promote NLRP3 activity [26], the group of Ulf Wagner found that manipulating cellular cAMP levels had no effect on Ca^{2+} mediated NLRP3 activation [23].

The role of ROS in NLRP3 activation is further confounded by research discounting any role for ROS in modulating NLRP3 activity [22], while other laboratories have linked cellular ROS to the inhibition of caspase-1 and IL-1β activation [50–52]. Over several years, the work by Anna Rubartelli and colleagues has attempted to reconcile some of these differences, and have suggested a model where the cells own anti-oxidant response to ROS production is responsible for determining the level of NLRP3 activity, and therefore both cellular oxidation and reduction reactions may participate in NLRP3 functioning [39].

1.4. Apoptosis and NLRP3 activation

Apoptosis is a mode of cell death caused through the activation of intracellular caspases. Extrinsic or death receptor induced apoptosis specifically activates caspase-8, an initiator caspase, which cleaves and activates downstream executioner caspases responsible for cellular dismantling. Intrinsic or mitochondrial apoptosis is signaled by cellular stress, such as ultra violet irradiation, and culminates in the formation of Bax and Bak oligomeric pores on mitochondria to cause irreversible mitochondrial membrane damage. The loss of mitochondrial membrane integrity allows the release of cytochrome c, where it binds Apaf1 to generate active caspase-9 and cause apoptotic cell death.

In 2012 the laboratory of Moshe Arditi delineated a model that unified how divergent structural and chemical molecules caused NLRP3 activation [31]. It was proposed that NLRP3 stimuli induce mitochondrial apoptosis to cause the release of oxidized mitochondrial DNA (mtDNA), and that oxidized mtDNA bound and directly activated NLRP3 (Fig. 3). Hence, if correct, this model predicts that genetic deletion of Bax and Bak, which are essential executioners of mitochondrial apoptosis, should prevent NLRP3 activation. However, in conflict with the above model, our laboratory has observed normal NLRP3 function in Bax and Bak doubly deficient bone marrow derived macrophages (our unpublished data). Using caspase-1 deficient cells, recent work shows that the NLRP3 and AIM2 inflammasomes do have the potential to induce apoptosis, but after inflammasome assembly and ASC binding to caspase-8, rather than via the mitochondria [53–55].

Augustine Choi's laboratory has also proposed that mtDNA release facilitates NLRP3 function in response to LPS and ATP treatment [34]. However, in this study inflammasome activation was required for mtDNA release and therefore, although mtDNA enhanced NLRP3 activation, it was not a prerequisite for NLRP3 inflammasome formation and activation. Data from this research also demonstrated that mtDNA

could efficiently activate the AIM2 inflammasome, and it remains unclear as to which inflammasome, NLRP3 or AIM2, mtDNA activates under physiological conditions. Because the mitochondrial permeability transition (MPT) inhibitor cyclosporine A prevented LPS and ATP activation of NLRP3, MPT pore opening was proposed as a requirement for mtDNA release into the cytoplasm (Fig. 3). However, genetic studies have yet to validate if the MPT pore contributes to NLRP3 activity.

1.5. Localization of the NLRP3 inflammasome to mitochondrial membranes

Research by Jurg Tschopp's group linking mitochondrial ROS to NLRP3 activation proposed that NLRP3 stimuli induced a re-localization of NLRP3 from the ER to ER-mitochondrial membranes where it formed a functional inflammasome with ASC and caspase-1 [30]. Subsequently, it was reported by Shizuo Akira's laboratory that dynein mediated microtubule transport of mitochondria was required for the clustering of NLRP3 and ASC on ER-mitochondrial membranes to drive inflammasome assembly (Fig. 3) [56]. NLRP3 binding to mitochondria has been reported by Robert Germain's group to require the mitochondrial protein MAVS [57]. The tethering of NLRP3 to mitochondria via MAVS appeared to be key to efficient NLRP3 activation, because MAVS deficient macrophages were shown to be defective in NLRP3 function [57]. However, the role of MAVS in regulating NLRP3 activity has been questioned, as other groups have been unable to detect differences in NLRP3 signaling between wild type and MAVS deficient cells (Gabriel Nunez, personal communications).

Further ambiguities linking NLRP3 localization to the mitochondria are several studies demonstrating that active AIM2, NLRC4 or NLRP3 inflammasomes all result in the formation of strong, probably cytosolic, ASC-caspase-1 foci [11,18,53,58–61]. A recent study by Bing Sun and colleagues examined ASC localization in peritoneal macrophages following NLRP3 stimulation by immuno-fluorescence microscopy, and compared it to organelles such as mitochondria, Golgi, ER, endosomes, phagosomes, and lysosomes. Consistent with active inflammasomes being cytosolic, ASC foci showed no significant co-localization with any of the organelles studied [58].

It is perceivable that NLRP3 mitochondrial-ER associations may be transient in nature and occur to facilitate NLRP3-ASC-Caspase-1 interactions and post-translational modifications, but that active inflammasomes are subsequently released into the cytosol to efficiently process pro-IL-1β. However, this possibility has yet to be explored.

1.6. Other inflammasome ambiguities

A number of other critical regulators of NLRP3 function have been proposed in recent years. Below we review a few of the more interesting, and often surprising, issues and conflicts in the NLRP3 inflammasome literature that have yet to be resolved.

1.7. Lysosomal perturbation

One mechanism for NLRP3 activation that has gained credence is through phagolysosomal rupture (Fig. 2). Particulate and crystalline NLRP3 activators, including MSU crystals, alum, silica, amyloid-β, calcium pyrophosphate dehydrate, as well as bacteria (i.e. Mycobacterium tuberculosis) are phagocytosed by antigen presenting cells and cause disruption of the phagolysosome. It has been suggested that the release of lysosomal cathepsin B activates NLRP3 via an undefined mechanism. While inhibition of cathepsin B using CA-074 Methyl ester (CA-074-Me) blocks particulate induced NLRP3 activation [62,63], genetic deletion of cathepsin B has been reported to have no effect [22,64], or only a partial effect [62], on NLRP3 function. Therefore there may be redundant or additional lysosomal enzymes involved in NLRP3 activation, such as cathepsin L [65]. Complicating the use of CA-074-Me are reports showing that at higher doses it can inhibit NLRP3 stimuli, such as nigericin, that activate NLRP3 independent of lysosomal disruption [66]. While typical concentrations of 10–20 μM of CA-074 Me are used to examine whether a particular NLRP3 stimulus acts by lysosomal perturbation, it is possible that effects observed at doses higher than 1 μM are off-target [67]. A recent report has also highlighted that rapid phagolysosomal rupture may have an inhibitory effect on inflammasome activation by causing cathepsin (CA-074-Me blockable) mediated caspase-1 degradation as well as necrosis [68,69].

The potent vacuolar-type H^{+}-ATPase (V-ATPase) inhibitor bafilomycin A1 limits lysosomal acidification and function. In addition to CA-074-Me it is therefore often used as an additional tool to investigate lysosomal mediated NLRP3 activation. However, bafilomycin A1 inhibits V-ATPase's located throughout the endomembrane system, including the ER, Golgi apparatus and plasma membrane. Furthermore, V-ATPase blockade by bafilomycin A1 induces a multitude of cellular events, such as increasing lysosomal pH and inducing lysosomal enzyme secretion into the extracellular environment, inhibiting autophagy, inducing apoptosis and causing a loss of mitochondrial function and membrane potential [70–72]. In fact, in human cells enhanced LPS induced IL-1β or NLRP3 activation is observed in response to agents that increase lysosomal pH and/or decrease cytosolic pH, including bafilomycin A1 and ammonium chloride, and this may reflect a role for endo-lysosomal mediated exocytosis in the secretion of activated inflammasomes and IL-1β [73,74]. Studies have also used the lysosomotropic agent L-leucyl-L-leucine methyl ester (LeuLeuOMe) to cause lysosome rupture and activate NLRP3 [63]. However, even here the polymerization of LeuLeuOMe within lysosomes may impart pore-forming capabilities [75] that could, in theory, activate NLRP3 when released into the cytosol. Therefore, whether the release of phagolysosomal particulate and crystalline NLRP3 stimuli into the cytosol causes NLRP3 activation upon lysosomal disruption, or if it is the release of lysosomal enzymes (or calcium stores) that activate NLRP3, still appears a valid question.

1.8. TXNIP

Thioredoxin (TRX)-interacting protein (TXNIP) has been linked to the regulation of cellular redox reactions and lipid and glucose metabolism. Work by Jurg Tschopp's group proposed that TXNIP dissociates from TRX and binds to NLRP3 upon excess ROS production to allow for NLRP3 activation, and consistent with this, TXNIP deficient macrophages did not activate NLRP3 [76]. This was a significant observation because NLRP3 signaling has emerged as a potential culprit in the development of obesity-induced insulin resistance, atherosclerosis and type 2 diabetes [77]. Disappointingly however the NLRP3–TXNIP link has not been reproducible in other laboratories, with the deletion of TXNIP reported to have no impact on NLRP3 activation [78].

1.9. IAP proteins

Mammalian cells, including macrophages, express the related inhibitor of apoptosis (IAP) proteins cellular IAP1 (cIAP1), cIAP2 and X-linked IAP (XIAP), and interestingly, an early study by David Vaux's group demonstrated that expression of XIAP or cIAP1 could prevent cell killing caused by caspase-1 overexpression [79]. However, more recently research by Maya Saleh and colleagues has suggested that cIAP1 and cIAP2 are, non-redundantly, required for caspase-1 activity [80]. It was proposed that cIAP1/2 and their binding partner TRAF2 regulate cIAP1/2 mediated K63-linked ubiquitylation of caspase-1 to promote its activation. Consistent with this model, it was shown that deletion of cIAP2 alone, or cIAP1, blunted NLRP3 activity. In contrast, using differently derived cIAP1 or cIAP2 null mice to those used by the Maya Saleh laboratory, or TRAF2 gene targeted myeloid cells, our group observed no detrimental effect on NLRP3 and caspase-1 activation in cIAP1, cIAP2, or TRAF2 deficient macrophages [45]. However, we did observe that when cIAP1/2 were co-deleted with the related IAP, XIAP, together they played a critical role in suppressing NLRP3

activation following LPS treatment. LPS stimulation of macrophages deficient in all three IAPs (cIAP1, cIAP2 and XIAP) activated a death signaling complex termed the ripoptosome [81,82], and promoted receptor-interacting protein 3 (RIP3) kinase activation of the NLRP3-caspase-1 inflammasome, as well as caspase-8 cleavage of pro-IL-1β (Fig. 2). Consistent with these findings, Fas mediated activation of IL-1β has recently been shown to occur by caspase-8 cleavage [83]. Similarly, several groups have reported RIP3 activation in IAP depleted cell lines or primary murine macrophages [81,82,84], while David Wallach's laboratory recently demonstrated that deletion of caspase-8, which negatively regulates RIP3, also resulted in RIP3 dependent activation of the NLRP3 inflammasome [85]. While RIP3 is critical for the execution of necroptotic cell death, these studies highlight its potential for directly modulating inflammatory cytokine activation.

1.10. TAK1

Regulation of cellular osmolarity has emerged as a determinant of NLRP3 function, with studies implicating potassium efflux [16] and increased intracellular calcium levels in NLRP3 activation [23–26]. It has been suggested that cell swelling followed by a regulatory volume decrease can act as a trigger for NLRP3 assembly and activation [86]. Although this conclusion has recently been challenged [22], it was proposed that decreased extracellular osmolarity can activate TAK1 (TGF-β activated kinase-1), and TAK1 inhibition by chemical compounds or siRNA silencing blocks NLRP3 activation [86,87]. However our group, and that of Rong-Fu Wang, has independently demonstrated that genetic deletion of TAK1 in macrophages does not reduce NLRP3 activity caused by stimuli such as ATP, nigericin or alum [45,88].

1.11. PKR

PKR, or double-stranded RNA-activated protein kinase, is a key regulator of several pathways involved in inflammation, cell death and the immune response to microbial infections. It has been reported by Kevin Tracey and colleagues that PKR deficient cells are defective for NLRP1, NLRP3, NLRC4 and AIM2 inflammasome function, and that autophosphorylation of PKR prompts its interaction with these specific NLRs to activate them [89]. However, the laboratory of Gabriel Nunez recently published that two independent lines of PKR deficient mice showed normal inflammasome activation following NLRP3, NLRC4 and AIM2 stimulation [90]. Remarkably, in another twist, a recent publication described 7-desacetoxy-6,7-dehydrogedunin (7DG) as a novel PKR inhibitor that prevented inflammasome formation and activation, as did siRNA silencing of PKR. In contrast to the previous study though, PKR phosphorylation was reported as being dispensable for PKR mediated inflammasome activation [91].

2. Conclusions

Much progress has been made in elucidating how NLRP3 is assembled into an active inflammasome complex, and the mechanisms that regulate its function. Yet several issues raised in this review clearly highlight the need for further work. Recent studies have indicated that some of the contradictory findings reported in the inflammasome field may result from chemical or gene knockdown off-target effects, differences in intestinal mircroflora between mouse colonies, or subtle differences in the genetic backgrounds of independently derived mutant mice. For example, while chemical or siRNA targeting of NADPH oxidases, TXNIP, cathepsin B, TAK1 and pannexin-1 initially implicated these proteins as critical in regulating NLRP3 function, the use of gene-targeted mice or primary human patient cells has questioned these conclusions [21,22,45,51,78,88,92]. It is feasible that the differential effects of various chemical redox modulators may also be explained by off-target actions (such as cell death) or the different metabolic landscapes and redox responses of immortalized monocyte and macrophage cell lines versus primary monocytes and macrophages.

Opposing findings for the role of NLRP3 and caspase-1 in murine colitis models appear to be caused, at least in part, by differences in intestinal microflora between independently housed mouse colonies, and the use, or not, of littermate controls [93]. In regards to the importance of mouse strain genetic background, the original caspase-1 null mice derived on the 129 mouse lineage [94] have recently been shown to harbor a caspase-11 inactivating mutation [8], calling for a re-evaluation of many studies using these mice that were published prior to this knowledge. This finding also has implications for other genes that are closely linked to caspase-11 and were targeted using the 129 mouse strain. For example, despite extensive back-crossing of 129 derived cIAP1 deficient mice onto a C57BL/6 background, the genomic proximity of cIAP1 (and cIAP2) to caspase-11 (2.5 Mbp) can fail to restore functional caspase-11 [95]. Different immune responses and altered expression of unrelated genes have also been observed in independently derived ASC deficient mice [96,97], and while the causes for this remain unclear, minor but significant variations in the genetic background of these mice may also explain some discrepancies.

Despite these ambiguities and unresolved issues surrounding NLRP3 regulation, a significant body of work has implicated redox reactions and ion fluxes in NLRP3 activation. However, their targets require clarification and further genetic evidence is needed to properly evaluate the importance of these processes in NLRP3 functioning. If, as recently proposed [22], potassium efflux is the critical inflammasome determinant required for NLRP3 activation by all stimuli, understanding mechanistically how potassium efflux occurs under different conditions, and how this triggers NLRP3 activation, will be important to elucidate.

Future work to resolve the intracellular localization of NLRP3 before and after activation is also needed. This is likely to require increased imaging resolution than is currently afforded by standard fluorescence and confocal microscopy techniques. The predominant active ASC foci reported by several laboratories suggest that this large oligomeric complex should be amenable to detailed immuno-electron microscopy analysis. Once the localization of the inactive and active NLRP3 inflammasome can be agreed upon, this will facilitate our understanding of what, if any, role the mitochondria might play in regulating its activity, be it ROS production, mtDNA induced activation or the coordination of NLRP3 protein interactions.

Acknowledgements

The authors wish to thank Gabriel Nunez and members of his group for sharing unpublished data. The authors' research is funded by an NHMRC grant (1051210) to J.V. and K.L. and an NHMRC CDF fellowship (1052598) to J.V. Our work is also made possible through Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRIISS.

References

[1] S.L. Masters, M. Gerlic, D. Metcalf, S. Preston, M. Pellegrini, et al., NLRP1 inflammasome activation induces pyroptosis of hematopoietic progenitor cells, Immunity 37 (2012) 1009–1023.
[2] E.A. Miao, I.A. Leaf, P.M. Treuting, D.P. Mao, M. Dors, et al., Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria, Nat. Immunol. 11 (2010) 1136–1142.
[3] P. Menu, J.E. Vince, The NLRP3 inflammasome in health and disease: the good, the bad and the ugly, Clin. Exp. Immunol. 166 (2011) 1–15.
[4] S.L. Masters, A. Simon, I. Aksentijevich, D.L. Kastner, Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease (*), Annu. Rev. Immunol. 27 (2009) 621–668.
[5] T. Strowig, J. Henao-Mejia, E. Elinav, R. Flavell, Inflammasomes in health and disease, Nature 481 (2012) 278–286.
[6] E. Latz, T.S. Xiao, A. Stutz, Activation and regulation of the inflammasomes, Nat. Rev. Immunol. 13 (2013) 397–411.
[7] S. Wang, M. Miura, Y.K. Jung, H. Zhu, E. Li, et al., Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE, Cell 92 (1998) 501–509.

[8] N. Kayagaki, S. Warming, M. Lamkanfi, L. Vande Walle, S. Louie, et al., Non-canonical inflammasome activation targets caspase-11, Nature 479 (2011) 117–121.
[9] V.A. Rathinam, S.K. Vanaja, L. Waggoner, A. Sokolovska, C. Becker, et al., TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria, Cell 150 (2012) 606–619.
[10] P. Gurung, R.K. Malireddi, P.K. Anand, D. Demon, L.V. Walle, et al., Toll or interleukin-1 receptor (TIR) domain-containing adaptor inducing interferon-beta (TRIF)-mediated caspase-11 protease production integrates Toll-like receptor 4 (TLR4) protein- and Nlrp3 inflammasome-mediated host defense against enteropathogens, J. Biol. Chem. 287 (2012) 34474–34483.
[11] P. Broz, T. Ruby, K. Belhocine, D.M. Bouley, N. Kayagaki, et al., Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1, Nature 490 (2012) 288–291.
[12] N. Kayagaki, M.T. Wong, I.B. Stowe, S.R. Ramani, L.C. Gonzalez, et al., Noncanonical inflammasome activation by intracellular LPS independent of TLR4, Science (2013), http://dx.doi.org/10.1126/science.1240248 (Epub ahead of print).
[13] Y. Aachoui, I.A. Leaf, J.A. Hagar, M.F. Fontana, C.G. Campos, et al., Caspase-11 protects against bacteria that escape the vacuole, Science 339 (2013) 975–978.
[14] C.N. Casson, A.M. Copenhaver, E.E. Zwack, H.T. Nguyen, T. Strowig, et al., Caspase-11 activation in response to bacterial secretion systems that access the host cytosol, PLoS Pathog. 9 (2013) e1003400.
[15] S. Mariathasan, D.S. Weiss, K. Newton, J. McBride, K. O'Rourke, et al., Cryopyrin activates the inflammasome in response to toxins and ATP, Nature 440 (2006) 228–232.
[16] V. Pétrilli, S. Papin, C. Dostert, A. Mayor, F. Martinon, et al., Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration, Cell Death Differ. 14 (2007) 1583–1589.
[17] M. Lamkanfi, J.L. Mueller, A.C. Vitari, S. Misaghi, A. Fedorova, et al., Glyburide inhibits the Cryopyrin/Nalp3 inflammasome, J. Cell Biol. 187 (2009) 61–70.
[18] T. Fernandes-Alnemri, J. Wu, J.-W. Yu, P. Datta, B. Miller, et al., The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation, Cell Death Differ. 14 (2007) 1590–1604.
[19] P. Pelegrin, A. Surprenant, Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor, EMBO J. 25 (2006) 5071–5082.
[20] T.-D. Kanneganti, M. Lamkanfi, Y.-G. Kim, G. Chen, J.-H. Park, et al., Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling, Immunity 26 (2007) 433–443.
[21] Y. Qu, S. Misaghi, K. Newton, L.L. Gilmour, S. Louie, et al., Pannexin-1 is required for ATP release during apoptosis but not for inflammasome activation, J. Immunol. 186 (2011) 6553–6561.
[22] R. Munoz-Planillo, P. Kuffa, G. Martinez-Colon, B.L. Smith, T.M. Rajendiran, et al., K+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter, Immunity 38 (2013).
[23] M. Rossol, M. Pierer, N. Raulien, D. Quandt, U. Meusch, et al., Extracellular Ca2+ is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors, Nat. Commun. 3 (2012) 1329.
[24] Z. Zhong, Y. Zhai, S. Liang, Y. Mori, R. Han, et al., TRPM2 links oxidative stress to NLRP3 inflammasome activation, Nat. Commun. 4 (2013) 1611.
[25] T. Murakami, J. Ockinger, J. Yu, V. Byles, A. McColl, et al., Critical role for calcium mobilization in activation of the NLRP3 inflammasome, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 11282–11287.
[26] G.S. Lee, N. Subramanian, A.I. Kim, I. Aksentijevich, R. Goldbach-Mansky, et al., The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP, Nature 492 (2012) 123–127.
[27] D. Arnoult, F. Soares, I. Tattoli, S.E. Girardin, Mitochondria in innate immunity, EMBO Rep. 12 (2011) 901–910.
[28] J.-M. Bruey, N. Bruey-Sedano, F. Luciano, D. Zhai, R. Balpai, et al., Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1, Cell 129 (2007) 45–56.
[29] M. Gerlic, B. Faustin, A. Postigo, E.C. Yu, M. Proell, et al., Vaccinia virus F1L protein promotes virulence by inhibiting inflammasome activation, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 7808–7813.
[30] R. Zhou, A.S. Yazdi, P. Menu, J. Tschopp, A role for mitochondria in NLRP3 inflammasome activation, Nature 469 (2011) 221–225.
[31] K. Shimada, T.R. Crother, J. Karlin, J. Dagvadorj, N. Chiba, et al., Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis, Immunity 36 (3) (2012) 401–414.
[32] K.E. Lawlor, A. van Nieuwenhuijze, K.L. Parker, S.F. Drake, I.K. Campbell, et al., Bcl-2 overexpression ameliorates immune complex-mediated arthritis by altering FcgammaRIIb expression and monocyte homeostasis, J. Leukoc. Biol. 93 (2013) 585–597.
[33] T. Saitoh, N. Fujita, M.H. Jang, S. Uematsu, B.G. Yang, et al., Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production, Nature 456 (2008) 264–268.
[34] K. Nakahira, J.A. Haspel, V.A. Rathinam, S.J. Lee, T. Dolinay, et al., Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome, Nat. Immunol. 12 (2011) 222–230.
[35] C.S. Shi, K. Shenderov, N.N. Huang, J. Kabat, M. Abu-Asab, et al., Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction, Nat. Immunol. 13 (2012) 255–263.
[36] J. Harris, M. Hartman, C. Roche, S.G. Zeng, A. O'Shea, et al., Autophagy controls IL-1{beta} secretion by targeting pro-IL-1{beta} for degradation, J. Biol. Chem. 286 (2011) 9587–9597.
[37] J. Tschopp, K. Schroder, NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nat. Rev. Immunol. 10 (2010) 210–215.
[38] C. Dostert, V. Pétrilli, R. Van Bruggen, C. Steele, B.T. Mossman, et al., Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica, Science 320 (2008) 674–677.
[39] A. Rubartelli, Redox control of NLRP3 inflammasome activation in health and disease, J. Leukoc. Biol. 92 (2012) 951–958.
[40] C. Lupfer, P.G. Thomas, P.K. Anand, P. Vogel, S. Milasta, et al., Receptor interacting protein kinase 2-mediated mitophagy regulates inflammasome activation during virus infection, Nat. Immunol. 14 (2013) 480–488.
[41] A.C. Bulua, A. Simon, R. Maddipati, M. Pelletier, H. Park, et al., Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS), J. Exp. Med. 208 (2011) 519–533.
[42] M.D. Cordero, E. Alcocer-Gomez, O. Culic, A.M. Carrion, M. de Miguel, et al., NLRP3 inflammasome is activated in fibromyalgia: the effect of coenzyme Q10, Antioxid. Redox Signal. (2013), http://dx.doi.org/10.1089/ars.2013.5198 (Epub ahead of print).
[43] F. Bauernfeind, E. Bartok, A. Rieger, L. Franchi, G. Nunez, et al., Cutting edge: reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 inflammasome, J. Immunol. 187 (2011) 613–617.
[44] O. Gross, A.S. Yazdi, C.J. Thomas, M. Masin, L.X. Heinz, et al., Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1, Immunity 36 (2012) 388–400.
[45] J.E. Vince, W.W. Wong, I. Gentle, K.E. Lawlor, R. Allam, et al., Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation, Immunity 36 (2012) 215–227.
[46] K. Schroder, V. Sagulenko, A. Zamoshnikova, A.A. Richards, J.A. Cridland, et al., Acute lipopolysaccharide priming boosts inflammasome activation independently of inflammasome sensor induction, Immunobiology 217 (2012) 1325–1329.
[47] C. Juliana, T. Fernandes-Alnemri, S. Kang, A. Farias, F. Qin, et al., Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation, J. Biol. Chem. 287 (2012) 36617–36622.
[48] B.F. Py, M.S. Kim, H. Vakifahmetoglu-Norberg, J. Yuan, Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity, Mol. Cell 49 (2013) 331–338.
[49] G. Lopez-Castejon, N.M. Luheshi, V. Compan, S. High, R.C. Whitehead, et al., Deubiquitinases regulate the activity of caspase-1 and interleukin-1beta secretion via assembly of the inflammasome, J. Biol. Chem. 288 (2013) 2721–2733.
[50] F. Meissner, K. Molawi, A. Zychlinsky, Superoxide dismutase 1 regulates caspase-1 and endotoxic shock, Nat. Immunol. 9 (2008) 866–872.
[51] F.L. van de Veerdonk, S.P. Smeekens, L.A. Joosten, B.J. Kullberg, C.A. Dinarello, et al., Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 3030–3033.
[52] F. Meissner, R.A. Seger, D. Moshous, A. Fischer, J. Reichenbach, et al., Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease, Blood 116 (2010) 1570–1573.
[53] V. Sagulenko, S.J. Thygesen, D.P. Sester, A. Idris, J.A. Cridland, et al., AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC, Cell Death Differ. 20 (2013) 1149–1160.
[54] R. Pierini, C. Juruj, M. Perret, C.L. Jones, P. Mangeot, et al., AIM2/ASC triggers caspase-8-dependent apoptosis in Francisella-infected caspase-1-deficient macrophages, Cell Death Differ. 19 (2012) 1709–1721.
[55] J. Masumoto, T.A. Dowds, P. Schaner, F.F. Chen, Y. Ogura, et al., ASC is an activating adaptor for NF-kappa B and caspase-8-dependent apoptosis, Biochem. Biophys. Res. Commun. 303 (2003) 69–73.
[56] T. Misawa, M. Takahama, T. Kozaki, H. Lee, J. Zou, et al., Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome, Nat. Immunol. 14 (2013) 454–460.
[57] N. Subramanian, K. Natarajan, M.R. Clatworthy, Z. Wang, R.N. Germain, The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation, Cell 153 (2013) 348–361.
[58] Y. Wang, C. Yang, K. Mao, S. Chen, G. Meng, et al., Cellular localization of NLRP3 inflammasome, Protein Cell 4 (2013) 425–431.
[59] K. Mao, S. Chen, M. Chen, Y. Ma, Y. Wang, et al., Nitric oxide suppresses NLRP3 inflammasome activation and protects against LPS-induced septic shock, Cell Res. 23 (2013) 201–212.
[60] P. Broz, K. Newton, M. Lamkanfi, S. Mariathasan, V.M. Dixit, et al., Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against Salmonella, J. Exp. Med. 207 (2010) 1745–1755.
[61] Y. Qu, S. Misaghi, A. Izrael-Tomasevic, K. Newton, L.L. Gilmour, et al., Phosphorylation of NLRC4 is critical for inflammasome activation, Nature 490 (2012) 539–542.
[62] A. Halle, V. Hornung, G.C. Petzold, C.R. Stewart, B.G. Monks, et al., The NALP3 inflammasome is involved in the innate immune response to amyloid-beta, Nat. Immunol. 9 (2008) 857–865.
[63] V. Hornung, F. Bauernfeind, A. Halle, E.O. Samstad, H. Kono, et al., Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization, Nat. Immunol. 9 (2008) 847–856.
[64] C. Dostert, G. Guarda, J.F. Romero, P. Menu, O. Gross, et al., Malarial hemozoin is a Nalp3 inflammasome activating danger signal, PLoS One 4 (2009) e6510.
[65] P. Duewell, H. Kono, K.J. Rayner, C.M. Sirois, G. Vladimer, et al., NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals, Nature 464 (2010) 1357–1361.
[66] H. Hentze, X.Y. Lin, M.S. Choi, A.G. Porter, Critical role for cathepsin B in mediating caspase-1-dependent interleukin-18 maturation and caspase-1-independent necrosis triggered by the microbial toxin nigericin, Cell Death Differ. 10 (2003) 956–968.
[67] R. Mihalik, G. Imre, I. Petak, B. Szende, L. Kopper, Cathepsin B-independent abrogation of cell death by CA-074-OMe upstream of lysosomal breakdown, Cell Death Differ. 11 (2004) 1357–1360.

[68] M.E. Guicciardi, G.J. Gores, Complete lysosomal disruption: a route to necrosis, not to the inflammasome, Cell Cycle 12 (2013).
[69] H. Lima Jr., L.S. Jacobson, M.F. Goldberg, K. Chandran, F. Diaz-Griffero, et al., Role of lysosome rupture in controlling Nlrp3 signaling and necrotic cell death, Cell Cycle 12 (2013).
[70] P. Boya, R.A. Gonzalez-Polo, N. Casares, J.L. Perfettini, P. Dessen, et al., Inhibition of macroautophagy triggers apoptosis, Mol. Cell Biol. 25 (2005) 1025–1040.
[71] H. Tapper, R. Sundler, Bafilomycin A1 inhibits lysosomal, phagosomal, and plasma membrane H(+)-ATPase and induces lysosomal enzyme secretion in macrophages, J. Cell Physiol. 163 (1995) 137–144.
[72] V.V. Teplova, A.A. Tonshin, P.A. Grigoriev, N.E. Saris, M.S. Salkinoja-Salonen, Bafilomycin A1 is a potassium ionophore that impairs mitochondrial functions, J. Bioenerg. Biomembr. 39 (2007) 321–329.
[73] C. Andrei, C. Dazzi, L. Lotti, M.R. Torrisi, G. Chimini, et al., The secretory route of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles, Mol. Biol. Cell 10 (1999) 1463–1475.
[74] K. Rajamaki, T. Nordstrom, K. Nurmi, K.E. Akerman, P.T. Kovanen, et al., Extracellular acidosis is a novel danger signal alerting innate immunity via the NLRP3 inflammasome, J. Biol. Chem. 288 (2013) 13410–13419.
[75] D.L. Thiele, P.E. Lipsky, Mechanism of L-leucyl-L-leucine methyl ester-mediated killing of cytotoxic lymphocytes: dependence on a lysosomal thiol protease, dipeptidyl peptidase I, that is enriched in these cells, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 83–87.
[76] R. Zhou, A. Tardivel, B. Thorens, I. Choi, J. Tschopp, Thioredoxin-interacting protein links oxidative stress to inflammasome activation, Nat. Immunol. 11 (2010) 136–140.
[77] S.L. Masters, E. Latz, L.A. O'Neill, The inflammasome in atherosclerosis and type 2 diabetes, Sci. Transl. Med. 3 (2011)(81 ps17).
[78] S.L. Masters, A. Dunne, S.L. Subramanian, R.L. Hull, G.M. Tannahill, et al., Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes, Nat. Immunol. 11 (2010) 897–904.
[79] A.G. Uren, M. Pakusch, C.J. Hawkins, K.L. Puls, D.L. Vaux, Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4974–4978.
[80] K. LabbÈ, C.R. McIntire, K. Doiron, P.M. Leblanc, M. Saleh, Cellular inhibitors of apoptosis proteins cIAP1 and cIAP2 are required for efficient caspase-1 activation by the inflammasome, Immunity 35 (2011) 897–907.
[81] T. Tenev, K. Bianchi, M. Darding, M. Broemer, C. Langlais, et al., The ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs, Mol. Cell 43 (2011) 432–448.
[82] M. Feoktistova, P. Geserick, B. Kellert, D.P. Dimitrova, C. Langlais, et al., cIAPs block ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms, Mol. Cell 43 (2011) 449–463.
[83] L. Bossaller, P.I. Chiang, C. Schmidt-Lauber, S. Ganesan, W.J. Kaiser, et al., Cutting edge: FAS (CD95) mediates noncanonical IL-1beta and IL-18 maturation via caspase-8 in an RIP3-independent manner, J. Immunol. 189 (2012) 5508–5512.
[84] S. McComb, H.H. Cheung, R.G. Korneluk, S. Wang, L. Krishnan, et al., cIAP1 and cIAP2 limit macrophage necroptosis by inhibiting Rip1 and Rip3 activation, Cell Death Differ. 19 (2012) 1791–1801.
[85] T.B. Kang, S.H. Yang, B. Toth, A. Kovalenko, D. Wallach, Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasome, Immunity 38 (2013) 27–40.
[86] V. Compan, A. Baroja-Mazo, G. Lopez-Castejon, A.I. Gomez, C.M. Martinez, et al., Cell volume regulation modulates NLRP3 inflammasome activation, Immunity 37 (2012) 487–500.
[87] Y.N. Gong, X. Wang, J. Wang, Z. Yang, S. Li, et al., Chemical probing reveals insights into the signaling mechanism of inflammasome activation, Cell Res. 20 (2010) 1289–1305.
[88] A.A. Ajibade, Q. Wang, J. Cui, J. Zou, X. Xia, et al., TAK1 negatively regulates NF-kappaB and p38 MAP kinase activation in Gr-1+CD11b+neutrophils, Immunity 36 (2012) 43–54.
[89] B. Lu, T. Nakamura, K. Inouye, J. Li, Y. Tang, et al., Novel role of PKR in inflammasome activation and HMGB1 release, Nature 488 (2012) 670–674.
[90] Y. He, L. Franchi, G. Nunez, The protein kinase PKR is critical for LPS-induced iNOS production but dispensable for inflammasome activation in macrophages, Eur. J. Immunol. 43 (5) (2013) 1147–1152.
[91] E.C. Hett, L.H. Slater, K.G. Mark, T. Kawate, B.G. Monks, et al., Chemical genetics reveals a kinase-independent role for protein kinase R in pyroptosis, Nat. Chem. Biol. 9 (2013) 398–405.
[92] R. van Bruggen, M.Y. Koker, M. Jansen, M. van Houdt, D. Roos, et al., Human NLRP3 inflammasome activation is Nox1-4 independent, Blood 115 (2010) 5398–5400.
[93] E. Elinav, J. Henao-Mejia, R.A. Flavell, Integrative inflammasome activity in the regulation of intestinal mucosal immune responses, Mucosal Immunol. 6 (2013) 4–13.
[94] D.B. Conze, L. Albert, D.A. Ferrick, D.V. Goeddel, W.C. Yeh, et al., Posttranscriptional downregulation of c-IAP2 by the ubiquitin protein ligase c-IAP1 in vivo, Mol. Cell Biol. 25 (2005) 3348–3356.
[95] N.S. Kenneth, J.M. Younger, E.D. Hughes, D. Marcotte, P.A. Barker, et al., An inactivating caspase 11 passenger mutation originating from the 129 murine strain in mice targeted for c-IAP1, Biochem. J. 443 (2012) 355–359.
[96] S.K. Ippagunta, R.K. Malireddi, P.J. Shaw, G.A. Neale, L.V. Walle, et al., Addendum: defective Dock2 expression in a subset of ASC-deficient mouse lines, Nat. Immunol. 13 (2012) 701–702.
[97] A.H. Ellebedy, C. Lupfer, H.E. Ghoneim, J. DeBeauchamp, T.D. Kanneganti, et al., Inflammasome-independent role of the apoptosis-associated speck-like protein containing CARD (ASC) in the adjuvant effect of MF59, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 2927–2932.