CHEST

Mitochondria and Critical Illness

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Classically, mitochondria have largely been believed to influence the development of illness by modulating cell metabolism and determining the rate of production of high-energy phosphate compounds (eg, adenosine triphosphate). It is now recognized that this view is simplistic and that mitochondria play key roles in many other processes, including cell signaling, regulating gene expression, modulating cellular calcium levels, and influencing the activation of cell death pathways (eg, caspase activation). Moreover, these multiple mitochondrial functional characteristics are now known to influence the evolution of cellular and organ function in many disease states, including sepsis, ICU-acquired skeletal muscle dysfunction, acute lung injury, acute renal failure, and critical illness-related immune function dysregulation. In addition, diseased mitochondria generate toxic compounds, most notably released mitochondrial DNA, which can act as danger-associated molecular patterns to induce systemic toxicity and damage multiple organs throughout the body. This article reviews these evolving concepts relating mitochondrial function and acute illness. The discussion is organized into four sections: (1) basics of mitochondrial physiology; (2) cellular mechanisms of mitochondrial pathophysiology; (3) critical care disease processes whose initiation and evolution are shaped by mitochondrial pathophysiology; and (4) emerging treatments for mitochondrial dysfunction in critical illness.

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Basics of Mitochondrial Physiology

The mitochondrion is a double-membrane organelle present in almost all eukaryotic organisms. Prevailing theory suggests that mitochondria are derived from bacteria that originally merged with proto-eukaryotic cells to form a combined symbiotic cellular organism. This theory explains the morphology of mitochondria (which are structurally similar to bacteria) and the fact that mitochondria have their own genetic code, mitochondrial DNA (mtDNA), which has similarity to the bacterial genetic code.[1]

The inner and outer layers of the mitochondrion membrane are separated by an intermembrane space (Fig 1). The outer membrane is permeable to molecules of

ABBREVIATIONS: ADP = adenosine diphosphate; ATP = adenosine triphosphate; DAMP = danger-associated molecular pattern; ETC = electron transport chain; FADH2 = flavin adenine dinucleotide; LPS = lipopolysaccharide; MAVS = mitochondrial antiviral signaling proteins; MPT = mitochondrial permeability transition pore; mtDNA = mitochondrial DNA; NADH = nicotinamide adenine dinucleotide; PINK1 = phosphatase and tensin homolog-induced kinase 1; rhTFAM = human recombinant transcription factor a, mitochondrial protein; ROS = reactive oxygen species; TLR = Toll-like receptor; VIDD = ventilator-induced diaphragm dysfunction

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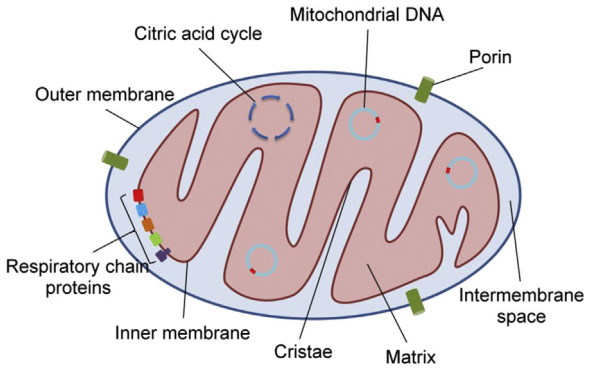

Figure 1 – *This schematic presents the general structure of a mitochondrion. This organelle has an outer and inner membrane. The inside of the inner membrane contains the matrix, and the space between the outer and inner membrane is termed the intermembrane space. The matrix contains the citric acid cycle components and mitochondrial DNA. The complexes of the electron transport chain are located on the inner membrane. (Reprinted with permission from Ralto and Parikh.[2])*

< 5,000 Da via the channel protein (porin).[2] The inner membrane, however, is highly impermeable to ions and molecules, and serves as an anchor for the components of the electron transport chain (ETC).[2,3]

A major function of mitochondria is to supply a large portion of the adenosine triphosphate (ATP) needed to meet cellular energy needs.[4] This is largely accomplished by the process of oxidative phosphorylation (aerobic respiration) in which nicotinamide adenine dinucleotide (NADH), supplied from the mitochondrial matrix, donates electrons to the mitochondrial ETC, which delivers these electrons to molecular oxygen, the final electron acceptor[4] (Fig 2).[5] Movement of electrons down the ETC (complexes I-IV) is linked to transport of hydrogen ions across the inner mitochondrial membrane, creating an electrochemical gradient across this membrane. The energy stored in this gradient is used to phosphorylate adenosine diphosphate (ADP) to ATP, via ATP synthase (complex V).

The net result of this process is to oxidize nutrients (represented by NADH delivery to the ETC), and energy is generated to convert ADP to ATP. As with all sophisticated metabolic processes, there are multiple steps that can be altered by disease processes with pathological results, including inhibition of electron transport due to depletion of critical ETC proteins, leakage of protons across the normally low permeability inner membrane, incomplete delivery of electrons to molecular oxygen with resultant generation of potentially toxic molecular species (ie, superoxide, hydrogen peroxide, hydroxyl radicals, peroxynitrite), and either over-delivery or under-delivery of NADH to the ETC.[6] The consequences of such alterations are explored in the Mechanisms of Mitochondrial Pathophysiology section.

As indicated earlier, NADH is a major source of electrons that fuel the electron transport and lead to generation of ATP by ATP synthase. In turn, the main source of NADH is the citric acid cycle contained in the mitochondrial matrix. Acetyl-CoA, derived from pyruvate or the beta-oxidation of fatty acids, is the principal substrate that enters the citric acid cycle.[7] The citric acid cycle oxidizes acetyl-CoA, generating NADH (flavin adenine dinucleotide [FADH2]) and guanosine triphosphate. NADH can then supply electrons to the ETC via complex I, while FADH2 supplies electrons to the ETC via complex II. Reducing equivalents can also be fed into the ETC via the glycerol phosphate shuttle.

Although generation of ATP by mitochondria is clearly dependent on adequate supply of oxygen to cells as an electron acceptor and adequate delivery of electron donors via NADH/FADH2, regulation of ATP generation to precisely match cellular ATP usage depends primarily on the activity of the mitochondrial ADP/ATP carrier, which imports ADP from the cytosol and exports ATP from the mitochondrial matrix.[8] This transport protein consists of three homologous domains, each composed of two transmembrane α-helices linked with a loop and short α-helix on the matrix side. The transporter cycles between a cytoplasmic and matrix state in which a central substrate binding site is alternately accessible to these compartments for binding of ADP or ATP. In this fashion, transport of ADP into the mitochondria is quantitatively linked to transport of ATP into the cytosol.

Optimal performance of the mitochondria depends on adequate levels of critical proteins required for electron transport, citric acid cycle function, and mitochondrial structure. Maintenance of these protein concentrations requires coordinated synthesis of proteins encoded by nuclear DNA and proteins transcribed from mtDNA. A sophisticated system regulates this coordinated protein synthesis, albeit research conducted the last 20 years indicates that a master regulator of mitochondrial biogenesis is a transcriptional coactivator, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.[9] Certain disease processes are now known to inhibit mitochondrial biogenesis, impairing maintenance of adequate levels of these critical proteins. In addition,

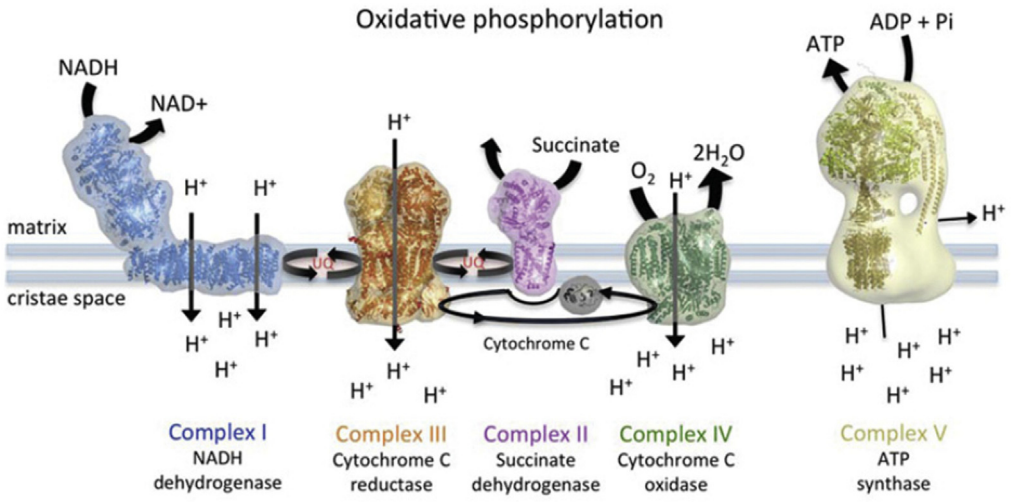

Figure 2 – *This diagram presents the five components of the mitochondrial electron transport chain. These include: (1) complex I, NADH/ubiquinone oxidoreductase (blue); (2) complex II, succinate dehydrogenase (pink); (3) complex III, cytochrome c reductase (orange); (4) complex IV, cytochrome c oxidase (green); and (5) complex V, mitochondrial ATP synthase (tan). Electrons are largely supplied to the chain by NADH (far left) and electrons subsequently flow along the chain until reacting with the final electron acceptor, oxygen, at complex IV. Electron flow causes pumping of protons (H^+ ions) from the mitochondrial matrix to the intermembrane space. The electrochemical energy stored by proton pumping is utilized by complex V to phosphorylate ADP to ATP (far right). ADP = adenosine diphosphate; ATP = adenosine triphosphate; NADH = nicotinamide adenine dinucleotide. (Reprinted with permission from Davies and Daum.[5])*

pharmacological therapies have been identified that can activate mitochondrial biogenesis, potentially inducing mitochondrial repair and reversal of disease-induced mitochondrial damage.[10] Discussion of pharmacological treatments to potentiate mitochondrial biogenesis are presented in the Therapies section.

Although mitochondria play a key role in cellular energy metabolism, these organelles also regulate several other cellular functions. Mitochondria maintain cellular calcium homeostasis[11] and prevent calcium-mediated toxicity to cytosolic processes. Mitochondria also play a central role in programmed cell death (ie, apoptosis). Excessive mitochondrial stress triggers activation of mitochondrial caspase, which, in turn, cleaves nuclear DNA and induces cell death. Mitochondria also regulate cell signaling via generation of free radicals and other reactive oxygen species (ROS).[12] For example, mitochondria superoxide generation is now understood to regulate hypoxia-inducible factor 1-alpha levels under normal physiological conditions, with hypoxia-inducible factor 1-alpha determining, in turn, cellular responses to hypoxemia.[13] Mitochondria also modulate cellular differentiation, cell cycle regulation, and cell growth.[14] Several proteins are uniquely synthesized in the mitochondrial matrix, most notably heme proteins (ie, the porphyrin ring).[15] Mitochondria are also required for synthesis of steroids.[16]

Mechanisms of Mitochondrial Pathophysiology

ETC Dysfunction

Mitochondria are an important source of superoxide- and superoxide-derived ROS (ie, hydrogen peroxide, hydroxyl radicals, peroxynitrite).[17] Under normal physiological conditions, low-level production of these molecular species is believed to contribute to normal cell signaling, but in pathological states, the level of production of these molecular species may rise, inducing damage to mitochondrial constituents, including the ETC itself.[18] In keeping with this concept, several disease states, including sepsis, have been shown to both increase mitochondrial ROS production in multiple organs and to induce ETC abnormalities in these same tissues.[19,20] One study found that reductions in ETC protein constituents associated with sepsis were largely confined to proteins containing or associated with iron sulfur centers, suggesting that superoxide-driven, iron-catalyzed Fenton reactions were largely responsible for ETC protein depletion.[21] Loss of mitochondrial ETC constituents have been reported in several disease states, and in these conditions impaired ATP production by mitochondria may promote disease pathogenesis.[22-25]

Mitochondrial Free Radical Production

In addition to damaging mitochondrial ETC proteins directly, mitochondrial-derived ROS have the capacity

to react with and alter the function of multiple other cellular constituents, including lipids, proteins, and DNA within mitochondria.[26-29] mtDNA is believed to be especially susceptible to damage by ROS due to lack of protective histones,[30] and oxidatively modified base content in mtDNA is generally 10- to 20-fold higher than that of nuclear DNA.[30,31]

Although superoxide generated within mitochondria is believed to have a limited ability to directly exit mitochondrial membranes, superoxide can react to form molecular species (eg, hydrogen peroxide) that more readily cross membranes and can react with and alter cellular constituents in the cytosol.[32,33] Moreover, in some pathological states, nitric oxide generation is markedly increased and nitric oxide can combine with superoxide to generate peroxynitrite, which is a potent reactant capable of severely damaging proteins and modifying lipids.[34]

Mitochondrial Calcium Transport Alterations

Mitochondria usually play a role in maintaining normal cellular calcium homeostasis, taking up excess cytosolic cellular calcium in quiescent cells and thereby preserving low, nontoxic cytosolic calcium levels.[35] Several factors can lead to increases in mitochondrial calcium levels, however, including an increase in cytosolic calcium levels due to release from intracellular organelles (ie, the endoplasmic reticulum and the sarcoplasmic reticulum). In addition, mitochondrial calcium transport is a regulated process, with calcium influx dependent on the activity of the calcium uniporter and mitochondrial calcium release determined, in part, by the activity of the mitochondrial sodium/calcium ion channel.[36,37] Under normal circumstances, enhanced mitochondrial calcium levels as a result of increased cytosolic calcium (eg, in muscle as the result of sarcoplasmic reticulum calcium release) and increased uniporter activity can act to enhance mitochondrial ATP generation by stimulating the citric acid cycle to generate higher levels of NADH and, also, to directly activate ATP synthase.[38] This mechanism permits coupling of ATP production to ATP demand in skeletal muscles during contraction.

When mitochondrial calcium concentrations rise to exceedingly high levels, however (eg, following ischemia/reperfusion in organs), mitochondrial formation of superoxide and other ROS can increase, leading to enhanced mitochondrial-dependent ROS-mediated cell damage.[39] In addition, increases in mitochondrial calcium levels may act synergistically with increased mitochondrial ROS to trigger membrane permeability transition (MPT) pore opening, leading to release of cytochrome c from mitochondria and subsequent activation of mitochondrial cell death pathways.[40] Once damaged, the ability of the mitochondria to store calcium can diminish, leading to lost mitochondrial calcium-buffering capacity, increased cytosolic calcium levels, and calcium-mediated cellular damage.[30]

Mitochondrially Induced Apoptosis

Mitochondria play an important role in mediating regulated cell death (ie, apoptosis). This process is usually triggered by opening of the MPT pore.[41] This pore is composed of several proteins on the inner mitochondrial membrane and includes the adenine nucleotide translocator, cyclophilin D, and the voltage-dependent anion channel.[42] Opening of the pore can be triggered by several factors, including increasing mitochondrial calcium levels, matrix alkalinization, a large negative voltage across the inner membrane, and oxidative modification of the protein constituents of the pore. Opening of the pore allows release of cytochrome c into the cytosol and its interaction with cytosolic pro-apoptotic members of the B-cell lymphoma 2/Bcl-2-associated X protein family. Cytochrome c, in turn, induces caspase 9 activation, caspase 3 activation, cleavage of nuclear DNA, and cell death by the caspase apoptotic pathway. In addition, opening of the pore allows influx of solutes into the mitochondria, initiating mitochondrial rupture.[43,44]

Alterations in Mitochondrial Shape, Fusion, and Fission

Mitochondria are dynamic structures that can change morphological characteristics (eg, shape, position) in response to a variety of stimuli. In addition, mitochondria can both divide (fission) and merge with (fusion) adjacent mitochondria. Fission seems to be mediated by formation of a multimeric complex containing dynamin-related protein 1, which wraps around the outer mitochondrial membrane and exerts mechanical force, cutting the mitochondrion into two pieces.[45] Fusion is mediated by two distinct enzyme complexes, mitofusin 1 and 2, and optic atrophy 1, which fuse, respectively, the outer and inner mitochondrial membranes.

These properties are believed to allow damaged portions of mitochondria to be removed, to allow mitochondria to combine with newly formed and better-functioning mitochondrial components, and to permit movement of mitochondria to cellular areas of high metabolic

demand. Factors that trigger morphological changes include substrate oversupply (which promotes fragmentation), substrate undersupply (inducing elongation), adenosine 5′-monophosphate-activated protein kinase signaling, and adrenergic signaling.[46-51] Factors that block mitochondrial movement/fission/fusion are believed to prevent optimization of mitochondrial structure and performance, leading to mitochondrial dysfunction, cellular instability, and cell death.[52,53]

Mitophagy

When mitochondria become dysfunctional, as the result of cellular senescence or pathological processes, these organelles can damage cells both by failing to perform their critical functions (eg, ATP generation, maintaining normal cell signaling) and by actively stimulating hazardous processes (eg, release of toxic ROS, apoptosis, increasing cellular calcium levels).[45] To defend against these dysfunctional properties of damaged mitochondria, cells have evolved mechanisms to sequester and remove these damaged organelles; this form of autophagy has been termed mitophagy.[54] This process is triggered by a loss of mitochondrial membrane potential that then initiates accumulation of two proteins, phosphatase and tensin homolog-induced kinase 1 (PINK1), and the E3 ubiquitin ligase Parkin on the outer surface of the damaged mitochondrion. In response to this event, an isolation membrane surrounds the mitochondrion,[55] forming an autophagosome that then fuses with a lysosome, degrading the enclosed organelle.[56,57] Evidence largely suggests that mitophagy is a beneficial process and that mutations which alter the function of the PINK1/Parkin proteins result in severe disease.[58-60]

Mitochondrially Mediated Critical Care Disease Processes

Sepsis

Perhaps the best example of the role of mitochondrial dysfunction in modulating organ failure and death is sepsis. Although macrocirculatory failure (ie, reductions in arterial pressure and cardiac output due to third spacing of fluid via leaky capillary beds and impaired cardiac contractility) does occur in patients with sepsis, many patients still die when adequately resuscitated and with normal to increased levels of cardiac output.[61] A second process contributing to sepsis-induced organ failure is believed to be microcirculatory abnormalities.[62] Several pieces of evidence suggest, however, that organ failure and lactate production can occur even when cellular levels of oxygen remain adequate,[63,64] suggesting that oxygen delivery alone does not entirely account for sepsis-induced alterations in tissue metabolism.

Conversely, multiple studies have now reported on alterations in mitochondrial function and mitochondrially driven cellular pathways in sepsis, and this research suggests that sepsis-induced mitochondrial alterations may play a pathophysiological role in the induction and propagation of sepsis-induced organ failure.[65-69] According to Miksa et al,[70] initial increases in sympathetic outflow during the early stages of sepsis result in the massive activation of liver Kupffer cell cytokine production, which, in turn, contributes to systemic organ failure. It is now known that Kupffer cell cytokine production depends on mitochondrial generation of free radicals and that pharmacological suppression of radical formation reduces both cytokine generation and mortality in animal models.[71] As sepsis progresses, mitochondria in multiple organs develop evidence of both increased free radical generation and alterations in various aspects of mitochondrial function. For example, in the heart, mitochondrial free radical generation seems to activate mitochondrially driven activation of the caspase 9 apoptotic pathway, inducing caspase-mediated cardiac dysfunction.[72] By such mechanisms, sepsis-induced mitochondrial alterations can have widespread effects on organ function that are independent of alterations in cell energetics.

In addition, there is ample evidence that sepsis induces damage to the mitochondrial ETC in many organs, impairing oxidative phosphorylation and ATP generation.[73] This phenomenon impairs the ability of mitochondria to both maintain ATP levels and utilize oxygen, potentially explaining the observation that lactate levels can remain high or increase in sepsis even when delivery of oxygen to tissues seems to be adequate. The first evidence of this problem in human patients was reported by Brealey et al,[73] who found that mitochondrial ETC activity was severely impaired in patients with septic shock, with an inverse correlation of complex I activity with shock severity. In addition, mitochondrial abnormalities predicted survival in this study, with poor mitochondrial function (ie, low ATP levels) correlated with an increased risk of death.

In addition to directly damaging ETC components, sepsis alters other cellular processes that regulate mitochondrial function. For example, several studies

indicate that sepsis increases mitochondrial calcium levels, an effect that can have deleterious consequences.[74] In keeping with this possibility, one report found that animals with lower cardiac mitochondrial calcium levels had a reduced mortality compared with animals with higher levels. In addition, studies have shown that sepsis had reduced expression of multiple mitochondrial proteins in patients who died as the result of infection.[75] In contrast, increased mitochondrial biogenesis has been reported in patient survivors of sepsis.[76]

Mitochondrially Mediated Lung Disease

It is well known that alveolar cell mitochondrial function is reduced in animal models of acute lung injury.[77] It is also known that infusion of bone marrow-derived stromal cells reduces lung damage in acute lung injury.[78] One study found, moreover, that bone marrow-derived stromal cells have the capacity to directly transfer mitochondria to pulmonary alveolar cells and that these high-functioning donated mitochondria may be responsible for the beneficial effects of stromal cells. For this work, Islam et al[78] injected bone marrow stromal cells into mouse lungs with lipopolysaccharide (LPS)-induced acute lung injury, and they found that the stromal cells formed gap junctional channels with alveolar epithelial cells, with subsequent transfer of mitochondria containing microvesicles via these channels (Fig 3). Mitochondrial transfer subsequently increased alveolar ATP levels and reduced animal mortality.

Mitochondrial-dependent processes may also play a role in mediating lung dysfunction in chronic lung diseases such as interstitial fibrosis. One of the factors believed to contribute to the pathogenesis of pulmonary fibrosis is heightened transforming growth factor-β signaling, which increases expression of profibrotic genes in lung fibroblasts.[79,80] Importantly, one report found that lung fibroblasts from patients with pulmonary fibrosis generated more mitochondrial ROS than normal human lung fibroblasts.[79] In addition, these authors found that transforming growth factor-β increased mitochondrial free radical generation in fibroblasts and that administration of mitochondrially targeted antioxidants attenuated transforming growth factor-β induction of fibrotic gene expression.[80] Taken together, these findings argue that increased fibroblast mitochondrial free radical generation may be a major mechanism driving the development of interstitial lung disease.

Critical Illness-Induced Skeletal Muscle Dysfunction

Clinical data indicate that diaphragm dysfunction is common in ICU patients who are mechanically ventilated and that diaphragm weakness is a major risk factor for prolonged mechanical ventilation.[81,82] Diaphragm weakness is also associated with a high ICU mortality. Two processes are believed to be the major causes of ICU-associated diaphragm dysfunction, including sepsis-induced weakness and ventilator induced diaphragm dysfunction (VIDD). Animal models have shown that both of these mechanisms of diaphragm weakness may be linked to alterations in mitochondrial properties. For example, sepsis increases diaphragm superoxide generation, reduces diaphragm mitochondrial function, produces selective reductions in seven ETC proteins (two subunits of complex I, three subunits of complex III, one subunit of complex IV, and one subunit of complex V), and acutely reduces gene expression of multiple ETC components.[21,83-85] In addition, diaphragm force-generating capacity is massively reduced in response to even short durations of sepsis (ie, 24-48 h) in animals.[86,87] Research indicates that all of these abnormalities may be due to excessive sepsis-related mitochondrial generation of free radicals,

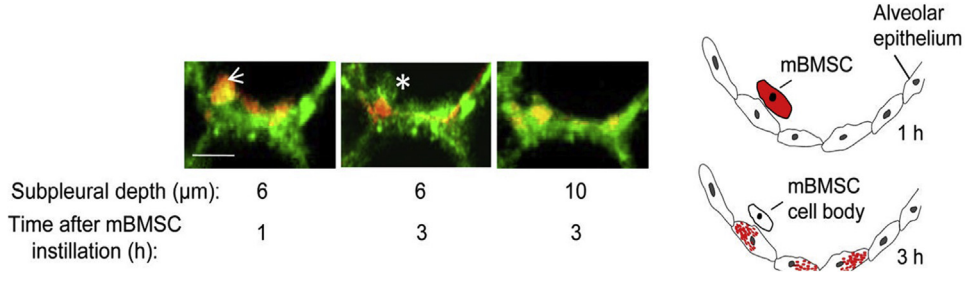

Figure 3 – *Transfer of mitochondria from bone marrow stem cells to alveolar cells. In this experiment, intrapulmonary mBMSC were administered to animals. These images represent lung and show that an mBMSC (far left, arrow) has lodged next to alveolar epithelial cells (green) at 1 h following systemic administration. By the next time point (3 h), images show that mitochondria (orange) have been transferred from the mBMSC into the alveolar cell.* The cartoon on the far right schematically depicts these events. mBMSC = mouse bone marrow stem cells. (Reprinted with permission from Islam et al.[78])*

which impair mitochondrial function and activate proteolytic pathways, inducing force loss.[21,83-87] Similar processes and pathways have been shown to be evoked in the diaphragm by VIDD.[88] Importantly, several studies have shown that it is possible to prevent diaphragm derangements in response to both sepsis and VIDD by administration of antioxidants.[21,83-88]

Sepsis also produces significant reductions in limb muscle function. This was first demonstrated by Brealey et al,[73] who found severe sepsis-induced alterations in both ETC composition in limb muscle biopsy samples and reported severe reductions in limb muscle mitochondrial function. It has been widely assumed that sepsis-induced muscle dysfunction is largely related to increases in circulating cytokine levels, but it is possible that circulating mitochondrial-related danger-associated molecular patterns (DAMPs) may either contribute to the development of skeletal muscle alterations in this syndrome, or, alternatively, muscle may be a source of DAMPs that affects the function of other organs.

As indicated in the Mechanisms of Mitochondrial Pathophysiology section, one of the mechanisms by which mitochondrial pathology alters organ function is via the induction of cellular apoptosis. Some research suggests that sepsis induces apoptosis of nuclei in skeletal muscle, particularly in muscle satellite cells.[89] The impact of this phenomenon, however, is of uncertain significance, because myocytes are multinucleated, and loss of small numbers of myocyte nuclei would not be expected to cause cell death. Although it has been traditionally taught that muscle nuclei have well-regulated myonuclear domains, with loss of nuclei resulting in muscle atrophy and accretion of nuclei absolutely required for cell growth, recent studies have called this dogma into question.[90] Additional research is thus needed to clarify the consequences of sepsis-induced skeletal myocyte and satellite cell nuclei apoptosis on muscle function.

Mitochondrially Modulated Alterations in Immune Function

Mitochondria play a role in modulating immune cell function by several mechanisms. First, a major feature of many inflammatory processes is activation of immune cells (ie, neutrophils, macrophages) in response to cytokines, LPS, other ligands (eg, fibronectin), and components of infecting organisms.[91] Many of these signals have their effects mediated by activation of cell surface Toll-like receptors (TLRs). It is now known that mitochondria ROS generation can potentiate macrophage TLR activation, increasing the ability of immune cells to destroy bacteria and viruses. Second, mitochondria also contain specific proteins termed mitochondrial antiviral signaling proteins (MAVS) that aggregate at the mitochondrial surface.[92]

Double-stranded RNA viruses interact with the cytoplasmic helicase RIG-1, which binds to MAVS and then initiates nuclear factor kappa-light-chain-enhancer of activated B cells and interferon regulatory factor 3-mediated generation of interferon beta, a key regulator of viral defenses. In this manner, mitochondria play a critical role in cell defense against viruses.[93,94] Third, inflammation can also induce cellular production of mitochondrial-derived vesicles, which result in presentation of mitochondrial antigens at the cell surface with activation of major histocompatibility complex-dependent signaling.[95] Fourth, differentiation of macrophages into pro-inflammatory (M1) and antiinflammatory (M0) phenotypes, a process critical for mediating lung responses to inflammation and infection, is dependent on alterations in mitochondrial bioenergetic function.[96] Through all these mechanisms, mitochondrial-dependent processes in immune cells play a major role in modulating host defenses to invading bacteria and viruses.

Mitochondrial DAMPs

When cells are dying, they can release intracellular components into the extracellular space. Some of these cellular components are toxic, and release of these compounds, termed DAMPs, can activate immune processes and cell death pathways throughout the body. One particular cellular compound with significant toxicity is mtDNA, which contains components (unmethylated CpG and formylated peptides) only otherwise found in bacteria[97-100] (Fig 4).[101] Release of mtDNA from the mitochondria seems to be linked to opening of the MPT pore.[102,103] As reviewed earlier, opening of this pore is linked to increases in mitochondrial ROS production and reductions in the mitochondrial membrane potential.[103,104] There are two major mechanisms by which mtDNA induces cellular toxicity. First, mtDNA can interact with and activate the NLRP3 inflammasome, which triggers caspase 1 activation and cellular production of inflammatory cytokines (IL-18 and IL-1β).[105] A second mechanism is via activation of TLR9, which recognizes and binds to unmethylated CpG motifs within DNA and thus recognizes both bacterial DNA and bacterial-like mtDNA.[106-108] This

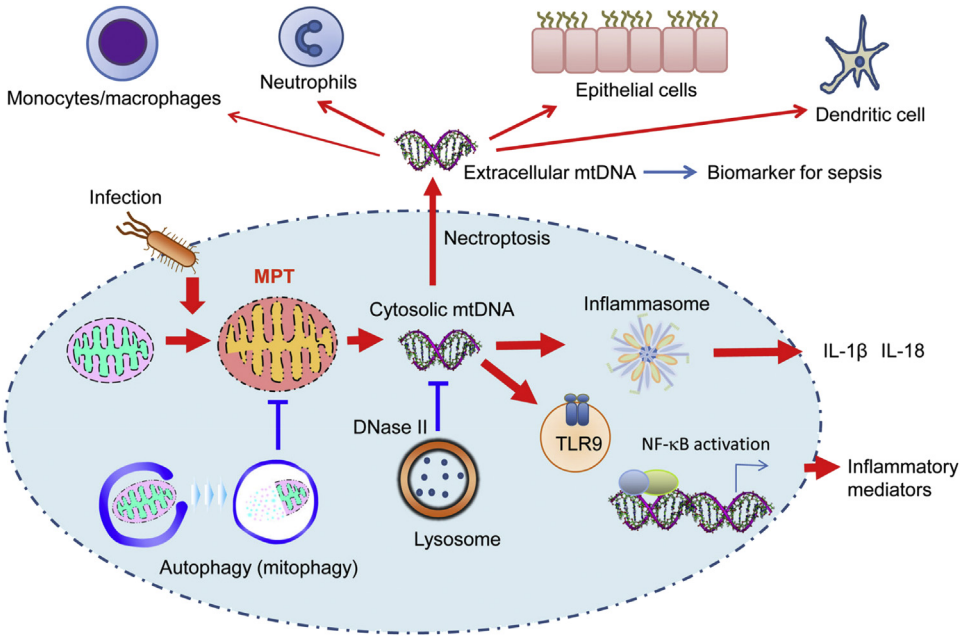

Figure 4 – *This diagram presents a role for mtDNA as a DAMP. In this diagram, an infection triggers the MPT leading to release of mtDNA into the cytosol. Cytosolic mtDNA then activates TLR9 and the inflammasome to initiate pro-inflammatory processes. Cell death (necroptosis) leads to release of mtDNA into the extracellular space (and circulation) to stimulate the immune system and epithelial cells, contributing to systemic inflammation and tissue damage. DAMP = danger-associated molecular pattern; IL = interleukin; MPT = mitochondrial potential transition; mtDNA = mitochondrial DNA; NF-κB = nuclear factor kappa B; TLR9 = Toll-like receptor 9. (Reprinted with permission from Harrington et al.[101])*

process has been shown to be responsible for the development of acute lung injury following administration of mtDNA to mice.[109] The clinical importance of these processes is illustrated by the findings of Nakahira et al,[110] who found mtDNA plasma levels to be significantly higher in critically ill patients who died within 28 days of admission than patients who survived.

Therapies

Antioxidants

Many previous attempts to treat mitochondrial diseases with antioxidants have failed to achieve clinical success primarily because of the nonspecific cellular localization of traditional antioxidants and the inability of these agents to be transported across multiple biological barriers to achieve therapeutic effects in the cells of interest.[111] For these reasons, several antioxidants have been chemically modified to facilitate selective accumulation within mitochondria. This approach is based on the fact that the mitochondrial matrix has a negative potential compared with the cytosol and the extracellular space, and thus large diameter cations remain selectively sequestered within the mitochondrial matrix. In addition, use of lipophilic side chains facilitates movement of molecules across mitochondrial membranes. Attachment of lipophilic cations to antioxidants increases mitochondrial concentrations of these molecules by a hundred-fold over vascular levels.

Several drugs have been developed by using this approach, including mitoquinone (ubiquinone attached to a triphenylphosphonium cation), mitotempol (tempol attached to a triphenylphosphonium cation; a similar structured related molecule is mitotempo), and SKQ1 (plastoquinonyl decyltriphenyl phosphonium). A related agent is SS31, a small mitochondrially targeted peptide.[112] One study found that mitoquinone reduced ROS formation and maintained mitochondrial membrane potential in an in vitro endothelial cell model of sepsis and, moreover, that mitoquinone administration in vivo to septic animals reduced liver and renal injury.[113] Mitoquinone has also been reported to reduce cardiac mitochondrial and contractile dysfunction in an animal model of sepsis (Fig 5).[72] Mito-TEMPO has been found to reduce renal injury in an animal model of sepsis.[114] In addition, SS31 has been shown to prevent VIDD. In this latter study, animals treated with SS31 were protected against mechanical ventilation-induced diaphragm mitochondrial dysfunction, oxidative stress, and contractile dysfunction.[88]

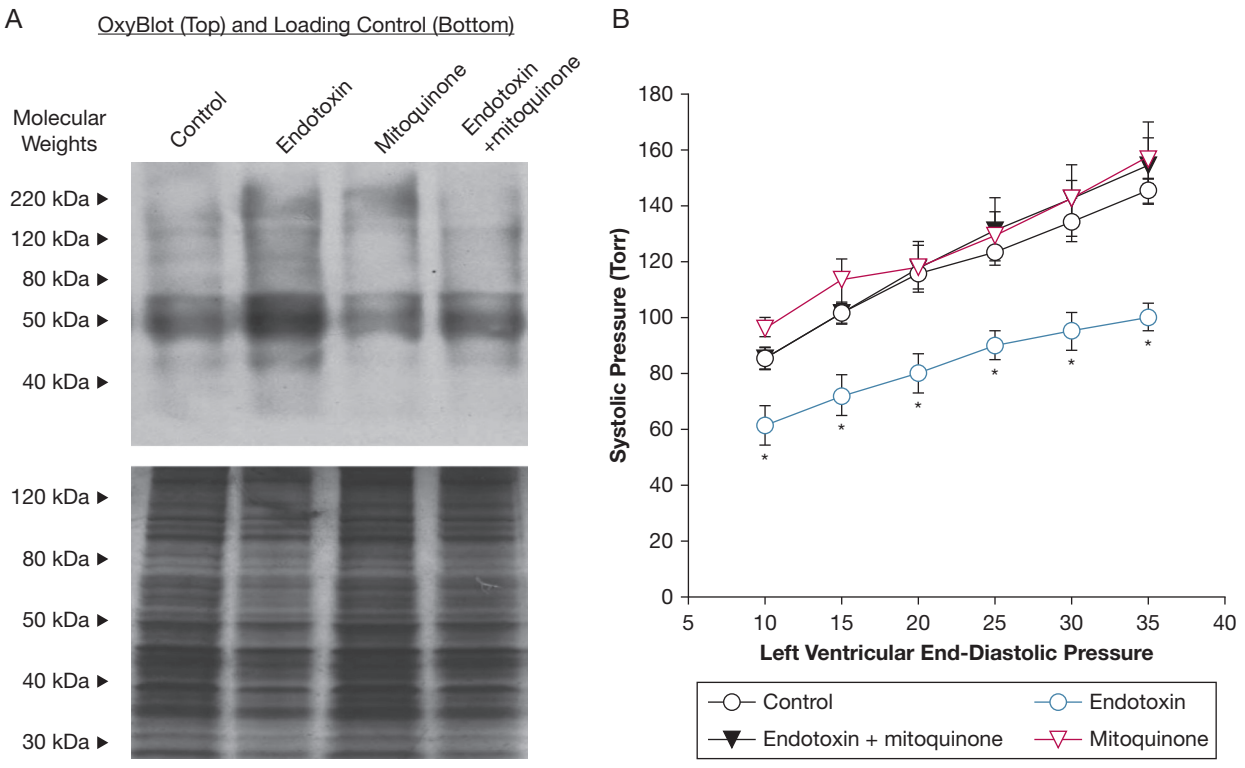

Figure 5 – *In this experiment, endotoxin administration to rats induced an increase in the oxidative modification of cardiac proteins (A, OxyBlot technique) and induced a reduction in cardiac function as exemplified by a downward shift in the heart systolic pressure-end diastolic pressure curve (B). Administration of a mitochondrially targeted antioxidant (mitoquinone) reduced protein oxidative modification and preserved cardiac function. (Reprinted from Supinski et al.*[72]*)*

Antioxidants without mitochondrial targeting have also been shown to protect against some diseases affecting critically ill patients, and it is likely that these effects may be mediated, at least in part, by reduction in levels of mitochondrial oxidative stress. For example, *N*-acetyl cysteine seems to be effective in reducing the level of injury in patients with acute hepatic failure[115] due to stresses known to cause oxidative injury to mitochondria. In other research, animal and patient studies suggest that administration of vitamin C may ameliorate the development of arteriolar hyporeactivity and vasogenic shock in sepsis.[116] In keeping with this possibility, one study found that administration of the combination of hydrocortisone, vitamin C, and thiamine to patients with sepsis reduced mortality, decreased an index of organ failure, and decreased the need for pharmacological administration of vasopressor therapy compared with historical control subjects.[117]

Non-antioxidant Mitochondrial Therapies

Melatonin has significant effects on inflammation, including an action to act as a scavenger for oxygen and nitrogen-derived reactive species (eg, superoxide, nitric oxide).[115,118,119] In animal studies of sepsis (LPS and cecal ligation puncture induced), melatonin prevented mitochondrial structural damage, prevented mitochondrial complex I and IV inhibition, and improved mitochondrial generation of ATP.[120,121] In another study, melatonin administration to newborns with sepsis produced lower concentrations of lipid peroxidation products.[122]

Another therapy is the administration of cesium nanoparticles.[71] Administration of a single dose (0.5 mg/kg) of cesium nanoparticles intravenously to septic rats diminished cellular ROS generation, restored BP, and significantly improved survival rates. This group found that the effects of cesium nanoparticles were mediated, in part, by suppression of mitochondrial free radical generation, which, in turn, reduced production of cytokines by Kupffer cells and macrophages.

Induction of Mitochondrial Biogenesis

The preceding sections described therapies to prevent mitochondrial injury and dysfunction, but another logical approach to treat mitochondrial dysfunction is to

activate cell programs to replace damaged proteins and enhance mitochondrial biogenesis. Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, a transcriptional coactivator that interacts with the nuclear receptor peroxisome proliferator-activated receptor gamma, is now recognized as the major regulator determining cellular production of mtDNA-dependent mitochondrial proteins. A variety of agonists of peroxisome proliferator-activated receptor gamma have been identified, including drugs such as pioglitazone and rosiglitazone. Studies have shown that both of these agents potently induce mitochondrial biogenesis in animals and humans and that these agents can prevent cell dysfunction and death in response to stimuli that damage mitochondria.[123]

Another group of agents that increase mitochondrial biogenesis are activators of sirtuins. An example of this class of agents is resveratrol, a potent sirtuin 1 activator, which enhances mitochondrial biogenesis, augments oxidative metabolic capacity, and has been shown to be protective in animal models of cardiovascular disease, metabolic syndrome, and muscle disease.[124]

An extremely novel treatment to augment mitochondrial biogenesis is the administration of human recombinant transcription factor a, mitochondrial protein (rhTFAM), a human recombinant TFAM protein with a mitochondrial targeting sequence. TFAM has several actions, including regulation of mtDNA replication. Treatment of aged mice with rhTFAM stimulated mitochondrial biogenesis in multiple tissues.[125] In addition, rhTFAM has been shown to reduce mortality in an animal model of sepsis.[126]

Mitochondrial Transplantation

Another technique to restore mitochondrial function is by direct transplantation of high-quality mitochondria into targeted tissues. The most experience with use of this technique is to restore function to diseased hearts.[127] These studies show that mitochondria injected or perfused into cardiac tissue are rapidly internalized by cardiac cells in vivo. Most importantly, these studies indicate that mitochondrial transplantation into ischemic cardiac tissue markedly augments cardiac function, increases energy production, improves myocardial contractility, and reduces cell death. More recent studies show that mitochondrial transplantation is also capable of rescuing other organs.[128]

Conclusions

The last 20 years have led to a massive increase in our understanding of the importance of mitochondria as regulators of multiple aspects of cellular function. Key recent discoveries indicate that alterations in the properties and function of mitochondria play a role in modulating the development of many forms of critical illness. Diseases are now known to alter regulation of mitochondrial ETC function, affect generation of free radicals (including superoxide) by mitochondria, substantially change regulators of mitochondrial calcium transport and mitochondrial calcium concentrations, affect activation of mitochondrially driven apoptotic pathways, change the dynamics of mitochondrial shape/fission/fusion, and activate mitophagy pathways. These pathophysiological processes, in turn, are now known to influence the progression of dysfunction in many forms of organ injury, including sepsis-related organ failure, acute and chronic lung disease, skeletal muscle dysfunction, and the regulation of immune cell function in a variety of diseases. In addition, release of mtDNA is now recognized as an important trigger of systemic inflammation, damaging multiple organs and determining mortality in critically ill patients.

Novel therapies are currently being studied with the potential to prevent and reverse mitochondrial dysfunction, including a variety of mitochondrial-targeted drugs, agents that induce mitochondrial biogenesis, and novel techniques to transplant normal mitochondrial into damaged cells. We anticipate that translation of these emerging therapies to the bedside may lead to major advances in critical care medicine.

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