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MITOCHONDRIAL MECHANISMS IN IMMUNITY AND INFLAMMATORY CONDITIONS: BEYOND ENERGY MANAGEMENT

Silvia Alvarez[1,2], Virginia Vanasco[1,2], Juan Santiago Adán Areán[1,2], Natalia Magnani[1,3], Pablo Evelson[1,3]

[1] Universidad de Buenos Aires. CONICET. Instituto de Bioquímica y Medicina Molecular (IBIMOL), Facultad de Farmacia y Bioquímica. Junín 946, C1113AAD CABA, Argentina.

[2] Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Químicas. Cátedra de Fisicoquímica. Junín 946, C1113AAD CABA, Argentina.

[3] Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Ciencias Químicas. Cátedra de Química General e Inorgánica. Junín 946, C1113AAD CABA, Argentina.

Corresponding author: Silvia Alvarez
University of Buenos Aires
School of Pharmacy and Biochemistry
Junín 956, 2nd floor
C1113AAD CABA
Argentina
tel. +5411 5287 4239
email: salvarez@ffyb.uba.ar

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ABSTRACT

Significance: The growing importance of mitochondria in the immune response and inflammation is multifaceted. Unraveling the different mechanisms by which mitochondria have a relevant role in the inflammatory response beyond the energy management of the process, is necessary for improving our understanding of the host immune defense and the pathogenesis of various inflammatory diseases and syndromes.

Critical issues: Mitochondria are relevant in the immune response at different levels, including releasing activation molecules, changing its structure and function to accompany the immune response, and serving as a structural base for activating intermediates as NLRP3 inflammasome. In this scientific journey of dissecting mitochondrial mechanisms, new questions and interesting aspects arise, such as the involvement of mitochondrial-derived vesicles in the immune response with the putative role of preventing uncontrolled situations.

Recent advances: Researchers are continuously rethinking the role of mitochondria in acute and chronic inflammation and related disorders. As such, mitochondria have important roles as centrally positioned signaling hubs in regulating inflammatory and immune responses. In this review, we present the current understanding of mitochondrial mechanisms involved, beyond the largely known mitochondrial dysfunction, in the onset and development of inflammatory situations.

Future directions: Mitochondria emerge as an interesting and multifaceted platform for studying and developing pharmaceutical and therapeutic approaches. There are many ongoing studies aimed to describe the effects of specific mitochondrial targeted molecules and treatments to ameliorate consequences of exacerbated inflammatory components of pathologies and syndromes, resulting in an open area of increasing research interest.

KEYWORDS

mitochondria; immunity; inflammation; mitochondrial dynamics; mitochondrial-targeted therapeutics

1.INFLAMMATION AND MITOCHONDRIA

Inflammation is commonly defined as a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells and toxic compounds (Chen et al, 2017), inducing acute or chronic responses and potentially leading to tissue damage or disease. Two complementary immune systems coexist in vertebrates that recognize and eliminate pathogens: innate and adaptive immune systems. Within a short period after activation of the innate immune system, the acute inflammatory response is initiated by immune cells (mainly including neutrophils, natural killer cells, monocytes, and macrophages) enabling secretion of various cytokines and chemokines to recruit other immune cells (as activated T lymphocytes) at the site of infection (Chaplin, 2010). However, if its capacity is exceeded, the adaptive immune system is engaged, and specific T and B cells are activated. If this process is prolonged or inefficient, it progresses to a chronic state of inflammation. In this sense, chronic inflammation is a prolonged, dysregulated, and maladaptive response that involves persistent inflammation and, often, tissue damage (Medzhitov, 2008). This is the basis by which inflammation is also viewed as a driving factor in many diseases (Nathan and Ding, 2010), including atherosclerosis, cancer, heart disorders, autoimmunity, and it is a major contributor to age-related conditions (Netea et al, 2017).

Mitochondria are major players in the innate immune response in antimicrobial defense and sterile inflammation (West, 2017), as active actors in different mechanisms, where they not only provide a platform for various signaling events but also contribute to the effector response (dela Cruz and Kang, 2018; Brokatzky and Hacker, 2022). For example, mitochondria play an important role in the regulation of major elements of innate immune receptor signaling pathways such as RIG I-like helicases receptors (RLR), Toll-like-receptors (TLR), Nuclear oligomerization domain (NOD)-like receptors (NLR). In this sense, mitochondria have several structural and functional characteristics that justify the relevant role of this organelle in immunity and inflammation. They have their own genome (mtDNA) and a characteristically double membrane, being the inner membrane intimately ligated to O_2 metabolism and redox processes through de respiratory chain and production of reactive oxygen species (ROS), and ATP production through the F_1F_oATP synthase. Apart from being the powerhouse of the cell, the mitochondrion has emerged as a signaling hub intimately involved in the modulation of the inflammatory response (Andrieux et al, 2021). At this stage, is relevant to point out that they are evolutionary endosymbionts that were derived from bacteria, and so might bear bacterial molecular motifs (Zhang et al, 2010).

Although there is consensus about the important role of oxidants in inflammatory processes, mechanisms remain largely unclear. The intricate relationship between oxidative stress, redox

based-mechanisms and inflammation is an area of intense research and evolution. The set of principles that define redox signaling and redox regulation in different processes is nicely and systematically defined in the review published by Jones and Sies (Jones and Sies, 2015). A challenging issue is the involvement of mitochondria, as energy and redox cellular hubs, on processes that may be the cause or consequences of inflammatory situations (Andrieux et al, 2021). The complex role of mitochondria can be analyzed in pathologies where inflammation is relevant actor as in sepsis and endotoxemia. In these situations, mitochondria have been described as not only involved in the initiation of inflammation (through damage-associated molecular patterns -DAMPs- release), but also functionally affected by the inflammatory response leading to dysfunctional mitochondria and decreased ATP production (Vico et al, 2019; Adan-Arean et al, 2021). Also, this elusive insight is trying to be uncovered, for example, in neurodegenerative conditions linked to inflammatory states (dela Cruz & Kang, 2018; Newman & Shadel, 2018; Tang et al., 2014). Considerable efforts are made by redox researchers to decipher these linked areas that are constantly evolving. In this review we discuss emerging research on how the immune response may be regulated and affected trough mechanisms involving mitochondria, and the profound link between inflammation and mitochondrial function and structure. A special highlight is provided to the role of DAMPs and the therapeutic potential of targeting mitochondria in inflammatory situations. In this sense, current understanding of mitochondrial mechanisms involved, beyond the largely known mitochondrial dysfunction, in the onset and development of inflammatory situations is presented.

2.TRIGGERING IMMUNE RESPONSES: THE ROLE OF DAMPs

Immune system activation occurs not only due to external stimuli, but also due to endogenous molecules (Netea et al, 2020). As such, mitochondrial constituents might play as DAMPs that could trigger innate immune responses during pathological insults (Zhang et al, 2010; dela Cruz and Kang, 2018; Grazioli and Pugin, 2018). Mitochondrial DAMPs (mtDAMPs) are potent immunological activators probably due to the bacterial ancestry of mitochondria, and include not only proteins but also DNA or lipids (Zhang et al, 2010). In other words, when mitochondria are stressed or dysfunctional, they may produce a variety of DAMPs which may function as drivers of inflammatory responses (Fig. 1), and if their presence is sustained in time they may lead to pathological situations. In this sense, current evidence indicates that uncontrolled and excessive release of mitochondrial DAMPs is associated with the process severity, has prognosis value in human diseases, and contributes to the dysregulated process observed in numerous inflammatory and autoimmune conditions (Grazioli and Pugin, 2018). Interestingly, Nicolas-Avila et al described a

non-canonical route for elimination of damaged mitochondria through a network of resident macrophages surrounding cardiomyocytes thus preventing DAMPs release (Nicolas-Avila et al, 2020). Failure to proper eliminate them results in inflammasome activation and autophagy arrest.

Mitochondrial DAMPs include mtDNA, ATP, mitochondrial transcription factor A (TFAM), N-formyl peptide, succinate, cardiolipin (CL), and cytochrome c (cyt c) (Fig. 1). In the last years, the *in vivo* occurrence of mitochondrial double-stranded RNA led to its description as DAMPs triggering interferon type I response (Dhir et al, 2018; Pajak et al, 2019). Considering mtDNA as an example, it contains 37 genes coding for 2 ribosomal nucleic acids, 22 transfer RNAs, and 13 essential protein subunits of the oxidative phosphorylation system (Andersson et al, 2003). If compared with nuclear DNA, it presents low or absent methylation levels and increased susceptibility to oxidative damage (Andersson et al, 2003; Mechta et al 2017; Guitton et al, 2022). Its association into protein-DNA complexes (nucleoids) under the control of TFAM (Grazioli and Pugin, 2018) renders protection from oxidative damage. MtDNA release pathways include passive mechanisms, as in necroptosis and apoptosis (Linkermann and Green, 2014), and active mechanisms, as through mitochondrial-derived vesicles (MDV) (Islam et al., 2012). Free mtDNA can be recognized by three important pattern recognition receptors (PRR) of the innate immune system: TLR9, inflammasomes and type I interferon response receptor (Grazioli and Pugin, 2018; Patergnani et al, 2020). In these cases, interaction of mtDNA with its receptors trigger a pro-inflammatory response (de Gaetano et al, 2021). Another interesting example is CL, a lipid dimer consisting of two phosphatidyl groups bridged by glycerol localized in the inner mitochondrial membrane. It has relevant functions in mitochondrial respiration and mitochondrial biogenesis (Chicco and Sparagna, 2007; Claypool and Koehler, 2012). Moreover, several reports highlighted the close interaction that exists between CL and cyt c (Rice et al, 2021). ROS-mediated CL peroxidation causes the detachment of cyt c from the inner membrane, probably inducing apoptosis. CL can induce inflammation by its binding to NLRP3 inflammasomes (Iyer et al, 2013). In the same way, interference with CL synthesis has been shown to inhibit NLRP3 inflammasome activation (Liu et al, 2019). Additionally, CL can stimulate the activation and proliferation of CL-responsive γδ T cells (Dieudé et al, 2011). As a whole, it has become increasingly evident that mtDAMPs play a critical role in sterile inflammation and in various diseases such as those characterized by chronic inflammation. Although an increasing number of studies have demonstrated that mitochondrial DAMPs can be actively released by non-necrotic cells exposed to an external stimulation (Katsumi et al, 2019; Murao et al, 2021), the mechanisms for extracellular release *via* MDV or autophagy requires further clarification. Is important to point out that therapeutics designed to target mitochondrial DAMPs and their related signaling pathways are promising in various inflammatory and auto-immune diseases. Moreover, the measurement of

mitochondrial DAMPs in body fluids as a biological marker of a disease may also be useful for prognostic value (Fucikova et al, 2015; Huckriede et al, 2021). The future direction in this idea is to analyze mtDAMPs impact in the initiation and progression of related diseases. Recent advances in mitochondrial biology have enabled researchers to rethink the mitochondrial role in acute and chronic inflammation and related disorders. As such, mitochondria have important roles as centrally positioned signaling hubs (besides the classical view as cellular energy hubs) in regulating inflammatory and immune responses.

3.MITOCHONDRIAL REGULATORY POINTS AND IMMUNE CELL FATE

3a. Mitochondrial regulatory points

Recent studies have shown that mitochondrial metabolism has an essential role in controlling the fate of immune cells. From T cells to macrophages, mitochondria play a key role in the various metabolic pathways that define each immune cell subset (van der Windt et al, 2012; O´Neall et al, 2016; Mehta et al, 2017; Sugiura et al, 2022). At this point, it is important to analyze how mitochondrial metabolism dictates the function and fate of immune cells beyond its role in generating ATP. In this central position, mitochondria emerge with various metabolic points plausible to be importantly regulated (Fig. 2) and that may determine immune cell function. The knowledge and analysis of mitochondrial hot points plausible of being regulated open important directions for developing directed therapeutics based on functional regulation.

One of the regulatory points is represented by the glycolysis pathway (producing pyruvate from glucose). In a further step, pyruvate can either be secreted as lactate or enter mitochondria and be oxidized to acetyl-CoA by pyruvate dehydrogenase. This last process can be negatively regulated by pyruvate dehydrogenase kinase. To enter the tricarboxylic acid (TCA) cycle, acetyl-CoA needs to be condensed with oxaloacetate to produce citrate. In the final step, reducing equivalents are formed (NADH and $FADH_2$) allowing the regeneration of oxaloacetate. T cell activation by the stimulation T cell receptor (TCR), activates signaling pathways and transcription factors that, in turn, increase the flux of metabolites through glycolysis and the TCA cycle (Wang et al, 2011). It has been shown that pharmacological or genetic inhibition of glycolysis limits TCR-dependent T cell proliferation (Chang et al, 2013). Macrophage polarization is also important at this point. In this sense, M_1 macrophages display an increased glycolytic flux that is characterized by a high rate of lactate production that has been shown to be necessary for its pro-inflammatory functions (Griffiths et al, 2017; Haschemi et al, 2012). It was observed that increased lactate produced in M_1 macrophages, can cause epigenetic modifications leading to a M_2 phenotype and resolution of the

inflammatory situation (Zhang et al, 2019). Moreover, M_1 macrophages have a unique TCA cycle that is described to be broken at two points: at isocitrate dehydrogenase and at complex II, allowing not only the occurrence of reverse electron transfer (RET) but also the net increase of NO production from glutamate. On the contrary, M_2 macrophages display lower levels of glycolytic flux and an unbroken TCA cycle (Jha et al, 2015). One interesting situation to highlight is that in activated macrophages the occurrence of the production of itaconate from aconitate, blocks succinate-mediated inflammatory processes (O´Neill et al, 2019).

Another regulatory point includes the exportation of citrate to cytoplasm to regenerate acetyl-CoA and oxaloacetate through the citrate shuttle, which in turn produces *de novo* fatty acids through fatty acid synthase (FAS). Alternatively, fatty acid oxidase (FAO) can catalyze the inverse process, a reaction taking place in mitochondria (Almeida et al, 2021; Zhu et al, 2022). This division of fatty acid catabolism in mitochondria and fatty acid anabolism in the cytoplasm helps to compartmentalize reactions and to prevent futile cycles. Regarding this decision point, it is worth to note that T cells increase citrate transport into the cytoplasm through the citrate shuttle to increase the cytosolic levels of acetyl-CoA (Mehta et al, 2017), a required situation for histone acetylation and interferon γ (IFNγ) production. Fatty acid trafficking is necessary for M_2 macrophages activation as the treatment with etoxomir (pharmacological inhibitor of FAO) prevents its activation (Vats et al, 2006). Also, it has been suggested that fatty acid acquirement by M_2 macrophages could be used to activate this type of macrophages specific gene expression profiles in an independent manner from mitochondrial metabolism (Mehta et al, 2017). Interestingly, it was shown that FAO positively regulates mitochondrial spare respiratory capacity in $CD8^+$ T cells and, therefore, favors cellular survival (Nomura et al, 2016).

Another interesting regulatory point is the electron transport chain (ETC) (that is analyzed in the following item) and the production of mtROS from it, through the partial reduction of O_2 to superoxide. The important role of mtROS in T cell activation is supported by evidence indicating that T cell activation requires the recruitment of mitochondria to the immune synapse (Sena et al, 2013). Moreover, Zhang et al. showed that activated T cells inhibit the formation of the mitochondrial permeability transition pore (mPTP) probably to prevent increased mtROS leakage from mitochondria (Zhang et al, 2016). An interesting aspect of this regulatory point is the concept called mitohormesis, where a mild increase of ROS described as the hormetic stressor can lead to a sublethal stress (Calabrese et al, 2007), thus triggering the activation of a retrograde mitochondria-to-nucleus signaling mechanisms and the vitagene network (Calabrese et al, 2010; Yun and Finkel, 2014; Fischer and Ristrow, 2020). This process was described to be plausible to occur in macrophages

leading to metabolism reprogramming as a mechanism to restrain inflammation through tolerance (Timblin et al, 2021).

And last but not least, ATP production and maintenance of the mitochondrial inner membrane potential also represent an important regulatory point that is plausible to be regulated during the activation and fate of immune cells (Faas et al, 2017), and is discussed in the following section.

3b. The relevance of electron transport chain in immune cells

In immunometabolism, the importance of the ETC has drifted from bioenergetics to its regulatory and signaling pathways (Glancy et al, 2020; Yin and O'Neill, 2021; Preau et al, 2021). The origin of this change was the description of cyt c, part of the ETC, as a driver of apoptosis. This finding resignified the importance of the ETC beyond energy management. In Fig. 3, the main roles and involvement are summarized for each respiratory complex. The description and characteristics of the ETC is beyond the scope of this review and has been largely reviewed elsewhere (Acín-Pérez et al, 2020; Nolfi-Donegan et al, 2020; Vercellino and Sazanov, 2022).

The ROS produced by *complex I* significantly impact the immune system. For example, in response to LPS macrophages can shift from oxidative phosphorylation to glycolysis while also increasing succinate levels (Mills et al, 2016). This pathway can activate hypoxia-inducible factor 1α (HIF1α), a key transcription factor in the expression of proinflammatory genes, that in turn can lead to the increased production of interleukin 1β (IL-1β) (Mills et al, 2016). In this way, treatment with metformin or rotenone (complex I inhibitors) was shown to reduce pro-IL-1β production and to increase the anti-inflammatory IL-10 production in macrophages (Xian et al, 2021). Complex I is also implicated in lymphocyte activation, proliferation, and function. Its activity is required in proliferating cells as it maintains an appropriate NAD^+/NADH ratio to support aspartate and nucleotides biosynthesis (Sullivan et al, 2015). Moreover, blocking complex I with rotenone inhibited proliferation in T effector cell subsets, and aspartate supplementation partially restored the proliferative capacity of rotenone pretreated T_H1 cells (Bailis et al, 2019). In another study, it was observed that in $CD8^+$ T cells, rotenone inhibited T cell cytotoxicity based on the measurement of decreased production of IFNγ and tumor necrosis factor α (TNFα) and degranulation (Yi et al, 2006).

As previously stated, *complex II* has been implicated in M_1 macrophage activation. Also, accumulated succinate can also be exported from mitochondria to cytosol where it can inhibit PHD activity to stabilize HIF1α (Tannahill et al, 2013). This effect of complex II is strengthened by the use of dimethyl malonate, a potent inhibitor of succinate oxidation by complex II, inhibiting LPS-activated macrophages IL-1β production but increasing IL-10 production (Mills et al, 2016). Interestingly,

reduced IFNγ production by T_H1 cells was observed through complex II activity inhibition using, for example, thenoyltrifluoroacetone, 3-nitropropionic acid or atpenin 5 (Bailis et al, 2019).

Complex III is another important mtROS producer besides complex I in the ETC. MtROS produced through Q site of complex III are important activators of HIF1α (Mansfield et al, 2005), which is widely expressed in innate and adaptive immune cells including macrophages, neutrophils, and lymphocytes. Analyzing the specific importance of complex III, it has been described that the loss of RISP (Rieske iron-sulfur protein) leads to impaired differentiation of hematopoietic stem cells with histone hypoacetylation due to reduced citrate levels (Ansó et al, 2017). Also, functional complex III is required for antigen specific $CD4^+$ T cell activation (Weinberg et al, 2019). A specific mutation of complex III in T cells allowed Sena et al. to demonstrate that mtROS produced at complex III were required for IL-2 production through the activation of NFAT (nuclear factor of activated T cells), and antigen-specific proliferation. In this way, complex III seems to be essential for the correct development of T cells (Sena et al, 2013).

Knockdown of cytochrome oxidase or *complex IV* in macrophages was shown to increase the production of IL-1β, IL-6, and TNFα, and M_1 polarization and phagocytosis processes (Angireddy et al, 2019). Importantly, it has been shown that in NK cells complex IV is required for antigen-specific amplification (Mah-Som et al, 2021). The non-structural component COX10 is commonly studied to unravel the mechanism by which complex IV is associated with the immune system. T cell specific knockdown of this component was described to impair T cell activation, demonstrating that is important for T cell exit from quiescence acting as a metabolic checkpoint for cell fate decisions following activation (Tan et al, 2017).

Although *F_1F_o-ATP synthase* is not strictly part of the mitochondrial respiratory chain, it is important to briefly discuss its importance based on its main activity as ATP producer. For example, in polarized macrophages, inhibition of ATP synthesis or decreased ATP levels can suppress both M_1 and M_2 activity (Mills et al, 2016; Qin et al, 2020). Also, it was shown that oligomycin (F_1F_o-ATPase synthase inhibitor) can decrease LPS-induced IL-1β levels in M_1 macrophages and suppress arginase activity in M_2 macrophages (Mills et al., 2016). At this point, ATP production and the maintenance of membrane potential are necessary in a context of immune cell activation and differentiation, processes that are directed by energy expenditure and maintained mitochondrial function (Mehta et al, 2017; Stanzani et al, 2019; Vico et al, 2019).

Considering the mitochondrial respiratory chain as a whole, it is important to recognize its therapeutic potential. This observation is based on its importance in the activation and modulation of the inflammatory response, as center of cellular energy metabolism, regulating the levels of NADH, FADH and ATP associated to the Krebs cycle, fatty acid oxidation, aminoacid oxidation, and

pyrimidine biosynthetic pathway, and the maintenance of cellular redox balance (Yin and O'Neill, 2021). Nevertheless, researchers should not set aside the importance of the spatial organization of the respiratory chain in supercomplexes for the functional and regulatory aspects on the activation of the immune response.

4. THE ROLE OF MITOCHONDRIA ON NLRP3 INFLAMMASOME ACTIVATION

The NLRP3 inflammasome is a multiprotein complex, composed of three protein subunits: a sensor molecule (NLRP3), an adaptor protein (ASC), and an effector protein (caspase-1) (Rathinam and Fitzgerald, 2016) (Fig. 4). While NLRP3 inflammasome activity is a necessary component of the innate immune response, its excessive activation can lead to the inflammatory form of cell death called pyroptosis (Swanson et al, 2019). The discovery of the NLRP3 inflammasome and pyroptotic cell death in the last decade has shown the increasing relevance of mitochondria as key players in the inflammatory response (Jo et al, 2016; Huang et al, 2022). Mitochondria are actively involved not only in activation pathways, but also as docking sites in the activation process (Swanson et al, 2019). In this sense, DAMPs act as one of the links between mitochondria and inflammation, and are recognized by the cytoplasmic nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs) (Gong et al, 2020). These NLRs recognize a wide spectrum of intracellular inflammatory stimuli. There are four classes of NLRs (NLRP1, NRLP3, NLRC4 and AIM1) that sense a variety of inflammatory signals to mediate caspase-dependent activation of cytokines (Sandhir et al, 2017). The most common and largely studied is NLRP3 and is normally localized in the cytosol. NLRP3 inflammasome structure is a complex association of cytosolic NLRP3, ASC (apoptosis associated speck-like protein containing a CARD), and caspase 1 (Gilkerson and Materon, 2014); Fig. 4 shows its structure and activation, emphasizing the activating signals involved and the main consequences observed. Briefly, upon activation, NLRP3 recruits ASC and pro-caspase 1, which in turn becomes activated in caspase 1 being the responsible for the activation of IL-1β and IL-18 (Yang et al 2019; Huang et al, 2021).

One of the most important interactions of mitochondria with NLRP3 inflammasome is its role in the signaling events for proper activation (Billingham et al, 2022). It has been described that DAMPs leaked from damaged mitochondria can activate the innate immune response through the assembly of the multimeric protein complex inflammasome, ultimately leading to the generation of biologically active cytokines (IL-1β and IL-18) (Ge et al, 2020; Liu et al, 2019; Pandey et al, 2021; Sugiura et al, 2014). This, in turn, may lead to pyroptosis (Bouhamida et al, 2022; Sandhir et al, 2017). Interestingly, since mtDNA and mtRNA metabolism are implicated in NLRP3 inflammasome activation (Shimada et al, 2012; Zhong et al 2019; Hsu et al, 2023), their involvement in the

pathogenesis of inflammatory syndromes (as sepsis) is not surprising. Moreover, NLRP3 polymorphisms can be used as a risk estimate for the development of sepsis and inflammatory associated pathologies, highlighting the relevant role of inflammasome activation (López-Armada et al, 2013; Schunk et al, 2021).

Mitochondrial dynamics emerges as a key regulator for NLRP3 inflammasome activation. It has been shown that Drp1 knockdown that results in aberrant mitochondrial elongation, increases NLRP3-dependent caspase 1 activation and IL-1β secretion in mouse bone marrow-derived macrophages (Park et al, 2015). Moreover, in the presence of carbonyl cyanide m-chlorophenyl hydrazone (chemical inducer of mitochondrial fission) effectively decreases NLRP3 inflammasome assembly and activation. Moreover, it has been shown that mitofusin 2 (Mfn 2, mediator of mitochondrial fusion) is involved in its activation (Ichinohe et al, 2013). It is interesting to note that SESN2 (sestrin 2, a stress-inducible protein) has been described to control NLRP3 inflammasome activation probably by increased removal of damaged mitochondria through mitophagy (Kim et al, 2016). In line with this observation, it was described that autophagy can control inflammation through macrophage-disruption of apoptotic corpses or inhibiting NLRP3 inflammasome activation by removing damaged mitochondria (López-Armada et al, 2013). Conversely, inhibition of mitophagy results in spontaneous inflammasome activation (Nakahira et al, 2011).

In addition to the role of mitochondria in signaling events for inflammasome activation, there is increasing evidence that mitochondria are also important as docking sites in the activation process. This contribution of mitochondria in NLRP3-mediated inflammation is demonstrated by the recruitment in the cytoplasm of macrophages in a process mediated by microtubules (Misawa et al, 2013). In this sense, microtubule-driven apposition of ASC on mitochondria to NLRP3 on the endoplasmic reticulum was observed to contribute to inflammasome activation (Misawa et al, 2013). Nakahira and col. observed that NLRP3 relocalizes to mitochondria following stress (Nakahira et al, 2015). During activation NLRP3 and ASC redistribute to the perinuclear space where endoplasmic reticulum colocalize with mitochondria-associated membranes (MAMs). Taking these observations together, it is viewed that MAMs allow physical interaction between Inflammasome and its ligands. The emerging role of MAMs as modulators of innate immunity is further supported by evidence showing that abnormalities on ER-mitochondria relationships correlates with the inflammation severity in pathologies with an inflammatory component such as cancer, diabetes, and obesity (Pereira et al, 2022).

Regarding the physical interaction between NLRP3 and mitochondria for inflammasome activation, it is worth to describe the implication of three molecules as connection points. One of these relevant molecules appears to be the phospholipid CL that, apart from serving as binding site

for molecules related to autophagy and apoptosis (Dudek, 2017), also binds NLRP3 and caspase 1 (Swanson et al, 2019). Another example is the mitochondrial antiviral signaling protein (MAVS), which is an adaptor in RNA sensing pathways. Its presence is important for NLRP3 inflammasome activation during RNA viral infections (Swanson et al, 2019). MAVS recruits NLRP3 and locates it to mitochondria for inflammasome activation (Subramanian et al, 2013). Importantly, MAVS appear to be a non-essential protein for inflammasome activation induced by other stimuli. Lastly, Mfn 2 was found to be important in this type of mechanism (Swanson et al, 2019). It was shown that during viral infection, Mfn 2 is required for the formation of a complex with MAVS to support the localization of NLRP3 to mitochondria (Ichinohe et al, 2013).

This item reveals the relevance of the nature of the different types of involvement of mitochondria in the mechanism of NLRP3 inflammasome activation. A greater understanding of the underlying mechanisms on the activation process as docking site for assembly and further activation of NLRP3 inflammasome opens an exciting target for pharmacological interventions. Moreover, mitochondria are also involved in the activation (through mtDNA) of others signal transduction cascades involved in inflammation as the one including cyclic GMP-AMP synthase (cGAS) and the stimulator of the interferon response cGAMP interactor 1 (STING1) (Marchi et al, 2022).

5. MITOCHONDRIA QUALITY CONTROL AND MITOCHONDRIAL ARCHITECTURE

The importance of maintaining a healthy and functional mitochondrial population and the close relationship between structure and ultrastructure with mitochondrial function is evidenced by the coexistence of multiple interconnected mechanisms that manage mitochondrial homeostasis to guarantee the maintenance of cellular bioenergetics in the face of different stimuli. Relevant mechanisms responsible for maintaining mitochondrial homeostasis (in number and quality) are mitochondrial biogenesis, fusion, and fission events, mitophagy, and production of MDVs, and are the basis of the global process of mitochondria quality control (Giacomello et al, 2020; Diao and Gustafsson, 2022; Xia et al, 2022). Mitochondrial shape is fundamental for mitochondrial metabolic activity (Giacomello et al, 2020). For example, in fused organelles ATP production and matrix content exchange are facilitated. On the contrary, fissioned organelles may produce more ROS and may be metabolized through mitophagy. Also, mitochondrial fragmentation is necessary for equivalent distribution of components during cell division. It should be noted that these events do not occur independently and that their correct integration will ultimately preserve the mitochondrial functional activity in cells (Fig. 5). Current evidence places mitochondrial function and molecules involved in its homeostasis at the center of the immune response in inflammatory situations, either

related to effector cells of this response, such as macrophages (Sancho et al, 2017) and T cells (Steinert et al, 2021), or in injured cells due to the inflammatory condition.

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5.a Mitochondrial biogenesis

This process can be described as the event by which a cell increases its mitochondrial mass, a process that is not only linked to cell division but can also be induced by different stimuli such as high energy requirements, physical training, increased NO levels, and increased ROS production among others. Mitochondrial biogenesis requires the coordinated interaction of nuclear DNA and mtDNA through the activation of different transcription factors to carry out the *de novo* synthesis of mitochondrial proteins and mtDNA. The key transcription factor that regulates mtDNA transcription corresponds to the peroxisome proliferator-activated receptor-gamma coactivator (PGC1) family of transcription factors. Briefly, PGC1α is activated either by phosphorylation via AMPK (Ke et al, 2021), or by deacetylation by SIRT1 (Majeed et al, 2021). This situation, in turn, leads to the activation of several transcription factors, being the most relevant the nuclear respiration factors (Nrf1 and Nrf2), PPAR and TFAM, finally leading to mtDNA transcription and replication. Specifically, Nrf1 and Nrf2 are the main transcription factors that activate the expression of nuclear genes related to mitochondrial respiratory function (Ke et al, 2021), while TFAM regulates mtDNA replication and transcription, coordinates the assembly of the mitochondrial DNA, and regulates mtDNA copy number (Luo et al, 2021).

Although the relevance of mitochondrial biogenesis during the immune response in inflammatory events is constantly increasing, the unraveling of the molecular mechanisms that activate and drive this process is still ongoing. It has been shown that during bacterial infection PGC1β is activated in mouse macrophages, increasing mitochondrial function and mtROS production (Sancho et al, 2017), with ultimately bactericidal activity. In contrast, PGC1β-deficient macrophages showed decreased bactericidal capacity (Sancho et al, 2017). In line with this observation, it was described the importance of PGC-1β for the efficient organization and packing of ETC components in dendritic cells (Guak et al, 2022). More recently, Dumauthioz et al. showed that PGC1α overexpression favors the formation of resident CD8 memory T cells (Dumauthioz et al, 2021). It has been described that TFAM is not only involved in the initiation of mtDNA transcription, but also preserving the structural stability of mtDNA as well (West et al, 2016; Li et al, 2019). Interestingly, TFAM deficiency in mice has been shown to induce mtDNA stress, which stimulates cytosolic antiviral innate immune responses in both mouse embryonic fibroblasts and bone marrow-derived macrophages (West et al, 2016; Luo et al, 2021). Finally, inflammatory processes such as endotoxemia have been described to affect mitochondrial biogenesis in cells of the innate or

acquired response. For example, increased expression of PGC1α and TFAM has been observed in a model of acute endotoxemia both in rat cardiomyocytes (Vanasco et al, 2014) and rat brain cortex (Adán Areán et al, 2021), once again underlining the role of mitochondrial homeostasis as response to inflammatory injury.

5.b Fusion and fission

Mitochondria form networks that during cell life undergo continuous antagonistic fusion and fission events (Bereiter-Hahn and Vöth, 1994). As detailed description of these processes is not the aim of this review, only a brief and introductory reference is included. In mammals, mitochondrial fusion is mainly regulated by the proteins Opa1 (optic atrophy 1, an inner mitochondrial membrane GTPase) (Cipolat et al, 2004), and by the outer membrane GTPases or Mfn 1 and 2 (Santel and Fuller, 2001); while mitochondrial fission is mainly controlled by the cytosolic protein Drp1 (dynamin-related protein 1, a GTPase), where its translocation from the cytoplasm to the mitochondria is an essential step in organelle fragmentation (Smirnova et al, 2001). Although pro-fission proteins (such as Drp1, Fis1) and pro-fusion proteins (such as Mfn1, Mfn2, and Opa1) have been described to act independently in fission and fusion mechanisms (Detmer and Chan, 2007), emerging evidence suggests the existence of crosstalk between fission and fusion machinery. In this sense, the importance of Fis1 in the bidirectional regulation of mitochondrial fusion and fission machinery has been demonstrated, since it is not limited to mitochondrial fission promotion; it can also actively inhibit mitochondrial fusion (Yu et al, 2020). Since Opa1 is also involved in the biogenesis of mitochondrial cristae and respiration, it has been a relevant subject of study in recent years. Sanchez Rodriguez et al (Sánchez-Rodríguez et al, 2022) found that selective removal of Opa1 causes important alterations in macrophage activation and polarization, through decreased IL-6 and TNFα release, reduced NO burst, and macrophage polarization towards M_1. Moreover, Drp1 silencing in macrophages decreased mitochondrial fragmentation and production of TNFα after stimulation with LPS, while the production of IL-6 and IL-1β was observed increased (Gao et al, 2021). Generalizing, infection with bacterial pathogens is generally associated with a fragmented mitochondrial population, while viral infection leads to mitochondrial fusion in macrophages (Tiku et al, 2020). However, both mitochondrial fission and fusion in macrophages are promoted in a context-dependent manner, setting the importance to unravel these complex mechanisms and the molecules involved that interact during the immune response and inflammatory processes.

Interestingly, efferocytosis, defined as the clearance of apoptotic cells by macrophages is necessarily related to mitochondrial fission (Doran et al, 2020). Apoptotic cell uptake by macrophages triggers mitochondrial fission induced by Drp1, enabling efficient apoptotic cell

degradation in phagosomes, and release of calcium-mediated vesicular trafficking, ultimately leading to the resolution of the inflammatory situation (Wang et al, 2017).

5.c Mitophagy

Mitophagy (or autophagy of damaged mitochondria), is viewed as a mechanism involved in mitochondrial quality control by selectively degrading non-functional mitochondria (Vincow et al, 2013). Several studies, using different cell models, have shown that the dissipation of the mitochondrial membrane potential would be the signal selectively recognized by PINK1 (PTEN-induced kinase 1, a protein involved in initiating this selective autophagy process) (Jin et al, 2010; Kondapalli et al, 2012). Briefly, under basal conditions PINK1 protein n-terminal region is transferred across the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM), with the kinase domain located closer to the C-terminal region protruding towards cytosol. PINK1 is then cleaved by IMM-linked proteases and subsequently degraded by the proteasome, leading to undetectable basal levels of PINK1 under physiological conditions (Ge et al, 2020). Stressors such as membrane depolarization, or mitochondrial complexes dysfunction lead to accumulation of PINK1 in the OMM by impairing intermembrane transport from the N-terminal domain to the IMM. Subsequent homodimerization of PINK1 in OMM leads to autophosphorylation, promoting activation and recruitment of E3-Parkin ubiquitin ligase, initiating the mitophagic pathway of these mitochondria (Narendra et al, 2008; Suen et al, 2010; Wai and Langer, 2016). Defective removal of damaged mitochondria leads to hyperactivation of inflammatory signaling pathways and subsequently to chronic systemic inflammation and development of inflammatory diseases. Although the crosstalk between mitophagy mechanisms and host defense has been established only recently, a growing body of evidence supports the importance of their coordination (Gkikas et al, 2018). Interestingly, it has been described that the nitrosylation of PINK1 reduces the translocation of Parkin to the mitochondrial membrane, producing the accumulation of damaged mitochondria, observed in Parkinson's disease with chronic inflammatory characteristics (Oh et al, 2017). In recent years, new actors in mitophagy processes have been described. Among them, BCL2 interacting protein 3 (BNIP3) has been described as an important inducer of mitophagy in inflammatory, hypoxic and exercise conditions. However, the regulatory role of BNIP3 in mitochondrial dysfunction and mitophagy is complex and multifaceted and is currently under study (Gao et al, 2020). Also, it has been described that vacuole membrane protein-1 (VMP1) would be involved as a necessary agent to trigger mitophagy in the inflammatory process occurring in acute pancreatitis (Vanasco et al, 2021). It is worth to note that the accumulation of damaged mitochondria as a consequence of imbalance of quality control mechanisms, leads to the release of DAMPs (Zhang et al, 2010) as

analyzed in previous sections of this review. Consequently, pathways involved in the synthesis of different early-phase inflammatory mediators such as TNFα, ILs, IFNγ and ROS/RNS are activated (Geto et al, 2020; Roh and Sohn, 2018). For example, Irazoki et al. found that alterations in mitophagy flux and lysosomal function caused by decreased BNIP3 led to the accumulation of autolysosomes with undegraded cargo, favoring the interaction between mtDNA and TLR9 leading to NFκB induction and inflammation (Irazoki et al, 2022). Macrophages deficient in LC3 and Beclin1 have been reported to display an accumulation of defective mitochondria, disturbing cytosolic levels of mtDNA that induce NLRP3 activation, and IL-1β secretion (Nakahira et al, 2011; Zhou et al, 2011). In this direction, Zhong et al. found that mitophagy deficiency associated with aging in macrophages, caused by the decrease in mitochondrial ubiquitination mediated by the PINK1/Parkin pathway, produces the accumulation of damaged mitochondria, favors the release of mtDNA to the cytosol and increases STING activation that signals for age-associated chronic sterile inflammation (Zhong et al, 2022).

5d. Mitochondrial-derived vesicles

It is worth discussing the important emerging role of MDVs in mitochondrial quality control and its potential involvement in the immune response and inflammatory situations. These vesicles were firstly identified by Neuspiel in 2008 (Neuspiel et al, 2008) and were observed as budding from mitochondria as single or double membrane structures including mitochondrial outer and inner membrane with matrix content. It was described that its main function is to carry specific material between mitochondria and other organelles including lysosomes where they are degraded (Sugiura et al, 2014; Todkar et al, 2021). To undoubtedly define these structures, they must be formed in the absence of Drp1, its diameter should be between 70 and 150 nm, and they have to carry selective cargo (Neuspiel et al, 2008; Schumann and Subramani, 2008). Interestingly, MVDs are early formed during mitochondrial stress, before the occurrence of mitophagy, thus regulating mitochondrial mass in a rapid fashion (Peng et al, 2022).

It has been previously presented that mitochondria can release DAMPs thus activating the innate immune system. Taking this into account, MDVs not only participate as actors in mitochondrial quality control processes and communication between cells (Sugiura et al, 2014), but prevents the secretion of DAMPs by wrapping them for degradation as well. However, when lysosomes are overwhelmed with the degradation process, immune responses can be triggered. In the same way, Todkar et al. showed that the cargo selectivity depends on the different MDV targeting pathways; Opa1 and sorting nexin 9 (Snx9)-dependent MDVs are required to target mitochondrial proteins to extracellular vesicles, while parkin (in Parkinson´s disease with a chronic

inflammatory component) blocks this process by directing damaged mitochondrial content to lysosomes (Todkar et al, 2021). This observation shows that the formation of MDVs containing, for example, damaged oxidized proteins is stimulated by the PINK1/Parkin pathway (McLelland et al, 2014).

6. THERAPEUTIC TARGETING

Throughout the present review mitochondria have been identified to play a central role in a wide range of mechanisms associated with cellular homeostasis relevant for immune processes, such as (a) provide the majority of the cellular energy coupling the oxidation of fatty acids and pyruvate with the production of ATP by the ETC, (b) production and regulation of mtROS which are highly involved in the redox metabolism, (c) maintain the Ca^{2+} levels, (d) cell fate and differentiation through the mtPTP, among others (Wallace, 2005; Magnani et al, 2020; Marchi et al, 2022; Hu et al, 2022). Failure of those mitochondrial functions are relevant from the pathophysiological perspective as they are involved in the initiation and progression of numerous inflammatory diseases. Therefore, maintenance of mitochondrial network integrity and function arose as a hallmark in the development of new therapeutic strategies aiming to ameliorate an exacerbated inflammatory response (van der Windt et al, 2012; Chellappan et al, 2022). The present section intends to overview recent progress on the pharmacological approaches and the mitochondria-targeted strategies proposed to improve its function, to highlight their potentially clinical impact in inflammation and inflammatory-associated syndromes.

6a. Modulation of the ETC function

Mitochondrial-targeted treatments aiming to restore or enhance electron transfer have been proposed. Increasing the reducing equivalents feeding into the electron transfer chain may help to bypass complexes dysfunction in order to improve mitochondrial energy production. For example, succinate enhance electron transfer from complex II to complex III when complex I is blocked, or the use of menadione allows the direct transfer of reducing equivalents directly to complex IV in patients with a complex III defect (Wallace et al, 2010; Saeb-Parsy et al, 2021). Based on similar mechanisms, reduced cofactors NADH and $FADH_2$ availability could be increased by treatment with dichloroacetate, and thiamine (vitamin B1). Both compounds act as a mitochondrial pyruvate dehydrogenase kinases (PDK) inhibitor, reducing PDK function and leading to pyruvate dehydrogenase complex (PDHC) activity. This effect increases the catabolism of pyruvate to acetyl-CoA, entering the Krebs cycle and generating reduced cofactors, resulting in a characteristic reversed glycolytic phenotype of cancer cells (El-Hattab et al, 2017; Zeng et al, 2021; Yang et al, 2022; Lee et

al, 2022). Also, PDHC increased activity exert a protective strategy through the release regulation of inflammatory mediators switching the metabolism in response to stressors such as sepsis (Zeng et al, 2021).

Regarding the mitochondrial respiratory chain complexes dysfunction, partial inhibition of the respiratory chain complexes activity with small molecules emerged as a promising mitochondrial-related therapeutic strategy. Dimethyl malonate (DMM) is a cell permeable complex II inhibitor that directly inhibits succinate oxidation. Accumulation of succinate has been proved to limit the anti-inflammatory cytokine IL-10 production in macrophages (Scialò et al, 2017). Administration of DMM leads to a decrease mtROS production from complex I by reverse electron transport, which are required for the expression of IL-1β and a range of proinflammatory cytokines (Mills et al, 2017; Xu et al, 2018; Yin and O'Neill, 2021), showing the importance of mitochondrial complex II in the oxidative damage mechanisms and initiation and progression of an inflammatory response under different pathologies like cardiac arrest or liver injury (Yin and O'Neill, 2021). Metformin presents similar outcomes as it inhibits mitochondrial complex I blocking also the reverse electron transport from complex II boosting the anti-inflammatory response (Tsuji et al, 2020). Metformin, a widely utilized type 2 diabetes treatment, is a safe and inexpensive drug, and these features have led investigators to consider evaluating this drug in several studies to extend its potentially beneficial anti-inflammatory effects (Mehta et al, 2017). Interestingly, it was described that its mechanism may be related to the occurrence of mitohormesis and the consequent activation of peroxiredoxins by a mild increase in mitochondrial ROS production (Renis et al, 1996; De Haes et al, 2014).

Another therapeutic strategy aiming to compensate for lack of efficiency in ATP production by increasing cofactors oxidation, decreasing ROS production, and increasing AMPK expression is the uncoupling approach, by use of small molecule mitochondrial uncouplers, genetic methods to increase the expression of uncoupling proteins or compounds that can modulate the activity of uncoupling proteins (Hass and Barnstable, 2021). Protonophores such as DNP and FCCP, are able to pass through the mitochondrial inner membrane and increase the proton transport into the matrix initiating changes in the membrane potential required for the ATP production (Childress et al, 2018) leading to metabolic parameters improvement. Considering that off-target effects, such as depolarization of the plasma membrane, were described after the administration of this compounds (Goedeke and Shulman, 2021), research has focused on controlled release formulation plus specific targeting in order to reduce toxicity (Childress et al, 2018).

6b. Mitochondrial antioxidants and ROS scavengers

Supplementation with several natural antioxidants or compounds with antioxidant activity, such as Coenzyme Q (CoQ), vitamins E, C, and lipoic acid were proved to ameliorate oxidative damage as they provide an electron sink (Vanasco et al, 2008; Oliver and Reddy, 2019). To improve efficiency, compounds like CoQ and vitamin E were covalently linked to the lipophilic triphenylphosphonium cation (MitoQ, MitoVit E) which is electrostatically attached to the negatively charged mitochondrial matrix which confers the ability to cross the phospholipid bilayers redirecting it to the matrix that leads to its accumulation many hundred-fold (Bhatti et al, 2017; Capeloa et al, 2022; Sang et al, 2022). Once inside the mitochondria their mechanism of action has been described as reduction of mtROS levels resulting in not only a decreased oxidative insult to macromolecules but affecting the redox signaling pathways as well (Yan et a., 2020; Weissman and Maack, 2021). Metabolic antioxidants like lipoic acid have shown relatively low toxic effects as they can be included in the cellular redox homeostasis response. However, this also represents a disadvantage as it results in a short period of time where these antioxidants can act as mtROS scavengers (Wallace, 2005; El-Hattab et al, 2017). Preventing oxidative damage using mitochondrial-targeted antioxidants has also been extensively proved as an effective therapy for diabetes and its related chronic inflammation (Rovira-Llopis et al, 2018). Of note, mitohormesis and vitagenes activation should be taken into account when analyzing the effect of the supplementation with antioxidants as it might represent an innovative approach in therapeutic intervention (Calabrese et al, 2008; Calabrese et al, 2010).

In the immunometabolism research field, a new generation of mtROS suppressors are also being developed, showing their ability to improve redox signaling (Mehta et al, 2017). S1QELs and S3QELs, which are novel compounds that selectively prevent the excess of complex I¬dependent and complex III¬dependent ROS production, respectively (Scialo and Sanz, 2021). Therefore, they emerge as potential therapeutic molecules for the treatment of diseases that involve mitochondrial ROS-triggered aberrant inflammation.

6c. Regulation of mitochondrial biogenesis and turnover

As previously described, mitochondrial biogenesis is a fundamental pathway required to maintain a healthy mitochondrial population and proper organelle function with PGC1α as nodal regulator. If intracellular ROS levels increase, PGC1α is directly phosphorylated by AMPK thus mediating mitochondrial biogenesis (Palikaras et al, 2017; Whitley et al, 2019). For example, decreased mRNA expression of several genes associated with oxidative phosphorylation has been described in diabetic patients, including genes regulated by PGC1α and nuclear respiratory factors (Rocha et al, 2020). Metformin promotes the activation of AMPK and PGC1α, and was observed

associated to the reduction of mitochondrial complex I-ROS production (Yan et al, 2020). Besides biogenesis, mitochondrial dynamics also offers regulatory targets. Proper control of mitochondrial dynamics regulating mitochondrial fission and fusion provides a window for therapeutic intervention. Small molecules can modulate Drp1, Opa1 or Mfn activity. Drp1 inhibitor mdivi-1 is the most studied molecule that specifically inhibited the GTPase activity of Dnm1 and abolished its assembly. Treatment with mdivi-1 rebalance mitochondrial morphology in disease models associated with excessive mitochondrial division, improving mitochondrial metabolic function, and reducing the cell death (Bordt et al, 2017; Li et al, 2018; Hernandez-Resendiz et al, 2020;). On the other hand, regulation of the mitochondrial fragmentation machinery with chemical therapeutic approaches such as BGP-15 can also be useful to restore normal mitochondrial dynamics in cells (Szabo et al., 2018). Data obtained in numerous studies suggest that targeting mitochondrial dynamics with small molecules that may directly or indirectly increase mitochondrial fusion activity leads to decreased cell death (Whitley et al, 2019).

There is sufficient evidence *in vitro* and *in vivo* that mitochondria have a relevant role in a wide range of inflammatory diseases. Therefore, as mitochondria can be seen as the powerhouse and regulatory center of immunity, the current aim of pharmacological research studies is to design and evaluate small molecules (Table 1) which are able to modulate the mitochondrial mechanisms involved in cellular immunometabolism. In conclusion, it is important to consider targeting compounds towards mitochondria as the main challenge in order to increase treatments specificity for reducing side effects.

7. CONCLUSIONS AND PERSPECTIVES

Unraveling the different mechanisms by which mitochondria have a relevant role in the inflammatory response beyond energy management of the process, is important for improving our understanding of the host immune defense and the pathogenesis of various inflammatory diseases and syndromes. The relevance of mitochondria as a necessary actor in the inflammatory response should be interpreted and based in its complex nature and the existence of other required actors. Consequently, new questions and interesting aspects arise, such as the involvement of MDV in the immune response with the putative objective of preventing uncontrolled situations.

One intriguing aspect is that mitochondria can be important for the immune response at different levels, including releasing activation molecules, changing its structure and function to accompany the immune response, and serving as a structural base for activating intermediates as in NLRP3 inflammasome. In line with this appreciation, this organelle is plausible of being regulated

and, consequently, emerges as an interesting and multifaceted platform for studying and developing pharmaceutical and therapeutic approaches. There are many ongoing studies aimed to describe the effects of specific mitochondrial targeted molecules and treatments to ameliorate consequences of exacerbated inflammatory components of pathologies and syndromes, resulting in an open area of increasing research interest.

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CONFLICT OF INTEREST

The authors do not present any conflict of interest.

LIST OF ABBREVIATIONS

AIM1 Apoptosis inhibitor of macrophages 1

AMPK Adenosine monophosphate-activated protein kinase

ASC Apoptosis associated speck-like protein containing a CARD

ATP Adenosine triphosphate

BNIP3 BCL2 interaction protein 3

CL Cardiolipin

CoQ Coenzyme Q

cGAS cyclic GMP-AMP synthase

cyt c Cytochrome c

DAMPs Damage-associated molecular patterns

DMM Dimethyl malonate

DNP 2,4-dinitrophenol

dsRNA double stranded RNA

Drp1 Dynamin-related protein 1

ETC Electron transport chain

FAO Fatty acid oxidase

FAS Fatty acid synthase

FCCP p-trifluoromethoxyphenylhydrazone

Fis 1 Mitochondrial fission 1 protein

HIF1α Hypoxia inducible factor 1α

IFNγ Interferon γ

IL Interleukin

Antioxidants and Redox Signaling
MITOCHONDRIAL MECHANISMS IN IMMUNITY AND INFLAMMATORY CONDITIONS: BEYOND ENERGY MANAGEMENT (DOI: 10.1089/ars.2023.0367)

IMM Inner mitochondrial membrane

LC3 Microtubule-associated protein 1A/1B-light chain 3

LPS Lipopolysaccharide

MAMs Mitochondrial-associated membranes

MAVS Mitochondrial antiviral signaling protein

MDVs Mitochondrial-derived vesicles

Mfn Mitofusin

mtDNA Mitochondrial deoxyribonucleic acid

mtPTP Mitochondrial transition permeability pore

mtROS mitochondrial reactive oxygen species

NFkB Nuclear factor kappa-light-chain-enhancer of activated B cells

NFAT Nuclear factor of activated T cells

NLR Nuclear oligomerization domain-like receptor

NLRP Nod–Like Receptor Protein

NLRC4 Nod-like receptor card domain 4

Nrf1/2 Nuclear respiratory factor 1/2

OMM Outer mitochondrial membrane

Opa1 Optic atrophy 1

PDHC Piruvate dehydrogenase complex

PDK Piruvate dehydrogenase kinase

PGC1α Proliferator-activated receptor coactivator 1α

PHD Prolyl hydroxylase

Antioxidants and Redox Signaling
MITOCHONDRIAL MECHANISMS IN IMMUNITY AND INFLAMMATORY CONDITIONS: BEYOND ENERGY MANAGEMENT (DOI: 10.1089/ars.2023.0367)

PINK1 PTEN-induced kinase 1

PRR Pattern recognition receptor

RET Reverse electron transfer

RISP Rieske iron-sulfur protein

RLR RIG I-like helicases receptor

ROS Reactive oxygen species

SESN2 Sestrin 2

SIRT1 Sirtuin 1

STING Stimulator of interferon genes

TCA Tricarboxylic acid cycle

TCR T cell receptor

TFAM Mitochondrial respiratory factor A

TLR Toll-like receptors

TNFα Tumor necrosis factor α

VMP1 Vacuole membrane protein 1

REFERENCES

Acín-Pérez R, Hernansanz-Agustín P and Enríquez JA. Analyzing electron transport chain supercomplexes. Methods Cell Biol 2020;155:181–197; doi: 10.1016/BS.MCB.2019.12.002.

Adán Areán JS, Vico TA, Marchini T, et al. Energy management and mitochondrial dynamics in cerebral cortex during endotoxemia. Arch Biochem Biophys 2021;705:108900-108910; doi: 10.1016/j.abb.2021.108900.

Almeida L, Dhillon-LaBrooy A, Carriche G, et al. $CD4^+$ T-Cell differentiation and function: unifying glycolysis, fatty acid oxidation, polyamines NAD mitochondria. J Allergy Clin Immunol 2021;148(1):16–32; doi: 10.1016/J.JACI.2021.03.033.

Andersson SGE, Karlberg O, Canbäck B, et al. On the origin of mitochondria: a genomics perspective. Philos Trans R Soc Lond B Biol Sci 2003;358(1429):165–179; doi: 10.1098/RSTB.2002.1193.

Andrieux P, Chevillard C, Cunha-Neto E, et al. Mitochondria as a celular hub in infection and inflammation. Int J Molec Sci 2021; 22:11338-11367; doi: 10.3390/ijms222111338.

Angireddy R, Kazmi HR, Srinivasan S, et al. Cytochrome c oxidase dysfunction enhances phagocytic function and osteoclast formation in macrophages. FASEB J 2019;33(8):9167–9181; doi: 10.1096/FJ.201900010RR.

Ansó E, Weinberg SE, Diebold LP, et al. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. Nat Cell Biol 2017;19(6):614–625; doi: 10.1038/ncb3529.

Bailis W, Shyer JA, Zhao J, et al. Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. Nature 2019;571(7765):403–407; doi: 10.1038/s41586-019-1311-3.

Bereiter-Hahn J and Vöth M. Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 1994;27(3):198–219; doi: 10.1002/jemt.1070270303.

Bhatti JS, Bhatti GK and Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders — a step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis 2017;1863(5):1066–1077; doi: 10.1016/j.bbadis.2016.11.010.

Billingham LK, Stoolman JS, Vasan K, et al. Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. Nat Immunol 2022;23(5):692–704; doi: 10.1038/S41590-022-01185-3.

Bordt EA, Clerc P, Roelofs BA, et al. The putative Drp1 inhibitor Mdivi-1 is a reversible mitochondrial complex I inhibitor that modulates reactive oxygen species. Dev Cell 2017;40(6):583-594; doi: 10.1016/j.devcell.2017.02.020.

Bouhamida E, Morciano G, Perrone M, et al. The interplay of hypoxia signaling on mitochondrial dysfunction and inflammation in cardiovascular diseases and cancer: from molecular mechanisms to therapeutic approaches. Biology (Basel) 2022;11(2):300-332; doi: 10.3390/biology11020300.

Brokatzky D, Hacker G. Mitochondria: intracellular sentinels of infections. Med Microbiol Immunol 2022;211:161-172; doi: 10.1007/s00430-022-00742-9.

Buck MD, O´Sullivan D, Klein Geltink RI, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. Cell 2016;166:63-76; doi:10.1016/j.cell.2016.05.035.

Calabrese V, Mancuso C, Calvani M, et al. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nature Rev 2007;8:766-775; doi:10.1038/nm2214.

Calabrese V, Cornelius C, Dinkova-Kostova A, et al. Vitagenes, celular stress response, and acetylcarnityne: relevance to hormesis. Biofactors 2009; 35(2):146-160; doi:10.1002/biof.22.

Calabrese V, Cornelius C, Dinkova-Kostova A, et al. Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. Antioxid Redox Signal 2010;13(11):1763-1811; doi:10.1089/ars.2009.3074.

Capeloa T, van de Velde JA, d'Hose D, et al. Inhibition of mitochondrial redox signaling with MitoQ prevents metastasis of human pancreatic cancer in mice. Cancers (Basel) 2022;14(19):4918-4938; doi: 10.3390/cancers14194918.

Chang CH, Curtis JD, Maggi LB, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 2013;153(6):1239-1251; doi: 10.1016/j.cell.2013.05.016.

Chaplin DD. Overview of the immune response. J Allergy Clin Immunol 2010;125:S3-23; doi:10.1016/j.jaci.2009.12.980.

Chelliappan DK, Paudel KR, Tan NW, et al. Targeting the mitochondria in chronic respiratory diseases. Mitochondrion 2022; 67: 15-37; doi: 10.1016/j.mito.2022.09.003.

Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2017;9(6):7204–7218; doi: 10.18632/oncotarget.23208.

Chicco AJ and Sparagna GC. Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am J Physiol Cell Physiol 2007;292(1);33-44; doi: 10.1152/ajpcell.00243.2006.

Childress ES, Alexopoulos SJ, Hoehn KL, et al. Small molecule mitochondrial uncouplers and their therapeutic potential. J Med Chem 2018;61(11):4641–4655; doi: 10.1021/acs.jmedchem.7B01182.

Cipolat S, de Brito OM, Dal Zilio B, et al. OPA1 requires Mitofusin 1 to promote mitochondrial fusion. Proc Natl Acad Sci U S A 2004;101(45):15927–15932; doi: 10.1073/pnas.0407043101.

Claypool SM and Koehler CM. The complexity of cardiolipin in health and disease. Trends Biochem Sci 2012;37(1):32–41; doi: 10.1016/j.tibs.2011.09.003.

dela Cruz CS and Kang MJ. Mitochondrial dysfunction and damage associated molecular patterns (DAMPs) in chronic inflammatory diseases. Mitochondrion 2018;41:37–44; doi: 10.1016/j.mito.2017.12.001.

De Haes W, Frooninckx L, Van Assche R, et al. Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. Proc Natl Acad Sci U S A 2014;111(24):E2501-E2509; doi:10.1073/pnas.1321776111.

Detmer SA and Chan DC. Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 2007;8(11):870–879; doi: 10.1038/NRM2275.

Dhir A, Dhir S, Borowski LS, et al. Mitochondrial double-stranded RNA triggers antiviral signaling in human. Nature 2018; 560:238-242; doi: 10.1038/s41586-018-0363-0.

Diao R, Gustafsson AB. Mitochondrial quality surveillance: mitophagy in cardiovascular health and disease. Am J Physiol Cell Physiol 2022: 322:C218-C230; doi: 10.1152/ajpcell.00360.2021.

Dieudé M, Striegl H, Tyznik AJ, et al. Cardiolipin binds to CD1d and stimulates CD1d-restricted δ T cells in the normal murine repertoire. J Immunol 2011;186(8):4771–4781; doi: 10.4049/JIMMUNOL.1000921.

Doran AC, Yurdogul A, and Tabas I. Efferocytosis in health and disease. Nat Rev Immunol 2020; 20(4):254-267; doi:10.1038/s41577-019-0240-6.

Dudek J. Role of cardiolipin in mitochondrial signaling pathways. Front Cell Dev Biol 2017;5:90-107; doi: 10.3389/fcell.2017.00090.

Dumauthioz N, Tschumi B, Wenes M, et al. Enforced PGC-1α expression promotes CD8 T cell fitness, memory formation and antitumor immunity. Cell Mol Immunol 2021;18(7):1761–1771; doi: 10.1038/S41423-020-0365-3.

El-Hattab AW, Zarante AM, Almannai M, et al. Therapies for mitochondrial diseases and current clinical trials. Mol Genet Metab 2017;122(3):1–9; doi: 10.1016/j.ymgme.2017.09.009.

Faas MM, Sáez T and de Vos P. Extracellular ATP and adenosine: the yin and yang in Immune responses? Mol Aspects Med 2017;55:9–19; doi: 10.1016/j.mam.2017.01.002.

Fischer F, and Ristrow M. Endogenous metabolites promote stress resistance through induction of mitohormesis. EMBO Rep 2020;21(5):e50340; doi: 10.15252/embr.202050340.

Fucikova J, Moserova I, Urbanova L, et al. Prognostic and predictive value of DAMPs and DAMP-associated processes in cancer. Front Immunol 2015;6:402-419; doi: 10.3389/fimmu.2015.00402.

de Gaetano A, Solodka K, Zanini G, et al. Molecular mechanisms of mtDNA-mediated inflammation. Cells 2021;10(11):2898-2919; doi: 10.3390/cells10112898.

Gao A, Jiang J, Xie F, et al. Bnip3 in mitophagy: novel insights and potential therapeutic target for diseases of secondary mitochondrial dysfunction. Clin Chim Acta 2020;506:72–83; doi: 10.1016/j.cca.2020.02.024.

Gao F, Reynolds MB, Passalacqua KD, et al. The mitochondrial fission regulator DRP1 controls post-transcriptional regulation of TNF-α. Front Cell Infect Microbiol 2021;10:593805-593819; doi: 10.3389/fcimb.2020.593805.

Ge P, Dawson VL, Dawson TM. PINK1 and Parkin mitochondrial quality control: a source of regional vulnerability in Parkinson's disease. Mol Neurodegener 2020;15(1):20-38; doi: 10.1186/s13024-020-00367-7.

Geto Z, Molla MD, Challa F, et al. Mitochondrial dynamic dysfunction as a main triggering factor for inflammation associated chronic non-communicable diseases. J Inflamm Res 2020;13:97–107; doi: 10.2147/JIR.S232009.

Giacomello M, Pyakurel A, Glytsou C, et al. The cell biology of mitochondrial membrane dynamics. Nat Rev Mol Cell Biol 2020;21(4):204–224; doi: 10.1038/S41580-020-0210-7.

Gilkerson R, Materon L. Two roads converging: mitochondria and inflammatory signaling. Clin Immuno Immunother 2014;1(1):1–7; doi: 10.24966/ciit-8844/100004.

Gkikas I, Palikaras K, Tavernarakis N. The role of mitophagy in innate immunity. Front Immunol 2018;9:1283-1298; doi: 10.3389/fimmu.2018.01283.

Glancy B, Kim Y, Katti P, et al. The functional impact of mitochondrial structure across subcellular scales. Front Physiol 2020;11:541040-541064; doi: 10.3389/fphys.2020.541040.

Goedeke L, Shulman GI. Therapeutic potential of mitochondrial uncouplers for the treatment of metabolic associated fatty liver disease and NASH. Mol Metab 2021;46:101178-101192; doi:10.1016/j.molmet.2021.101178.

Gong T, Liu L, Jiang W, et al. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol 2020;20(2):95–112; doi: 10.1038/S41577-019-0215-7.

Grazioli S, Pugin J. Mitochondrial damage-associated molecular patterns: from inflammatory signaling to human diseases. Front Immunol 2018;9:832-849; doi: 10.3389/fimmu.2018.00832.

Griffiths HR, Gao D, Pararasa C. Redox regulation in metabolic programming and inflammation. Redox Biol 2017;12:50–57; doi: 10.1016/j.redox.2017.01.023.

Guak H, Sheldon RD, Bedows I, et al. PGC-1β maintains mitochondrial metabolism and restrains inflammatory gene expression. Sci Rep 2022; 12:16028; doi: 10.1038/s41598-022-20215-6.

Guitton R, Dölle Ch, Alves G, et al. Ultra-deep whole genome bisulphite sequencing reveals a single methylation hotspot I human brain mitochondrial DNA. Epigenetics 2022; 17(8):906-921; doi; 10.1080/15592294.2022.2045754.

Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J 2001;353:411–416; doi: 10.1042/0264-6021:3530411.

Haschemi A, Kosma P, Gille L, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metab 2012;15(6):813–826; doi: 10.1016/j.cmet.2012.04.023.

Hass DT, Barnstable CJ. Uncoupling proteins in the mitochondrial defense against oxidative stress. Prog Retin Eye Res 2021;83:100941-100966; doi: 10.1016/j.preteyeres.2021.100941.

Hernandez-Resendiz S, Prunier F, Girao H, et al. Targeting mitochondrial fusion and fission proteins for cardioprotection. J Cell Mol Med. 2020;24(12):6571-6585; doi:10.1111/jcmm.15384.

Hsu CG, Li W, Sowden M, et al. Pnpt mediates NLRP3 inflammasome activation by MAVS and metabolic reprogramming in macrophages. Cell Mol Immunol 2023; 20:131-142; doi: 10.1038/s41423-022-00962-2.

Hu H, Guo L, Overholser J, et al. Mitochondrial VDAC1: a potential therapeutic target of inflammation-related diseases and clinical opportunities. Cells 2022; 11: 3174-3200; doi: 10.3390/cells11193174.

Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. Cell Mol Immunol 2021;18(9):2114–2127; doi: 10.1038/S41423-021-00740-6.

Huckriede J, Anderberg SB, Morales A, et al. Evolution of NETosis markers and DAMPs have prognostic value in critically ill COVID-19 patients. Sci Rep 2021;11(1):15701-15719; doi: 10.1038/S41598-021-95209-X.

Ichinohe T, Yamazaki T, Koshiba T, et al. Mitochondrial protein Mitofusin 2 is required for NLRP3 inflammasome activation after RNA virus infection. Proc Natl Acad Sci USA 2013;110(44):17963–17968; doi: 10.1073/pnas.1312571110.

Irazoki A, Martinez-Vicente M, Aparicio P, et al. Coordination of mitochondrial and lysosomal homeostasis mitigates inflammation and muscle atrophy during aging. Aging Cell 2022;21(4):e13583; doi: 10.1111/acel.13583.

Islam MN, Das SR, Emin MT, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung Injury. Nat Med 2012;18(5):759–765; doi: 10.1038/nm.2736.

Iyer SS, He Q, Janczy JR, et al. Mitochondrial cardiolipin is required for NLRP3 inflammasome activation. Immunity 2013;39(2):311–323; doi: 10.1016/j.immuni.2013.08.001.

Jha AK, Huang SCC, Sergushichev A, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity 2015;42(3):419–430; doi: 10.1016/j.immuni.2015.02.005.

Jin SM, Lazarou M, Wang C, et al. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. J Cell Biol 2010;191(5):933–942; doi: 10.1083/JCB.201008084.

Jo EK, Kim JK, Shin DM, et al. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 2016;13(2):148–159; doi: 10.1038/cmi.2015.95.

Jones DP, Sies H. The redox code. Antioxid Redox Signal 2015;23(9):734–746; doi: 10.1089/ars.2015.6247.

Katsumi T, Guicciardi ME, Azad A, et al. Activated cholangiocytes release macrophage-polarizing extracellular vesicles bearing the DAMP S100A11. Am J Physiol Cell Physiol 2019;317(4):C788–C799; doi: 10.1152/ajpcell.00250.2019.

Ke L, Li Q, Song J, et al. The mitochondrial biogenesis signaling pathway is a potential therapeutic target for myasthenia gravis via energy metabolism. Exp Ther Med 2021;22(1):e13583; doi: 10.3892/etm.2021.10134.

Kim MJ, Bae SH, Ryu JC, et al. SESN2/Sestrin2 suppresses sepsis by inducing mitophagy and inhibiting NLRP3 activation in macrophages. Autophagy 2016;12(8):1272–1291; doi: 10.1080/15548627.2016.1183081.

Kondapalli C, Kazlauskaite A, Zhang N, et al. PINK1is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating serine 65. Open Biol 2012;2(5):120080-120097; doi: 10.1098/rsob.120080.

Lee H, Jeon J, Lee YJ, et al. Inhibition of pyruvate dehydrogenase kinase in $CD4^+$ T cells ameliorates intestinal inflammation. Cell Molec Gast Hepatol 2022 in press; doi: 10.1016/j.jcmgh.2022.09.016.

Li H, Slone J, Fei L, et al. Mitochondrial DNA variants and common diseases: a mathematical model for the diversity of age-related mtDNA mutations. Cells 2019;8(6):608-629; doi: 10.3390/cells8060608.

Li N, Wang H, Jiang C, et al. Renal ischemia/reperfusion-induced mitophagy protects against renal dysfunction via Drp1-dependent-pathway. Exp Cell Res 2018;369(1):27–33; doi: 10.1016/j.yexcr.2018.04.025.

Linkermann A, Green DR. Necroptosis. N Engl J Med 2014;370(5):455–465; doi: 10.1056/nejmra1310050.

Liu J, Wang T, He K, et al. Cardiolipin inhibitor ameliorates the non-alcoholic steatohepatitis through suppressing NLRP3 inflammasome activation. Eur Rev Med Pharmacol Sci 2019;23(18):8158–8167; doi: 10.26355/eurrev-201909-19036.

López-Armada MJ, Riveiro-Naveira RR, Vaamonde-García C, et al. Mitochondrial dysfunction and the inflammatory response. Mitochondrion 2013;13(2):106–118; doi: 10.1016/j.mito.2013.01.003.

Luo J, Liu H, Li DKJ, et al. Repression of the expression of proinflammatory genes by mitochondrial transcription factor A is linked to its alternative splicing regulation in human lung epithelial cells. BMC Immunol 2021;22(1):74-88; doi: 10.1186/s12865-021-00464-2.

Magnani ND, Marchini T, Calabró V, et al. Role of mitochondria in the redox signaling network and its outcomes in high impact inflammatory syndromes. Front Endocrinol (Lausanne) 2020;11:568305-568320; doi: 10.3389/fendo.2020.568305.

Mah-Som AY, Keppel MP, Tobin JM, et al. Reliance on Cox10 and oxidative metabolism for antigen-specific NK cell expansion. Cell Rep 2021;35(9):109209-109227; doi: 10.1016/j.celrep.2021.109209.

Majeed Y, Halabi N, Madani AY, et al. SIRT1 promotes lipid metabolism and mitochondrial biogenesis in adipocytes and coordinates adipogenesis by targeting key enzymatic pathways. Sci Rep 2021;11(1):8177-8196; doi: 10.1038/s41598-021-87759-x.

Mansfield KD, Guzy RD, Pan Y, et al. Mitochondrial dysfunction resulting from loss of cytochrome c impairs cellular oxygen sensing and hypoxic HIF-alpha activation. Cell Metab 2005;1(6):393–399; doi: 10.1016/j.cmet.2005.05.003.

Marchi S, Guilbaud E, Tait S, et al. Mitochondrial control of inflammation. Nat Rev Immunol 2022; 25:1-15; doi: 10.1038/s41577-022-00760-x.

McLelland GL, Soubannier V, Chen CX, et al. Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. EMBO J 2014;33(4):282–295; doi: 10.1002/embj.201385902.

Mechta M, Ingerslev LR, Fabre O, et al. Evidence suggesting absence of mitochondrial DNA methylation. Front Genet 2017; 8:166; doi: 10.3389/fgene.2017.00166.

Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454:428-535; doi:10.1038/nature07201.

Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. Nat Rev Immunol 2017;17(10):608–620; doi: 10.1038/nri.2017.66.

Mills EL, Kelly B, Logan A, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 2016;167(2):457-470; doi: 10.1016/j.cell.2016.08.064.

Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. Nat Immunol 2017;18(5):488–498; doi: 10.1038/NI.3704.

Misawa T, Takahama M, Kozaki T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol 2013;14(5):454–460; doi: 10.1038/NI.2550.

Murao A, Aziz M, Wang H, et al. Release mechanisms of major DAMPs. Apoptosis 2021;26(3–4):152–162; doi: 10.1007/s10495-021-01663-3.

Nakahira K, Haspel JA, Rathinam VAK, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 2011;12(3):222–230; doi: 10.1038/ni.1980.

Nakahira K, Hisata S, Choi AMK. The roles of mitochondrial damage-associated molecular patterns in diseases. Antioxid Redox Signal 2015;23(17):1329–1350; doi: 10.1089/ars.2015.6407.

Narendra D, Tanaka A, Suen DF, et al. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 2008;183(5):795–803; doi: 10.1083/jcb.200809125.

Nathan C, Ding A. Nonresolving inflammation. Cell 2010;140:871-882; doi:10.1016/j.cell.2010.02.029.

Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. Nat Immunol 2017;18(8):826–831; doi: 10.1038/ni.3790.

Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol 2020;20:375-388; doi:10.1038/s41577-020-0285-6.

Neuspiel M, Schauss AC, Braschi E, et al. Cargo-selected transport from the mitochondria to peroxisomes is mediated by vesicular carriers. Curr Biol 2008;18(2):102–108; doi: 10.1016/j.cub.2007.12.038.

Newman LE, Shadel GS. Pink1/Parkin link inflammation, mitochondrial stress, and neurodegeneration. J Cell Biol 2018;217(10):3327–3329; doi: 10.1083/jcb.201808118.

Nicolas-Avila JA, Lechuga-Vieco AV, Esteban-Martinez L, et al. A network of macrophages supports mitochondrial homeostasis in the heart. Cell 2020; 183:94-109; doi: 10.1016/j.cell.2020.08.031.

Nolfi-Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biol 2020;37:101674-101683; doi: 10.1016/j.redox.2020.101674.

Nomura M, Liu J, Rovira I, et al. Fatty acid oxidation in macrophage polarization. Nature 2016; 17:216-218; doi: 10.1038/ni.3366.

Oh CK, Sultan A, Platzer J, et al. S-Nitrosylation of PINK1 attenuates PINK1/Parkin-dependent mitophagy in HiPSC-based Parkinson's disease models. Cell Rep 2017;21(8):2171–2182; doi: 10.1016/j.celrep.2017.10.068.

Oliver DMA, Reddy PH. Small molecules as therapeutic drugs for Alzheimer's disease. Mol Cell Neurosci 2019;96:47–62; doi: 10.1016/j.mcn.2019.03.001.

O´Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. Nature Rev 2016;16(9):553-565; doi: 10.1038/nri.2016.70.

O´Neill LA, and Artyomov M. Itaconate: the poster child of metabolic reprogramming in macrophage function. Nature Rev 2019;19(5):273-281; doi: 10.1038/s41577-019-0128-5.

Pajak A, Laine I, Clemente P, et al. Defects of mitochondrial RNA turnover lead to the accumulation of double-stranded RNA in vivo. Plos Genet 2019; 15(7)e1008240; doi: 10.1371/journal.pgen.1008240.

Palikaras K, Daskalaki I, Markaki M, et al. Mitophagy and age-related pathologies: development of new therapeutics by targeting mitochondrial turnover. Pharmacol Ther 2017;178:157–174; doi: 10.1016/j.pharmthera.2017.04.005.

Pandey A, Shen C, Feng S, et al. Cell biology of inflammasome activation. Trends Cell Biol 2021;31(11):924–939; doi: 10.1016/j.tcb.2021.06.010.

Park S, Won JH, Hwang I, et al. Defective mitochondrial fission augments NLRP3 inflammasome activation. Sci Rep 2015;5:15489-15503; doi: 10.1038/srep15489.

Patergnani S, Vitto V, Pinton P, et al. Mitochondrial stress responses and “mito-inflammation” in cystic fibrosis. Front Pharmacol 2020; 11: 581114-581126: doi: 10.3389/fphar.2020.581114.

Peng T, Xie Y, Sheng H, et al. Mitochondrial-derived vesicles: gatekeepers of mitochondrial response to oxidative stress. Free Radic Biol Med 2022;188:185–193; doi: 10.1016/j.freeradbiomed.2022.06.233.

Pereira AC, de Pascale J, Resende R, et al. ER-mitochondria communication is involved in NLRP3 inflammasome activation under stress conditions in the innate immune system. Cell Mol Life Sci 2022;79(4):213; doi: 10.1007/s00018-022-04211-7.

Preau S, Vodovar D, Jung B, et al. Energetic dysfunction in sepsis: a narrative review. Ann Int Care 2021; 11:104-125; doi: 10.1186/s13613-021-00893-7.

Qin J, Zhang X, Tan B, et al. Blocking P2X7-mediated macrophage polarization overcomes treatment resistance in lung cancer. Cancer Immunol Res 2020;8(11):1426–1439; doi: 10.1158/2326-6066.cir-20-0123.

Rathinam VAK, Fitzgerald KA. Inflammasome complexes: emerging mechanisms and effector functions. Cell 2016:165:792-800; doi:10.1016/j.cell.2016.03.046.

Renis M, Calabrese V, Russo A, et al. Nuclear DNA strand breaks during ethanol-induced oxidative stress in rat brain. FEBS Lett 1996;390:153-156; doi:10.1016/0014-5793(96)00647-3.

Rice M, Wong B, Oja M, et al. A role of flavonoids in cytochrome c-cardiolipin interactions. Bioorg Med Chem 2021;33:116043; doi: 10.1016/j.bmc.2021.116043.

Rocha M, Apostolova N, Diaz-Rua R, et al. Mitochondria and T2D: role of autophagy, ER stress, and inflammasome. Trends Endocrinol & Metab 2020;31:725-741; doi:10.1016/j.tem.2020.03.004.

Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. Immune Netw 2018;18(4):e27; doi: 10.4110/in.2018.18.e27.

Ron-Harel N, Santos D, Ghergurovich JM, et al. Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation. Cell Metab 2016; 24:104-117; doi: 10.1016/j.cmet.2016.06.007.

Rovira-Llopis S, Apostolova N, Bañuls C, et al. Mitochondria, the NLRP3 inflammasome, and sirtuins in type 2 diabetes: new therapeutic targets. Antioxid Redox Signal 2018;29:749-791; doi:10.1089/ars.2017.7313.

Saeb-Parsy K, Martin JL, Summers DM, et al. Mitochondria as therapeutic targets in transplantation. Trends Mol Med 2021;27(2):185–198; doi: 10.1016/j.molmed.2020.08.001.

Sánchez-Rodríguez R, Tezze C, Agnellini AHR, et al. OPA1 drives macrophage metabolism and functional commitment via P65 signaling. Cell Death Differ 2022; doi: 10.1038/S41418-022-01076-y.

Sancho D, Enamorado M, Garaude J. Innate immune function of mitochondrial metabolism. Front Immunol 2017;8:527; doi: 10.3389/fimmu.2017.00527.

Sandhir R, Halder A, Sunkaria A. Mitochondria as a centrally positioned hub in the innate immune response. Biochim Biophys Acta Mol Basis Dis 2017;1863(5):1090–1097; doi: 10.1016/j.bbadis.2016.10.020.

Sang W, Chen S, Lin L, et al. Antioxidant mitoquinone ameliorates EtOH-LPS induced lung injury by inhibiting mitophagy and NLRP3 inflammasome activation. Front Immunol 2022; 13: 973108-97310826; doi: 10.3389/fimmu.2022.973108.

Santel A, Fuller MT. Control of mitochondrial morphology by a human mitofusin. J Cell Sci 2001;114(Pt 5):867–874; doi: 10.1242/jcs.114.5.867.

Schumann U, Subramani S. Special delivery from mitochondria to peroxisomes. Trends Cell Biol 2008;18(6):253–256; doi: 10.1016/j.tcb.2008.04.002.

Schunk SJ, Kleber ME, März W, et al. Genetically determined NLRP3 inflammasome activation associates with systemic inflammation and cardiovascular mortality. Eur Heart J 2021;42(18):1742–1756; doi: 10.1093/eurheartj/ehab107.

Scialò F, Fernández-Ayala DJ, Sanz A. Role of mitochondrial reverse electron transport in ROS signaling: potential roles in health and disease. Front Physiol 2017;8: 428-435; doi: 10.3389/fphys.2017.00428.

Scialo F, Sanz A. Coenzyme Q redox signalling and longevity. Free Radic Biol Med 2021;164:187–205; doi: 10.1016/j.freeradbiomed.2021.01.018.

Sena LA, Li S, Jairaman A, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 2013;38(2):225–236; doi: 10.1016/j.immuni.2012.10.020.

Shimada K, Crother TR, Karlin J, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity 2012;36:401-414; doi: 10.1016/j.immuni.2012.01.009.

Smirnova E, Griparic L, Shurland DL, et al. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol Biol Cell 2001;12(8):2245–2256; doi: 10.1091/mbc.12.8.2245.

Stanzani G, Duchen MR, Singer M. The role of mitochondria in sepsis-induced cardiomyopathy. Biochim Biophys Acta Mol Basis Dis 2019;1865(4):759–773; doi: 10.1016/j.bbadis.2018.10.011.

Steinert EM, Vasan K, Chandel NS. Mitochondrial metabolism regulation of T cell-mediated immunity. Annu Rev Immunol 2021;39:395–416; doi: 10.1146/annurev-immunol-101819-082015.

Subramanian N, Natarajan K, Clatworthy MR, et al. The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation. Cell 2013;153(2):348–361; doi: 10.1016/j.cell.2013.02.054.

Suen DF, Narendra DP, Tanaka A, et al. Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic cybrid cells. Proc Natl Acad Sci U S A 2010;107(26):11835–11840; doi: 10.1073/pnas.0914569107.

Sugiura A, Andrejeva G, Voss K, et al. MTHFD2 is a metabolic checkpoint controlling efector and regulatory T cell fate and function. Immunity 2022;55:65-81; doi:10.1016/j.immuni.2021.10.011.

Sugiura A, McLelland G, Fon EA, et al. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. EMBO J 2014;33(19):2142–2156; doi: 10.15252/embj.201488104.

Sullivan LB, Gui DY, Hosios AM, et al. Supporting aspartate biosynthesis is an essential function of respiration in proliferating cells. Cell 2015;162(3):552–563; doi: 10.1016/j.cell.2015.07.017.

Swanson K v., Deng M, Ting JPY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 2019;19(8):477–489; doi: 10.1038/s41577-019-0165-0.

Szabo A, Sumegi K, Fekete K, et al. Activation of mitochondrial fusion provides a new treatment for mitochondria-related diseases. Biochem Pharmacol 2018;150:86–96; doi: 10.1016/j.bcp.2018.01.038.

Tan H, Yang K, Li Y, et al. Integrative proteomics and phosphoproteomics profiling reveals dynamic signaling networks and bioenergetics pathways underlying T cell activation. Immunity 2017;46(3):488–503; doi: 10.1016/j.immuni.2017.02.010.

Tang X, Luo YX, Chen HZ, et al. Mitochondria, endothelial cell function, and vascular diseases. Front Physiol 2014;5; doi: 10.3389/fphys.2014.00175.

Tannahill GM, Curtis AM, Adamik J, et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature 2013;496(7444):238–242; doi: 10.1038/nature11986.

Tiku V, Tan MW, Dikic I. Mitochondrial functions in infection and immunity. Trends Cell Biol 2020;30(9):748; doi: 10.1016/j.tcb.2020.07.001.

Timblin GA, Tharp KM, Ford B, et al. Mitohormesis reprograms macrophage metabolism to enforce tolerance. Nat Metab 2021;3(5):618-635; doi: 10.1038/s42255-021-00392-w.

Todkar K, Chikhi L, Desjardins V, et al. Selective packaging of mitochondrial proteins into extracellular vesicles prevents the release of mitochondrial DAMPs. Nat Commun 2021;12(1):1971; doi: 10.1038/s41467-021-21984-w.

Tsuji G, Hashimoto-Hachiya A, Yen VH, et al. Metformin inhibits IL-1β secretion via impairment of NLRP3 inflammasome in keratinocytes: implications for preventing the development of psoriasis. Cell Death Discov 2020;6(1):11; doi: 10.1038/S41420-020-0245-8.

Van der Windt GJ, Everts B, Chang Ch, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 2012; 36:68-78; doi: 10.1016/j.immunol.2011.12.007.

Vanasco V, Cimolai MC, Evelson P, et al. The oxidative stress and the mitochondrial dysfunction caused by endotoxemia are prevented by α-lipoic acid. Free Radic Res 2008;42(9):815–823; doi: 10.1080/10715760802438709.

Vanasco V, Ropolo A, Grasso D, et al. Mitochondrial dynamics and VMP1-related selective mitophagy in experimental acute pancreatitis. Front Cell Dev Biol 2021;9:640094; doi: 10.3389/fcell.2021.640094.

Vanasco V, Saez T, Magnani ND, et al. Cardiac mitochondrial biogenesis in endotoxemia is not accompanied by mitochondrial function recovery. Free Radic Biol Med 2014;77:1–9; doi: 10.1016/j.freeradbiomed.2014.08.009.

Vats D, Mukundan L, Odegaard JI, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab 2006;4(1):13–24; doi: 10.1016/j.cmet.2006.05.011.

Vercellino I, Sazanov LA. The assembly, regulation and function of the mitochondrial respiratory chain. Nat Rev Mol Cell Biol 2022;23(2):141–161; doi: 10.1038/S41580-021-00415-0.

Vico TA, Marchini T, Ginart S, et al. Mitochondrial bioenergetics links inflammation and cardiac contractility in endotoxemia. Basic Res Cardiol 2019;114(5):38; doi: 10.1007/s00395-019-0745-y.

Vincow ES, Merrihew G, Thomas RE, et al. The PINK1-Parkin pathway promotes both mitophagy and selective respiratory chain turnover in vivo. Proc Natl Acad Sci USA 2013;110(16):6400–6405; doi: 10.1073/pnas.1221132110.

Wai T, Langer T. Mitochondrial dynamics and metabolic regulation. Trends Endocrinol Metab 2016;27(2):105–117; doi: 10.1016/j.tem.2015.12.001.

Walker MA, Slate N, Alejos A, et al. Predisposition to infection and SIRS in mitochondrial disorders: 8 year experience in an academic center. J Allergy Clin Immunol Pract 2014; 2(4):465-468; doi: 10.1016/j.jaip.2014.02.009.

Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet 2005;39:359–407; doi: 10.1146/annurev.genet.39.110304.095751.

Wallace DC, Fan W, Procaccio V. Mitochondrial energetics and therapeutics. Annu Rev Pathol 2010;5:297–348; doi: 10.1146/annurev.pathol.4.110807.092314.

Wang R, Dillon CP, Shi LZ, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 2011;35(6):871–882; doi: 10.1016/j.immuni.2011.09.021.

Wang Y, Subramanian M, Yurdagul A, et al. Mitochondrial fission promotes the continued clearance of apoptotic cells by macrophages. Cell 2017; 171(2):331-345; doi:10.1016/j.cell.2017.08.041.

Weinberg SE, Singer BD, Steinert EM, et al. Mitochondrial complex III is essential for suppressive function of regulatory T cells. Nature 2019;565(7740):495–499; doi: 10.1038/S41586-018-0846-Z.

Weissman D, Maack C. Redox signaling in heart failure and therapeutic implications. Free Radic Biol Med 2021;171:345–364; doi: 10.1016/j.freeradbiomed.2021.05.013.

West AP, Khaury-Hanold W, Staron M, et al. Mitochondrial DNA stress primes the antiviral innate immune response. Nature 2015;520:553-557; doi: 10.1038/nature14156.

West AP, Shadel GS. Mitochondrial DNA in innate immune responses and inflammatory pathology. Nat Rev Immunol 2017;17:363-375; doi:10.1038/nri.2017.21.

Whitley BN, Engelhart EA, Hoppins S. Mitochondrial dynamics and their potential as a therapeutic target. Mitochondrion 2019;49:269–283; doi: 10.1016/j.mito.2019.06.002.

Wong HS, Dighe PA, Mezera V, et al. Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions. J Biol Chem 2017; 292(41):16804–16809; doi:I 10.1074/jbc.R117.789271.

Xia Y, Gao B, Zhang X. Targeting mitochondrial quality control of T cells: regulating the immune response in HCC. Front Oncol 2022; 12: 993437-993451; doi: 10.3389/fonc.2022.993437.

Xian H, Liu Y, Rundberg Nilsson A, et al. Metformin inhibition of mitochondrial ATP and DNA synthesis abrogates NLRP3 inflammasome activation and pulmonary inflammation. Immunity 2021;54(7):1463-1477.e11; doi: 10.1016/j.immuni.2021.05.004.

Xu J, Pan H, Xie X, et al. Inhibiting succinate dehydrogenase by dimethyl malonate alleviates brain damage in a rat model of cardiac arrest. Neuroscience 2018;393:24–32; doi: 10.1016/j.neuroscience.2018.09.041.

Yan J, Jiang J, He L, et al. Mitochondrial superoxide/hydrogen peroxide: an emerging therapeutic target for metabolic diseases. Free Radic Biol Med 2020;152:33–42; doi: 10.1016/j.freeradbiomed.2020.02.029.

Yang J, Griffin A, Qiang Z, et al. Organelle-targeted therapies: a comprehensive review on system design for enabling precision oncology. Signal Transduct Target Ther 2022;7(1):379; doi: 10.1038/s41392-022-01243-0.

Yang Y, Wang H, Kouadir M, et al. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 2019; 10:128-139; doi: 10.1038/s41419-019-1413-8.

Yi JS, Holbrook BC, Michalek RD, et al. Electron transport complex I is required for $CD8^+$ T cell function. J Immunol 2006;177(2):852–862; doi: 10.4049/jimmunol.177.2.852.

Yin M and O'Neill LAJ. The role of the electron transport chain in immunity. FASEB Journal 2021;35(12):e21974; doi: 10.1096/fj.202101161r.

Yu R, Lendahl U, Nistér M, et al. Regulation of mammalian mitochondrial dynamics: opportunities and challenges. Front Endocrinol (Lausanne) 2020;11:1–21; doi: 10.3389/fendo.2020.00374.

Yun J and Finkel T. Mitohormesis. Cell Metab 2014;19:757-766; doi: 10.1016/j.cmet.2014.01.011.

Zeng Z, Huang Q, Mao L, et al. The pyruvate dehydrogenase complex in sepsis: metabolic regulation and targeted therapy. Front Nutr 2021;8:783164; doi: 10.3389/fnut.2021.783164.

Zhang B, Liu SQ, Li C, et al. MicroRNA-23a curbs necrosis during early T cell activation by enforcing intracellular reactive oxygen species equilibrium. Immunity 2016;44(3):568–581; doi: 10.1016/j.immuni.2016.01.007.

Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010;464(7285):104–107; doi: 10.1038/nature08780.

Zhang D, Tang Z, Huang H, et al. Metabolic regulation of gene expression by histone lactylation. Nature 2019; 574:575-580; doi: 10.1038/s41586-019-1678-1.

Zhong Z, Llang S, Sanchez-Lopez E, et al. New mitocondrial DNA synthesis enables NLRP3 inflammasome activation. Nature 2018; 560:198-203; doi:10.1038/s41586-018-0372-z.

Zhong W, Rao Z, Xu J, et al. Defective mitophagy in aged macrophages promotes mitochondrial DNA cytosolic leakage to activate STING signaling during liver sterile inflammation. Aging Cell 2022;21(6):e13622; doi: 10.1111/acel.13622.

Zhou R, Yazdi AS, Menu P, et al. A role for mitochondria in NLRP3 inflammasome activation. Nature 2011;469(7329):221–226; doi: 10.1038/nature09663.

Zhu X, Zhang W, Wu C, et al. The novel role of metabolism-associated molecular patterns in sepsis. Front Cell Infect Microbiol 2022; 12; 915099-915106; doi: 10.3389/ fcimb.2022.915099.

Table 1: Metabolic and pharmacological small molecules use to modulate mitochondrial metabolism as potential therapeutic approaches in mitochondrial-initiated inflammatory mechanisms.

Mechanism/Agent	Mitochondrial target	Mechanism	Reference
Modulation of the ETC function			
Succinate		Bypasses complex I	Saeb-Parsy et al, 2021;
Menadione	PDK inhibitor	Bypasses complex II. Increased reduce cofactors	Wallace et al, 2010
Dichloroacetate (DCA)	PDK inhibitor	Increasing PDHC, thereby increasing the catabolism of pyruvate to acetyl-CoA	Yang et al., 2022; Zeng et al., 2021
Thiamine (vitamin B1)		Cofactor of PDHC	El-Hattaba et al., 2017
Dimethyl malonate (DMM)	Complex II inhibitor	leads to a decrease ROS production from complex I by reverse electron transport, impaired proinflammatory cytokine	Scialò et al., 2017
Metformin	Complex I inhibitor	Blocks the reverse electron transport from complex II impairing complex I-ROS production Activates AMPK and PGC1α promoting mitochondrial biogenesis	Tsuji et al., 2020 Yan et al., 2020
2,4-dinitrophenol (DNP) Carbonyl cyanide p-trifluoro-methoxyphenyl (FCCP)	Protonophoric uncouplers	Modulate mitochondrial membrane potential by increasing the proton transport into the matrix to improve ATP production	Childress et al., 2018

Mitochondrial antioxidants and ROS scavengers			
MitoQ MitoVit E	Mitochondrial-targeted antioxidants Complex I and II	Transfers electrons to complex III. Preserve mitochondrial membrane potential, decreased ROS production and organelle damage avoiding excessive cell death	Bhatti et al., 2017
S1QELs and S3QELs	Mitochondrial ROS suppressors	Prevent excessive ROS production from complex I and complex III	Scialo and Sanz 2021
Regulation of mitochondrial biogenesis and turnover			
mdivi-1	Drp1 protein and complex I	Inhibitor Drp1 and complex I; less fission, improved mitochondrial function	Bordt et al., 2017; Hernandez-Resendiz et al., 2020
BGP-15	Opa1	Stimulated GTPase activity and assembly of Opa1, increasing mitochondrial fission; activated diverse other signaling pathways	Szabo et al., 2018

FIGURE LEGENDS

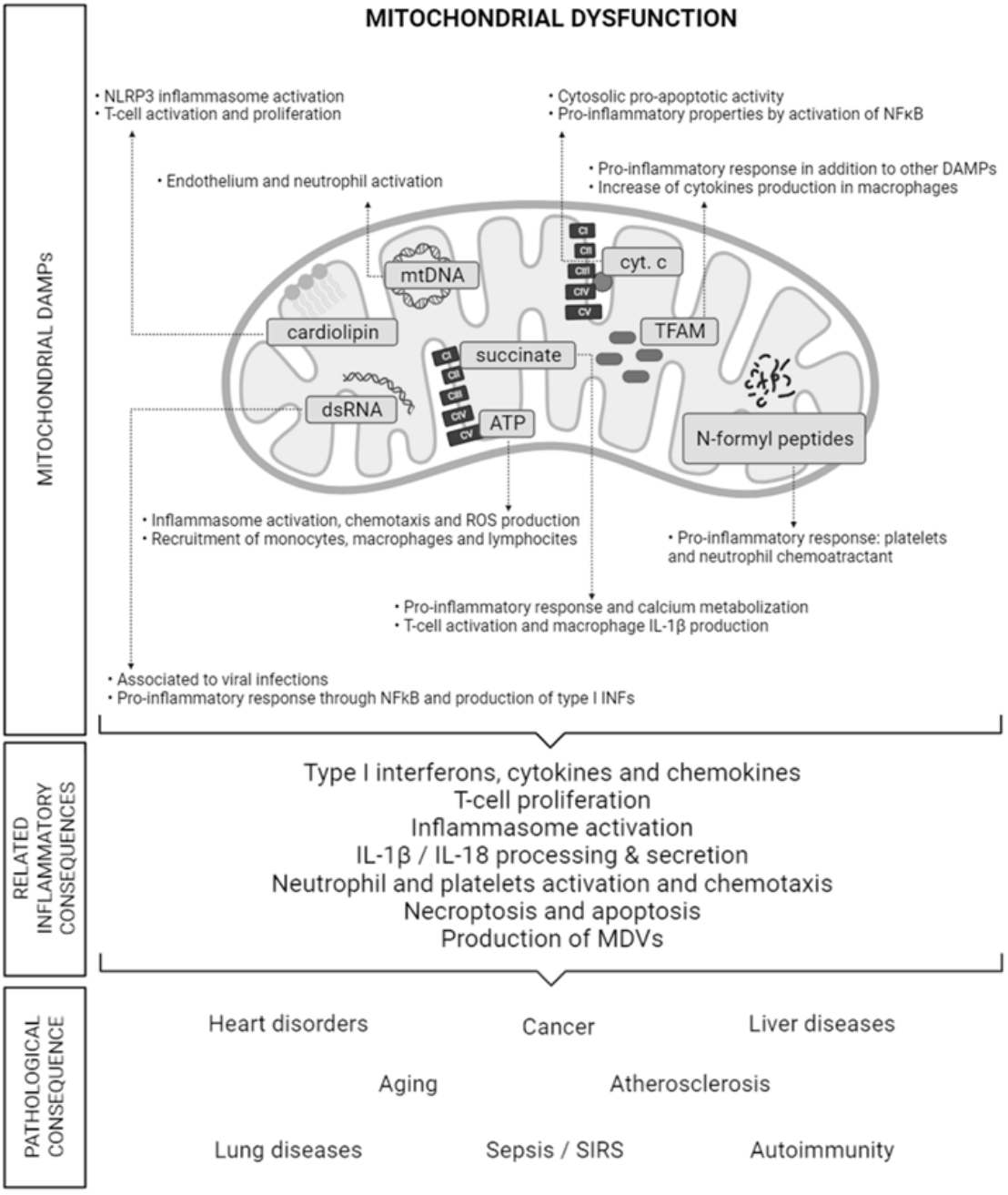

FIGURE 1. Mitochondrial dysfunction, release of DAMPs, and the consequences in the immune system and inflammatory response. The disruption of membrane integrity leads to the release of mitochondrial ligands and DAMPs, as N-formyl peptides, cardiolipin, mtDNA, dsRNA, ATP. Briefly, these DAMPs trigger a wide array of inflammatory responses, including neutrophil activation and migration, inflammasome activation resulting in IL-1β and IL-18 processing and secretion, and pro-inflammatory cytokine and chemokine production. If sustained in time, this situation may have pathological consequences as cancer, heart disorders, infection, aging, among others.

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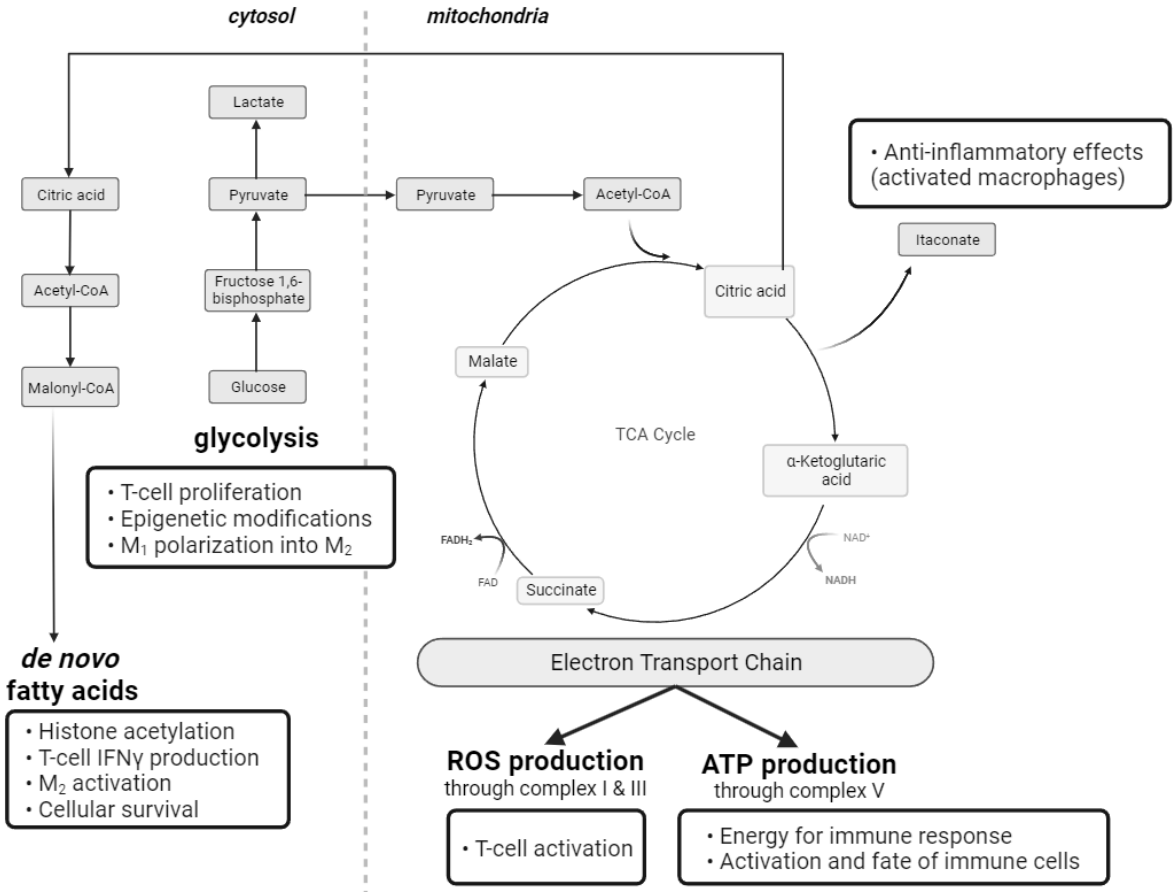

FIGURE 2. Mitochondrial hot points plausible to be regulated with relevant consequences for immune responses. These relevant points include: glycolysis (where pyruvate can either be secreted as lactate or oxidized to acetyl-CoA and enter the TCA cycle), TCA cycle and *de novo* synthesis of fatty acids (through the intermediate production of citrate), mtROS production (through the electron flux leakage and RET), and ATP production (that uses energy provided by the electron transport chain through the maintenance of the inner membrane potential). The importance of these regulatory points on the function and maturation of immune cells, and the inflammatory response are also included.

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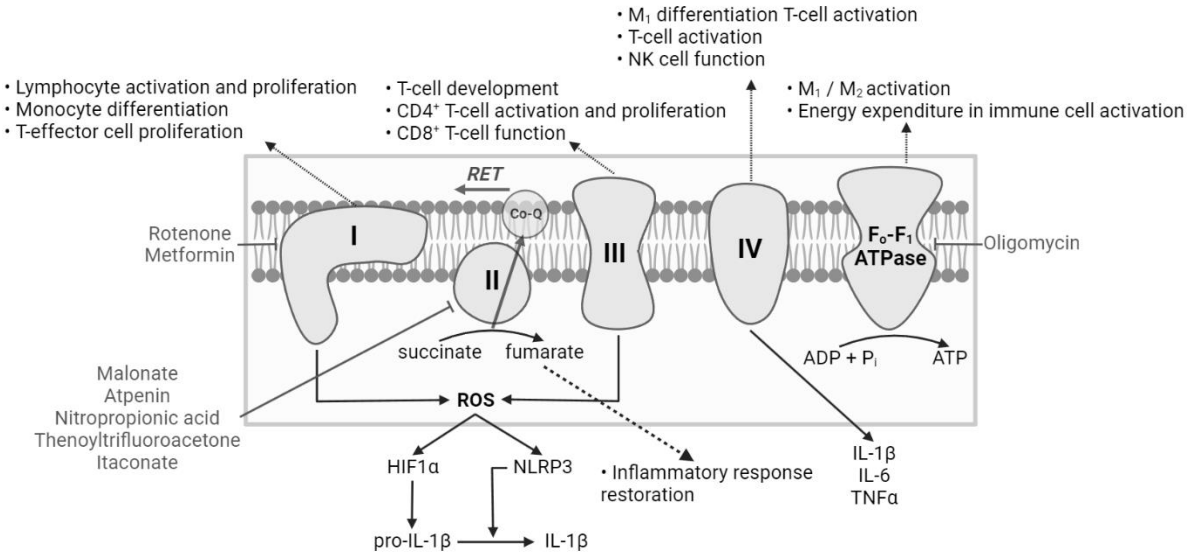

FIGURE 3. The electron transport chain and the regulation of the immune response. As extensively analyzed in this review, mitochondrial respiratory complexes have implications with immune consequences through the activation and regulation of different mechanisms. In activated macrophages ROS produced at *complex I* can a) activate NLRP3 inflammasome through oxidizing mtDNA and b) stabilize HIF1α through inhibition of PHD. By both mechanisms IL-1β production is induced. Complex I is also implicated in lymphocyte activation, proliferation, and function, T cell proliferation, and monocyte differentiation. Its activity is required in proliferating cells as it maintains an appropriate NAD^+/NADH ratio to support aspartate and nucleotides biosynthesis. Regarding *complex II*, oxidation of succinate to fumarate is decreased. However, in the case of pretrained macrophages this inhibition is not observed, and the inflammatory response is restored (preventing immune paralysis). ROS derived from *complex III* are another major activation signal for HIF1α that is a master regulator for various immune cell functions. For example, this complex regulates $CD4^+$ T cell activation and proliferation, and $CD8^+$T cell function. Inhibition of complex IV promotes M_1 differentiation and the production of proinflammatory cytokines, indicating the anti-inflammatory properties of complex IV. ATP synthase increases the production of IL-1β produced by M_1 macrophages and induces the differentiation of M_2 macrophages. Also, this complex is involved in T cell fate decisions, and NK cell function. Full lines indicate main effects, dashed lines indicate main effects while being inhibited, and main inhibitors of the respiratory chain are included in the figure.

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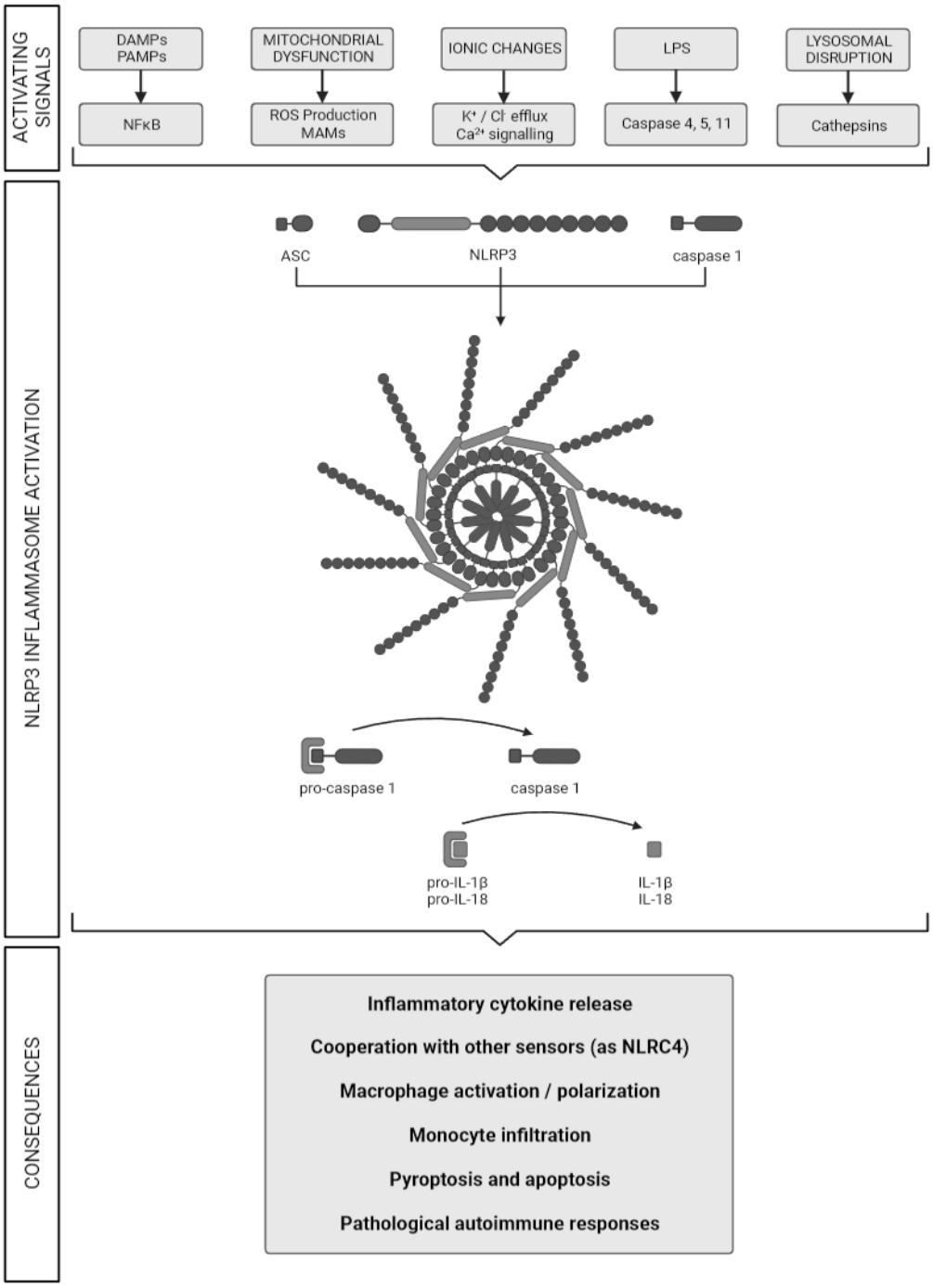

FIGURE 4. Formation of the NLRP3 inflammasome complex. Activation by different stressors of this complex involves constituent molecules as NLRP3, ASC and caspase 1. The activated inflammasome allows the cleavage of pro-caspase 1 into its active form (caspase 1), which in turn cleaves pro-IL-1β and pro-IL-18 to their active forms (IL-1β and IL-18, respectively). The immune consequences include: inflammatory cytokines release, macrophage activation and polarization, monocyte infiltration, and pyroptosis, among others.

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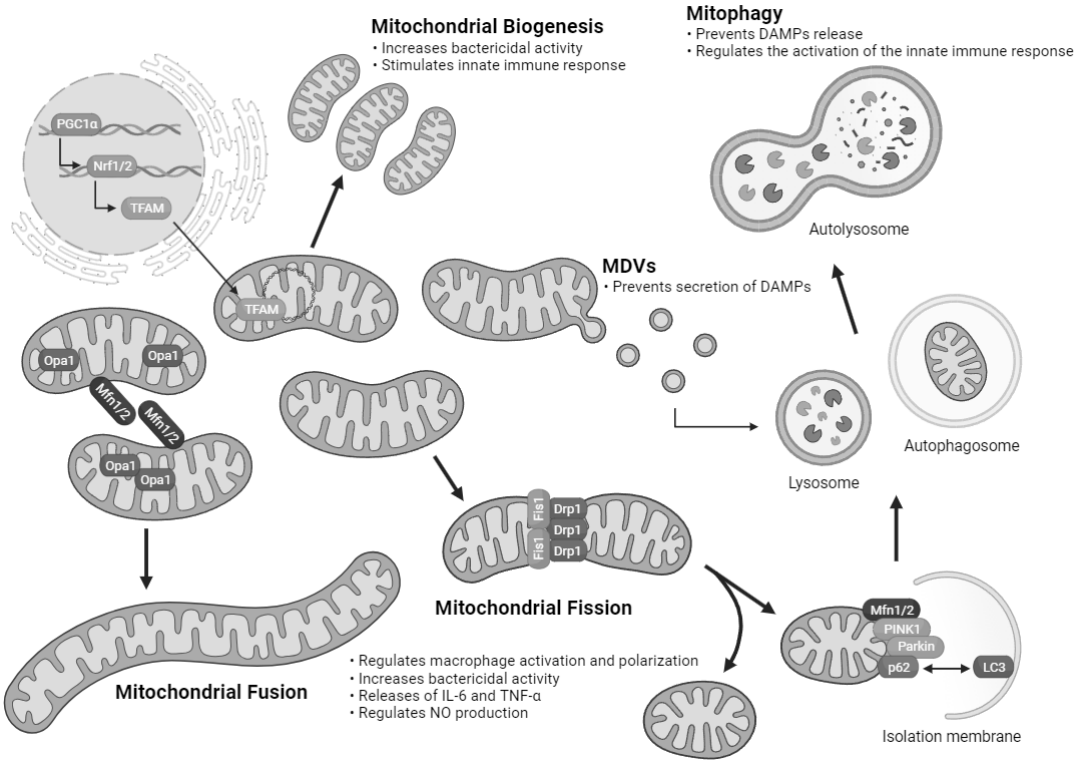

FIGURE 5. Mitochondrial quality control and the coordination of mitochondrial biogenesis, fusion and fission, mitophagy and MDV production. *Mitochondrial biogenesis* needs the activation of PGC1α. Once activated, PGC1α binds to Nrf 1/2 to promote expression of several mitochondrial proteins, including TFAM for mtDNA synthesis, ultimately resulting in increased mitochondrial number. In the *fusion* process, outer mitochondrial membrane (OMM) fusion is mediated by Mfn1 and Mfn2, whereas inner mitochondrial membrane (IMM) fusion is mediated by Opa1. Mitochondria *fission* requires the recruitment of Drp1 from cytosol to mitochondria. In Parkin-dependent *mitophagy*, when mitochondria are depolarized in response to various insults, Parkin is translocated to the outer membrane of mitochondria. This process is regulated by PINK1, which is stabilized on depolarized mitochondria. PINK1 either directly phosphorylates Parkin or ubiquitin to promote Parkin translocation or its ligase activity. Once Parkin translocates to mitochondria, it promotes selective mitophagy through mitochondrial ubiquitination and recruitment of autophagy receptor proteins such as p62, which further recruit LC3 positive autophagosomes. *Mitochondria derived vesicles* may be produced even before mitophagy is activated. The segregation of mitochondria to form MDVs is also regulated by PINK1 and Parkin. MDVs are fused with late endosomes and multivesicular bodies and then delivered to lysosomes where they are eventually degraded. Each of these processes, in individual or coordinated fashion, have important regulatory functions for activation, differentiation and function of immune cells.