

The sensing of mitochondrial DAMPs by non-immune cells

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ABSTRACT Mitochondria are the source of damage-associated molecular patterns (DAMPs), which are molecules that play a key modulatory role in immune cells. These molecules include proteins and peptides, such as N-formyl peptides and TFAM, as well as lipids, and metabolites such as cardiolipin, succinate and ATP, and also mitochondrial DNA (mtDNA). Recent data indicate that somatic cells sense mitochondrial DAMPs and trigger protective mechanisms in response to these signals. In this review we focus on the well-described effects of mitochondrial DAMPs on immune cells and also how these molecules induce immunogenic responses in non-immune cells. Special attention will be paid to the response to mtDNA.

Received originally: 01.10.2018

in revised form: 03.05.2019,

Accepted 10.05.2019,

Published 23.05.2019.

Keywords: mitochondria, DAMP, immunity, mitochondrial DNA, TLR9, cGAS.

Abbreviations:

cGAS – cyclic GMP-AMP synthase, **CL** – cardiolipin, **DAMP** – damage-associated molecular pattern, **ER** – endoplasmic reticulum, **FPR** – formyl peptide receptor, **IFN** – interferon, **IMM** – inner mitochondrial membrane, **MTD** – mitochondria-derived DAMP, **mtDNA** – mitochondrial DNA, **NFP** – N-formyl peptide, **OMM** – outer mitochondrial membrane, **PAMP** – pathogen-associated molecular pattern, **PRR** – Pattern Recognition Receptor, **ROS** – reactive oxygen species, **STING** – stimulator of interferon genes, **TLR** – Toll-like receptor.

MITOCHONDRIA ARE IMMUNOGENIC ORGANELLES

The efficiency of the innate immune system is determined by the capacity of distinct cell types to discriminate self from non-self structures. The dysregulation of this ability results in either immunodeficiency pathologies or autoinflammatory and autoimmune diseases. The immune system is primed to recognize pathogen-associated molecular patterns (PAMPs), derived from infection, through a variety of receptors. However, misplaced self-molecules can also trigger similar types of responses. Such molecules are called damage-associated molecular patterns (DAMPs). Mitochondria produce DAMPs and in fact, are relevant contributors to the cellular generation of these damage signals. In this regard, both the origin and features of these molecules account for the immunogenic capacity of the mitochondrion.

Mitochondria arose around two thousand million years ago, which makes them one of the most ancient endo-

membrane systems in eukaryotic cells. In 1967, Lynn Margulis rescued the long forgotten endosymbiont theory of organelle origin [1]. This proposes that eukaryotic cells derived from the engulfment of an α -proteobacterium by the eukaryotic progenitor. The resemblance of modern mitochondria to their bacterial ancestor supports this theory. Among other features, mitochondria are comprised of two functionally different and separate membranes that surround a matrix compartment that contains the un-methylated mitochondrial circular genome, which is organized as nucleoids throughout the matrix [2]. The bacterial-like characteristics of mitochondria also reinforce the notion of them being hubs of immunity. The proteins found in mitochondria are structurally similar to those in bacteria and enable their recognition by the same receptors of the immune system [3].

Mitochondria are pivotal organelles for many cellular functions and are the primary energy-generating system in

most eukaryotic cells. The architecture of mitochondria is essential for their proper function and also for the confinement of mitochondria-derived immunogenic molecules. At the ultrastructural level, mitochondria are comprised by two membranes, namely the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM). The OMM is structurally simple and highly permeable to small molecules and ions, while at the same time it protects the cell from noxious mitochondrial products, including reactive oxygen species (ROS), immunogenic mtDNA [4] and death signals. The IMM is morphologically more complex, and it creates an impermeable barrier between the matrix and the intermembrane space. This restrictive permeability and proper cristae morphology are the two major physical features that enable mitochondria to perform oxidative phosphorylation [5]. Additionally, these organelles participate in intermediary metabolism, the regulation of programmed cell death, calcium homeostasis, and the generation and control of ROS [2, 6, 7]. Mitochondrial functionality is synonymous with cellular homeostasis. In this regard, diverse molecules extruded from mitochondria alert neighboring cells, the immune system, and the producing cell itself about mitochondrial dysfunction. This signal triggers various mechanisms aimed to revert the defect and recover homeostasis, or, under chronic or more severe conditions, to induce a systemic response.

MITOCHONDRIA GENERATE DIFFERENT TYPE OF IMMUNOGENIC MOLECULES OR DAMPs

Mitochondria-derived DAMPs (hereafter MTDs) include not only proteins but also DNA, lipids and metabolites, and they show immunogenic capacity when misplaced or imbalanced. In this review we focus on the well-described effects of MTDs on immune cells and also how these molecules induce immunogenic responses in non-immune cells.

MTDs are recognized by Pattern Recognition Receptors (PRRs). Usually, the receptors that recognize specific mitochondrial molecules are those that engage to the pathogenic homologs of these molecules. Therefore, the response triggered by MTDs resembles pathogenic effects. Hence, MTDs in sterile conditions have been studied with the aim to unravel autoimmunity and autoinflammatory diseases.

Mitochondria produce several DAMPs, such as ATP, succinate, cardiolipin, N-formyl peptides, mtDNA and mitochondrial transcription factor A (TFAM), which serve as danger flags for immunological signaling (**Figure 1**) [4, 8–11].

In addition to being the source of DAMPs, mitochondria are also linked to immunity through their role as innate immune platforms that harbor the mitochondrial antiviral-signaling protein (MAVS) as a viral RNA sensor and the Nod-like receptor 3 (NLRP3) inflammasome as a multiple immunogenic receptor [12–16].

Adenosine triphosphate

ATP is the energetic currency in all living organisms. It is synthesized mainly in mitochondria by ATP synthase cou-

pled to the electron transport chain in the IMM. It is transported to the cytosol by the ATP-ADP translocase and used by many energy-demanding reactions in the cell. The conversion from adenosine triphosphate to adenosine diphosphate through the donation of a phosphate group enables biochemical reactions.

In addition to the crucial role of intracellular ATP as an energy source for the maintenance of cellular homeostasis, it is also a key player in extracellular signaling. ATP is released from cells by cell damage, exocytosis as well as by non-vesicular mechanisms, which include ATP release channels [17]. It has been demonstrated that extracellular ATP is sensed by P2XR and P2YR receptors in the plasma membrane [18, 19]. ATP is secreted from various types of cells under stress conditions. For instance, bacterial, hypotonic and mechanical insults on epithelial cells result in increased secretion of ATP [20–22]. High levels of extracellular ATP are sensed by leukocytes and promote relaxation, vasodilatation, neurotransmission, platelet aggregation, ion transport regulation, cell growth, and immune response—all processes triggered in response to tissue damage [23–25]. In particular, neutrophils activate chemotaxis, release IL-8 and elastase, increase adhesion capacity to endothelial cells, cause degranulation, and produce ROS and ATP to further enhance the innate immune response [26]. In monocytes and macrophages, extracellular ATP promotes the production of the pro-inflammatory cytokines IL-1 β and IL-18. ATP binds to P2X7 receptor and induces K⁺ efflux through the P2X7 channel. This results in caspase-1 cleavage in the NLRP3 inflammasome, which in turn promotes cytokine maturation and secretion [27, 28]. Both in monocytes and T lymphocytes, ATP induces shedding of L-selectin by P2X7R activation, thereby leading to transmigration through the endothelium. These observations thus indicate the involvement of ATP in both innate and adaptive immunity [29].

Succinate

Succinate is an intermediate of the tricarboxylic acid (TCA) cycle that is generated from succinyl-CoA via succinyl-CoA ligase. However, succinate has been shown to be secreted to the extracellular media *in vitro*, and this is stimulated by antimycin A treatment, which inhibits electron transfer between cytochrome b and c1 [30]. Indeed, extracellular succinate acts as a signaling molecule and is recognized by immune cells through its G-protein-coupled receptor, namely succinate receptor 1 (SUCNR1, also named GPR91) [31]. The activation of the receptor stabilizes hypoxia-inducible factor-1 alpha (HIF-1 α), which favors the pro-inflammatory differentiation of T lymphocytes [32]. Succinate is also described to have synergic effects with Toll-like receptor (TLR) ligands in dendritic cells for the production of cytokines.

Cardiolipin

Cardiolipin (CL) is a phospholipid that accounts for 20% of total lipid content in the IMM [33–35]. CL is composed of two phosphatidylglyceride backbones and a glycerol head

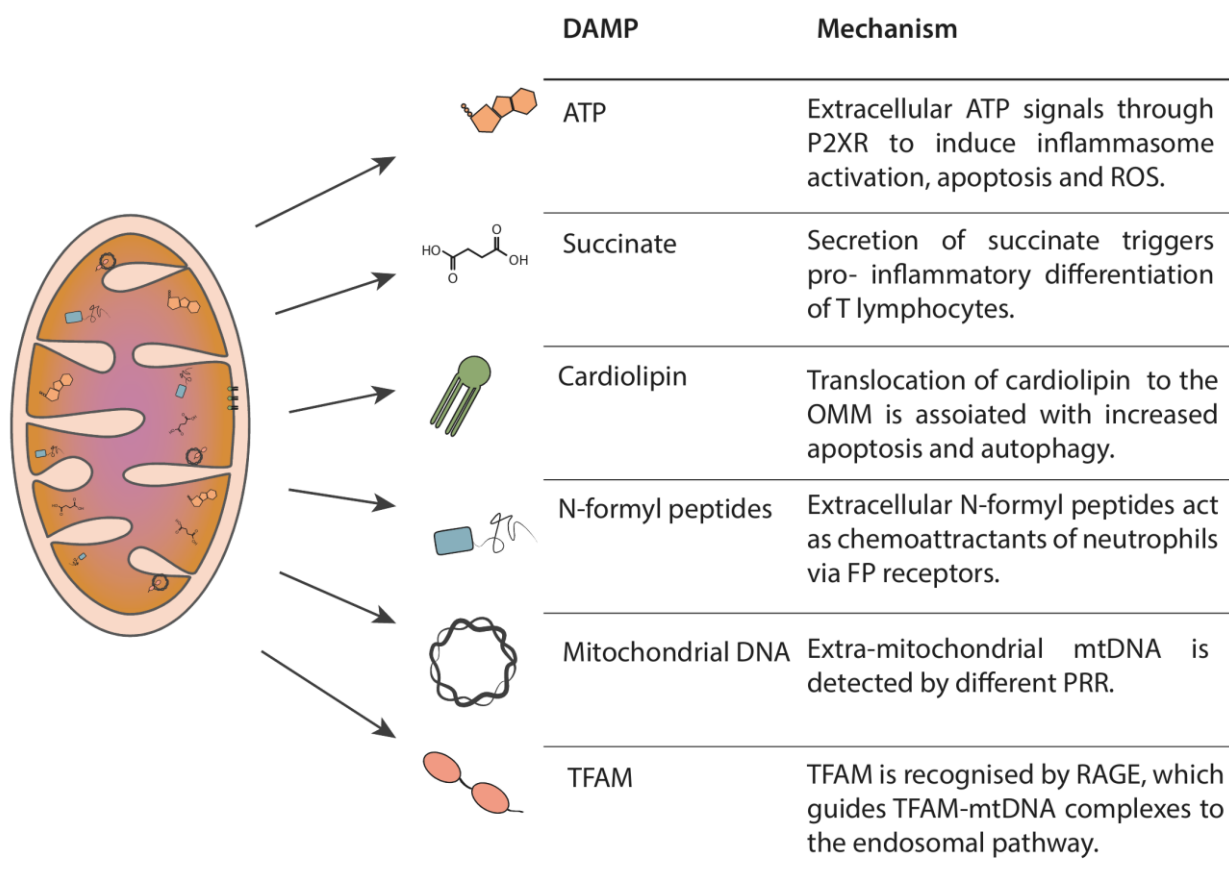


FIGURE 1: Mitochondria-derived DAMPs. Mitochondria generate immunogenic molecules, named damage associated molecular patterns (DAMPs). The scheme represents the localization of the different DAMPs in their non-immunogenic state, as well as a brief description of their immunogenic capacity when misplaced.

group. Four fatty acids chains, with different lengths and degrees of saturation, are bound to CL [36]. This phospholipid is pivotal in many mitochondrial processes, including protein import, dynamics, respiratory chain functionality, and metabolism [37, 38]. Cellular necrosis exposes CL to the extracellular media, which can be sensed by T cells through CD1d [39]. Also, CL can bind directly to NLRP3 and activate inflammasome-mediated immune response [40]. CL is increased in tracheal aspirates of human pneumonia patients, as well as in lung injury models [41]. However, like other MTDs, CL is found in both bacterial and mitochondrial membranes. Therefore, to date, it has been difficult to verify the origin of pathology-associated high levels of this phospholipid in the extracellular media.

N-formyl peptides

Bacteria use the addition of a formyl group, a carbonyl bonded to hydrogen, to methionine to initiate protein synthesis. Bacterial N-formyl peptides (NFPs) serve as chemoattractants to activate host phagocytes [42]. Polymorphonuclear and mononuclear phagocytes show high expression of formyl peptide receptors (FPRs), members of the seven families of transmembrane G protein-coupled receptors

[43]. Recognition of NFPs by FPR in the plasma membrane of the phagocyte initiates various defense responses of the immune cell, such as morphological polarization, locomotion, phagocytosis, ROS generation, and protease secretion [42]. NFPs were first described to present chemoattractant capacity for neutrophils and platelets [44, 45]. Mice unable to detect NFPs, by genetic knockout of formyl peptide receptor 1, show higher susceptibility to infection by *Listeria monocytogenes* [46]. In humans, localized juvenile periodontitis patients carry dysfunctional variant alleles of the FPR gene and present reduced neutrophil chemotaxis capacity to NFPs [47, 48]. Interestingly, NFPs are extruded not only by pathogens like *Escherichia coli* but also by the mitochondria of damaged or dying cells. Mitochondrial formylation of methionine is needed for translation initiation of mRNA transcribed from mtDNA, a mechanism reminiscent of bacteria [49]. Thus, mitochondria produce NFP due to the translation of mtDNA-encoded proteins within the mitochondrial matrix. Mitochondrial extracts of NFPs induce the chemotaxis of polymorphonuclear cells, whereas the non-formylated peptides are innocuous. Moreover, NFPs are secreted only by necrotic cells, thus excluding apoptotic cells [45]. These observations

suggest that NFPs have dual roles in tissue homeostasis. On the one hand, they promote the clearance of bacteria and infected cells. On the other hand, they enable the identification of damage cells undergoing necrosis, a process that will lead to extrusion of mitochondrial content. NFPs therefore allow the rapid clearance of these damaged cells by phagocytes.

Mitochondrial DNA

The mitochondrial genome is a double-stranded circular DNA molecule of around 16 kilobases, present in hundreds to thousands of copies per cell. It is packed with nucleoids, which are slightly elongated, irregularly shaped structures of approximately 80–100 nm. Nucleoids associate with the IMM and distribute throughout mitochondria [50]. They contain relatively high levels of TFAM (1 subunit every 16–17 bp of mtDNA), which is essential for mtDNA maintenance because it is responsible for mtDNA packaging [51–56]. There is some debate regarding the number of mtDNA copies per nucleoid. While some authors have described several copies of mtDNA per nucleoid [57], others have reported only one copy [56, 58]. The organization of mtDNA into nucleoids is essential for the correct distribution and segregation of mtDNA. The mitochondrial genome encodes for 22 tRNA, 2 rRNA and 13 essential subunits of the mitochondrial oxidative phosphorylation system: complex I (ND1–ND6), complex III (Cyt b), complex IV (COX I–COX III), and complex V (A8 and A6). It also contains a few non-coding sequences, the largest being the displacement loop (D-loop). This region of the mtDNA is also denominated the control region, because it contains the promoter for the transcription of both light and heavy strands (LSP and HSP) and the origin of replication for the heavy strand [59, 60].

Owing to the α -proteobacterial origin of mitochondria, mtDNA has unique features that are important for its role in innate immune responses and inflammation. Misplaced mtDNA has been widely shown to induce a pro-inflammatory state [4, 61–65]. Methylation of the CpG regions in mtDNA differs to that of the nuclear DNA and confers mtDNA with immunogenic potential due to its resemblance to DNA of pathogens, specially bacteria. Some authors have described the absence of methylation in mtDNA, while others report that the nuclear DNA methyltransferase (DNMT1) is found in mitochondria, thereby suggesting some degree of methylation in mammalian cells [66, 67]. In addition to hypomethylation, mtDNA can undergo oxidative damage, which is immunogenic and can be recognized by PRRs, independently of the degree of methylation [68, 69]. Extracellular mtDNA binds TLR9, which has also been described to recognize unmethylated or undermethylated CpG DNA. In macrophages, mtDNA engagement with TLR9 induces the production of pro-inflammatory cytokines, chemotaxis and phagocytic activation through a MyD88-dependent signaling cascade. TLR9 has been associated with the development of polymicrobial acute kidney injury through the recognition of mtDNA release [70].

High levels of circulating mtDNA have been linked to liver dysfunction and increased neutrophil-mediated inflammatory responses [71]. The injection of mtDNA induces lung injury and arthritis with infiltration of mononuclear cells in mice [72]. These data support the notion that mtDNA participates in the development of inflammatory responses *in vivo* [4, 73]. mtDNA also induces inflammation in microglial and neuronal cells or in mouse brains [74, 75]. Furthermore, it has been reported that mtDNA stress elicited by TFAM deficiency triggers cytosolic antiviral signaling, promoted by cytosolic mtDNA leakage [64]. Thus, the degree of packaging, the stability, the localization and the presence of oxidative damage modifications or mutations are implicated in mtDNA-innate immune signaling [12].

TFAM

Mitochondrial transcription factor A (TFAM) is member of the HMG box family of proteins. TFAM interacts with mitochondrial DNA and regulates both its transcription and replication, thus modulating mtDNA-encoded protein expression and mtDNA copy number [76, 77]. TFAM correct expression is crucial for mitochondrial function and thus cellular homeostasis [55]. The presence of extracellular TFAM is described to induce an inflammatory response, similarly to the action of another DAMP of the same family of proteins, namely HMGB1. TFAM also enhances the immunogenicity of mtDNA [78]. While bound to mtDNA, it can interact with the plasma membrane receptor RAGE and induces the internalization of mtDNA, thereby promoting its recognition by TLR9 [79]. Also, TFAM enhances cytokine secretion in combination with NFPs [80]. Treatment with TFAM increases the levels of IL6 and TNF in the serum of rats and in the media from RAW264.7 macrophage cultures [81].

NON-IMMUNE CELLS ALSO RESPOND TO MITOCHONDRIAL DAMPS

Mitochondrial DAMPs were initially reported in cells of the immune system [82]. However, we now know that somatic cells also respond to mitochondrial DAMPs to either trigger protective mechanisms or pathways to exacerbate the signal. In this regard, the release of mitochondrial DAMPs in ischemia/reperfusion during liver transplantation, together with pro-inflammatory cytokines, causes hepatic inflammation and cell death [83]. In contrast, some mitochondrial DAMPs may have a protective role. The activation of the P2X7 receptor, an ATP-gated trimeric membrane cation channel, induces plasmalemmal blebbing [84], which prevents cellular damage triggered by bacterial pore-forming toxins. On the basis of these data, it has been proposed that ATP modulates inflammation and prevents cell death upon activation of the P2X7 receptor.

Necrosis is not strictly necessary for the release of mitochondrial DAMPs. Duregatti and colleagues reported that neurons treated with presynaptic toxins release hydrogen peroxide, as well as mtDNA and cytochrome c, secondary to mitochondrial dysfunction. These DAMPs activate Schwann cells to initiate neural degeneration. The release

of mtDNA and cytochrome c results from the opening of the mitochondrial transition pore, and the maintenance of mitochondrial permeability was shown to be key to restraining mitochondrial DAMP release [85].

Cardiolipin is asymmetrically enriched in the IMM. Under stimuli determining cell or mitochondrial dysfunction such as membrane depolarization or tBid binding, CL is translocated to the OMM, a process facilitated by the phospholipid scramblase 3 (PLS3) [86]. The presence of CL in the OMM induces mitophagy [87], and apoptosis by reducing OMM permeability and thus enabling cytochrome c release. Regarding the immunogenic capacity of CL, it acts as a signaling platform in the recruitment of inflammasome particles and to induce the activation of these molecules [86, 88].

Mitochondrial DAMPs can play modulatory roles, for example, by increasing endothelial cell permeability, thus allowing the transmission of the immunogenic response to distal organs [89]. These studies indicate that mitochondrial DAMPs are important in the different levels of the immunogenic response in non-immune cells.

The regulatory role of succinate or N-formyl peptides in non-immune cells remains unclear. However, given that the expression of the succinate receptor, SUCNR1, is high in liver and kidney [90], it is likely that it mediates mitochondrial stress in these tissues. Similarly, N-formyl peptide receptors FPR1 and FPR2 show a relative broad expression so they may also play a modulatory role in various tissues [91].

MECHANISMS OF RESPONSE TO MITOCHONDRIAL DNA

Since nucleic acids are central for the replication and propagation of most pathogens, it is not surprising that their detection is covered by various kinds of PRRs localized in diverse cellular compartments. In particular, mtDNA is recognized by four innate immune receptors: cytosolic cyclic GMP-AMP synthase (cGAS), endosomal TLR9, and the two inflammasomes: Absent In Melanoma 2 (AIM2), and NOD, LRR and Pyrin domain-containing protein 3 (NLRP3) [92, 93]. Here we focus mainly on cGAS and TLR9 as mtDNA sensors since the mechanism through which mtDNA activates inflammasomes is poorly characterized.

Mitochondrial DNA and cGAS signaling

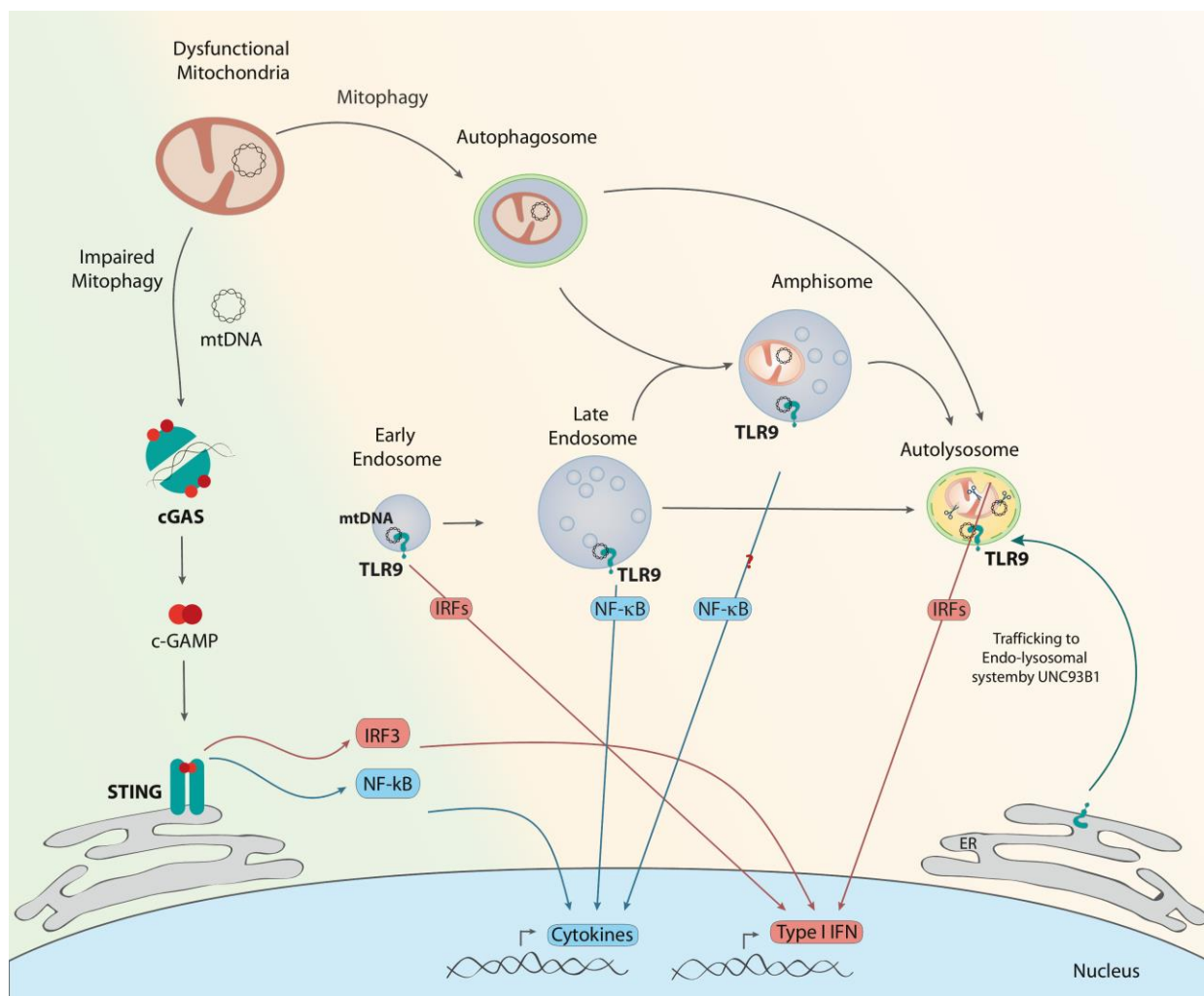
cGAS is the most recently described DNA sensor [94, 95]. Cytosolic double stranded (ds)DNA activates cGAS to form a dimeric cGAS-DNA complex that synthesizes cyclic GMP-AMP or cGAMP from ATP and GTP. This cGAMP functions as a second messenger because it is a high-affinity ligand for the endoplasmic reticulum (ER) membrane adaptor protein stimulator of interferon genes (STING) [96]. cGAMP induces conformational changes in STING, which results in the subsequent activation of the transcription factors NF- κ B and IRF3 through the kinases IKK and TBK1, respectively (Figure 2) [97–101]. Interestingly, cGAS induces autophagy independently of STING as a protective mechanism against ischemia-reperfusion injury in liver [102].

Under conditions in which DNA is bound to HMGB1 or TFAM and forms a protein-DNA ladder, cGAS signaling is promoted [103]. Specifically, mtDNA has been reported to trigger a type I interferon (IFN) response and expression of IFN-stimulated genes (ISG) in a *Tfam* heterozygous context [64]. West and colleagues observed mtDNA stress, characterized by reduced nucleoid number and increased nucleoid size. In these conditions, mtDNA was found in the cytosolic fraction in the context of mitochondrial hyperfusion. Interestingly depletion of mitochondrial fusion protein Mfn1 normalizes ISG expression. In obesity-induced insulin resistance, the release of mtDNA to the cytosol has been described as a major driver of the chronic inflammation associated with the disease, through the activation of the cGAS-STING pathway [104]. This pathway is also activated upon release of mtDNA in apoptotic conditions, although the apoptosis pathway silences the immunogenic response. McArthur and colleagues used apoptosis as a model situation of mtDNA release to the cytosol and found that the BAK/BAX macropore allows the IMM to herniate, creating a protrusion in the mitochondrial surfaces of naked IMM. The loss of membrane integrity then allowed exposure of mtDNA to the cytosol [105].

Mitochondrial DNA as a TLR9 agonist

TLR9 was the first protein of the TLR family to be described as a nucleic acid sensor (Figure 2). It is expressed mainly in immune system cells, including dendritic cells and macrophages. However, it is also found in other non-immune cells such as muscle and epithelial cells, among others [106]. It binds specifically to unmethylated CpG DNA, like the mtDNA, in the endolysosomal compartment [12, 107–109]. TLR9 signals through the myeloid differentiation primary response protein 88 (MyD88), which activates a number of kinases and transcriptional factors, namely mitogen-activated protein kinases (MAPK), nuclear factor- κ B (NF- κ B) and IRF7 to enhance pro-inflammatory and type I interferon responses, respectively. Nucleic acid-sensing TLRs are not detectable at the cell surface but instead reside within internal compartments. In particular, full-length TLR9 localizes in the ER under unstimulated conditions [110]. More recent studies have identified that, upon stimulation, full-length TLR9 traffics through the Golgi apparatus to the endolysosomal compartment, guided by the accessory protein UNC93B1, and it is then cleaved to become DNA sensing-competent [111–115]. The recruitment of TLR9 to the endolysosomal compartment is key for its function since proteolytic activation of TLR9 occurs in endosomes, and in addition, MyD88 localizes in this compartment.

There is some evidence suggesting that the specific endolysosomal compartment in which the interaction between DNA and TLR9 takes place determines the type of immune response generated [116, 117]. Thus, it has been proposed that TLR9 signaling from late endosomes leads to the activation of NF- κ B, whereas TLR9 signaling from a distinct population of endosomes brings about the recruitment of IRF and induction of type I IFN (IFN-a, IFN-b).



IN PRESS

FIGURE 2: cGAS and TLR9 activation by mtDNA. Under conditions of impaired mitophagy initiation (green background), thus accumulation of dysfunctional mitochondria, there is mtDNA leakage to the cytosol. cGAS homodimers recognize double stranded DNA as mtDNA and produce c-GAMP, which interacts with STING, triggering IRF3 or NF-κB, and in turn the expression of type I IFNs or cytokines, respectively. Under conditions in which mitophagosomal formation occurs normally but resolution is defective (orange background), mtDNA instability can lead to its recognition by TLR9 in different endosomal compartments. TLR9 is recruited from the ER to the endo-lysosomal system, guided by UNC93B1. Engagement of TLR9 in early endosomes or lysosomes results in type I IFN expression through various IRFs. Interaction of mtDNA with TLR9 in late endosomes results in NF-κB activation. Given the resemblance of amphisomes to late endosomes, TLR9 engagement in amphisomes could result in cytokine production.

This alternative compartment is hypothesized to be either early endosomes or lysosomes [118, 119]. Interestingly, TLR9 modulates energy metabolism in cardiomyocytes [120], reduces ATP, and enhances AMPK activity [121]. In all, distinct cellular responses can be elicited in different cell types through the activation of the TLR9 receptor system.

Regarding mtDNA, unmethylated CpG motifs of mtDNA trigger TLR9 signaling. Several studies have reported the relevance of circulating or extracellular mtDNA in TLR9-dependent inflammation in diseases such as rheumatoid arthritis [122], atherosclerosis [10, 123], acute liver injury [71], and *Streptococcus pneumoniae* infections [124], among others [10, 62, 125–129]. Rats subjected to vascular injury showed increased extracellular mtDNA, which led to

lung tissue damage through a TLR9-dependent pathway [130]. In pregnancy, high levels of cell death are associated with preeclampsia through a mechanism involving mtDNA and TLR9 [131]. Also, high mtDNA levels in non-alcoholic steatohepatitis patients are reported to activate TLR9 and exacerbate the inflammatory profile [129]. Moreover, high mobility group protein B1 (HMGB1), a nuclear DNA-binding protein released from necrotic cells, was found to be an essential component of DNA-containing immune complexes that stimulate cytokine production through a TLR9-MyD88 pathway involving the multivalent receptor RAGE [132]. Similarly, extracellular TFAM-bound mtDNA can induce a further stronger NF-κB activation since plasma membrane receptor RAGE interacts with TFAM and delivers mtDNA to TLR9 [133].

Mitochondrial DNA engages with TLR9 in the lysosomes of DNase II-deficient hearts, thereby suggesting that undegraded mtDNA escaping from autophagy induces TLR9 and causes inflammation in cardiomyocytes [61]. In parallel, De Leo *et al.* reported a non-inflammatory role of the TLR9-DNA interaction in the lysosome cargo response, which is required to sustain the autophagic flux [134]. In skeletal muscle, ablation of the mitochondrial fusion protein Opa1 leads to a severe mitochondrial inflammatory myopathy, which is caused by TLR9 activation through a mechanism that requires mtDNA [135]. This inflammatory process is a primary cell-autonomous response of muscle cells to Opa1 deficiency and it leads to NF- κ B activation [135]. Another less characterized response of TLR9 is the interaction with mtDNA and HMGB1 in the cytosol during hypoxia, which is involved in tumor growth [136]. In all, various mechanisms have been implicated in TLR9-associated pathologies. This is not surprising given the multiple factors involved in infection or injury [137–139].

Inflammasome

Mitochondrial DNA can also be recognized by two members of a superfamily of immunogenic receptors, namely the inflammasomes AIM2 and NLRP3. These are mainly cytosolic multiprotein oligomeric complexes that activate caspase-1, which in turn proteolytically cleaves IL-1 β and IL-18, thus generating mature active forms of the secreted cytokines [28]. The inflammasome is triggered not only by pathogen-associated molecular patterns (PAMPs) as flags of pathogen infection but also by DAMPs, which flag cellular malfunction or stress. The activation of the inflammasome involves two sequential signals. The priming signal leads to NF- κ B activation, which includes the expression of inflammasome components and inactive forms of the cytokines. This signal prepares the cell for a possible activation of the pathway in response to, for instance, pathological infection. Typically, this first signal is the engagement of a membrane PRR, for example TLR4, but it is also triggered by intracellular receptors like intraluminal TLRs, including TLR7 and TLR9. Experimentally, lipopolysaccharide (LPS) is widely used to prime cells for inflammasome activation.

The second signal is the trigger itself, which interacts with the recognition part of the complex and leads to the oligomerization and activation of the inflammasome particle. In addition to the recognition protein, which is specific for each type of inflammasome, a scaffold protein, the adaptor protein known as ASC, is common to all of them. This protein serves as a bridge from the upstream inflammasome sensor molecule to caspase 1. Inflammasome assembly and oligomerization results in the cleavage of caspase 1, which in turn causes rapid and efficient activation and secretion of large amounts of IL-1 β , which were already expressed in response to the priming signal. Inflammasomes fall into several categories. In this regard, Broz and Dixit classified them into the following groups: 1) the nucleotide-binding oligomerization domain (NOD); the leucine-rich repeat (LRR)-containing protein (NLR) or NLR inflammasomes; 2) the absent in melanoma 2 (AIM2) and pyrin inflammasomes, which are canonical in-

flammasomes; and 3) non-canonical inflammasomes [140]. NLRP3 is the most studied type of inflammasome, yet the mechanisms of activation are so diverse that there is no consensus regarding whether it has affinity for a wide range of molecules or whether there is an unknown intracellular signal that converges all the different triggers into NLRP3 activation [16, 141, 142]. Classically, the inflammasome has been addressed only in macrophages and other inflammatory cells. However, several studies have reported inflammasome activation in non-immune cells, like podocytes, hepatocytes, and cardiac and skeletal myocytes [143–149]. The involvement of mtDNA in the activation of NLRP3 was reported upon mitochondrial dysfunction, leading to mtDNA leakage to the cytosol in primed macrophages [68]. In fact, mitochondria are proposed to harbour NLRP3 and be able to regulate the activity of the inflammasome complex. On one hand, activation of mitophagy reduces inflammation by clearing mitochondrial-bound NLRP3 complexes. On the other hand, mitochondrial ROS can exacerbate inflammasome immunogenic signal [14].

CONCLUSIONS AND PERSPECTIVES

Mitochondrial DAMPs have been studied mainly in the context of the function of immune cells, and we now know that they play a key role in the activity of the innate immune system, as well as in pathologies associated with immunodeficiencies and autoimmune and autoinflammatory diseases. In this regard, the mechanism by which mitochondrial dysfunction leads to the release of the different DAMPs described and the conditions under which some molecules predominate over others remain elusive. Specific studies are required to clarify this point.

There is a fragmented understanding of the cellular mechanisms that delocalize mtDNA and generate mitochondrial DAMPs. In this context, further research is needed. The relative role of the different cytosolic sensors to mitochondrial DNA should be clarified. In short, a greater knowledge of the stability and targeting of mtDNA and its sensors will allow us to predict the response of given cells to the generation of mitochondrial DAMPs triggered by specific mitochondrial damage.

Finally, future research should seek to unravel how non-immune cells respond to mitochondrial damage caused by the release of DAMPs, and mechanisms involved in these responses. Of particular relevance is understanding those conditions in which the damage to non-immune cells leads to chronic inflammatory responses. The findings of such lines of research would contribute to shedding light on diseases that are only partially understood, as is the case of inflammatory myopathies, and would also help to define pharmacological treatments for the same.

ACKNOWLEDGMENTS

A.R-N. was the recipient of a FPI fellowship from the “Ministerio de Educación y Cultura”, Spain. This study was supported by research grants from the MINECO (SAF2016-75246R), Generalitat de Catalunya (grant 2014SGR48),

INFLAMES (PIE-14/00045) from the Instituto de Salud Carlos III, and CIBERDEM (Instituto de Salud Carlos III). A.Z. is a recipient of an ICREA “Academia” (Generalitat de Catalunya). We gratefully acknowledge institutional funding from the MINECO through the Centres of Excellence Severo Ochoa Award, and from the CERCA Programme of the Generalitat de Catalunya.

CONFLICT OF INTEREST

Authors show no conflict of interest in connection with this manuscript.

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Please cite this article as: Aida Rodríguez-Nuevo and Antonio Zorzano (2019). The sensing of mitochondrial DAMPs by non-immune cells. *Cell Stress*: *in press*.

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