

# Mitochondrial function and dysfunction in innate immunity

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The mitochondria play an important role in the activation of the innate immune system. This organelle modulates the metabolic reprogramming of the immune cell into proinflammatory or anti-inflammatory subtypes, which typically utilize very different metabolic pathways to fulfill their functions. It also acts as a signaling platform to activate immune routes in both immune and nonimmune cells, as it can generate agonists for inflammatory pathways, including toll-like receptors, inflammasomes, or the cyclic GMP–AMP synthase–stimulator of interferon genes pathway, which lead to the generation of proinflammatory cytokines and antiviral molecules such as type-I interferons. These novel functions of the mitochondria are important in the fight against pathogens, but also contribute to human disease when dysregulated. This review describes recent findings in this field and highlights the role of mitochondrial nucleic acids in the regulation of innate immune signaling pathways.

## Addresses

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## Introduction

Mitochondria are essential double-membrane-enclosed organelles that carry out a wide array of functions. In addition to ATP synthesis, mitochondria participate in heat production, calcium signaling, detoxification of reactive

oxygen species (ROS), synthesis of heme and assembly of iron–sulfur clusters [1], and the regulation of cell death by apoptosis, necrosis, necroptosis, ferroptosis, and pyroptosis [2]. Because of their endosymbiotic origin, they contain their own DNA, which is a circular molecule that contains hypomethylated stands for cytosine triphosphate deoxynucleotide (C) linked by a phosphodiester (p) bond to a guanine triphosphate deoxynucleotide (G) (CpG) islands and encodes 37 genes, including 13 messenger RNAs, 2 ribosomal RNAs, and 22 transfer RNAs [3].

Emerging functions include their role in the modulation of innate and adaptive immunity. This review focuses on the role of the organelle in innate immunity, which is dual: on one hand, they regulate metabolic reprogramming of immune cells to support their specific functions, a field called immunometabolism [4]. On the other hand, they function as signaling platforms, and mitochondrial components of prokaryotic origin act as damage-associated molecular patterns (DAMPs), recognized by pattern-recognition receptors (PRR) after loss of mitochondrial integrity in both immune and nonimmune cells. Mitochondrial ROS or nucleic acids, such as mitochondrial DNA (mtDNA) or double-stranded RNAs (dsRNAs), activate pathways that lead to the secretion of proinflammatory cytokines, a phenomenon called sterile inflammation [5].

In this article, we will review the role of mitochondria in innate immunity and will focus on the role of mitochondrial nucleic acids in the activation of the innate response.

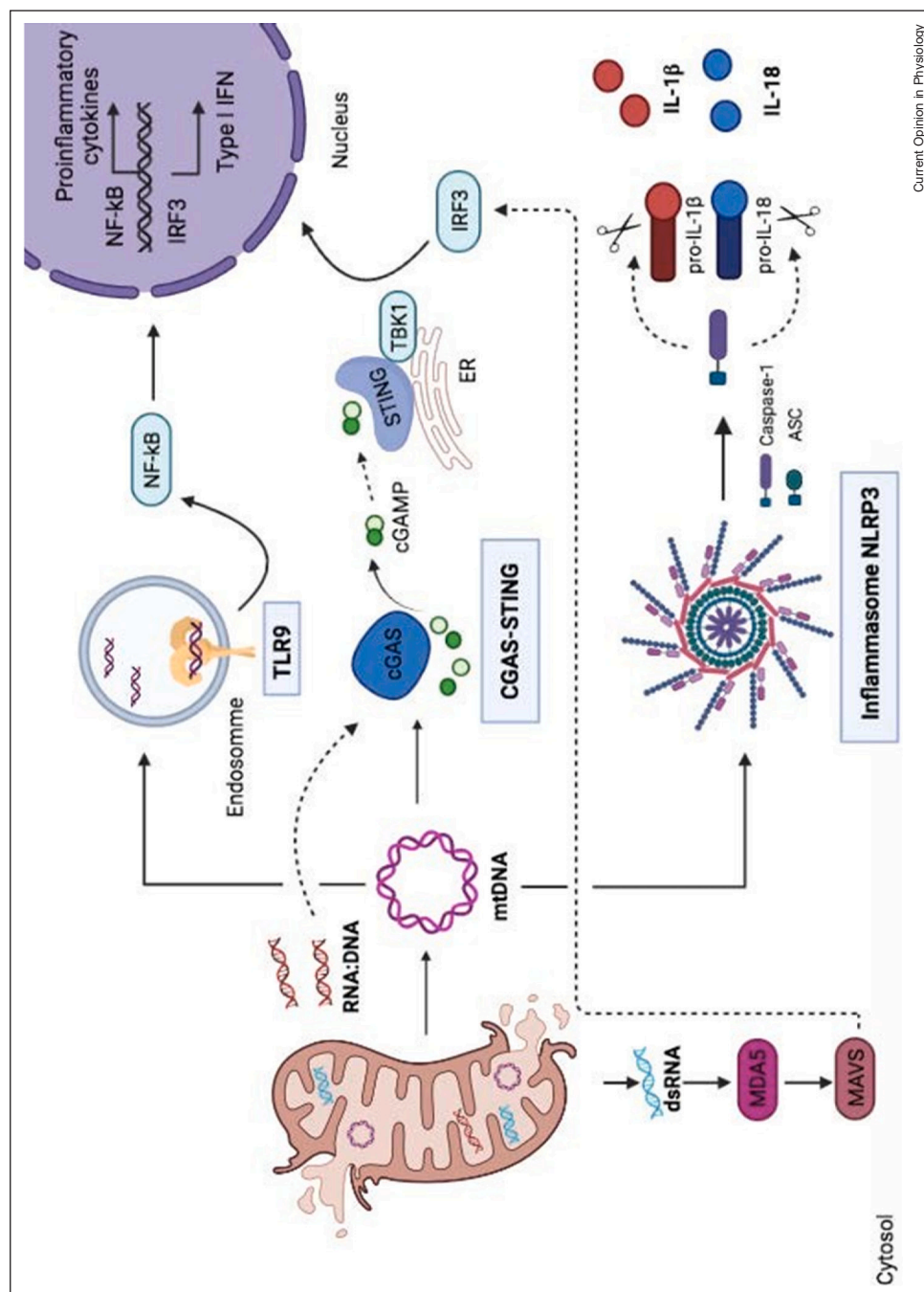
## Role of mitochondria as a source of damage-associated molecular patterns

Innate immune cells and other cell types recognize structures specific for microbes such as pathogen-associated molecular patterns (PAMPs), which bind to PRRs specifically, including retinoic acid-inducible gene-I (RIG-I)-like receptors, NOD-like receptors, or Toll-like receptors (TLR), to activate innate responses that are essential for the elimination of pathogens [6]. However, by sensing DAMPs that originate from within the cell itself, PRRs also trigger immune responses in the absence of infection.

## Mitochondrial DNA as an immunostimulator

Because of their endosymbiotic origin, mitochondria can generate potent immunostimulatory DAMPs, including mtDNA and other molecules (such as cardiolipin [7],

Figure 1



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Representation of proinflammatory signaling pathways triggered by mitochondrial DAMPs. Mitochondrial DAMPs activate several proinflammatory pathways: the release of mtDNA into the cytosol from dysfunctional mitochondria can trigger various proinflammatory signaling pathways, including TLR9, the cGAS-STING pathway, and the NLRP3 inflammasome. TLR9 recognizes mtDNA in the endosome, triggering the activation of NF- $\kappa$ B, and its transfer to the nucleus where it initiates the proinflammatory response. cGAS binds mtDNA or RNA:DNA hybrids in the cytosol and activates type-I interferon transcription. The NLRP3 inflammasome is activated by oxidized mtDNA and triggers the release of IL-1 $\beta$  and IL-18 cytokines through activation of caspase-1. Double-stranded RNA is sensed by MDA-5, which then activates MAVS and triggers the transcription of genes encoding type-I interferons. Damaged mitochondria are also capable of generating inflammatory signals in neighboring cells through exosomes, which contain mtDNA, and thus cause immune responses in other cells. Image created by BioRender.com.

ROS, or N-formyl peptides) (Figure 1). Therefore, when mitochondrial physiology is altered, dysfunctional mitochondria accumulate and produce a variety of DAMPs that engage the innate immune system [8]. Owing to its bacterial origin, mtDNA is structurally and chemically different from nuclear DNA and is recognized as foreign when outside of the mitochondrial space, where it is sensed by PRRs, including cyclic GMP–AMP synthase (cGAS), TLR9, and inflammasomes, to trigger proinflammatory cytokines and type-I interferon responses [8].

The presence of cytosolic or circulating mtDNA is indeed associated with inflammation and disease, including, diabetes, pulmonary hypertension [9], nonalcoholic steatohepatitis (NASH) [10], schizophrenia [11], COVID-19 [12], sickle cell disease [13], diabetes [14], and sepsis [15].

### Mitochondrial DNA and the activation of immune signaling

#### *Toll-like receptors*

TLRs, evolutionary conserved receptors expressed by all innate immune cells, detect a wide range of PAMPs and DAMPs: for instance, TLR4 detects lipopolysaccharide, a cell wall component of Gram-negative bacteria, TLR2 detects lipoteichoic acid, a cell wall component of Gram-positive bacteria, TLR3 recognizes double-stranded RNA, TLR7 recognizes single-stranded RNA in the endosomal system, and TLR9 detects unmethylated CpG DNA motifs (such as those present in mtDNA) in the endosomal system.

TLRs consist of a N-terminal domain that contains leukine-rich repeats and binds their ligand, a single-helix transmembrane domain, and a C-terminal domain that initiates downstream signaling. They typically function as homo- or heterodimers [16].

Activation of TLRs triggers signaling through myeloid differentiation primary response gene 88 (MyD88) and/or TIR-domain-containing adapter-inducing interferon- $\beta$  (TRIF). Signaling through MyD88 leads to activation of mitogen-activated protein kinases (MAPK) [16] and transcription factor nuclear factor kappa B (NF- $\kappa$ B), a heterodimer composed of RelA and p50, which translocates to the nucleus where it induces the expression of proinflammatory cytokines and chemokines [16], including interleukin (IL)-6, IL-1 $\beta$ , or tumor necrosis factor (TNF). Activation of NF- $\kappa$ B requires the phosphorylation and degradation of inhibitory  $\kappa$ B, which masks the nuclear localization signal of NF- $\kappa$ B. TRIF in turn signals through TANK-binding kinase 1 (TBK1) and transcription factor interferon regulatory factor 3 (IRF3) to drive a type-I interferon response.

#### *Inflammasomes*

Inflammasomes are cytosolic complexes whose function is the activation of caspase-1, a protease that in turn cleaves pro-IL-1 $\beta$  and pro-IL-18 into bioactive cytokines that amplify inflammation. Caspase-1 also cleaves and activates gasdermin D [17], a pore-forming protein involved in an immunogenic death type called pyroptosis. Thus, inflammasomes are central players in inflammation.

Multiple studies have demonstrated an important role for mitochondria in signaling mediated by the nod-like receptor protein-3 (NLRP3) inflammasome [18,19], a complex formed by NLRP3, the adapter apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and caspase-1. This inflammasome requires two steps for full activation: first, priming, which is triggered by TLR signaling and involves upregulation of NLRP3 and pro-IL-1 $\beta$ ; next, activation or assembly of the complex, which is triggered in vitro by structurally diverse and chemically unrelated compounds (such as ATP, K<sup>+</sup> ionophores, or particulate matter), none of which bind NLRP3 itself directly. Indeed, oxidized mtDNA released by dysfunctional mitochondria into the cytosol binds directly to and drives activation of the NLRP3 inflammasome [20–22].

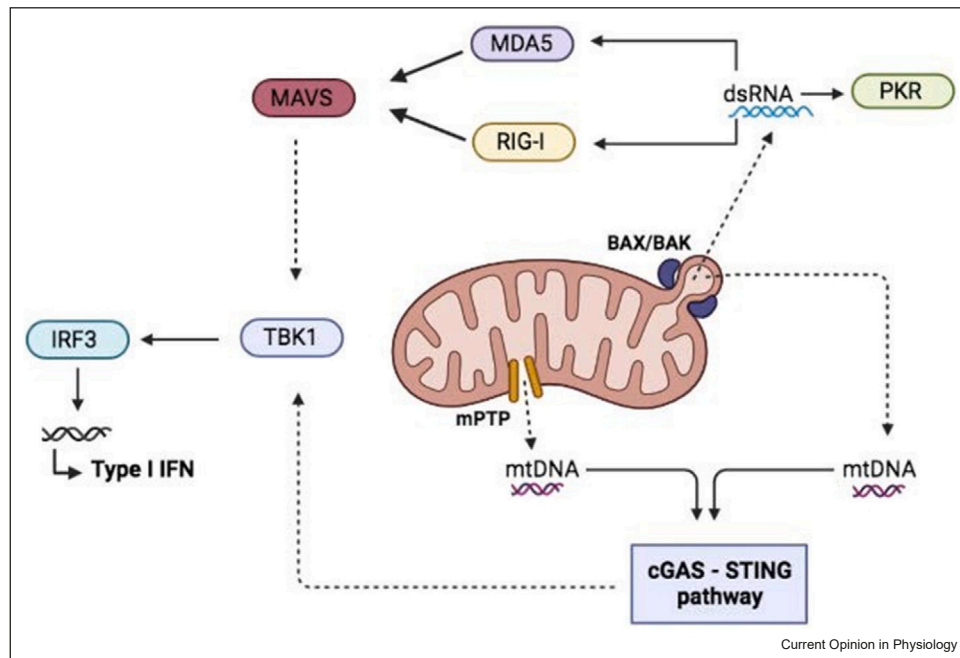
An additional inflammasome that senses double-stranded DNA (dsDNA) is absent in melanoma 2 (AIM2), a complex formed by AIM2, ASC, and caspase-1. AIM2 has been shown to bind mtDNA [20]. Circulating cell-free mtDNA contributes to AIM2 inflammasome-mediated chronic inflammation in type-2 diabetes patients [14].

#### *Cyclic GMP–AMP synthase–stimulator of interferon genes*

An important dsDNA sensor in the cytoplasm is cGAS. White et al. and Rongvaux et al. first showed that cGAS recognizes cytosolic mtDNA as a DAMP [23,24]. Then West and colleagues demonstrated that depletion of Transcription Factor A, Mitochondrial (TFAM), a histone-like mitochondrial transcription factor that controls mtDNA stability, results in the release of mtDNA into the cytosol and activation of cGAS [25].

Upon DNA binding, cGAS synthesizes the dinucleotide second messenger 2'3'-GMP-cyclic AMP (cGAMP) that in turn binds to and activates stimulator of interferon genes (STING). STING then traffics from the endoplasmic reticulum to the Golgi apparatus, where it initiates a phosphorylation cascade that includes TBK1 and IRF3, which initiates transcription of type-I interferons. Autocrine and paracrine signaling through the type-I interferon receptor results in the expression of hundreds of interferon-stimulated genes (ISG) through the Janus kinase / Signal transducer and activator of transcription proteins (JAK/STAT) signaling pathway

Figure 2



Immune signaling pathways triggered by mitochondrial nucleic acid sensing. Mitochondrial RNA (mtRNA) and mtDNA are released into the cytosol through BAX/BAK-mediated outer membrane permeabilization. mtRNA is recognized by MDA-5, RIG-I, and PKR, dsRNA sensors in the cytosol. MDA-5 and RIG-I interact with MAVS, which initiates transcription of type-I interferons. mtDNA can also be released from the mitochondria through the mPTP. Image created by BioRender.com.

[26]. Stimulation of the cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) pathway also activates NF- $\kappa$ B.

Interestingly, some pathogens such as some viruses (including dengue or influenza) activate cGAS through the release of mtDNA, which promotes an antiviral state [27,28], while other viruses such as zika block this pathway [29].

#### *Translocation of mitochondrial DNA to the cytosol*

The detailed mechanism by which mtDNA is translocated to the cytosol of the cell is still debated, although multiple routes probably exist, depending on the cell type or kind of stimuli (Figure 2).

It has been reported that the inner mitochondrial membrane can extrude into the cytosol through large mitochondrial outer membrane pores formed by the proapoptotic proteins BAX and BAK [30,31]. These membranes form vesicles that carry matrix components with them, including mtDNA. The inner mitochondrial membrane of this cytosolic vesicles might then lose its integrity to release the mtDNA to the cytosol, which leads to cGAS activation. Another form of mtDNA release involves passage of short mtDNA fragments through the outer membrane via voltage-dependent

anion channels (VDAC) [32]. The mechanism by which the inner membrane is permeabilized in this case is unknown, but might involve the mitochondrial permeability transition pore (mPTP), an unspecific inner mitochondrial membrane channel of elusive molecular composition [33].

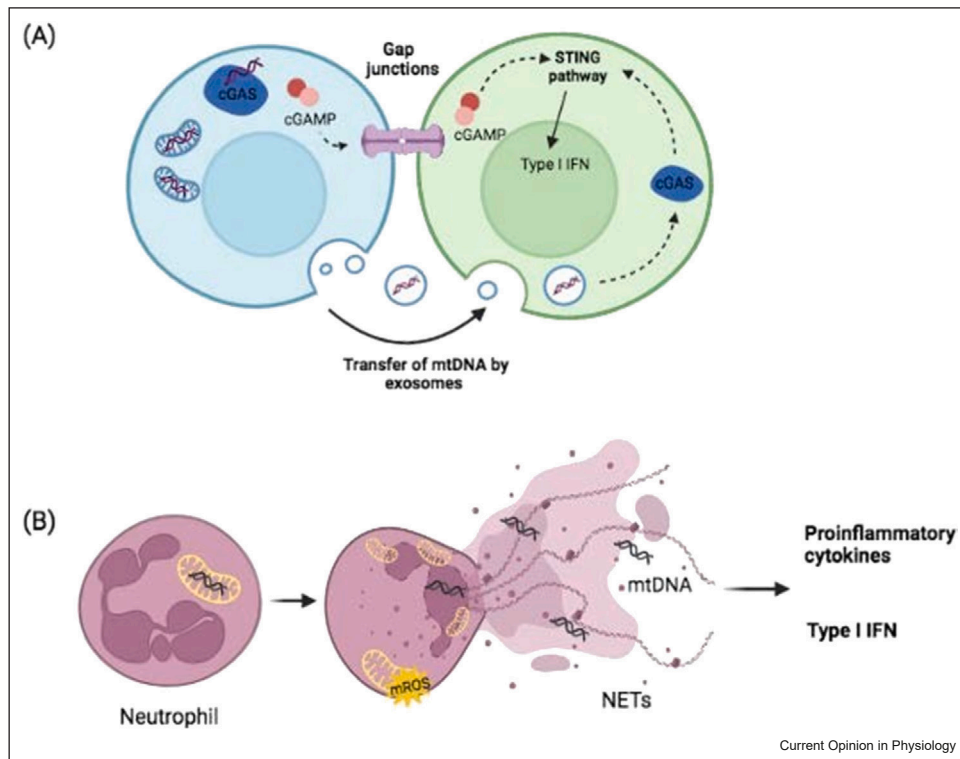
Indeed, some studies suggest that the mPTP might be involved in the permeabilization of the inner mitochondrial membrane. When this  $\text{Ca}^{2+}$ -regulated channel opens, there is an increase in the permeability to solutes with molecular masses up to 1.5 kDa, which leads to mitochondrial depolarization and swelling. Translocation of mtDNA and activation of the NLRP3 inflammasome seem to be inhibited by cyclosporin A (CsA), an inhibitor of the mPTP [18]. However, depletion of Cyclophilin D, a known regulator of the mPTP (and the molecular target of CsA) does not inhibit the NLRP3 inflammasome [34], which suggests that CsA inhibits NLRP3 activation by an mPTP-independent mechanism.

#### **Cell-to-cell signaling by mitochondrial DNA**

mtDNA extrusion affects not only the cell where it occurs, as mtDNA can be transferred from one cell to another (Figure 3), thus propagating the inflammatory signal [35].



Figure 3



Cell-to-cell signaling by mtDNA. **(a)** The extrusion of mtDNA to the cytosol can trigger inflammatory signaling in neighboring cells. Upon binding mtDNA, cGAS produces cGAMP, a second messenger that can be transferred to other cells via gap junctions, where it activates STING. mtDNA by itself can also be passed between cells by exosomes and, once in the cytosol of the target cells, be recognized by cGAS, leading to activation of STING. **(b)** Upon stimulation, neutrophils undergo NETosis. They produce NETs containing, among other molecules, DNA, proteins, and oxidized mtDNA. The presence of mtDNA elicits the secretion of proinflammatory cytokines and type-I interferons (IFNs) in the target cell. Image created by BioRender.com.

#### Transfer of mitochondrial DNA by exosomes

During immune responses, intercellular unions between cells called immune synapsis, whereby cells secrete a wide variety of active biomolecules, take place [36]. One of the mechanisms by which this transfer is carried out is the secretion of extracellular vesicles (EV), including exosomes [36]. They are of interest because they are enriched with bioactive molecules and genetic material, such as genomic DNA and mtDNA. It has been shown that mtDNA in EVs is able to activate type-I interferon signaling through the cGAS–STING pathway in target cells [36].

Horizontal transfer of mtDNA via extracellular vesicles is not limited to immune cells, and has been demonstrated in many cell types, including cancer cells [37,38].

#### Signaling through gap junctions

Interestingly, cGAMP can also be passed to other cells via gap junctions [39]. Direct transfer of this second messenger is advantageous compared with paracrine

interferon signaling, as it is faster and is independent of transcription and translation.

In the brain, breast or lung cancer cells transfer cGAMP to astrocytes via gap junctions, where it activates STING, secretion of type-I interferons, and proinflammatory cytokines, which in turn act as paracrine signals in the cancer cells to support brain metastasis [40].

#### Signaling through neutrophil extracellular traps

Upon stimulation by pathogens or in vitro by several compounds, neutrophils release extracellular net structures formed by DNA, proteins, and other molecules, a cell death process called NETosis. Formation of these neutrophil extracellular traps (NETs) requires ROS and successive work showed that upon stimulation, mitochondria are mobilized to the cell surface where they release oxidized mtDNA, and thus NETs are also enriched in mtDNA in systemic lupus erythematosus (SLE) and other conditions [41]. Released oxidized mtDNA is proinflammatory and induces secretion of

type-I interferons and other cytokines in a STING-dependent manner [41]. Interestingly, mitochondrial ROS inhibition using scavengers in vivo reduces disease severity and type-I IFN responses in a mouse model of lupus [41].

This process is also important in other diseases such as cancer. Neutrophils in hepatocellular carcinoma patients display high levels of mitochondrial ROS and form NETs that are enriched in oxidized mtDNA. These NETs stimulate an inflammatory response in tumor cells and promote metastasis [42].

Remarkably, extracellular traps that contain DNA are not limited to neutrophils, as other immune cells such as macrophages are also able to generate them in a ROS-dependent manner [43,44].

### Other mitochondrial nucleic acids in immune signaling

#### *Mitochondrial dsRNA*

The entire mitochondrial genome is transcribed as two genome-sized polycistronic transcripts, one on each strand. Mitochondrial dsRNA generated by this bidirectional mtDNA transcription triggers antiviral signaling mediated by melanoma differentiation-associated gene 5 (MDA-5), a sensor of long dsRNAs, mitochondrial antiviral signaling protein (MAVS), an outer mitochondrial membrane adapter protein, and BAX/BAK-dependent mitochondrial outer membrane permeabilization [45]. MDA-5 and MAVS interact via caspase activation and recruitment domains (CARDs) present on both proteins. After their interaction, MAVS forms aggregates that recruit TBK1 and IRF3, which initiates transcription of type-I interferons.

Interestingly, cells from patients carrying mutations in the gene that encodes polynucleotide phosphorylase, an enzyme of the mitochondrial RNA degradosome that prevents formation and release of dsRNA, exhibit greater accumulation of dsRNA and elevated expression of ISG [45].

Mitochondrial dsRNAs can also be detected by protein kinase RNA-activated (PKR), which is also called eukaryotic translation-initiation factor 2- $\alpha$  kinase 2, an ISG that inhibits protein translation [46].

Strikingly, double-strand breaks in mtDNA also trigger a type-I interferon response that requires RIG-I, a sensor of short dsRNAs, MAVS, and BAX/BAK-dependent mitochondrial outer membrane permeabilization [47]. This has led to suggest that these breaks in mtDNA lead to the generation of short single-stranded mitochondrial RNAs by the mitochondrial RNA polymerase (when it runs off the mtDNA at the breakpoint). These molecules may in turn form short dsRNAs by folding, and

then bind to RIG-I and activate interferon signaling [48]. RIG-I and MAVS also interact via CARDs. RIG-I is not activated by endogenous mRNAs because they are capped at the 5' triphosphate end [49,50].

#### *RNA:DNA hybrids*

RNA:DNA hybrids, which can occur during replication of mtDNA, can activate secretion of type-I interferons via the cGAS-STING pathway [51]. Binding of hybrids to cGAS induces synthesis of cGAMP, albeit at lower amounts than dsDNA.

### Role of reactive oxygen species and oxidized mitochondrial DNA in innate immunity

ROS directly damage and kill pathogens but also have signaling functions in innate immunity and are important modulators of cytokine secretion. Mitochondrial ROS promote proinflammatory signaling by stimulating the NF- $\kappa$ B pathway and the MAPK extracellular signal-regulated kinase [52]. The mechanism involves ROS-mediated formation of a disulfide bond between molecules of NF- $\kappa$ B essential modulator (NEMO), which is part of a complex that activates NF- $\kappa$ B. Indeed, ROS scavengers, such as N-acetyl-L-cysteine, decrease secretion of proinflammatory cytokines after TLR activation [53], and increased mitochondrial ROS levels in cells from TNF receptor-associated periodic syndrome patients lead to exacerbated release of proinflammatory cytokines.

Interestingly, the role of ROS in the activation of the NLRP3 inflammasome is still somewhat controversial. While it has been reported that mitochondrial ROS are essential for the activation of the inflammasome (possibly by leading to mtDNA oxidation and translocation to the cytosol) [54,55], some groups have reported that ROS are not essential at all [46] or that they only control priming (which requires NF- $\kappa$ B signaling) but not activation of the NLRP3 inflammasome [47]. We and others found that the requirement for mitochondrial damage and/or ROS during NLRP3 activation depends on the strength and duration of the stimulus [56,57], which might help reconcile these contradictory observations. ROS also stimulate the antiviral signaling pathways, as it triggers aggregation of MAVS (see below).

An interesting point is whether oxidized mtDNA is more immunogenic than nonoxidized mtDNA. Indeed, it has been shown that NLRP3 binds preferentially oxidized mtDNA, while NLRC4 binds regular mtDNA [20]. Also, mitochondrial ROS promote mtDNA fragmentation [58], which might increase their release via small channels such as the mPTP or VDAC [32]. Oxidized mtDNA also seems to be a more potent activator of TLR9 and to be more interferogenic than regular mtDNA, although the mechanisms are poorly understood [59].

### Quality-control programs in the mitochondria

Quality-control programs regulate mitochondrial fitness and thus can affect the release of mtDNA (and other DAMPs) to the cytosol. These processes include mitochondrial biogenesis, sirtuin-mediated protein deacetylation [56,60], mitochondrial dynamics, mitophagy, and protein degradation [5], and they interplay with each other. We will only describe mitochondrial dynamics and mitophagy in this review.

#### *Mitochondrial dynamics*

The mitochondrial network is highly dynamic and can undergo fusion and fission events, dynamic morphological changes responsible for the maintenance of mitochondrial homeostasis in physiological conditions and in response to stress and infections [61]. Unbalanced dynamics lead to abnormal immune responses and pathological conditions. Recent roles of mitochondrial dynamics in innate immunity are being described [62].

Mitofusins are outer membrane proteins responsible for the coordination of mitochondrial fusion. Mitofusin 2 (MFN2) interacts with MAVS and inhibits MAVS-mediated IFN- $\beta$  signaling, and thus blocks signaling through RIG-I and MDA-5, the dsRNA sensing pathways [63]. As a result, MFN2 deficiency potentiates antiviral responses. Some viruses, such as the enterovirus D68, upregulate the expression of MFN2, which leads to decrease IFN- $\beta$  expression to promote viral replication [64]. Interestingly, cells devoid of both mitofusins display fragmented mitochondria and are defective in the antiviral response to RNA viruses [65], and MFN1 positively regulates antiviral immunity to RNA viruses [66], highlighting the differences between both MFN isoforms. These results also suggest that modulation of antiviral immunity by MFN2 is independent of its role in mitochondrial dynamics. Indeed, the interaction of MFN2 with MAVS has been shown both to prevent its oligomerization [67] and to promote its polyubiquitination and degradation [68].

The dynamin-related protein 1 (Drp1) is the main protein involved in mitochondrial fission. Nitric oxide release as a response to viral infection seems to activate Drp1 and induce mitochondrial fission, leading to an inhibition of the mitochondrial antiviral signaling and thus lowering the innate immune response. This seems to constitute an inhibitory loop for the innate immune response regulation [69]. Accordingly, knockdown of Drp1 or that of another fission factor, mitochondrial fission-1 protein, promotes mitochondrial fusion and enhances the antiviral pathway [70].

#### *Mitophagy*

Mitophagy is a type of autophagy that removes damaged or depolarized mitochondria. The most studied mitophagy pathway depends on phosphatase and tensin

homologue (PTEN)-induced putative kinase 1 (Pink1), a mitochondrial membrane potential-dependent kinase, and the E3 ubiquitin ligase, Parkin [71]. Defective removal of dysfunctional mitochondria leads to hyperactivation of inflammation.

Since mitophagy eliminates dysfunctional mitochondria and prevents release of mtDNA to the cytosol, its inhibition promotes NLRP3 inflammasome activation [18,19]. Mitophagy inhibition, by absence of Parkin or Pink1 or by absence of immunity-related GTPase family M protein 1, also stimulates the cGAS–STING pathway [72,73]. Indeed, some viruses promote mitophagy to decrease the production of type-I interferons and inhibit the antiviral response [74,75]. Interestingly, some recent reports have shown that, in some cellular models, mitophagy inhibition suppresses rather than augments type-I interferon signaling [73,76]. This apparent contradiction is explained by the fact that in some cell types, defective mitochondrial nucleic acids are sensed by endosomal TLR pathways instead of by cGAS–STING [73].

### Role of mitochondria in immune disease and therapies

In this section, we briefly describe some recent reports of innate immune disease associated with mitochondrial dysfunction, such as the type-I interferonopathies rheumatoid arthritis (RA), Perrault syndrome, SLE, and amyotrophic lateral sclerosis (ALS), which are caused by defective DNA clearance [77].

Chronic TNF stimulation induces mitochondrial dysfunction due to a block in mitophagy and this leads to mtDNA extrusion and activation of the cGAS–STING pathway in a mouse model of RA [78]. Inhibition of cGAS ameliorates the symptoms and could potentially be translatable to patients.

Perrault syndrome, an autosomal-recessive condition characterized by sensorineural hearing loss and ovarian failure, is caused by recessive mutations in ATP-dependent Clp protease proteolytic subunit (ClpP), a serine protease that degrades misfolded and damaged proteins. ClpP deficiency in mouse triggers altered mtDNA homeostasis, which leads to cGAS-dependent interferon responses and increased resistance to RNA and DNA viruses [79].

Lupus is a disease that displays type-I interferon dysregulations. BIT-4, a novel and potent inhibitor of the oligomerization of VDAC, decreases mtDNA release, interferon- $\beta$  release, NET formation, and disease severity in a mouse model of SLE [32]. Nicotinamide riboside, a precursor of the coenzyme NAD<sup>+</sup> that alters the metabolome of the cell, also decreases interferon- $\beta$  release in monocytes from SLE patients [76]. Oxidative

stress in peripheral blood mononuclear cells from SLE patients can cause MAV oligomerization in the absence of dsRNA or RNA viruses, which leads to spontaneous secretion of type-I interferons [80]. Interestingly, aggregated MAVs can be released and act in a prion-like manner to further amplify the production of proinflammatory cytokines. A mitochondria-targeted antioxidant such as mitoQ prevents MAVs aggregation, suggesting a therapeutic potential for SLE.

Some cases of ALS are caused by mutations in TDP-43, a nuclear DNA/RNA-binding protein. ALS-associated mutations trigger its localization in the mitochondria, where it enhances mtDNA release via mPTP [81]. Blockade of STING prevents inflammation in an ALS mouse model of TDP-43 overexpression.

In addition to autoimmune diseases, mtDNA is involved in other diseases that have an inflammatory component. For example, in chronic liver disorders such as NASH, mtDNA seems to activate both TLR9 and STING, and their ablation is protective [10,82]. TLR9 activation is also involved in pulmonary hypertension [9]. Mitochondrial dysfunction and activation of cGAS-STING has been observed in acute kidney injury mouse models and patients [83]. Finally, mtDNA is also involved in cardiovascular diseases: in mouse, mtDNA activates TLR9 in the heart after transverse aortic constriction, which promotes heart failure [84], and this is prevented by a TLR9 inhibitor [85]; ablation of TLR9 also protects from ischemia/reperfusion injury in the heart [86].

Preventing inflammation during these acute or chronic conditions may be advantageous to treat the disease.

## Conclusions and perspectives

The mitochondria are master regulators of metabolism and emerge now as new therapeutic targets in immune diseases. They play a central role in immune cell differentiation and function and in immune and non-immune cell signaling. Strategies to increase mitochondrial fitness will be instrumental in treating autoimmune diseases and degenerative diseases (including cancer, neurodegeneration, and diabetes) that have a low-grade chronic inflammatory component and display aberrant mtDNA release. Indeed, circulating mtDNA is a hallmark of many of these diseases.

The studies shown here leave open questions that need to be addressed. For example, the mechanism of release of mitochondrial nucleic acids to the cytosol is not completely understood. We do not understand why under some circumstances mtDNA uses Bax/Bak channels to cross the outer membrane, while in others, it travels through VDAC channels. It would be interesting to address whether the oxidation status of

mtDNA (which is probably a key factor in determining its length) controls which route it preferentially takes. Intact mtDNA does not fit through VDAC channels or through the mPTP, while oxidized and fragmented mtDNA probably does. Although there is a large body of evidence that shows that mtDNA is involved in the NLRP3 inflammasome activation (including inhibition of the inflammasome in cells devoid of mtDNA [18]), some reports question this hypothesis. It is possible that different experimental conditions are responsible for these discrepancies, but nevertheless, this shows that more work is required to clarify the matter.

Another topic that requires further study is the pathway by which mtDNA signals in different cell types. The cGAS-STING pathway appears to be expressed in most cell types, but TLR9 and the NLRP3 inflammasome seem to be restricted to innate immune cells only. These pathways also show interplay, as, for example, inflammasome activation triggers cleavage of cGAS and dampening of the antiviral pathway [87], while dsRNA sensing may activate NLRP3 inflammasome via MAVS-dependent and -independent routes [88,89].

Oxidized mtDNA appears to be more immunogenic than regular mtDNA. Does the level of oxidation of mtDNA increase binding to its receptors? Or does it change the likelihood of it being released or its binding partners. These are important questions that need to be addressed.

Clearly, more work is required to translate this emerging knowledge into the clinic to benefit patients.

## Conflict of interest

The authors have no conflicts of interest to disclose

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