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Review

Mitochondrial DNA and Mitochondrial-Derived Vesicles as Immunometabolic Modulators of Innate Immunosurveillance through Control of the Mitochondrial Permeability Transition Pore

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Abstract: Mitochondria are multifunctional organelles that integrate bioenergetics with innate immune regulation, acting as central hubs of immunometabolic control. Beyond ATP production, mitochondrial dysfunction promotes the release of mitochondrial damage-associated molecular patterns (mtDAMPs), among which mitochondrial DNA (mtDNA) represents a key inflammatory signal due to its bacterial features, susceptibility to oxidative damage, and capacity to activate innate immune pathways. Once displaced from mitochondria, mtDNA engages the cGAS–STING axis, the NLRP3 inflammasome, and Toll-like receptor 9 signaling, thereby driving type I interferon responses, NF-κB activation, and IL-1β maturation in sterile inflammatory conditions. A critical determinant of mtDNA release is the mitochondrial permeability transition pore (mPTP), a Ca^{2+}- and ROS-sensitive channel whose sustained opening promotes mitochondrial depolarization, structural collapse, and escape of oxidized mtDNA into cytosolic and extracellular compartments. In parallel, mitochondrial quality control (MQC) systems, including mitophagy and mitochondrial-derived vesicles (MDVs), regulate the selective sequestration and removal of damaged mitochondrial components. MDVs constitute an early and highly selective MQC pathway that traffics oxidized proteins, lipids, and in some contexts nucleic acids toward lysosomal or extracellular destinations. The balance between mPTP-dependent mitochondrial rupture, mtDNA oxidation and release, and MDV-mediated selective cargo sorting defines whether mitochondrial stress is resolved through adaptive quality control or converted into inflammatory signaling. Importantly, MDVs may either limit inflammation by removing damaged mitochondrial components or contribute to immune modulation depending on cargo composition and cellular context. Together, the mPTP-mtDNA-MDV axis emerges as an integrated framework linking mitochondrial integrity to innate immune activation, immunometabolic rewiring, and sterile inflammation, providing mechanistic insight into how mitochondrial stress is translated into inflammatory disease pathways.

Keywords: mitochondrial DNA; mitochondrial permeability transition pore; mitochondrial-derived vesicles; innate immunity; mitochondrial damage-associated molecular patterns; sterile inflammation

1. Introduction

Mitochondria are multifunctional organelles traditionally recognized as the principal sites of ATP generation through oxidative phosphorylation (OXPHOS). However, their biological relevance extends well beyond bioenergetics. They regulate calcium buffering, redox homeostasis, apoptosis, oxidative metabolism, and the production of metabolites that directly influence transcriptional and immune programs. In immune cells, these functions position mitochondria as central hubs of immunometabolism, coordinating the metabolic adaptations required for immune surveillance, inflammatory activation, resolution, and tissue repair [1,2]. Recent evidence has therefore reframed mitochondria not only as metabolic organelles, but also as immune signaling platforms that determine whether cellular stress remains adaptive or progresses toward inflammation [3,4].

This dual role is partly rooted in the bacterial ancestry of mitochondria. Mitochondria retain molecular features that resemble microbial pathogen-associated molecular patterns, including mitochondrial DNA (mtDNA) [5]. Under physiological conditions, the mitochondrial macromolecules are compartmentalized and immunologically silent. When mitochondrial integrity is compromised, however, they can be released into the cytosol or extracellular space and function as mitochondrial damage-associated molecular patterns (mtDAMPs) [6]. These mtDAMPs are sensed by innate immune receptors, including Toll-like receptors, formyl peptide receptors, the NLRP3 inflammasome, and the cGAS-STING axis, thereby initiating type I interferon responses, inflammasome activation, cytokine production, and sterile inflammation [7]. Among mtDAMPs, mtDNA has received particular attention because of its immunogenic structural features, including circularity, relative hypomethylation, CpG enrichment, and susceptibility to oxidative modification, in particular in the D-loop region of mtDNA. Cytosolic mtDNA can activate cyclic GMP-AMP synthase (cGAS)-stimulator of interferon gene (STING) signaling, leading to TBK1-IRF3 and NF-κB activation, while oxidized mtDNA has been implicated in NLRP3 inflammasome activation and IL-1β maturation [8,9]. Thus, mtDNA operates as a molecular bridge between mitochondrial dysfunction, antiviral-like innate immune signaling, and sterile inflammatory disease. Experimental studies have shown that mitochondrial stress can promote mtDNA-dependent innate immune activation [10].

To prevent uncontrolled exposure of mitochondrial immunogenic material, cells rely on mitochondrial quality control (MQC) mechanisms [11]. These include fusion-fission dynamics, proteostasis, mitophagy, and the selective vesicular trafficking pathway mediated by mitochondrial-derived vesicles (MDVs) [12]. MDVs bud from mitochondria and deliver selected mitochondrial cargo to lysosomes, peroxisomes, endolysosomal compartments, or extracellular vesicle pathways. Unlike mitophagy, which removes whole mitochondria, MDVs provide a more selective and often earlier response to mitochondrial stress, allowing damaged or oxidized mitochondrial components to be segregated without eliminating the entire organelle. This places MDVs at the interface between mitochondrial maintenance, intracellular trafficking, antigen presentation, and intercellular inflammatory communication [13]. However, a key determinant of whether mitochondrial stress culminates in cytoprotection or inflammation is the mitochondrial permeability transition pore (mPTP). The mPTP is a high-conductance permeability pathway of the inner mitochondrial membrane whose sustained opening disrupts mitochondrial membrane potential, impairs ATP synthesis, promotes matrix swelling, and can lead to outer membrane rupture and cell death. Transient or regulated pore activity may participate in calcium and metabolic homeostasis, whereas persistent mPTP opening is associated with mitochondrial injury and inflammatory signal amplification. In this context, the mPTP represents a critical checkpoint controlling mitochondrial membrane integrity and the probability that mtDNA and other mtDAMPs escape into immune-sensing compartments [14]. The balance between mPTP opening, mtDNA release, mitophagy, and MDV formation has been proposed to influence distinct immunometabolic outcomes, although the mechanistic relationship between mPTP activity and MDV biogenesis remains incompletely understood [12,13]. A schematic representation of this mitochondrial stress-response axis is illustrated in Figure 1, highlighting the divergence between MDV-mediated quality control and mtDNA-driven inflammatory signaling depending on mPTP status.

However, MDVs are not uniformly anti-inflammatory: depending on their cargo, cellular origin, and recipient cell, they may either support MQC or propagate inflammatory and antigenic signals. How mtDNA and MDVs function as mitochondrial signaling entities in innate immunosurveillance, with particular emphasis on the regulatory role of mPTP in shaping mitochondrial stress responses will be discussed [15,16]. Rather than considering mPTP opening, mtDNA release, and MDV biogenesis as independent events, we discuss these processes within an integrated framework in which mPTP activity may influence the balance between mitochondrial quality control and innate immune activation. In particular, although direct mechanistic links remain incompletely defined, current findings support a conceptual framework in which mitochondrial permeability, vesicular trafficking, and mtDNA mobilization are functionally interconnected processes. We propose that mPTP

activation constitutes a key decision point within mitochondrial quality control. Under conditions of limited stress, mitochondrial integrity is sufficiently preserved to permit selective MDV biogenesis and cargo disposal [17,18].

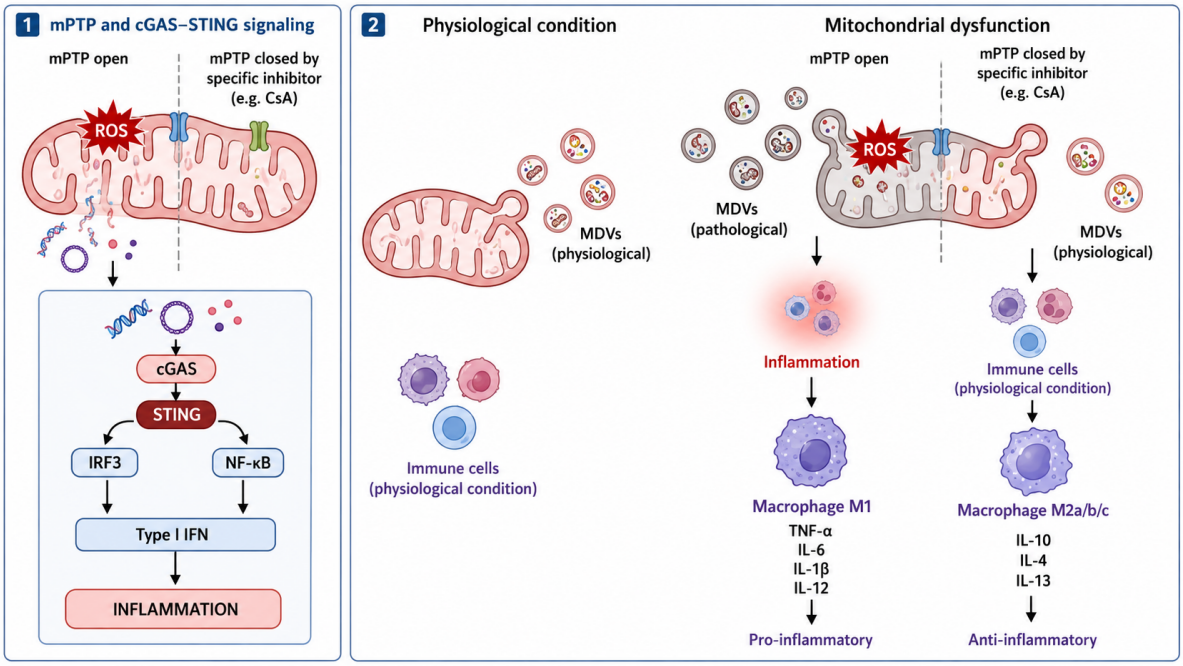

Figure 1. Role of the mitochondrial permeability transition pore (mPTP) in the release of mitochondrial components and modulation of the immune response. The **left panel** (**1**) illustrates the mechanism by which opening of the mPTP promotes the release of mitochondrial components, including ROS and mitochondrial DNA (mtDNA), into the cytoplasm. Cytosolic mtDNA activates the cGAS-STING signaling pathway, resulting in activation of the transcription factors IRF3 and NF-κB and induction of the production of type I interferons (Type I IFN) and pro-inflammatory mediators. In the proposed model, pharmacological closure of the mPTP using specific inhibitors, such as cyclosporine A (CsA), is hypothesized to limit mtDNA release and attenuate inflammatory activation. The **right panel** (**2**) represents the physiological condition, in which mitochondria release mitochondrial-derived vesicles (MDVs) in a controlled manner. In this context, MDVs participate in maintaining cellular homeostasis and contribute to normal communication with immune cells without inducing pathological inflammation. The **right panel** (**2**) shows the condition of mitochondrial dysfunction. Within this working model, persistent opening of the mPTP and excessive production of ROS are thought to promote the release of pathological MDVs and mitochondrial damage signals, favoring the activation of the inflammatory response. This state is associated with the polarization of macrophages toward the pro-inflammatory M1 phenotype, characterized by the secretion of cytokines such as TNF-α, IL-1, IL-6, IL-12, and IL-18. Conversely, closure of the mPTP limits mtDNA release and inflammatory signaling. Whether preservation of mitochondrial integrity promotes physiological MDV-mediated quality-control responses represents a proposed working model that requires further experimental validation.

As mitochondrial stress intensifies, sustained mPTP opening promotes mitochondrial depolarization, matrix swelling, and loss of membrane integrity, conditions that favor mtDNA release and innate immune activation. Under such conditions, selective vesicular quality-control pathways may become insufficient to fully contain mitochondrial damage.

Understanding this axis may clarify how mitochondrial dysfunction is translated into immunometabolic rewiring, sterile inflammation, and potentially targetable inflammatory disease mechanisms.

2. Mitochondrial DNA as an Inflammatory Signal

MtDNA is a compact, circular, double-stranded genome organized into protein-associated nucleoids and encoding 13 essential oxidative phosphorylation subunits, 2 ribosomal RNAs, and 22 transfer RNAs. Although normally confined within the mitochondrial matrix, mtDNA has intrinsic immunogenic properties that become relevant when mitochondrial integrity is compromised. Its bacterial ancestry, relative enrichment in unmethylated CpG motifs, limited histone-like protection, and close spatial relationship with the electron transport chain make mtDNA particularly vulnerable to oxidative injury and recognizable by innate immune sensors once displaced from mitochondria [19]. Oxidative stress promotes mtDNA base lesions, including 8-oxo-deoxyguanosine, strand

breaks, replication stress, and fragmentation. These events not only impair mitochondrial gene expression and respiratory efficiency but also convert mtDNA into a potent mitochondrial damage-associated molecular pattern. Oxidized mtDNA is especially inflammatory because it can engage cytosolic nucleic-acid and inflammasome pathways: early work showed that oxidized mtDNA binds and activates the NLRP3 inflammasome, promoting IL-1β secretion, whereas later studies demonstrated that fragmented oxidized mtDNA can activate both NLRP3 and cGAS-STING signaling [16,20].

The biological outcome depends largely on how mtDNA exits mitochondria. Several non-mutually exclusive routes have been described. First, mPTP opening provides a rapid, calcium- and ROS-sensitive mechanism for inner membrane permeabilization. In activated macrophages, oxidized mtDNA fragments activate NLRP3, cGAS-STING, and extracellular pro-inflammatory DNA signaling [16]. Second, during apoptosis or sublethal apoptotic stress, BAX/BAK-dependent mitochondrial outer membrane permeabilization can produce expanding macropores, allowing inner membrane herniation or permeabilization and subsequent mtDNA efflux into the cytosol [21]. When caspase activity is insufficient to silence this pathway, mtDNA engages cGAS-STING and promotes type I interferon responses [22]. Third, defective mitophagy permits the persistence of damaged mitochondria, increasing the probability of mtDNA oxidation, leakage, and chronic innate immune activation [23]. Finally, MDVs add a more selective trafficking route: they can remove damaged mitochondrial cargo for lysosomal disposal or deliver mitochondrial constituents, including mtDNA or mitochondrial RNA, to other intracellular or extracellular compartments. Thus, mtDNA is not merely a genetic molecule but a context-dependent immunometabolic signal. When MQC remains effective, mtDNA-containing material may be sequestered, repaired, degraded, or packaged into MDVs with limited inflammatory consequences [24,25].

In contrast, sustained oxidative stress, mPTP opening, BAX/BAK pore formation, or impaired mitophagy favors mtDNA mislocalization and conversion into an inflammatory alarmin. Among these pathways, mPTP opening is particularly relevant because it directly couples mitochondrial calcium overload, ROS generation, membrane permeability, mtDNA fragmentation, and innate immune activation, making it a critical checkpoint between mitochondrial stress adaptation and mito-inflammatory signaling [16,26].

3. From Mitochondrial Dysfunction to Innate Immune Signaling

Even if it is known that mPTP is a Ca^{2+}-sensitive, redox-regulated, high-conductance permeability pathway of the inner mitochondrial membrane that functions as a decisive checkpoint between reversible mitochondrial stress and irreversible mitochondrial damage, its precise molecular architecture remains debated. Two major classes of models have been proposed to explain the molecular identity of the mPTP. The ATP synthase model is supported by biochemical and structural studies suggesting that permeability transition may arise from conformational rearrangements involving ATP synthase dimers and the F_O sector, particularly under conditions of calcium overload and oxidative stress [27]. Within this framework, pore formation has been proposed to occur either at ATP synthase dimer interfaces or through structural rearrangements involving the c-ring of the F_O domain. An alternative model proposes that the adenine nucleotide translocator (ANT), a carrier responsible for ADP/ATP exchange across the inner mitochondrial membrane, can adopt channel-forming conformations and contribute directly to pore formation, as supported by genetic and pharmacological evidence [26]. However, neither model fully explains all experimental observations. While ATP synthase- and ANT-dependent mechanisms are supported by substantial evidence, permeability transition-like events have been reported even after disruption of specific ATP synthase or ANT components, suggesting that no single model fully accounts for all experimental findings. Consequently, current views increasingly suggest that mPTP activity may arise from context-dependent mechanisms involving multiple molecular entities, including ATP synthase, ANT, and regulatory factors such as cyclophilin D, rather than from a single universally conserved pore structure [26]. Cyclophilin D (CypD) is widely regarded as a major regulatory sensitizer of pore opening rather than a pore-forming subunit. Through its interaction with inner mitochondrial membrane components, CypD lowers the threshold for permeability transition in response to calcium overload and oxidative stress.

Sustained mPTP opening is favored by mitochondrial Ca^{2+} overload, reactive oxygen species (ROS), thiol oxidation, inorganic phosphate accumulation, adenine nucleotide depletion, and loss of membrane potential. Once activated, the pore permits solute flux across the inner mitochondrial membrane, inducing depolarization, osmotic matrix swelling, cristae remodeling, rupture or destabilization of the outer mitochondrial membrane, and release of mitochondrial constituents into the cytosol [26–28].

These events are particularly relevant because they allow mtDNA to function as an endogenous DAMP and initiate inflammatory responses. Recent evidence indicates that oxidative stress generates oxidized mtDNA, which can either be repaired by OGG1 or processed by Flap Endonuclease-1 into short fragments. In activated

macrophages, these oxidized fragments exit mitochondria through mPTP- and VDAC-dependent routes, thereby connecting mitochondrial Ca^{2+} uptake, ROS stress, pore opening, and cytosolic DNA sensing within a single inflammatory axis [16]. Persistent or uncontrolled mPTP opening therefore represents not only a bioenergetic catastrophe but also a pro-inflammatory event, because it increases the probability that oxidized mtDNA will enter immune-sensing compartments rather than being repaired, degraded, or removed through non-lytic mitochondrial quality-control mechanisms. In this context, pharmacological or genetic inhibition of mPTP regulators may preserve mitochondrial membrane integrity and limit mtDNA leakage. Whether this condition directly promotes MDV biogenesis or shifts mitochondrial stress responses toward MDV-mediated quality-control pathways remains to be established experimentally. Once released into the cytosol, mtDNA is mainly sensed by cGAS, which binds double-stranded DNA independently of sequence and promotes the synthesis of the second messenger 2′3′-cGAMP. This signal activates STING at the endoplasmic reticulum (ER) and ER-Golgi intermediate compartments, leading to TBK1-mediated IRF3 phosphorylation and concomitant NF-κB activation. Consequently, cytosolic mtDNA induces type I interferons, interferon-stimulated genes, TNF, IL-6, and other inflammatory mediators [7]. This pathway is especially relevant in sterile inflammation because mtDNA acts as a self-derived mimic of microbial DNA, converting mitochondrial damage into antiviral-like innate immune signaling [29].

In parallel, oxidized mtDNA can bind and activate the NLRP3 inflammasome, promoting ASC speck formation, caspase-1 activation, gasdermin D cleavage, and maturation of IL-1β and IL-18; this provides a second inflammatory output that is more closely linked to inflammasome-dependent cytokine maturation and pyroptotic amplification [16]. Extracellular or endosomal mtDNA can also engage Toll-like receptor 9 (TLR9), particularly when released during cell injury, extracellular vesicle trafficking, or inflammatory cell death, thereby activating MyD88-dependent NF-κB and interferon-regulatory pathways [30]. Overall, these pathways position mtDNA as a multilayered mitochondrial alarmin. Opening of the mPTP facilitates the escape of oxidized mtDNA fragments, whereas cGAS-STING converts cytosolic mtDNA detection into type I interferon and NF-κB-dependent inflammatory signaling. At the same time, oxidized mtDNA can activate NLRP3, leading to IL-1β and IL-18 maturation, while endosomal or extracellular mtDNA can be sensed by TLR9, further amplifying inflammatory cytokine production. Thus, the mPTP-mtDNA axis represents a mechanistic bridge between mitochondrial stress, impaired mitochondrial quality control, and innate immunosurveillance. Its biological outcome is shaped by the duration of pore opening, the oxidative state and size of mtDNA fragments, the efficiency of mitophagy and MDV-mediated cargo handling, and the cellular context in which DNA-sensing pathways are engaged. [31,32].

4. Mitochondrial-Derived Vesicles in Inflammatory Signaling

MDVs are increasingly recognized as an adaptive arm of MQC, positioned between local protein/lipid repair and wholesale organelle removal by mitophagy. They are typically described as small vesicular carriers of approximately 70–150 nm that bud from mitochondrial subdomains and selectively package mitochondrial cargo, including outer-membrane proteins, inner-membrane or matrix proteins, oxidized respiratory-chain components, lipids such as cardiolipin-related species, and, in some contexts, mitochondrial nucleic acids [33,34]. Their formation was initially distinguished from canonical mitochondrial fission and mitophagy by the observation that mitochondria can generate vesicles while remaining otherwise functional and respiring; early work also showed selective trafficking of mitochondrial cargo to peroxisomes or lysosomes rather than nonspecific mitochondrial fragmentation [12,35]. The concept was strengthened by studies demonstrating that oxidative stress stimulates MDV production and that damaged or oxidized mitochondrial proteins can be segregated into vesicular carriers for lysosomal degradation, providing a first-line quality-control response before the energetic and structural cost of mitophagy becomes necessary [36]. This is important because mitochondria are not homogeneous structures: oxidative damage may affect only restricted respiratory-chain complexes, import machineries, or lipid microdomains, and MDVs allow such damaged material to be excised while preserving the remaining mitochondrial network. Mechanistically, MDV biogenesis appears heterogeneous and is influenced by the nature and intensity of mitochondrial stress [37]. Therefore, the model discussed here should be regarded as a conceptual framework that integrates currently fragmented observations and highlights key areas requiring experimental validation. Some MDVs carry exclusively outer-membrane cargo, whereas others contain inner-membrane or matrix-derived components, implying that single- and double-membrane MDV subtypes coexist. Functionally, the cargo selectivity makes MDVs more than passive debris: they are triage organelles that determine whether mitochondrial stress is repaired, disposed of, presented immunologically, or exported [38,39]. Mild oxidative stress generally favors MDV-mediated sequestration of damaged cargo, whereas severe or persistent mitochondrial injury shifts the system toward permeability transition, outer-membrane rupture, mtDNA leakage, inflammasome

activation, and cell death. The relationship between MDVs and the mPTP is therefore conceptually important, although still incompletely resolved experimentally. Several studies support the concept that MDVs function as an adaptive quality-control pathway that limits the accumulation or release of damaged mitochondrial components, whereas mPTP opening promotes mtDNA release and inflammatory signaling. These observations provide a rationale for hypothesizing that preservation of mitochondrial integrity may favor MDV-mediated stress responses. However, direct experimental evidence linking mPTP inhibition to enhanced MDV biogenesis remains limited.

Sustained mPTP opening increases the probability of mitochondrial damage-associated molecular pattern release. Conversely, preservation of membrane integrity may permit mitochondria to engage non-lytic MQC pathways, including MDV production, cargo sorting, and lysosomal disposal [40]. Thus, rather than viewing MDVs and mPTP opening as isolated events, it may be useful to conceptualize them as alternative outcomes along a mitochondrial stress continuum, although direct experimental evidence supporting a causal transition between mPTP inhibition and physiological MDV formation is still lacking. Within this continuum, controlled homeostatic vesiculation preserves organelle function and compartmentalizes mitochondrial danger signals, whereas uncontrolled permeability transition shifts the outcome toward mtDNA escape, pathological MDV shedding, and inflammatory amplification [41]. Beyond intracellular MQC, MDVs and mitochondrial extracellular vesicles (EVs) are emerging as signaling entities that connect mitochondrial state to tissue-level immunometabolism. Extracellular MDVs or mitoEVs represent a specialized subset within the broader extracellular vesicle landscape, distinguished by mitochondrial cargo rather than by vesicle size alone. Their biological effect depends on cargo composition, route of biogenesis, cell of origin, recipient cell, and inflammatory context [42].

Vesicular packaging may allow mitochondrial material to be delivered in a more regulated and spatially restricted manner than free mtDNA release, but this does not make all MDVs anti-inflammatory. In dendritic cells and macrophage-lineage cells, mitochondrial cargo can participate in immune surveillance, antigen presentation, inflammatory priming, or tolerance-like remodeling depending on how it is processed [43]. The clearest immunological example is mitochondrial antigen presentation. Inflammatory conditions can promote MDV-dependent presentation of mitochondrial antigens on MHC class I molecules, a process repressed by PINK1 and Parkin, thereby linking MQC machinery to adaptive immune activation [44]. However, MDVs are also regulators of innate immune pathways, because they can either remove damaged mitochondrial components that would otherwise act as DAMPs or convey mitochondrial molecules to immune compartments where pattern-recognition receptors are activated. This duality is especially relevant for macrophages, whose effector programs are tightly coupled to metabolic state [44]. Classically activated inflammatory macrophages commonly display increased aerobic glycolysis, a disrupted tricarboxylic-acid cycle, succinate and itaconate accumulation, mitochondrial ROS production, NF-κB/HIF-1α activation, and high production of TNF, IL-6, IL-1β, and other inflammatory mediators [45]. Alternatively antiinflammatory state of macrophages more often rely on intact mitochondrial respiration, fatty-acid oxidation, OXPHOS, mitochondrial biogenesis, and transcriptional programs linked to IL-4/STAT6/PGC-1β signaling [46], although the binary M1/M2 framework is an oversimplification and tissue macrophages often occupy mixed or context-specific states Transcriptome-based network analysis reveals a spectrum model of human macrophage activation [47]. Within this framework, MDVs generated under conditions of preserved mitochondrial integrity could plausibly contribute to macrophage metabolic rewiring by transferring mitochondrial enzymes, respiratory-chain subunits, lipids, metabolites, or nucleic-acid cargo that affect mitochondrial respiration, ROS buffering, antigen processing, or inflammatory thresholds in recipient cells [48].

Evidence from the broader EV field supports the principle that extracellular vesicles can reprogram macrophage polarization and inflammatory tone [49], while newer mitoEV could support mitochondrial cargo transfer as a mechanism of intercellular metabolic communication. However, direct evidence that endogenous MDVs generated specifically by mPTP inhibition drive macrophages toward a stable OXPHOS-dependent anti-inflammatory phenotype remains limited and should be presented as a working model rather than an established conclusion [50]. Future studies will be required to determine whether distinct MDV populations actively instruct macrophage phenotypes or instead reflect the metabolic state of the donor cell. Thus, the balance between MDV-mediated mitochondrial signal packaging and free mtDNA leakage may act as a rheostat for macrophage inflammatory tone: MDV-dominant responses favor compartmentalized repair, selective disposal, antigen routing, or metabolic adaptation, whereas permeability-transition-dominant responses favor DAMP exposure and inflammatory amplification. This distinction is essential for interpreting MDVs therapeutically, because enhancing MDV formation without defining cargo, recipient cell, and inflammatory context could either suppress or propagate inflammation [42].

5. Conclusions and Future Perspectives

Mitochondria act as central hubs coordinating metabolism, cell survival, and innate immunity. The controlled handling of mitochondrial stress signals determines whether cells engage inflammatory responses or adaptive resolution mechanisms. mtDNA release and MDV-mediated quality-control responses may represent alternative mitochondrial outputs influenced by mitochondrial integrity and mPTP activity. Understanding how mPTP governs the balance between mtDNA-driven inflammation and MDV-mediated metabolic communication opens new conceptual and therapeutic avenues for mitigating chronic inflammatory diseases rooted in mitochondrial dysfunction. From a translational perspective, the mPTP-mtDNA-MDV axis may also offer opportunities for biomarker development and therapeutic intervention (Table 1). Circulating cell-free mtDNA, oxidized mtDNA, and EV-associated mtDNA could serve as measurable indicators of mitochondrial damage, inflammatory burden, or defective mitochondrial quality control, particularly when combined with downstream immune readouts such as type I interferon signatures, inflammasome activation, IL-1β/IL-18 release, and NF-κB-dependent cytokines [51]. In parallel, MDV-associated mitochondrial proteins, lipids, and nucleic acids are emerging as potential markers of compartmentalized mitochondrial stress and intercellular mitochondrial communication [52]. Therapeutically, the mPTP-mtDNA-MDV axis could be targeted at multiple levels: by stabilizing mitochondrial membranes and limiting pathological mPTP opening, by reducing oxidized mtDNA release, by modulating cGAS-STING or NLRP3 inflammasome signaling [53,54], or by redirecting mitochondrial stress toward selective vesicular disposal rather than uncontrolled mtDAMPs exposure [55]. However, clinical translation will require careful discrimination between protective MDV-mediated quality control and pathogenic vesicular release of immunogenic mitochondrial cargo. Thus, future studies should integrate mitochondrial biomarkers, vesicle-cargo profiling, and immune pathway activity to identify disease contexts in which modulation of this axis may have therapeutic value.

Overall, future work should address several core questions: What is the precise molecular structure of the mPTP in immune cells? Which biochemical features make released mtDNA inflammatory? Does mPTP inhibition directly promote MDV generation, or simply prevent catastrophic mtDNA leakage? Which MDV subtypes are protective, immunogenic, or metabolically active? Can extracellular MDV cargo reprogram macrophage metabolism *in vivo*? Which circulating mitochondrial biomarkers best reflect disease-relevant mitochondrial stress? Finally, can the mPTP-mtDNA-MDV axis be therapeutically tuned without suppressing essential immune surveillance? Resolving these issues will transform the axis from a conceptual framework into a testable platform for biomarker discovery and targeted intervention in sterile inflammatory diseases.

Table 1. illustrates the translational relevance of the mPTP-mtDNA-MDV axis by outlining candidate biomarkers, therapeutic targets, and potential clinical implications related to mitochondrial damage, vesicle-associated cargo, and downstream innate immune activation.

Translational Aspect	Candidate Biomarkers/Targets	Potential Implication
Downstream immune activation [51]	Type I interferon signatures, inflammasome activation, IL-1β/IL-18 release, NF-κB-dependent cytokines	May strengthen the interpretation of mtDNA-related biomarkers by linking them to innate immune activation
Compartmentalized mitochondrial stress [52]	MDV- or mitoEV-associated mitochondrial proteins, lipids, and nucleic acids	Emerging markers of compartmentalized mitochondrial stress and intercellular mitochondrial communication
mPTP-mtDNA-MDV therapeutic axis [54,55]	Stabilization of mitochondrial membranes; reduction of oxidized mtDNA release	Potential strategies to limit uncontrolled mitochondrial mtDAMPs exposure
Inflammatory pathway modulation [53,55]	cGAS-STING and NLRP3 inflammasome signaling	Therapeutic modulation may reduce mtDNA-driven inflammatory signaling

Author Contributions

S.N.: conceptualization, supervision; validation; writing—original draft; P.A.G.: writing—reviewing and editing; A.C.: writing—reviewing and editing; F.T.: writing—reviewing and editing; M.F.: writing—reviewing and editing; C.A.: validation; writing—original draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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During the preparation of this work, the authors used Microsoft Copilot to grammar correction, and text editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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