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Advances in the understanding of mitochondrial DNA as a pathogenic factor in inflammatory diseases [version 1; referees: 3 approved]

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Abstract
Mitochondrial DNA (mtDNA) has many similarities with bacterial DNA because of their shared common ancestry. Increasing evidence demonstrates mtDNA to be a potent danger signal that is recognised by the innate immune system and can directly modulate the inflammatory response. In humans, elevated circulating mtDNA is found in conditions with significant tissue injury such as trauma and sepsis and increasingly in chronic organ-specific and systemic illnesses such as steatohepatitis and systemic lupus erythematosus. In this review, we examine our current understanding of mtDNA-mediated inflammation and how the mechanisms regulating mitochondrial homeostasis and mtDNA release represent exciting and previously under-recognised important factors in many human inflammatory diseases, offering many new translational opportunities.
Introduction

Mitochondria are intracellular double-membrane-bound organelles (“cellular powerhouses”) with many essential physiological roles in energy production, programmed cell death, calcium homeostasis, and the synthesis of lipids, amino acids, and haem. In addition, they are involved in antibacterial, antiviral, and stress responses to hypoxia and tissue injury. Mitochondria are evolutionarily derived from energy-producing alpha-bacteria, engulfed by archaean cells approximately 2 billion years ago leading to a symbiotic relationship that forms the basis of the eukaryotic cell. The mitochondria share several features with bacteria, including the double-membrane structure, a circular genome that replicates independently of nuclear DNA, and the synthesis of N-formylated proteins. As the innate immune system recognises conserved bacterial molecules, mitochondrial constituents are similarly immunogenic, acting as damage-associated molecular patterns (DAMPs) when released into the cytosol and extracellular environment, triggering innate immune responses, and promoting inflammation. In this review, we focus particularly on the role of mitochondrial DNA (mtDNA) as a specific inflammatory factor, the mechanisms behind its abnormal release, and its effects on downstream inflammatory pathways in human inflammatory diseases.

Elevated circulating mtDNA in human diseases

Freely circulating mtDNA can be detected, and over 60 studies have quantified mtDNA by quantitative polymerase chain reaction (PCR) in plasma and serum in human diseases (Table 1). In general, they are increased in conditions with acute tissue injury such as trauma, acute myocardial infarction, and sepsis, implicating major cellular stress and uncontrolled cell death as key factors in the release of mtDNA (Figure 1). In cancer, where its role as “liquid biopsies” is a topic of considerable interest, the pattern is less clear, and relatively lower circulating levels are found in some cancers.

Systemic inflammatory response syndrome

Systemic inflammatory response syndrome (SIRS) is a serious condition associated with high mortality, and affected individuals display progressive signs or symptoms of systemic upset reflecting widespread inflammation, often involving multiple organ dysfunction and failure (for example, lungs, kidneys, and brain). SIRS is often a result of major sepsis but also commonly occurs in the context of injury such as trauma. An early study by Lam et al. found that individuals admitted for blunt traumatic injury had increased plasma nuclear DNA and mtDNA levels. Subsequently, Hauser et al. made the seminal observation that it is the freely circulating mtDNA following traumatic injury which possesses the distinct ability to trigger and drive the clinical manifestation of SIRS. Several studies have confirmed the observation of elevated plasma mtDNA in trauma and SIRS. A number of studies have found correlations with injury severity in trauma and higher mtDNA in non-survivors compared with survivors. Furthermore, Gu et al. found that elevated plasma mtDNA was an independent predictor of SIRS in trauma patients. In sepsis, elevated levels of circulating mtDNA have also been found in multiple studies. De Curo et al. found higher mtDNA in the plasma of critically ill paediatric patients who were septic compared with similarly unwell but non-septic patients. The one negative study in sepsis may be explained by numerous factors, including a relatively well patient cohort, only one “spot” measurement being taken at presentation, and the potentially confounding factor of cellular content/debris. Studies of patients in the intensive care setting have found that higher mtDNA levels are associated with poorer outcomes.

Acute single-organ injury: liver, heart, and brain

High levels of mtDNA are present in the serum and plasma of patients with acute injury to a variety of single organs. Acetaminophen overdose induces massive hepatocyte necrosis and in severe cases can lead to multi-organ failure and remains one of the commonest indications for liver transplantation. In fulminant liver failure secondary to acetaminophen overdose, mtDNA in the serum was found to be 30 to 40 times higher than normal, and non-survivors had higher levels than survivors; a separate study of drug-induced acute liver failure found serum mtDNA levels to be 10,000-fold higher. Serum mtDNA of acetaminophen overdose patients with derangement in the liver enzyme alanine aminotransferase (a marker of hepatocyte damage) is significantly higher than that of overdose patients who had normal liver enzymes, suggesting that the extent of mtDNA release into the circulation depends on the extent of hepatocyte necrosis. Similarly, extensive cardiomyocyte necrosis is found in acute myocardial infarction, which is also associated with elevated mtDNA in multiple studies and falls after angioplasty or coronary stent insertion to restore blood flow to the damaged myocardium. Patients with diabetes mellitus and coronary artery disease have higher mtDNA levels than those with diabetes but without coronary artery disease. mtDNA is also higher in acute cerebral ischaemia, caused by a reduction in cerebral blood flow by embolus or local thrombosis, and plasma levels gradually drop over time after the initial tissue injury. Interestingly, studies by the same group relating to plasma mtDNA in subarachnoid haemorrhage and spontaneous intracerebral haemorrhage found no significant difference compared with healthy controls, although both were small studies. Higher mtDNA is found in the cerebrospinal fluid of patients with subarachnoid haemorrhage and traumatic brain injury and is associated with worse clinical outcomes. Overall, in these conditions, significant mtDNA release following massive tissue or cellular injury is evident and likely contributes to the uncontrolled inflammatory response.

Chronic inflammatory and immune-mediated diseases

The role for mtDNA in immune-mediated inflammatory diseases, unlike conditions relating to injury, is now also emerging. In rheumatoid arthritis, a chronic relapsing autoimmune condition affecting the joints, mtDNA was present in the plasma and synovial fluid of most patients but undetectable in healthy controls. Similarly, higher plasma mtDNA is found in granulomatosis with polyangiitis, an autoimmune disease whose features include necrotising granulomatous inflammation and vasculitis. Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease with hallmarks including excessive type I interferon (IFN) and antibodies against nucleic acids. Caielli et al. explored the potential pathogenic importance of oxidised mtDNA in SLE. They showed that there is a defect in mitochondrial clearance that leads to abnormal extrusion of oxidised mtDNA, which triggers a subsequent interferogenic
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This table lists studies reporting mitochondrial DNA (mtDNA) analysed by polymerase chain reaction (PCR) on serum or plasma—that is, circulating as a damage-associated molecular pattern (DAMP)—in human diseases. *Letter* *PCR* rather than quantitative PCR used. Earlier study in 2010 not included. AMI, acute myocardial infarction; CAD, coronary artery disease; ED, emergency department; HC, healthy control; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen–antigen D related; ICU, intensive care unit; MODS, multiple organ dysfunction syndrome; PCI, percutaneous coronary intervention; SIRS, systemic inflammatory response syndrome; T2DM, type 2 diabetes mellitus; TLR9, Toll-like receptor 9.
Figure 1. The contribution of mitochondrial DNA to disease pathogenesis. Medical conditions are in italics. Where and how mitochondria are released are indicated in red. Box in dotted line frames mitochondrial DNA (mtDNA) sensor target. cGAS, cyclic GMP-AMP synthetase; IFN, interferon; IL, interleukin; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mtROS, mitochondria-derived reactive oxygen species; NET, neutrophil extracellular trap; NFκB, nuclear factor kappa B; pDC, plasmacytoid dendritic cell; SIRS, systemic inflammatory response syndrome; SLE, systemic lupus erythematosus; STING, stimulator of interferon genes; TLR9, Toll-like receptor 9.

Elevated anti-mtDNA antibodies were found in a separate study of SLE, particularly in lupus nephritis, where levels correlated with the lupus nephritis activity index better than anti-double-stranded DNA (anti-dsDNA) antibody levels did. In a further study of SLE, neutrophil extracellular traps (NETs) released from the inflammatory subset of low-density granulocyte were highly enriched in mtDNA compared with NETs from healthy control neutrophils. NETs are networks of extracellular fibres that are primarily composed of DNA and that are strikingly expelled following a form of neutrophil cell death (NETosis) with an aim to control pathogens; however, this study demonstrates that mtDNA-enriched NETs are pro-inflammatory in nature. Similar findings are reported in chronic granulomatous disease in this study. Higher levels of mtDNA have been found in the chronic inflammatory states of HIV (although not in all studies), end-stage renal failure, and diabetes mellitus (Table 1). In obese individuals with steatohepatitis, mitochondria enclosed in microparticles can also be detected in plasma. These findings suggest that mtDNA, otherwise a “self-signal”, may be an active component in the aberrant immune or inflammatory response in chronic diseases and in autoimmunity.

mtDNA contributes to inflammatory response

mtDNA was first directly implicated as a key factor in the development of inflammatory pathology over a decade ago when intra-articular injection of oxidised mtDNA, but not nuclear DNA, triggered inflammatory arthritis in mice. There are now numerous studies using in vivo injection of mtDNA to provoke local or systemic inflammation or both. Moreover, there are now several in vivo studies to show that genetic deletion or pharmacologic interference of these pathways reduces the inflammatory effect of mtDNA (as will be discussed in the next section). Hence, it is clear that mtDNA release is not an epiphenomenon but directly contributes to the genesis of inflammation (Figure 1). Current evidence shows that mtDNA-mediated inflammation is predominantly driven by the Toll-like receptor 9 (TLR9), inflammasome, and, more recently, stimulator of interferon genes (STING) pathways.

Toll-like receptor 9

TLR9 is located in the endoplasmic reticulum (ER) of various immune cells and translocates to the endosome upon sensing of hypomethylated DNA with CpG motifs, such as bacterial DNA.
Given its high frequency of unmethylated CpG dinucleotide repeats, it is postulated that mtDNA mediates inflammation dependent on the TLR9 pathway and potentially exerts a similar effect as on bacterial CpG. TLR9 recognises a variety of types of oligodeoxynucleotides (ODNs); for example, class A ODNs preferentially activate plasmacytoid dendritic cells whilst class B CpG ODNs activate B cells\(^5\). Some of our understanding of how mtDNA may interact with TLR9 is extrapolated from work with class A ODNs, although they do not necessarily have the same effect. After activation of TLR9 by CpG DNA, inflammatory cytokine induction and Th1 immune responses occur\(^26\) and TLR9 is necessary in CpG DNA-driven responses\(^25\). TLR9 ligands can preferentially activate downstream pathways, including pro-inflammatory nuclear factor kappa B (NF\(\kappa\)B), nucleotide-binding domain and leucine-rich repeat (NLR) pyrin domain containing 3 (NLRP3) inflammasomes, and interferon regulatory factor (IRF)-dependent type 1 IFN, which can upregulate IL-1 receptor antagonist\(^27\).

Most tissue injury models show better outcomes when the tlr9 gene is deleted. Wei \textit{et al.} recently observed that \text{tlr9}\(^{-/-}\) mice have improved survival outcome in a necrotic lung model of cationic nanocarrier-induced necrosis and mtDNA release \textit{in vivo}\(^27\). Furthermore, the pulmonary inflammation seen after injection of mtDNA was significantly reduced in \text{tlr9}\(^{-/-}\) and \text{Myd88}\(^{-/-}\) mice, underlining the importance of the TLR9–MyD88 pathway\(^26\). Intravenous injection of mitochondrial debris with substantial amounts of mtDNA into mice induced a systemic inflammatory response in wild-type mice that was significantly attenuated in \text{tlr9}\(^{-/-}\) mice\(^28\). \text{tlr9}\(^{-/-}\) mice also have better survival compared with wild-type counterparts in severe renal ischaemia reperfusion injury with associated decreased circulating mtDNA\(^28\). A similar protective effect is seen in \text{tlr9}\(^{-/-}\) mice with acute acetaminophen overdose with observed lower serum mtDNA and an absence of lung inflammation in contrast to the findings of wild-type mice\(^28\). Nevertheless, the reduction in mtDNA in \text{tlr9}\(^{-/-}\) mice is intriguing and could be explained by the reduced inflammation with lower resultant cellular necrosis. Alternatively, it is possible that TLR9 is somehow involved in mtDNA release into the extracellular circulation. In a recent study using a murine model of non-alcoholic steatohepatitis (NASH), mtDNA from NASH hepatocytes resulted in greater activation of TLR9 than did mtDNA from control livers\(^29\). This suggests that mtDNA that is selectively modified during pathologic disease processes can augment the ensuing inflammatory response. Similarly, the level of TLR9 expression (due to various factors) appears to be important. In those with high mtDNA levels, higher TLR9 expression is associated with increased mortality in the intensive care unit (ICU), as discussed earlier\(^28\).

Neutrophils have received the most attention in studies on mtDNA–TLR9 signalling in several different inflammatory settings. Zhang \textit{et al.} found that mtDNA activates neutrophil p38 mitogen-activated protein kinase (MAPK) through TLR9 with release of matrix metalloproteinase 8 (MMP8) and MMP9\(^30\), a finding confirmed in a study in which phosphorylated p38 and MMP9 increased after mtDNA treatment of neutrophils\(^31\). A separate study reported similar findings where pre-treatment with TLR9 inhibitor ODN2088 inhibited the activation of p38 MAPK and release of MMP8\(^32\). Gu \textit{et al.} also found that intratracheal administration of mtDNA provokes lung inflammation through TLR9–p38 MAPK\(^33\). Hip fracture in rats resulted in mtDNA release into the circulation as well as higher TLR9 and NF\(\kappa\)B p65 activation and subsequent lung injury\(^34\). The role of other MAPKs such as extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs) remains unclear and, to our knowledge, unexamined in this context. These data suggest a pathway where mtDNA activates neutrophils through TLR9 binding and activation of the MAPK pathway with subsequent MMP8 and MMP9 release (Figure 1).

When mtDNA is considered vis-à-vis the site and location of TLR9 receptor, mtDNA must be either displaced from whole mitochondria and moved into the cytosol or, when extracellular, internalised by some mechanism(s) to act on endosomal TLR9. The endosomal location of TLR9 is most likely a mechanism to avoid unwanted activation\(^35\). It is unclear how extracellular mtDNA is internalised, but possibilities include endocytosis, transmembrane diffusion, phagocytosis, and receptor-mediated endocytosis\(^36\). Transmembrane diffusion is unlikely because of the highly (negatively) charged nature of DNA, which makes it difficult to pass through the cellular membrane. A recent study found that monocyte-derived macrophages can take up whole mitochondria released from necroptosis, suggesting that phagocytosis could be a relevant mechanism\(^37\). The macrophage has a clear role in resolving inflammation by clearing up cellular debris and apoptotic bodies by phagocytosis. When mitochondria are not cleared during non-apoptotic cell death, the macrophage may phagocytose cellular corpses with mtDNA still abundantly present. Typically, apoptotic corpses can suppress the transcription of pro-inflammatory cytokine genes, promote the secretion of anti-inflammatory cytokines by phagocytes, and cause antigen-presenting cells to present dead cell antigen in a manner that promotes immunological tolerance (reviewed by Zitvogel \textit{et al.}\(^38\)). It will be of interest to consider the fate of mtDNA when macrophages or dendritic cells phagocytose cellular corpses with mtDNA. Does this clear the mtDNA or does it regulate subsequent functions (for example, immune responsiveness) in these cell types? This has yet to be studied in detail. It is also possible that binding to additional co-factors facilitates the internalisation into immune cells, and, in this instance, high-mobility group box 1 (HMGB1) and receptor for advanced glycation end products (RAGE) have been implicated\(^39\). In this study, HMGB1–CpG (class A) complexes resulted in TLR9/RAGE association and recruitment of MyD88 in B cells\(^36\). Here, RAGE was visualised as associating with the DNA and was internalised with some sequestered in endosome-like structures. However, this possible mechanism requires further investigation. It has also been proposed that activation of autoreactive B cells by CpG DNA occurs after B-cell receptor engagement, leading to the delivery of CpG DNA to endosomal TLR9\(^40\).

Although nucleic acid-sensing TLRs on immune cells are found mainly within cells, cell surface expression has also been described. Via flow cytometry, TLR9 has been detected on the surface of resting B lymphocytes\(^41,42\) and peripheral blood mononuclear cells\(^43,44\). One functional \textit{ex vivo} study found primary human and mouse TLR9 surface expression in neutrophils, which are upregulated by a variety of stimuli, including TLR9 agonists\(^45\). However, it remains unclear whether TLR9 is able to signal from the cell
that, given the diversity of NLRP3 activators, current literature suggests that the precise mechanism of NLRP3 activation is still under debate. Although the role of the inflammasome is often considered separately from TLR9 here, there is evidence that TLR9/NFκB activation is a necessary priming step leading to NLRP3 upregulation and subsequent downstream signalling. NFκB-activating stimulus is required for cells to express pro-IL-1β and NLRP3. Imeada et al. showed that stimulation of TLR9 by DNA fragments during early acetaminophen-induced cell death can lead to the transcriptional activation of the IL-1β gene, resulting in the formation of pro-IL-1β. Using the acetaminophen hepatotoxicity model, they showed that NLRP3 deletion and related inflammasome components ASC and Caspase-1 were protective against induced liver failure. A further study, however, did not show any effect of NLRP3 deletion on the outcomes of acetaminophen-induced liver failure. Hence, in the context of liver necrosis, the role for NLRP3 inflammasome remains controversial.

STING pathway
The role of mtDNA in innate immunity through the STING pathway has also been a focus of recent studies. STING is a cytosolic protein anchored to the ER. STING can be activated either by direct association with dsDNA or by cyclic dinucleotides, which can be derived from intracellular bacteria or viruses or produced by a DNA sensor, cyclic GMP–AMP (cGAMP) synthetase (cGAS). This, in turn, activates IRF3, which ultimately translocates to the nucleus and transcribes type I IFN genes, and also the NFκB pathway.

Two independent groups recently discovered that the STING-mediated IFN response can also be activated by mtDNA. They first observed that deficiency of apoptotic caspases (3, 7, and 9) resulted in upregulation of type I IFN genes. This response was dependent on Bak/Bax, pro-apoptotic proteins responsible for mitochondrial outer membrane permeabilisation leading to mtDNA release, and the release of cytochrome C, which activates the intrinsic apoptotic pathway. Typically, apoptosis is considered immunologic-silent; for example, it does not trigger an inflammatory response. However, these studies demonstrated that, when caspases (9 and 3/7) responsible for the completion of apoptotic process are inhibited or deleted, cytosolic mtDNA goes on to activate cGAS/STING-mediated type I IFN signalling. Hence, these caspases serve as a “brake” on the mtDNA-inflammatory effect during cell death. mtDNA released during cell death has been previously reported to provide a second signal that cooperates with an additional inflammatory signal (for example, LPS) to activate the NLRP3 inflammasome and induce IL-1β production in murine macrophages. Further evidence of an mtDNA role in STING-mediated IFN responses comes from West et al. Here, partial deficiency of the mtDNA-binding protein mitochondrial transcription factor A (TFAM) was associated with increased concentrations of cytosolic mtDNA and enhanced type I IFN response, which was attenuated by knockdown of components of the STING pathway.

Aberrant mtDNA-STING signalling has also been implicated in human inflammatory diseases, such as SLE. As discussed earlier, Lood et al. showed that treatment of human neutrophils with SLE-abundant ribonucleoprotein immune complexes induces mtROS, mtDNA oxidation, and translocation of the mitochondria to the
plasma membrane41. Oxidised mtDNA is then released extracellularly as a component of NETs. Transfection of NET-derived mtDNA results in expression of IFN-β in human peripheral mononuclear cells. Systemic injection of oxidised mtDNA increases IFN-stimulated gene expression in the spleen of wild-type but not STING-deficient mice. Similar to inflammasomes, uncontrolled mtROS production promoting cytosolic mtDNA release is important in STING activation and potentially in the case of autoimmunity. These studies highlight the importance of the innate cellular functions to handle mtDNA release during the initiation of cell death, which ultimately will decide whether the ensuing fate will be that of a silent or inflammatory outcome.

Mechanisms for mtDNA release

Two levels of mtDNA release—cytosolic and then extracellular—are critically important steps (Figure 1). In the former, the mechanism of release of mtDNA from mitochondria relies on the opening of mitochondrial permeability transition (MPT) pores in the inner mitochondrial membrane90. Inhibition of pore opening with cyclosporine A resulted in lower mtDNA in the cytosol after stimulation with LPS and ATP91. Ding et al. showed that the induction of ROS using oxidised low-density lipoprotein (ox-LDL) increased mtDNA leakage into the cytosol in a dose-dependent manner, and this effect was ameliorated with blockade of the ox-LDL receptor or a ROS inhibitor91.

In terms of extracellular release, cellular stress and necrosis are primary factors in the non-discriminatory liberation of a host of mitochondrial components such as mtDNA, N-formyl peptides, ATP, TFAM, and mitochondrial lipids. These mitochondrial constituents also exert their respective effects, which are wide-ranging, on key inflammatory pathways (extensively reviewed by Nakahira et al.81). Aside from this non-selective release after uncontrolled cell death, several studies have suggested additional mechanisms such as necroptosis (or programmed necrosis)92. Blood transfusion-induced endothelial necroptosis was recently found to increase extracellular mtDNA as a potential mechanism to explain transfusion-related lung injury93. A recent study suggested that, during necroptosis, mitochondria were released before plasma membrane rupture and then phagocytosed by monocyte-derived macrophages or dendritic cells, triggering an inflammatory response as shown by cytokine production and cell maturation, respectively61. Thus, ingestion of intact mitochondria may represent a distinct uptake mechanism following necroptosis. In a separate study, platelets were also found to be a source for free extracellular mitochondria release and then to act as an endogenous substrate for bacterial secreted phospholipase A,IIA (sPLA,A,IIA) leading to mitochondrial membrane hydrolysis, loss of mitochondrial structural integrity, and mtDNA release84. Intriguingly, Xin et al. found lower levels of mtROS production when metformin was added to activated platelets, and this was associated with decreased extracellular mtDNA release85. The authors found lower complex I activity of the platelet mitochondrial respiratory chain and suggested this as a mechanism for the observed suppressed mitochondrial dysfunction.

Whether there is an active element in mtDNA release is an interesting point of consideration. Active cellular transfer of mitochondria from stromal cells to rescue stricken lung alveoli cells in acute lung injury has been demonstrated86. Extracellular vesicles are important modes of intercellular communication and comprise exosomes (endosomal) and microvesicles (plasma membrane-derived) and are directed by exocytosis. Both chromosomal DNA77,78 and mtDNA99,100 have been observed in extracellular vesicles; it has been suggested that the transfer of altered mtDNA between cells may play a role in Alzheimer’s disease and skeletal muscle diseases99,100. As described earlier, in patients with NASH, a greater percentage of mitochondria was found inside extracellular microparticles and a higher percentage of microparticles contained mitochondria compared with lean subjects82. Furthermore, subjects with NASH had a higher level of oxidised mtDNA in microparticles. Further clarification is required on the concentration and significance of mtDNA in extracellular vesicles and whether this has different immunostimulatory effects compared with cell-free or surface-bound mtDNA. As previously mentioned, the pro-inflammatory effects of mtDNA are dependent on its oxidation51,101. The highly oxidative extracellular milieu at sites of tissue inflammation in patients with chronic inflammatory disease may overwhelm anti-oxidant systems, further potentiating the inflammatory potential of DAMPs such as mtDNA5.

mtDNA degradation and clearance

Several well-described clearance mechanisms limit the pro-inflammatory nature of mtDNA. Autophagy as discussed earlier is important87. Defective autophagy has been implicated in several chronic inflammatory human diseases, including Crohn’s disease103. A proportion of circulating DNA in the bloodstream appears to cross the kidney barrier and be excreted in the urine104. Indeed, mtDNA has been detected in the urine at elevated levels in patients with progressive acute kidney injury105. This may be due to the inflammatory state associated with this condition, the increased clearance with a disturbed kidney barrier, or both. Another possible mechanism of mtDNA clearance is phagocytosis by macrophages in a manner similar to the ingestion of the structurally similar bacterial DNA98. As described earlier, the outcome of phagocytosis of intact mitochondria may be pro- rather than anti-inflammatory; these divergent effects may also be dependent on the phenotype of the phagocytosing cells (for example, inflammatory versus pro-resolution macrophages/monocytes, neutrophils, and red blood cells69,93).

In general, non-host DNA in the circulation is digested in part by circulating nucleases, and mtDNA may be affected by a similar mechanism87. Intracellularly, DNases found in the autophagylosome play a vital role in degrading mtDNA102,106. Oka et al. showed that cardiac-specific deletion of Dnase II resulted in mtDNA accumulation in cardiomyocytes and the development of heart failure102. In human umbilical vein endothelial cells, lysosomal DNases protect cells against inflammation from mtDNA damage induced by ox-LDL93. Here, small interfering RNA (siRNA) knockdown of Dnase II amplifies the mtDNA–TLR9-mediated inflammatory response5. It is unclear whether nucleases have a similar action on mtDNA in the extracellular space or are relevant in the physiological setting, especially when mtDNA is present in microvesicles or housed within intact mitochondria, which protect against
DNase II. Intriguingly, DNase pre-treatment abolished renal mitochondrial injury that was observed after injection of mitochondrial debris (including mtDNA) in mice. However, the precise role of DNase and its effect on the immunostimulatory effects of mtDNA is likely to be more complex, as illustrated by a recent study which showed that DNase II was required for TLR9 activation by bacterial genomic DNA.

Conclusions: translational opportunities for mtDNA-mediated inflammation

mtDNA contributes to inflammation at multiple levels when tissue or cellular homeostasis is perturbed. Damaged mtDNA released into the cytosol has a functional short-range effect on immediate “alarm” systems such as the inflammasome and NFκB. Uncontrolled release of mtDNA into the circulation in conditions with significant tissue injury generates a more systemic effect whilst de-regulation of local mitochondrial homeostatic mechanisms such as autophagy or mtROS detoxification contributes to organ-specific pathology as observed in the heart and liver. Failure of such mechanisms may also give rise to a more wide-ranging consequence (for example, in autoimmune diseases such as SLE).

Our review shows that mtDNA-mediated inflammation is important and relevant to many human inflammatory diseases. However, this remains an underexplored field and more insights will likely emerge in the near future. The current evidence offers a rich realm of translational opportunities to target mtDNA-mediated inflammation. There are many plausible approaches, which include targeting cytosolic mtDNA release (for example, directly at MPT using cyclosporine or by specific mitochondrial anti-oxidant strategies, such as MitoQ10, to reduce mtROS), augmenting clearance (for example, using autophagy activators or correcting factors leading to impaired autophagy), diverting the cellular response following mitochondrial damage (for example, induction of pro-apoptotic caspases), and reducing the inflammatory potential of mtDNA (for example, DNases to digest NET-bound mtDNA and reducing oxidation of mtDNA).

Given that mtDNA can be measured and used as a biomarker, it offers a unique opportunity to stratify and identify individuals who may benefit from specific therapeutic targeting of downstream inflammation pathways (for example, TLR9, NLRP3, or STING pathways). As discussed earlier, there are numerous studies in sepsis, trauma, and acute single-organ injury that have already demonstrated that individuals with high mtDNA levels and TLR9 expressions have worse prognosis. Therefore, there are clear groups in which stratification is useful. However, a number of challenges exist to its implementation as a biomarker, such as the variation in which mtDNA is measured (for example, serum versus plasma, mtDNA-specific PCR primers) and reported in the literature. Standardisation of these protocols, including the identification of “normal” and “abnormal” ranges, will be important prior to clinical use. Furthermore, many studies have failed to include clinically relevant predictive statistics; further studies reporting such statistics in a variety of inflammatory conditions are required.

In conclusion, multiple lines of data show that innate responses to mtDNA, which is similar to and evolutionarily derived from bacteria, are hard-wired into our biology and drive the development of inflammation with pathologic consequences in many diseases.

Competing interests

The authors declare that they have no competing interests.

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References


12. Gu X, Yao Y, Wu G, et al. The plasma mitochondrial DNA is an...
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