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Mitochondria: Sensors and mediators of innate immune receptor signaling

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Abstract

By integrating stress signals with inputs from other cellular organelles, eukaryotic mitochondria are dynamic sensing systems that can confer substantial impact on innate immune signaling in both health and disease. This review highlights recently discovered elements of innate immune receptor signaling (TLR, RLR, NLR, CLR) associated with mitochondrial function and discusses the role of mitochondria in the initiation and/or manifestation of inflammatory diseases and disorders. We also highlight the role of mitochondria as therapeutic targets for inflammatory disease.

Introduction

Mitochondria are maternally inherited double membrane organelles that possess their own genome, transcriptome and proteome [1]. Mitochondria form a dynamic interconnected intracellular network, moving through the use of cytoskeletal motors and changing size and shape via processes such as fission and fusion. Mitochondrial fusion and fission facilitates mitochondrial DNA (mtDNA) protection, alteration of cellular energetics, and regulation of cell division [2]. Damaged or defective mitochondria are removed by selective encapsulation into double membraned autophagosomes and delivered to the lysosome for degradation by a process called mitophagy. Each mitochondrion has the ability to carry out oxidative phosphorylation (OXPHOS) using its electron transport chain (ETC), where the metabolic products generated from the Krebs cycle drive the generation of a proton gradient at the inner mitochondrial membrane (IMM), providing the energy needed for ATP generation. As well as being the main intracellular producers of energy (heat and ATP), mitochondria are sensors of oxygen, calcium and fuel (carbohydrates, fatty acids), manufacturers of metabolites and reactive oxygen species (ROS) and are effective inducers of cell death (apoptosis) [1]. Importantly for the purpose of this review, mitochondria can also sense danger signals and induce inflammation by activating and controlling the innate immune system.

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Mitochondria and the innate immune response

The innate immune response relies on pattern recognition receptors (PRRs) for the detection of infectious agents, cellular stresses or tissue damage. PRRs are a series of germline-encoded receptors that recognize conserved sets of molecular targets called pathogen-associated molecular patterns (PAMPs) and include retinoic acid inducible gene (RIG-1)-like receptors (RLRs), C-type lectin receptors (CLRs), Toll-like receptors (TLRs) and nuclear oligomerization domain (NOD)-like receptors (NLRs) [3]. PRRs allow for the rapid induction of inflammatory responses mediated by various cytokines and chemokines facilitating the eradication of pathogens. PRRs can also recognize damage-associated molecular patterns (DAMPs) that arise from endogenous molecules secreted or released from intracellular or extracellular sources as a result of tissue injury[1] (Fig.1 and Table 1). Emerging literature on the role of mitochondria in RLR, NLR and TLR signaling is discussed below. To date, little or no information exists on the role of mitochondria in CLR signaling pathways.

Mitochondria and RIG-1-like receptors

Cytoplasmic double stranded viral RNA is primarily detected by the RLRs, RIG-I and melanoma differentiation-associated gene 5 (MDA-5). These receptors activate nuclear factor B (NF- κ B) and interferon regulatory factor 3 (IRF-3), resulting in the production of type I interferons (IFNs) and other proinflammatory cytokines that promote adaptive antiviral immunity [4] (Fig.1). Mitochondria provide an integral platform (termed the mitoxosome) [5]for RLR signaling and are involved in the pathogenesis of numerous RLR-related inflammatory diseases (Table.1). The mitoxosome is composed of the nuclear encoded outer mitochondrial membrane (OMM) protein, termed mitochondrial antiviral signaling protein (MAVS) or IFN β promoter stimulator 1 (IPS1), CARD adaptor inducing IFN β (CARDIF) or virus-induced signaling adaptor (VISA). MAVS interacts with RIG-I resulting in the induction of antiviral and inflammatory responses mediated by the interaction with the tumor necrosis factor receptor-associated factor (TRAF) family (Fig.2). MAVS, which is expressed ubiquitously in various cell types, also coordinates apoptotic and metabolic functions by associating with peroxisomes, endoplasmic reticulum (ER) and autophagosomes[6].

RLR signaling relies on intact healthy mitochondria and functional elongated mitochondrial network. Cells with a dissipated mitochondrial membrane potential (ψ m) are deficient in MAVS-mediated antiviral signaling [6] and mimicking mitochondrial elongation favors the binding of MAVS to stimulator of interferon genes (STING), an ER protein involved in the RLR pathway [7]. In mammals, mitochondrial fusion is controlled by mitofusins 1/2 (MFN1, MFN2) and optic atrophy 1 (OPA1), whereas fission is controlled by dynamin-related protein 1 (DRP1) and fission 1 (FIS-1) [4]. Mouse embryonic fibroblasts (MEFs) deficient in MFN1 or and MFN2 display impaired induction of type I IFNs and other proinflammatory cytokines in response to viral infection [4]. Knockdown of MFN1 or OPA1 decreases virus-induced activation of the transcription factors, NF- κ B and IRF-3, whereas knockdown of DRP1 or FIS-1 increases RLR signaling [8]. Interestingly, MFN2 can also act as an inhibitor of antiviral signaling, a function that may be distinct from its role in

mitochondrial dynamics [4]. Recently, another mitochondrial fusion/fission protein, mitochondrial E3 ubiquitin protein ligase 1 (MUL1), has been shown to directly interact with MAVS and modulate RLR responses [9] (Fig.2). Taken together, these data demonstrate that impaired fission or fusion leading to mitochondrial fragmentation dampens RLR signaling.

The process of removing fragmented mitochondria by mitophagy is also a regulator of RLR signaling. The mitophagy related genes ATG5 and ATG12 inhibit RLR signaling [10] and the mitophagy regulator, SMAD specific E3 ubiquitin protein ligase 1 (SMURF1), also a mediator of viral autophagy [11], is involved in the inhibition of MAVS [12] (Fig.2). Additional mitochondrial proteins have also been implicated in modulating RLR signaling pathways, including uncoupling protein 2 (UCP2) (Fig.3) [13], immunity-related GTPase family M (IRGM)[14], matrix metalloproteinase-2 (MMP-2) [15], translocase of the mitochondrial outer membrane 70 (TOM70) [16], Nod like receptor X1 (NLRX1) [17] and receptor for globular head domain of complement component (C1q gC1qR) [18].

Mitochondria and Toll-like receptors

TLR family members operate on the plasma membrane (TLR1/2/4/5/6/1) or on the endosome/ER (TLR3/7/8/9) to initiate the innate immune response to PAMPs from bacteria, fungi, parasites, and viruses. All TLRs are type I transmembrane receptors that recruit various adaptor molecules to activate downstream pro-inflammatory cytokines including interleukin (IL)-1 β (IL-1 β), IL-6 and tumor necrosis factor α (TNF α) [3] (Fig.1). Recognition of PAMPs by TLRs (TLR3/7/8/9 and probably TLR4) takes place inside endosomes and lysosomes, with the ER controlling the transport of TLRs to their appropriate locations.

Mitochondrial regulator of fission and morphology protein, MARCH5 is an essential and positive modulator of TLR7 signaling [19] and the mitochondrial protein, ECSIT (evolutionarily conserved signaling intermediate in Toll pathways), a complex I ETC subunit, binds to the TLR signaling protein, TRAF6 leading to enhanced TLR signaling [19]. Recently, ECSIT has also been shown to interact with tripartite motif (TRIM) 59, a protein required for the TLR-mediated transduction pathway [20]. TRIM59 negatively regulates TLR signaling [20] (Fig.2).

With the emergence of metabolic profiling and a better understanding of complex metabolic process, a considerable amount of literature is now emerging for the role of mitochondrial metabolism in the control of PRR signaling, in particular, TLR signaling. Mitochondria are at the hub of cellular metabolism regulating the aerobic oxidation of fatty acids (FAs) and consuming the end products of glucose, glutamine and amino acid degradation to aerobically produce ATP from oxygen and water. TLR4 stimulation of macrophages causes significant remodeling of the subcellular lipidome [21] and mitochondrial-derived lipid biomarkers drive obesity-associated inflammation [22]. Obese and Type 2 Diabetes (T2D) patients have high TLR4 expression saturated free FAs (FFAs) stimulate surplus amounts of proinflammatory cytokines in adipose tissue (via TLR4) [23]. FFAs also induce β cell dysfunction in vivo by activating the TLR4/MyD88 pathway within islets, subsequently

leading to the recruitment of proinflammatory monocytes/macrophages [24]. Initially, saturated FAs (such as palmitate) were thought to act as direct ligands for TLR4 [25], however, new evidence shows that direct interactions between saturated FAs and TLR4 are unlikely [26,27] and that other molecules like the glycoprotein, Fetuin-A (FetA), act as adaptors between FFAs and TLR4 [28] (Fig.1).

Mitochondrial regulated glutamine, the most abundant amino acid in the human body has been shown to protect mitochondrial structure and function in oxygen toxicity and is anti-inflammatory in a number of human and murine models (Table.1) [29–35]. Glutamine modulates the expression of TLR (independently of MyD88) by regulating the expression of TRIF (TIR-domain-containing adapter-inducing interferon- β)[36] (Fig.2).

TLR signaling may also be able to reciprocally control mitochondrial dynamics. LPS stimulation reduces the expression of a number of mitochondrial enzymes involved in the Krebs cycle, inducing a metabolic ‘switch’ or transition from OXPHOS to aerobic glycolysis (Warburg effect) [37]. Citrate release from the mitochondrion into the cytosol, upon LPS stimulation produces acetyl-CoA to generate arachidonic acid for prostaglandin production [38] and LPS challenge increases the MyD88 and TRIF-dependent expression of the mitochondrial transcription factor A (T-FAM) and cytochrome c oxidase (COX) subunits I and IV [39]. LPS strongly increases the mitochondrial Krebs cycle intermediate, succinate [40]. In this study, Tannahill *et al* propose that succinate acts as an endogenous danger signal to stabilize hypoxia-inducible factor 1 α (HIF-1 α), which in turn regulates gene expression of IL-1 β and other HIF-1 α -dependent genes.

TLR3 induces mitochondrial dysfunction eventually leading to apoptosis [41] and sterile α - and heat armadillo-motif-containing protein (SARM), the most conserved TLR adaptor, localizes to the mitochondria and triggers intrinsic apoptosis by generating ROS and depolarizing ψ_m [42] (Fig.2). TLR2/4 signaling regulates mitochondrial biogenesis through the transcription factors NF- κ B, cAMP response element-binding protein (CREB), nuclear factor erythroid 2-related factor 2 (NRF2), and interferon response factors (IRF-3, IRF-7) [43]. The serine/threonine kinase AMP-activated (AMPK) promotes mitochondrial biogenesis and opposes inflammation by interfering with NF- κ B-dependent cytokine expression [43]. Sirtuin 1 (SIRT1) a regulator of mitochondrial energy homeostasis is anti-inflammatory and nuclear receptor interacting protein-1 (RIP140), which suppresses mitochondrial biogenesis co-activates certain cytokine genes [43]. Not surprisingly, mitochondria are now thought to play an important role(s) in many TLR associated diseases/disorders (Table.1). Finally, mitochondria may provide a direct link for the interplay of TLR and NLR signaling via the mitochondrial protein SMAC-DIABLO [44].

Mitochondria and (NOD)-like receptors

Cytosolic NLR family (NLRP1, NLRP3, and NLRC4) members act as fundamental components of the multiprotein inflammasome complex [45] that assemble in response to a number of physically and chemically diverse triggers. This in turn promotes the activation of caspase-1 leading to the maturation and secretion of IL-1 β , IL-18, and IL-33 (Fig.1). The best-characterized inflammasome is that consisting of NLRP3, apoptosis-associated speck-

like protein containing a CARD (ASC) and caspase-1. Previously, several models for NLRP3 inflammasome activation, such as potassium efflux and lysosomal destabilization were proposed. Emerging data now suggests that NLRP3 activation is dependent on the efficient localization of NLRP3 to mitochondria [46–47]. and on a deubiquitination mechanism initiated by TLR signaling and mitochondrial ROS (mROS) [48].

As in the case of RLR signaling, NLRP3 function appears to rely on a viable mitochondrial network for its activation. Under resting conditions NLRP3 protein is associated with the ER, but upon activation translocates to mitochondria and mitochondria-associated ER membranes (MAMs) [49]. Acetylated α -tubulin mediated dynein-dependent transport of mitochondria is essential for interaction of NLRP3 on the ER with ASC on mitochondria [47]. In addition, the RLR signaling protein, MAVS mediates the recruitment of NLRP3 to mitochondria, promoting production of IL-1 β [46]. NLRP3 inflammasome activation is impaired in mitochondrial-DNA depleted macrophages, OXPHOS-deficient cells, cells treated with mitochondrial antioxidants and in macrophages where the voltage dependent anion channel (VDAC), a major mitochondrial channel is removed [49]. NLRP3 also interacts with the mitochondrial protein, thioredoxin-interacting protein (TXNIP) [50]. Preventing mitochondrial removal by mitophagy through the impairment of autophagy or through the deletion of the mitophagy genes, PINK1 or PARKIN results in increased NLRP3 inflammasome activation, mROS production and enhanced IL-1 β secretion [45,49,51]. NLRX1 (also known as NOD5), a NLR localizes to the mitochondrial matrix where it interacts with complex III of the ETC and with mitochondrial Tu translation elongation factor (TUFM), a mediator of viral-induced autophagy, both of which may regulate MAVS [17,52] (Fig.2).

Mitochondrial dysfunction is associated with a number of NLR related diseases (Table.1) and some evidence exists for metabolic control of NLR signalling [53–55]. While it remains to be determined if these process are directly mediated by mitochondria, saturated FAs directly prime the NLRP3 inflammasome via TLR4, implicating NLR signalling with insulin resistance [53,54], fructose induces renal NLRP3 inflammasome activation [55] and low levels of cyclic AMP (cAMP) activate the NLRP3 inflammasome via the calcium-sensing receptor (CASR) [56] (Fig.1).

Mitochondrial DAMPS

Mitochondria compartmentalize a wide variety of molecules and upon their release from their double membraned enclosures behave as mitochondrial DAMPS (mtDAMPs) activating inflammatory responses. ATP is released from dying cells and recognized by the P2X7 receptor [1]. mtDNA that is released from dysfunctional mitochondria contributes to macrophage inflammasome activation [45]. Impaired removal of damaged mitochondria leads to the production of more mtDNA and increased inflammasome activation [45,49]. More recently, Shimada *et al* demonstrate that oxidized mtDNA released into the cytosol directly binds and activates the NLRP3 inflammasome and is related to the activation of apoptosis [57] (Fig.2).

mROS are proposed as mtDAMPs critical for activation of the inflammasome. Metabolic alterations in substrate supply to mitochondria, and potentially other post-translational modification of mitochondrial proteins, could act to modulate mROS production. Initial investigations into the role of ROS in inflammasome activation pointed to a role for NADPH oxidases (NOX), however emerging evidence suggests that NOXs may not as important as once thought and mitochondria, in particular the ETC, maybe the major generators of inflammasome –related ROS [58]. The established sites for mitochondrial ROS production are complex I, II and III (CI-III) of the ETC (Fig.3). Although most electrons flowing down the ETC redox gradient ultimately reach complex V, 1– 3% of electrons (leak) prematurely react with oxygen, at CI and CIII, to form superoxide and other ROS', collectively known as mROS [58,59](Fig.3). The superoxide anion (O_2^-) in the matrix is highly reactive and can damage mtDNA and proteins, including the high-iron-sulfur-containing ETC complexes themselves. The IMM enzyme glycerol-3-phosphate dehydrogenase (GPDH) is also capable of superoxide and the electron transferring flavoprotein ubiquinone oxidoreductase (ETF-QOR) can release ROS in the matrix during β -oxidation of fatty acids (Fig.3). Their contribution to the generation of mitochondrial ROS in vivo is uncertain but may be significant [58].

While major questions still remain about how mROS are generated and regulated, a number of studies have identified programmed mechanisms for the generation of mROS at the OMM. Superoxide may control its own production through activating UCPs that allow protons to leak back through the IMM and reduce the production of superoxide by the ETC [58] (Fig.3). Genetic ablation of UCP2 increases baseline mROS and increases pro-inflammatory cytokine production, antibacterial activity and confers resistance to *Toxoplasma gondii* infection highlighting the potential therapeutic benefits of targeting UCP2 function to enhance immunity [58]. TRAF6 ubiquitination of ECSIT at the mitochondrion promotes CI assembly and enhances mROS production. ECSIT- or TRAF6-deficient macrophages produce less ROS when stimulated by TLRs and have diminished ability to kill Salmonella [58]. Interestingly, mROS negatively regulates innate immune signaling pathways triggered by a DNA virus, but not by RNA viruses [60] and mROS may have a differential role in acute and persistent low-grade inflammation with sub-clinical doses of LPS inducing TLR-mediated generation of mROS, allowing mild induction of proinflammatory mediators [61].

Mitochondria are key regulators of Ca^{2+} signalling and Ca^{2+} stimulates mitochondrial biogenesis [43]. By taking up Ca^{2+} from the ER or the extracellular space, mitochondria regulate spatiotemporal patterns of Ca^{2+} signalling, Ca^{2+} mobilization and the activation of Ca^{2+} binding proteins resulting in the control of a diverse range of cellular processes [62]. Recent evidence now suggests that activation of the NLRP3 inflammasome requires Ca^{2+} signalling [56,62] via CASR suggesting that Ca^{2+} may act as a mtDAMP in conditions of stress. Several NLRP3 inflammasome activators mobilize Ca^{2+} and C/EPB homologous protein (CHOP), a protein known to regulate Ca^{2+} release from the ER, amplifies NLRP3 inflammasome activation [62].

Conclusion

Full elucidation of the role of mitochondria in innate immune signaling and assessing how immune cells may use these mitochondrial derived molecules to their advantage is of paramount importance. Quality control of mitochondrial integrity and function is essential not only in normal mammalian physiology, but also in the pathogenesis of major inflammatory disease states (Table.1). During inflammation, the cell faces a unique set of challenges and relies on efficient mitochondrial turnover and tight control of mitochondrial dynamics and biogenesis. In chronic conditions that display mitochondrial dysfunction and an inflammatory phenotype, including cancer and certain metabolic, neurodegenerative, and cardiovascular diseases, understanding the links between inflammation, mitochondrial biogenesis, and metabolism, is important. Agents that enhance mitophagy, modulate intracellular Ca^{2+} signaling, improve mitochondrial fusion and mitochondrial quality control (by removing mROS and cytosolic mtDNA) may confer beneficial anti-inflammatory effects and have the potential to mitigate a wide range of inflammatory diseases. Several of these approaches are under investigation, including the use of antioxidant compounds (resveratrol), development of specific mitophagy-inducing therapies (rapamycin), inhibitors of mitochondrial fission (MDIVI [63]), manipulation of mROS (UCPs) and the pharmacological manipulation of mitochondrial SIRT6 [64]. However future studies should be approached with caution. To date, the majority of literature has been limited to in vitro or ex vivo strategies and must be translated to in vivo models or human disease to fully support a role for mitochondrial dysfunction in the development of the inflammatory pathologies.

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Highlights

- Mitochondrial fission/fusion and mitophagy regulators modulate RLR responses.
- NLRP3 activation is dependent on mitochondrial localization and mROS.
- Cyclic AMP activates the NLRP3 inflammasome via the calcium-sensing receptor.
- LPS increases succinate to stabilize HIF-1 α -mediated regulation of IL-1 β .
- ECSIT interacts with TRIM59 negatively regulating TLR signaling.

Figure.1. Innate immune signaling by PRRs

Cytosolic viral RNA is recognized by the RIG I-like receptors that activate MAVS. MAVS interacts with RIG-I via TRAF, resulting in the induction of antiviral and inflammatory responses including NF-κB and IRF signaling pathways. TLRs recognize PAMPs from viruses, bacteria, parasites and fungi. TLRs are responsible for the recruitment of various adaptor molecules to activate downstream signaling pathways, including NF-κB, leading to the transcription of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α. The glycoprotein, Fetuin-A acts as an adaptor between saturated FFAs and TLR4. Members of the cytosolic NLR family act as central components of the multiprotein inflammasome complex. The best-characterized inflammasome is that consisting of NLRP3, ASC and caspase-1. Inflammasome components assemble, by a yet undefined mechanism in response to a number of physically and chemically diverse triggers including endogenous DAMPS,

ATP, lysosomal rupture and calcium. This in turn promotes the activation of caspase-1 leading to the maturation and secretion of IL-1 β , IL-18, and IL-33.

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Figure. 2. Mitochondrial and innate immune signaling

The TLR signaling protein, TRAF6 bind to the mitochondrial protein, ECSIT, a complex I ETC subunit enhancing TLR signaling. ECSIT also interacts with TRIM59, which negatively regulates TLR signaling. The mitochondrial protein, MARCH5, a regulator of mitochondrial fission and fusion positively regulates TLR7 signaling. Mitochondrial regulated glutamine modulates the expression of TLR by regulating the expression of TRIF. LPS stimulation reduces the expression of mitochondrial enzymes involved in the Krebs cycle and increases T-FAM, cytochrome c oxidase subunits I and IV and cytoplasmic citrate and succinate levels. SARM, a conserved TLR adaptor localizes to the mitochondria and triggers intrinsic apoptosis by generating ROS and depolarizing ψ_m . The fusion regulators, MFN1 or OPA1 increase the activation of the transcription factors, NF-κB and IRF-3, whereas the fission regulators DRP1 and FIS-1 decrease RLR signaling. Another mitochondrial fusion/fission protein, MUL1 directly interacts with MAVS and modulates RLR responses. The mitophagy related genes ATG5/12 and SMURF1 inhibit RLR signaling.

MMP-2 and TOM70 activate RLR signaling. Upon activation, NLRP3 translocates to mitochondria and to MAMs. mtDNA that is released from dysfunctional mitochondria contributes to macrophage inflammasome activation. The mitochondrial channel VDAC, ATP and mROS originating from the mitochondria are important for NLRP3 activation. NLRP3 interacts with the mitochondrial protein TXNIP. Mitophagy inhibits NLRP3 inflammasome activation, mROS production and IL-1 β secretion

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Figure 3. Mitochondria and ROS production

The established sites for mitochondrial ROS production at the IMM are complex I, II and III (CI-III) of the ETC. Electrons flow down the ETC redox gradient reaching complex V with 1–3% of electrons leaking to prematurely react with oxygen, at CI and CIII, to form superoxide and other ROS' (mROS). The IMM enzyme glycerol-3-phosphate dehydrogenase (GPDH) also produces superoxide and ROS are released in the matrix during β-oxidation of fatty acids. UCPs allow protons to leak back through the IMM and reduce the production of superoxide by the ETC. TRAF6 ubiquitination of ECSIT at the mitochondrion promotes CI assembly and enhances mROS production.

Table 1**Mitochondrial proteins and Innate Immune Receptor Signaling**

Mitochondrial Protein	Immune Target	Immune Response	Association with Disease?
RLR signaling			
MAVS [65]	+RIG-I	IFN-1, NF- κ B IRF3-antiviral signaling	Nonalcoholic steatohepatitis (mice) [66], Hepatitis virus A/B/C (cells, mice, human)[67], Vaccinia virus (cells) [68], syncytial virus (RSV) (mice)[69], herpes virus (cells, mice) [70], systemic lupus erythematosus (SLE)[71], colitis (mice) [72], Japanese encephalitis virus (JEV) (cells, mice) [73], influenza A virus (cells) [74], Chronic Obstructive pulmonary disease (cells, mice) [75]
TOM70 [16]	+MAVS		Reduces replication of vesicular stomatitis virus (VSV) and Newcastle disease virus (cells) [76]
STING [7]	+ MAVS		Increased susceptibility to (VSV) and herpes simplex virus type 1 infection (cells) [67].
NLRX1 [17]	–MAVS	Anti-inflammatory	Decreases the binding of RIG-I and MAV S during Sendai virus infection (cells) [67].
gC1qR [18]	–MAVS		Increased RLR-induced IFN β in response to virus, and less susceptible to VSV infection [67].
MFN2 [8]	+/-MAVS	Shown to activate and inhibit antiviral signaling	Ambiguous, as associated with heightened MAVS signaling and increased IFN β production but also essential for RLR antiviral responses [67]. MFN2 associated with T2D, Charcot Marie Tooth, Huntington's Disease [2]
MFN1 [8]	+ MAVS	IFN-1, NF- κ B IRF3	Decreased virus-induced NF κ B and IRF3 activation [66]
MUL1 [9,12]	–MAVS	Anti-inflammatory	
MMP-2 [15]	?	NF- κ B, NFAT, IRF7	Cardiac dysfunction (ischemia and systolic failure) (cells, mice) [15]
IRGM [14]	+ Autophagy	Autophagic clearance	Crohn's disease (cells, mice, human) [77], tuberculosis (cells, mice human) [77], inflammatory Bowel Disease (genome wide association study, GWAS) [77]
TLR signalling			
MARCH5 [19]	+TLR7	NF- κ B	Multiple disease phenotypes; examples include; Systemic inflammatory syndrome (human) [78], atherosclerosis (cells, mice, human) [79,80] Crohn's Disease (human) [81], tuberculosis [82,83], severe sepsis (cells, mice, humans) [1,45], SLE (human) [84], chronic chagas cardiomyopathy (mice) [85], T2D [3], Alzheimer's disease (in silico prediction) [86] and sepsis-associated acute kidney injury (AKI) (cells, mice)[1].
ECSIT[76]	+TLR1/2/4	TRAF6, NF- κ B, IRF3	
	+TRIM59	Inhibits NF- κ B IRF-3/7	
HABP [87]	+TLR4	NF- κ B, IRF3	
SMAC[44]	–TLR4	Anti-inflammatory	
SARM [42]	+ROS	Pro-apoptotic	
NLR signalling			

Mitochondrial Protein	Immune Target	Immune Response	Association with Disease?
mtNLRP3	+NLRP3	IL-1β, IL-18	Atherosclerosis (cells, mice, humans) [80], Alzheimer’s disease (AD)(mice, humans) [88], insulin resistance and T2D (cells, mice, human) [1] cryopyrinopathies (cells, mice, human)[1]
mtDNA [45]	+NLRP3,+TLR9		Elevated in trauma and RA patients, in patients with femur fracture (see [1] and below)
TXNIP[50]	+NLRP3		Improved glucose tolerance and insulin sensitivity (cells, mice) [50]
ATP	+NLRP3		Marker of injury, released from dying cells (cells, mice, human) [1]
VDAC 1/2	+NLRP3		Impaired activation of NLRP3 inflammasome[1]
Mt DAMPS, mitophagy and metabolism			
mtDNA	++NLRP3, +TLR9	IL-1β, IL-18 Pro-apoptotic	Myocarditis, dilated cardiomyopathy (cells, mice)[89] and pre-eclampsia (PE) (cells, rats) [90]. mtDNA is elevated in patients with renal failure femur fracture, cancer, trauma, RA [91]. Lower mtDNA levels are found in patients with metabolic diseases (T2D), HIV, multiple sclerosis and infertility [91].
mROS	+NLRP3, +TLR1/2/4 +TXNIP	NF-κB, IRF3, IL-1β, IL-18	Multiple disease phenotypes; examples;Dyslipidemia (cells, mice, human) [1], cardiovascular disease (cells, mice, humans) [1], T2D [92], TNFR1-associated periodic syndrome (TRAPS) (cells) [64], COPD (human)[93] nonalcoholic steatohepatitis (NASH) (cells, mice, human) [94], sepsis (cells, mice) [1], AD (cells, mice, humans) [88], Parkinson’s disease (PD) (cells, mice, human) [95], age-related macular degeneration (cells, mice, humans) [96]
UCP2 [97]	–ROS	Anti-inflammatory	UCP2 deficient mice are resistant to <i>Toxoplasma gondii</i> infection, and cells from UCP2-deficient mice display increased toxoplasma activity and intracellular killing of <i>Salmonella enterica</i> and <i>Listeria monocytogenes</i> . <i>Leishmania</i> infection increases UCP2, preventing ROS-mediated macrophage defense mechanisms facilitating parasite survival [58]
OXPHOS	+ ROS +P2X7 +NLRP3,+TLR1/2/4 +TXNIP	NF-κB IRF3, IL-1β, IL-18	High OXPHOS ATP leads to over activation of immune response in TRAPS [98]
Citrate	+ROS	Acetyl Co-A? [38], prostaglandins?	Citrate synthase activity is lower in obese patients[99]
Succinate	+ HIF-1α	IL-1β, IL-18	Succinate concentrations have been detected in the plasma of patients with peritonitis, and in the urine and plasma of diabetic and metabolic disease rodent models [40]
MtTFAM [39]		Monocyte activation and cytokine secretion	AD (human) [100]
CASR/cAMP/ Calcium[56,62]	+NLRP3		CASR is also pathogenically associated with cryopyrin-associated periodic syndromes (CAPS), a collection of

Mitochondrial Protein	Immune Target	Immune Response	Association with Disease?
			autoinflammatory diseases also connected with NLRP3 gene mutations [56].
NOD2/ATG16 L1		IL-1 β	Crohn's disease (human) [1]
ATG5/Beclin1 LC3	–NLRP3 –MAVS	Inhibit IL-1 β , IL-18, RLR	Spontaneous activation of NLRP3, <i>Mycobacterium tuberculosis</i> has been shown to suppress host innate immune defenses by modulating autophagy (cells) [101]
PINK1	–NLRP3	Inhibit IL-1 β , IL-18	Parkinson's disease; increased mROS production, NLRP3 inflammasome activation and enhanced IL-1 β secretion (cells, mice, human) [51].
PARKIN	–NLRP3	Inhibit IL-1 β , IL-18	
Unsaturated FA's	–TLR4	Inhibition of NF- κ B, IRF3	Suppressing TLR4 signaling and reducing the inflammatory response and mortality rate in septic patients with acute lung injury (ARDS) [56].
Saturated FAs	+TLR4 + NLRP3	NF- κ B, IRF3, IL-1 β /18	Dyslipidemia, cardiovascular disease, obesity, T2D [23]
Glutamine	–TLR4	NF- κ B, IRF3	Decreases oxidative stress in the kidneys in mice [31], decreases endotoxaemia in LPS-treated rats [30], antiinflammatory colitis (mice) [32] and abolishes the up-regulation of TLR4 in a murine model of irritable bowel syndrome (IBS). Conflicting human studies [33–35].