Autophagy, NLRP3 inflammasome and auto-inflammatory/immune diseases

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Received and accepted on June 30, 2016.

Clin Exp Rheumatol 2016; 34 (Suppl. 98): S12-S16.

Key words: autophagy, mitophagy, mitochondria, mtDNA, mtROS, NLRP3 inflammasome, auto-inflammatory diseases, immune diseases

Funding: Z. Zhong was supported by Cancer Research Institute Irvington postdoctoral fellowship;
E. Sanchez-Lopez was supported by Sara Borrell fellowship under ISCIII/MICINN program; Research was supported by grants from the NIH (AI043477 and CA163798) and a Leukemia and Lymphoma Society SCOR (20132569) to M. Karin, who is an American Cancer Society Research Professor and holder of the Ben and Wanda Hildyard Chair for Mitochondrial and Metabolic Diseases.

Competing interests: none declared.

ABSTRACT

Loss of homeostasis, as a result of pathogen invasion or self imbalance, causes tissue damage and inflammation. In addition to its well-established role in promoting clearance of pathogens or cell corpses, inflammation is also key to drive tissue repair and regeneration. Conserved from flies to humans, a transient, well-balanced inflammatory response is critical for restoration of tissue homeostasis after damage. The absence of such a response can result in failure of tissue repair, leading to the development of devastating immunopathologies and degenerative diseases. Studies in the past decade collectively suggest that a malfunction of NLRP3 inflammasome, a key tissue damage sensor, is a dominant driver of various autoinflammatory and autoimmune diseases, including gout, rheumatoid arthritis, and lupus. It is therefore crucial to understand the biology and regulation of NLRP3 inflammasome and determine its affect in the context of various diseases. Of note, various studies suggest that autophagy, a cellular waste removal and rejuvenation process, serves an important role as a macrophage-intrinsic negative regulator of NLRP3 inflammasome. Here, we review recent advances in understanding how autophagy regulates NLRP3 inflammasome activity and discuss the implications of this regulation on the pathogenesis of autoinflammatory and autoimmune diseases.

Introduction

Tissue damage can be caused by foreign insults (*e.g.* pathogen invasion) or self imbalance (*e.g.* metabolic dysfunction), both of which can breach normal tissue homeostasis (1). When damage occurs, our body senses deviation from homeostasis and initiates repair and regeneration processes to restore tissue function. Although inflammation is needed for driving tissue repair and regeneration, its dysregulation often results in severe immunopathologies. It is thought that a transient, well-balanced inflammatory response favours restoration of tissue homeostasis whereas prolonged, uncontrolled inflammation prevents it (1).

Inflammation is initiated by host recognition of foreign pathogens or self-danger signals. This is controlled by engagement of pattern recognition receptors (PRR) with pathogen associated molecule patterns (PAMP) or damage-associated molecule patterns (DAMP) (2). Studies in the past decade have suggested that a PRR called NLRP3, which belongs to the Nod-like receptor (NLR) family, is the key sensor of tissue damage. By sensing microbial pathogens and self-danger signals, NLRP3 interacts with the adaptor protein ASC which further recruits pro-caspase-1 to form a large protein complex, called the NLRP3 inflammasome. This results in autocleavage and activation of caspase-1, which converts pro-IL-1β and pro-IL-18 into their mature forms (2).

Aberrant NLRP3 inflammasome activation is associated with a number of human immune disorders (3, 4). For example, gain-of-function mutations in the gene encoding NLRP3 are associated with development of autoinflammatory syndromes collectively called the cryopyrin-associated periodic syndromes (CAPS) (5), which include familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and neonatal-onset multisystem inflammatory disease (NOMID) (6). All these syndromes are characterised by systemic inflammation caused by overproduction of IL-1β and IL-18. In addition, hyperactivation of NLRP3 inflammasome, independent of genetic alterations, was found in peripheral blood mononuclear cells

(PBMC) of patients with systemic lupus erythematosus (SLE) or diabetes (7, 8), although the precise mechanism by which NLRP3 inflammasome become hyperactivated remains unclear. Several studies suggest that mitochondrial damage dictates NLRP3 inflammasome assembly and that autophagy, a waste removal process, restrains inflammation through clearance of damaged mitochondria (9-12).

In this review, we summarise recent advances on the control of NLRP3 inflammasome activation by mitochondria and autophagy, and how this affects inflammation and tissue repair.

The NLRP3 inflammasome and auto-inflammatory/immune diseases

Production of bioactive IL-1β requires two distinct steps: "priming" and "activation" (2, 13). At "priming" step, recognition of the DAMP or PAMP by Toll-like receptors (TLR) leads to rapid activation of NF-κB, which induces *de novo* synthesis of pro-IL-1β and also increases the amount of NLRP3 protein. The second step involves formation of the NLRP3 inflammasome, which is comprised of the sensor NLRP3, the adaptor ASC (apoptosis associated speck-like protein containing a caspase activation and recruitment domain (CARD)), and the effector procaspase-1. Among NLR, NLRP3 is the most promiscuous sensor protein because it responds to a wide panoply of stimuli, including ATP, uric acid, lipid particles, microcrystals and toxins, that are structurally and functionally distinct from each other. Although the precise mechanism by which NLRP3 senses all of these stimuli remains elusive, it is proposed that ion fluxes, disruption of membrane integrity, and mitochondrial damage play key roles in NLRP3 inflamamsome activation (13).

Evidence for the pathogenic role of NLRP3 was obtained after discovery of *Nlrp3* gain-of-function mutations that are inherited with an autosomal-dominant pattern. Such mutations are responsible for a spectrum of auto-inflammatory diseases, collectively known as CAPS (6, 14). To date, more than 90 heterozygous *Nlrp3* mutations associated with CAPS have been described (6, 14). Individuals with CAPS suffer from chronic inflammation manifested by spontaneous fever episodes, blood and tissue neutrophilia, and localised inflammation in skin, joints, muscle and cerebrospinal fluid. Knock-in mice carrying *Nlrp3* associated with two specific CAPS phenotypes, FCAS and MWS, demonstrated that these pathologies are mediated by IL-1β-dependent innate immune responses (3). Although it is not clear how these diseases arise and progress, PBMC from patients with CAPS have hyperactive NLRP3 inflammasome and produce more IL-1β and IL-18 than healthy individuals under resting conditions or after minimal stimulation with either lipopolysaccharide or hypothermia (15, 16). Furthermore, PBMC carrying CAPS-associated mutations exhibit spontaneous formation of ASC specks which can be released extracellularly and function as DAMP (17). Importantly, the use of IL-1β signalling blockers has shown favourable clinical outcomes in CAPS patients, suggesting that termination of the inflammatory response mounted by NLRP3 inflammasome is essential to reverse disease development and restore tissue homeostasis (5, 18-20).

Interestingly, elevated circulating IL-18 has been detected in SLE patients (21). SLE is characterised by chronic production of autoantibodies which form immunecomplexes (IC) with self-antigens (*e.g.* DNA). These IC are deposited at distal organs (*e.g.* kidney), and cause immunopathology and tissue damage. Intriguingly, PBMC from SLE patients have hyperactive NLRP3 inflammasome (possibly due to mutations in *Atg5* and/or *Beclin1*), indicating that increased NLRP3 inflammasome activity in either autoimmune (SLE) or autoinflammatory (5) diseases might be a common pathogenic mechanism (8, 22).

Mitochondrial control of the NLRP3 inflammasome

Understanding the molecular mechanism responsible for NLRP3 inflammasome activation in health and disease has been the subject of intense research. Importantly, all of the diverse NLRP3 activators that have been examined do not bind to NLRP3 directly. Rather, all of these stimuli lead to mitochondrial damage and the release of signals that trigger inflammasome assembly. A role for mitochondria in regulating NLRP3 inflammasome activation was first shown by Jurg Tschopp's group (9). Inspired by their previous work on the role of reactive oxygen species (ROS) in NLRP3 inflammasome activation, Tschopp and colleagues found that the critical source of inflammasome activating ROS is not NADPH oxidases but mitochondria instead. Indeed, inhibitors that block mitochondrial respiration and ROS production as well as depletion of mitochondria prevented NLRP3 inflammasome activation. In addition, genetic ablation of VDAC channels (namely VDAC1 and VDAC3), located on the mitochondrial outer membrane and responsible for exchanging ions and metabolites with the cytoplasm, led to diminished mitochondrial (mt) ROS production and inhibition of NLRP3 inflammasome activation. Furthermore, overexpression of Bcl-2, a protein that inhibits VDAC activity, also leads to inhibition of IL-1β secretion. These results collectively established the mitochondrion as a key intermediate in NLRP3 inflammasome activation.

However, it is still not clear how mtROS activate the NLRP3 inflammasome. Given the fact that mtROS can affect many cellular targets, it is not even clear whether they act on NLRP3 directly. One possible mechanism involves gating of a plasma membrane ion channel called TRPM2 (transient potential melastatin 2), whose opening results in calcium influx and generation of an ionic environment favourable for NLRP3 inflammasome assembly and further mitochondrial damage. The removal of extracellular calcium or genetic ablation of TRPM2 significantly attenuates NLRP3 inflammasome activation (23). Elevated cytosolic calcium further promotes mtROS production, leading to damage of healthy mitochondria, thereby establishing a feed-forward loop that maximises mtROS production.

Moreover, mtROS can also lead to

oxidation of mtDNA, which is released from damaged mitochondria to the cytosol (24). Oxidised mtDNA, but not its reduced form, was proposed to serve as a direct ligand for NLRP3. However, further studies using combination of genetic, imaging, and biochemical tools are still needed to conclusively validate the role of oxidised *versus* non-oxidised mtDNA in NLRP3 activation. In addition to mtDNA, cardiolipin, which, upon stimulation with NLRP3 activators translocates from the inner to outer membrane of mitochondria, is thought to provide a mitochondrial "docking" site for NLRP3, which prior to macrophage stimulation, is located in the endoplasmic reticulum (ER) (25).

Autophagy/mitophagy keeps NLRP3 inflammasome in check.

As mentioned above, dysregulation of the NLRP3 inflammasome contributes to a number of major human diseases. It is therefore of utmost importance to keep activation of NLRP3 inflammasome in check. As NLRP3 inflammasome activation seems to be an "all-or-none" event, one way to regulate it is by keeping mitochondrial damage and release of mitochondria-derived activators under control. Indeed, several landmark studies, first published by Shizuo Akira and then followed by two reports from Jurg Tschopp and Augustine Choi, have collectively shown that the autophagy machinery constitutes a key cellular monitoring system that prevents excessive NLRP3 inflammasome activation (9, 11, 12). Although it was speculated that autophagy might inhibit NLRP3 inflammasome activation by promoting clearance of damaged mitochondria, the precise molecular events of this process were only discovered recently.

NF-κB is critical for inducing expression of pro-IL-1β and upregulating the amounts of NLRP3, surprisingly, however, pharmacologic or genetic inhibition of NF-κB resulted in unexpected exacerbation of NLRP3-dependent inflammation in preclinical animal models and human patients (26). This has led to the termination of a number of drug development programs aiming at blocking IKK-driven NF-κB activation as a way to prevent inflammation. Intrigued by these striking observations, we decided to further examine the molecular mechanism through which NF-ϰB restricts NLRP3 inflammasome activation. Since NF-κB was also reported to stimulate autophagy (27), we decided to interrogate the role of NF-ϰB regulated autophagy proteins. This investigation led us to focus on the autophagy adaptor p62/SQSTM1, whose expression is strongly induced by LPS priming of macrophages via an NF-κB-dependent manner (10).

In addition to being an autophagy adaptor, p62 is a multifunctional signalling hub that controls cellular homeostasis and functionality. However, its role in macrophage function was unexplored. We found that upon exposure to diverse NLRP3 activators, macrophage p62 forms aggregates that are located next to mitochondria (10). Further biochemical, genetic and imaging analysis revealed that exposure to NLRP3 inflammasome activators results in Parkin-dependent ubiquitination of damaged mitochondria resulting in formation of polyubiquitin chains to which p62 binds via its ubiquitin-association (UBA) domain. Mitochondria-bound p62 then interacts with the autophagosome docking protein LC3 via its LC3-interacting domain to shuttle damaged mitochondria into newly formed autophagosomes. The cargo, damaged mitochondria in this case, is thus delivered to autophagosome by p62 and eventually degraded within the lysosome (Fig. 1). In consistence with this notion, macrophages deficient in Parkin, p62 or Atg7 exhibit excessive NLRP3 inflammasome activation, and mice with myeloid-specific ablation of p62 or Atg7 develop severe IL-1β mediated immunopathologies. Importantly, we demonstrated that elimination of mitochondrial signals (*e.g.* mtROS and mtDNA) prevented excessive IL-1β production, confirming that the "NF-κB-p62-mitophagy" signalling axis represents a key macrophage-intrinsic regulatory mechanism that keeps NLRP3 inflammasome activation in check (10).

In summary, NF-κB controls two opposing signalling events: one of which facilitates the initiation of NLRP3 inflammasome activation, and the other prevents its overactivation. Thus, the newly identified "NF-κB-p62-mitophagy" signalling axis represents a macrophage-intrinsic mechanism by which NF-κB restricts its own inflammation-promoting activity to secure a beneficial inflammatory response that promotes pathogen clearance and favours tissue repair.

Concluding remarks

There is no doubt that dysregulated NLRP3 inflammasome accounts for the pathogenesis of numerous autoinflammatory and autoimmune diseases. Therefore, it is of utmost importance to understand the molecular mechanisms that control both the initiation and termination of NLRP3 inflammasome activation. Such mechanisms ensure the generation of a well-balanced inflammatory output that favours restoration of tissue homeostasis and avoids excessive collateral damage that would otherwise lead to autoimmune and autoinflammatory pathologies. Given the recent advances on understanding the roles of mitochondria and autophagy in regulating NLRP3 inflammasome activation, we are confident that by manipulating mitochondrial status and/or autophagy/mitophagy activity, we will be able to approach the development of proper NLRP3 inflammasome inhibitors that can be used to treat and prevent the different autoimmune and autoinflammatory disorders discussed above.

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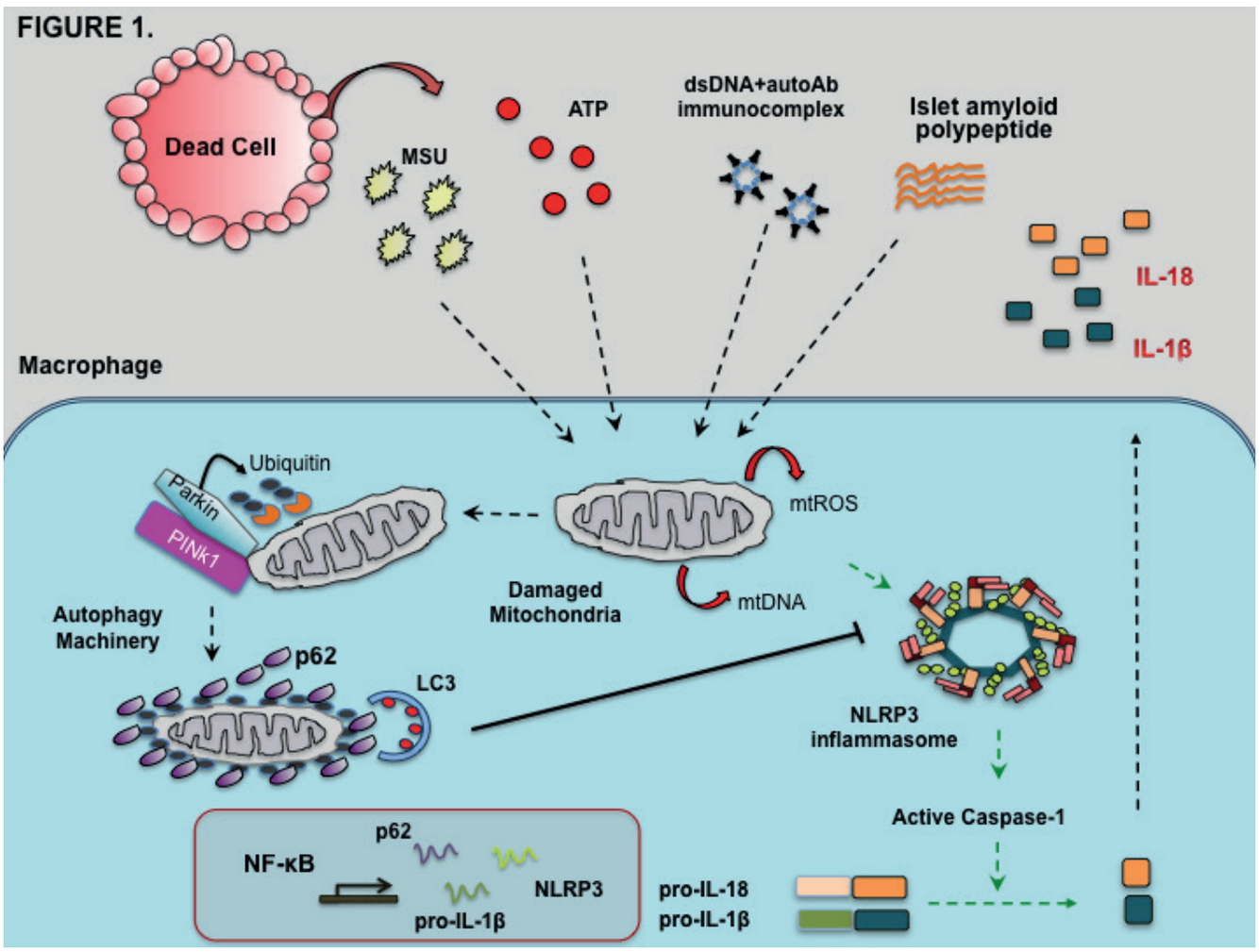

Fig. 1. Regulation of NLRP3 inflammasome by mitochondria and autophagy/mitophagy. Stimulation of macrophages with DAMP (*e.g.* ATP, uric acid), metabolic products (*e.g.* islet amyloid polypeptide) or autoantigen-antibody immunocomplexes, leads to mitochondrial damage and release of mtDNA and mtROS, that activate NLRP3. Meanwhile, mitochondria damage also results in Parkin activation and p62 mitochondrial recruitment. p62 delivers damaged mitochondria to autophagosome for eventual degradation, thereby eliminating NLRP3 activation signals derived from mitochondria.
MSU: monosodium urate crystal; PINK1: PTEN-induced putative kinase 1; LC3: microtubule-associated protein 1A/1B light chain 3; NF-κB: nuclear factor-kappa B; NLRP3: Nod-like receptor pyrin domain containing 3.

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