

Mitochondrial mechanisms of sepsis-induced organ failure

Mitochondria in Sepsis

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1. ABSTRACT

Sepsis is the leading cause of death in medical intensive care units. Though progress has been made in the early treatment of sepsis associated with hemodynamic collapse (septic shock), little is known about the pathogenesis of delayed organ dysfunction during sepsis. A growing body of data indicates that sepsis is associated with acute changes in cell metabolism, and that mitochondria are particularly susceptible. The severity of mitochondrial pathology varies according to host and pathogen factors, and appears to correlate with loss of organ dysfunction. In this regard, low levels of cell apoptosis and mitochondrial turnover are normally observed in all metabolically active tissues; however, these homeostatic mechanisms are frequently overwhelmed during sepsis and contribute to cell and tissue pathology. Thus, a better understanding of the mechanisms regulating mitochondrial damage and repair during severe sepsis may provide new treatment options and better outcomes for this deadly disease (30-60% mortality). Herein, we present compelling evidence linking mitochondrial apoptosis pathways to sepsis-induced cell and organ failure and discuss the implications in terms of future sepsis research.

2. INTRODUCTION

Sepsis, a systemic inflammatory response to infection (1), is a burgeoning public health problem. Sepsis is the 10th leading cause of death in the United States (2) accounting for an estimated 215,000 deaths and 16.7 billion dollars annually (3, 4). Alarming, the incidence of sepsis increased 5.6% annually between 1993 and 2003 with an increase in the number of patients with severe sepsis and attendant increase in overall sepsis mortality nationally (5).

Despite the pervasive nature of this disease, our understanding of the pathophysiology of sepsis is still in its infancy. The putative mechanisms of sepsis-induced organ failure and death were derived from the obvious clinical manifestations, including an overwhelming inflammatory response to an infectious insult (6) and hemodynamic instability (7). However, this view of sepsis has dimmed in recent years due to the fact that patients with sepsis do not appear to die either directly from their infection or the acute inflammatory response *per se* (8), and optimization of hemodynamic parameters, although beneficial in the early phase of sepsis (9), has no impact on organ failure or mortality in the context of established sepsis (10, 11).

Additionally, a series of clinical trials aimed at reducing the host's inflammatory response to infection have thus far been unsuccessful in improving outcomes in sepsis (12). Thus, progress towards more efficacious treatments for sepsis is contingent upon a better understanding of the pathogenesis of impaired organ function.

Patients that die from sepsis appear to fall into two distinct phenotypes. A minority of patients succumb to the overwhelming inflammatory burst and attendant hemodynamic embarrassment during the acute phase of sepsis. This subgroup of patients appears to benefit most from anti-inflammatory therapy (13) and aggressive correction of shock (9). The majority of the patients survive the initial inflammatory surge only to experience progressive loss of organ function during which they develop secondary nosocomial infections (8, 14). With the advent of mechanical ventilators, hemodialysis units, and medications to support the cardiovascular system, septic patients with organ failure (severe sepsis) may "survive" for many days, culminating in the withdrawal of supportive care by their physicians and families due to persistence of their sepsis-induced organ failure and inability to be liberated from life-support devices (15). If there were a way to reverse or prevent this process, tens of thousands of patients might be saved.

2.1. Oxygen delivery in sepsis

Significant progress in the management of sepsis has been realized with the development of protocolized algorithms for sepsis care aimed at early treatment of shock. The current recommendations for the treatment of sepsis focus on prompt treatment of the infectious insult (early antibiotics and source control) and early resuscitation aimed at improving tissue oxygen delivery (16). Despite significant improvement in outcomes attendant to early goal directed therapy to prevent shock, mortality in these patients remains unacceptably high (30.5%) (9). Clearly, though important, there is a ceiling to the mortality benefit realized by resuscitative efforts aimed at improving oxygen delivery in septic patients. Furthermore, attempts to aggressively increase oxygen delivery to super physiologic levels in the setting of established sepsis have failed to either demonstrate benefit (10) or lead to increased mortality (11). Thus, organ failure occurring in the subacute and chronic phases of severe sepsis is unrelated to energy substrate delivery (e.g., blood flow) (17).

2.2. Apoptosis in sepsis

Why septic patients fail to improve despite apparent clearing of their initial infection and restoration of hemodynamic stability is unclear; however, fundamental changes observed at the tissue level may provide the answer. Hotchkiss and colleagues were among the first to describe organ pathology at the time of death in patients with sepsis. They performed immediate autopsies on patients who had died of sepsis-induced organ failure, which typically occurred several days or even weeks after the onset of sepsis. At this late time point, they observed limited degrees of programmed cell death (apoptosis) or necrosis in vital organs; however, exceedingly high rates of apoptosis were documented in the spleen, particularly

splenic lymphocytes (56.3%), and intestinal epithelium (47.1%) compared to a non-septic critically ill control cohort (18). This finding of lymphocyte apoptosis in sepsis has been subsequently validated in a number of human and animal studies (19-23).

The consequences of selective apoptosis in "non-vital" organs during sepsis remain unclear; however, several theories have been advanced. In contrast to necrotic cells, which promote a vigorous pro-inflammatory response, apoptotic cells result in a suppression of the immune response. Exposure of the immune system to apoptotic cells triggers the release of immune-suppressing cytokines, including interleukin-10 (IL-10), and blunts the release of inflammatory cytokines interleukin-1 β (IL-1 β), tumor necrosis factor α (TNF α), and interleukin-18 (IL-18) in response to infectious antigens (24). Evidence supporting the notion that apoptotic cells contribute to sepsis-mediated immune suppression is provided by Hotchkiss *et al*, who show that inhibition of apoptosis in the spleen and gut improves sepsis survival and restores the integrity of the immune system (25). Furthermore, autologous transplantation of apoptotic lymphocytes into septic animals actually increases mortality from sepsis, and is reversed by administration of interferon gamma (INF γ), a potent pro-inflammatory cytokine (26). Thus, apoptotic cells promote "immune paralysis" by suppressing pro-inflammatory and stimulating anti-inflammatory cytokine production. These immune-suppressing consequences are further complicated by the depletion of specific components of the immune system (e.g., lymphocytes) as a direct consequence of apoptotic cell death. Thus, selective apoptosis of cells in non-vital organs renders the host susceptible to secondary, nosocomial infections, which are often lethal (14). These and other investigations have created enthusiasm for the development of anti-apoptotic therapies for sepsis (27), but this paradigm fails to explain why organs "shut down" during sepsis.

3. MITOCHONDRIA IN SEPSIS

3.1. Mitochondria in apoptosis

Apoptosis occurs through two distinct pathways, the extrinsic and the intrinsic, both of which have been shown to be active in sepsis (28), and are regulated significantly by mitochondrial events (29) (Figure 1). The extrinsic pathway results from an extracellular death signal to a member of the tumor necrosis factor receptor superfamily (TNFR), such as Fas-ligand or TNF α (30). TNFR engagement results in the activation of caspase-8, which cleaves and thereby activates other effector caspases to promote terminal steps toward cell death (i.e., DNA cleavage by endonucleases). Cross-talk between the extrinsic and intrinsic apoptotic pathways is provided by pro-apoptotic Bcl-2 proteins, particularly Bid, which reinforces apoptosis by promoting the release of pro-apoptotic mediators from the space between the inner and outer mitochondrial membranes (intra-membranous space), through mechanisms described below (31, 32).

The intrinsic apoptotic pathway relies primarily on the mitochondria to effect cell death. It is a response to

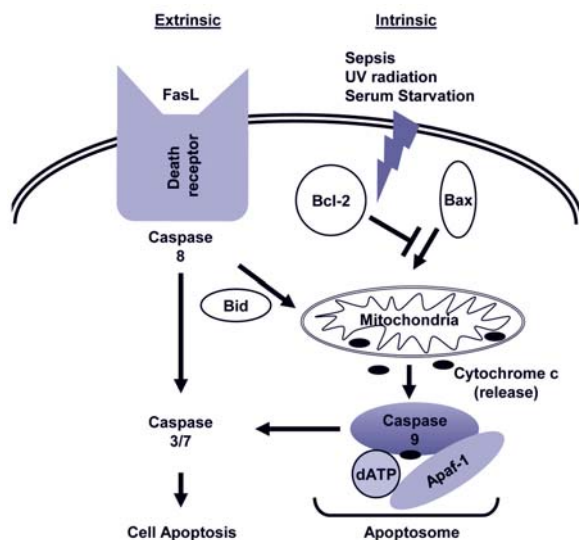


Figure 1. Pathways of apoptosis. Initiation of the extrinsic pathway begins with an extracellular death signal by a member of the tumor necrosis factor superfamily to their associated receptor. The receptor then activates caspase-8 via the Fas-associated death domain (FADD). Caspase-8 activates downstream apoptotic caspases, -3 and -7. Bid, a pro-apoptotic member of the Bcl-2 family, is also activated and thereby initiates the mitochondrial apoptotic machinery. The intrinsic pathway is activated by internal cellular damage, such as free radical generation during sepsis. Membrane permeabilization occurs either secondary to pro-apoptotic Bcl-2 proteins (Bax and Bak) associating with the outer mitochondrial membrane, this association is inhibited by Bcl-2, or though the inner mitochondrial permeability transition leading to osmotic swelling of the mitochondria and outer membrane rupture. Outer membrane permeabilization allows pro-apoptotic proteins, such as cytochrome c, to escape into the cytosol and form the apoptosome with Apaf-1, dATP, and caspase-9. The apoptosome, in turn, activates downstream caspases such as caspase-3 and -7 committing the cell to apoptosis.

irreversible cellular damage from stimuli including serum starvation, ionizing radiation, or DNA damage from free radical formation. The central event in the intrinsic pathway, as elegantly described by Green and Kroemer (33), is mitochondrial outer membrane permeabilization (MOMP). MOMP is promoted by the association of specific pro-apoptotic members of the Bcl-2 family, including Bax and Bak, with the outer mitochondrial membrane (34). Through unclear mechanisms, Bcl-2 proteins then induce increased permeability of the mitochondrial membrane resulting in the release of various pro-apoptotic mediators, some of which are larger than any known mitochondrial pore or channel. Alternatively, outer mitochondrial membrane rupture can occur without MOMP secondary to expansion of the mitochondrial matrix attendant to changes in the permeability of the inner membrane and movement of solutes and ions, particularly calcium, along their electrochemical gradients caused by the opening of high conductance pores spanning the inner membrane, a phenomenon referred to as the mitochondrial

permeability transition (MPT). As water and solutes equilibrate across the inner mitochondrial membrane, the mitochondrial matrix expands causing rupture of the relatively non-compliant outer mitochondrial membrane. By either mechanism, enhanced outer mitochondrial membrane permeability leads to the escape of pro-apoptotic mitochondrial proteins, such as cytochrome c, pro-caspase -2, -3, and -9, and apoptosis-inducing factor (AIF) (35), which are components of the “apoptosome” (36). The apoptosome, in turn, activates downstream caspases and endonucleases, which represent the committed step towards cell apoptosis (33). Apoptosis is an ATP-dependent form of cell death. Under extreme conditions (i.e., simultaneous induction of MPT in all mitochondria), there is an acute drop in ATP levels resulting in cell necrosis rather than apoptosis.

Therapies aimed at reducing apoptosis have shown efficacy in animal models of sepsis; however, the apoptotic cell death paradigm does not explain all the physiology perturbations associated with sepsis nor is the etiology of apoptosis in sepsis clear. Severe sepsis is commonly associated with acute loss of function of the central nervous system, heart, lungs, liver, and kidneys; however, with the exception of those who die acutely of refractory septic shock (37), minimal cell death is observed in vital organs during sepsis (38-40). Moreover, delayed apoptosis of pro-inflammatory cells (e.g., neutrophils) may contribute to increased organ damage and mortality during sepsis (23, 41-43). Thus, the effect of sepsis on the rate of apoptosis is cell and tissue-specific, with minimal cell death observed in vital organs even in fatal cases of sepsis, which strongly supports the notion that cell death does not primarily explain why organs fail during sepsis. Nonetheless, mitochondrial apoptosis pathways are required for the removal of damaged and dysfunctional mitochondria, and are thereby linked to sepsis-induced organ failure.

3.2. Mitochondrial dysfunction during sepsis

Recent studies have revived the notion that cell and tissue metabolism is fundamentally altered in vital organs during the course of sepsis, and incriminates mitochondria as central players in this process. The mitochondria are the so-called “powerhouse” of cellular metabolism and are responsible for 90% of cellular oxygen utilization and high-energy phosphate production (ATP) (44). Mitochondrial damage or depletion leads to increased reliance on anaerobic metabolism, which favors lactic acid production. Lactic acid production also occurs in settings of low oxygen tension; however, it has been demonstrated that most septic patients in the ICU actually have increased tissue oxygen tension (45). This phenomenon could be ascribed to derangement of microvascular blood flow (e.g., shunting) and resultant hypoxia; however, when regional perfusion is held constant, the ability of cells to utilize oxygen remains impaired (46, 47). Since aerobic respiration provides most of the energy supply for metabolically active tissues, it seems likely that altered tissue oxygen utilization, “cytopathic hypoxia”, at the mitochondrial level contributes significantly to organ dysfunction during sepsis (48).

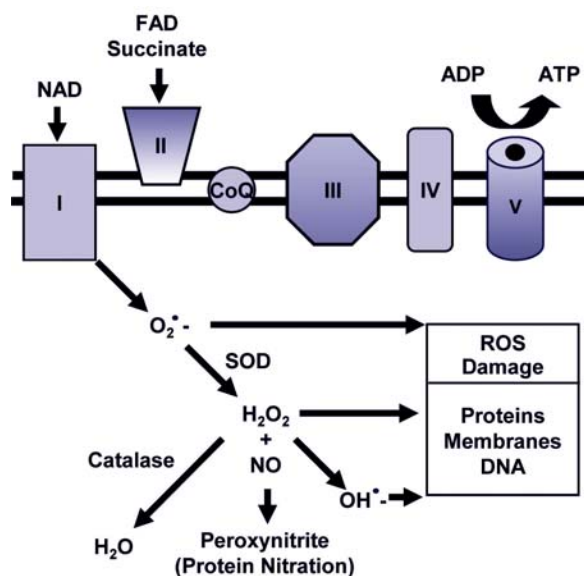


Figure 2. Free radical generation during sepsis. Free electrons generated during the ETC pass through the Q-cycle (CoQ) to oxygen generating superoxide. During sepsis, mitochondrial ROS production increases and disequilibrium develops between catalase and superoxide dismutase (SOD) such that ROS are not detoxified to form water, as occurs under “normal” conditions. Instead, mitochondrial generated ROS are available to react with other oxidants, such as nitric oxide (forming peroxynitrite (ONOO[•]) and other reactive nitrogen species) or free iron (to form hydroxyl radical (OH[•])). Ultimately, ROS modify the function of proteins and lipid membranes, and damage DNA, resulting in impaired mitochondrial function (see text).

Mitochondrial dysfunction was first described in sepsis and endotoxemia over 30 years ago (49, 50), but was disregarded as a significant cause of organ failure based upon a series of ill-conceived studies, which employed inadequate techniques to test the function of mitochondria. These studies fell into the category of improper handling of mitochondria (i.e., the mitochondria were dysfunctional in the control animals) (51) or selective isolation of a “normal” subpopulation of mitochondria with the exclusion of damaged ones, such as occurs when density gradients are employed for mitochondrial isolation (52). The sepsis model employed complicates the situation. In the endotoxemia model, widespread mitochondrial damage is acutely observed in vital organs (49, 50), and the severity of mitochondrial damage correlates with impaired tissue oxygen consumption (46, 53). By contrast, mitochondrial pathology is more subtle in bacterial sepsis models, because damaged mitochondria are effectively taken “off line” through the process of auto-digestion (autophagy) and lysosomal degradation (autolysis). This results in acute depletion of functioning mitochondrial populations (reduced tissue oxygen consumption); however, the qualitative function of the respiring mitochondria remains intact (54).

In support of the notion that mitochondrial dysfunction contributes to sepsis mortality, recent studies indicate that the severity of mitochondrial dysfunction during sepsis correlates with adverse outcomes, including hemodynamic instability, reduced tissue ATP levels, and dramatically increased mortality (55). The cause of mitochondrial dysfunction in sepsis is not entirely elucidated. However, several possible mechanisms deserve mention: mitochondrial dysfunction secondary to free radical damage, depletion of mitochondrial populations due to autophagy, and the inability of mitochondrial biogenesis to keep pace with mitochondrial loss during sepsis.

3.3. Mitochondrial oxidative damage

ATP production by the mitochondria occurs through the electron transport chain (ETC) localized to the inner mitochondrial membrane. The ETC is composed of five protein complexes I-IV and ATP synthase, which converts ADP to ATP through oxidative phosphorylation utilizing the proton gradient, which is generated across the inner mitochondrial membrane consequent to the displacement of hydrogen ions from the matrix to the intra-mitochondrial space during electron transport. Mitochondrial respiration is normally rapid in the presence of excess ADP and oxygen (state 3 respiration) and is much slower when ADP is completely consumed (state 4 respiration). State 4 respiration increases when oxygen consumption is “uncoupled” from oxidative phosphorylation (ATP formation) and reflects proton leak back into the mitochondrial matrix without cellular energy production. Sepsis is shown to reduce mitochondrial state 3 respiration in some animal models, reflecting inhibition of the ETC (56); whereas elevated state 4 respiration, reflecting abnormal permeability of the inner mitochondrial membrane, is observed in others (57). The function of mitochondria in vital organs in the context of human sepsis is unknown; however, recent studies show depletion and reduced function of mitochondrial ETC components in skeletal muscle (55) and liver (58). Strong evidence suggests that oxidative stress may be responsible for mitochondrial damage during sepsis and other acute illnesses.

During respiration under normal physiologic conditions free radicals including superoxides (O₂^{•-}), hydrogen peroxides H₂O₂, and hydroxyl radicals (OH[•]) are generated as toxic byproducts of the electron transport chain (59) (Figure 2). To protect against the threat of oxidative damage caused by these reactive oxygen species (ROS) mitochondria are replete with high concentrations of antioxidants such as reduced-glutathione. During bacterial sepsis there is an increase in the ratio of superoxide dismutase (the enzyme catalyzing the production of H₂O₂ from O₂^{•-}) to catalase, the enzyme which converts H₂O₂ to water (60). This leads to an overproduction of H₂O₂ and the consequent depletion of endogenous antioxidants (e.g. glutathione), resulting in a net increase in free radical formation (i.e., oxidant stress), including peroxynitrite and OH[•] (61, 62). This effect is potentially augmented by inflammatory cytokines such as IL-1β and TNF-α, which promote mitochondrial oxidant production (63). In turn, these free radicals can damage mitochondrial proteins,

including components of the ETC (64) and mitochondrial DNA (65). The latter inhibits mitochondrial biogenesis (66), which perpetuates a net loss of mitochondrial function.

Of particular interest during sepsis is the interaction between free radicals and nitric oxide (NO). Nitric oxide has long been implicated in the pathological changes in blood flow occurring at the macro- and micro-vascular levels during sepsis (67) and new evidence suggest that mitochondrial function is dramatically influenced by NO during sepsis (39). Under baseline conditions, NO is responsible for protein nitration (68), regulation of oxygen consumption and the rate of ATP generation by the cell through its capacity to regulate electron transport (e.g., inhibit cytochrome oxidase) (69), and promote new mitochondrial biogenesis (70). NO is produced by various isoforms of the enzyme nitric oxide synthase: neuronal (n)NOS, inducible (i)NOS, and endothelial (e)NOS (71). In addition, NO is potentially produced by a mitochondrial specific isoform (mt)NOS (72). During sepsis, overall NO production is dramatically increased, mostly due to the up-regulation of iNOS (73). This increase in NO, coupled with increased superoxide generation by mitochondria favors the formation of the peroxynitrite ion (74). Peroxynitrite modifies complex I of the ETC resulting in respiratory inhibition, decreased cellular energy, and loss of cell function, such as is observed in the heart and skeletal muscle in a rodent model of sepsis (39). This may be protective, in that proximal inhibition of the ETC is expected to reduce mitochondrial ROS formation, which occurs primarily through diversion of electrons into the “Q-cycle” via complex II (75). As shown by Crouser *et al*, inhibition of protein nitration is associated with restoration of mitochondrial activity and contractility in the heart in setting of endotoxemia, but leads to increased oxidant stress (54), which presumably would cause progressive myocardial damage. Mechanistically, in the absence of protein nitration, electron flow is greater in the ETC and respiration is more tightly coupled to ATP formation, which favors increased electron flow into the Q-cycle and greater mitochondrial ROS formation. The diversion of electrons to the Q-cycle is further exacerbated when there is inhibition of electron flow through the ETC distal to Complex II or when the capacity for ATP formation is suppressed, such as occurs in the context of sepsis (76). By contrast, uncoupling of electron transport from ATP formation, which reduces electron flow through the ETC, reduces the formation of ROS by way of the Q-cycle (77). Since mitochondria are the largest producers of ROS in most cells, and the ETC is susceptible to the damaging effects of ROS (78), sepsis-induced increases in mitochondrial ROS formation creates a vicious cycle of escalating mitochondrial damage (61). The role of NO in this process is controversial, with some studies reporting NO to be a mitochondrial toxin (75, 76), and others claiming NO to be protective (54, 79). Thus, it is likely that the role of NO differs from one experimental condition to another, from one cell type to another, and depending on the source and concentration of NO.

The controversy relating to the role of NO during sepsis is apparent when one considers the consequences of blocking NO synthesis. Inhibition of NO formation with specific iNOS inhibitors such as melatonin (73, 80) or in iNOS knockout mouse experiments (81) is shown to improve sepsis outcomes. Attenuation or elimination (genetic knockouts) of iNOS reportedly improves mitochondrial respiration and increases cellular energy stores (82), enhances myocardial function (83), and improves sepsis survival (84). However, NO is also essential for preservation of mitochondrial function during oxidant stress. By reacting with superoxide to form nitrotyrosine, NO serves as an effective “sink” for superoxide. In contrast to irreversible protein damage resulting from byproducts of superoxide (e.g., carbonylation), protein nitrosylation is potentially reversible. When nitrosylation is inhibited, there is a shift to protein carbonylation and permanent damage to mitochondrial ETC function (48, 85). In addition, endothelial NOS (eNOS) is a critical regulator of mitochondrial biogenesis (70). Thus, non-specific NOS inhibitors likely reduce overall mitochondrial density secondary to inhibition in eNOS and other NOS isoforms, thereby inhibiting mitochondrial biogenesis. The pleiotropic effects of NO may explain the results of a large clinical trial wherein a potent, nonspecific inhibitor of NOS isoforms resulted in increased mortality in septic patients (86). Thus, while massive NO overproduction is clearly detrimental during sepsis due to the hypotension and oxidative damage it can cause, a lack of NO is likewise deleterious (87). Further clarification of the role of NOS isoforms and NO metabolites in the pathogenesis of sepsis-induced mitochondrial dysfunction will require additional research.

3.4. Autophagy in sepsis

Damage to the mitochondria, as discussed above, can be lethal to the cell either through acute ATP depletion, which promotes necrotic cell death, or by induction of mitochondrial apoptotic cell death pathways. During the initiation of mitochondria-mediated apoptosis, there is dissolution of the inner membrane potential caused by the opening of high conductance pores (i.e., the MPT). The same mechanism is engaged in the selective removal of unneeded or unwanted mitochondria. Namely, damaged and senescent mitochondria tend to have lower transmembrane potentials, and at some point, a critical level of deenergization is reached, which promotes the activation of endogenous enzymes (88). This autodigestion (autolysis) of mitochondria appears to precede the definitive removal or recycling of mitochondria through a process called autophagy (89). Autophagy begins when the organelle designated for removal is surrounded by a new membrane structure derived from endoplasmic reticulum or consequent to *de novo* membrane production (90). The new vesicle, the autophagosome, then fuses with a lysosome for digestion and recycling of the organelle (91). Autophagy occurs continuously under normal physiologic conditions and is essential for organelle turnover and cell survival. During episodes of physiologic stress such as starvation, autophagy may serve a protective role to remove damaged organelles and conserve essential cellular resources.

However, autophagy may ultimately lead to cell death due to critical depletion of cellular organelles (92).

Evidence for the role of autophagy during sepsis has only recently been documented. In septic rats subjected to severe (fatal) peritonitis Watts *et al* observed decreased cardiac function, depletion of cellular energy stores, and approximately 30% reduction of mitochondrial density. Ultrastructural analysis of the heart reveals two distinct populations of morphologically normal mitochondria among degraded mitochondria, suggesting selective autophagy (93). Likewise, selective mitochondrial autophagy occurs in the liver in the absence of cell apoptosis or necrosis, and is associated with impaired tissue (mitochondrial) oxygen consumption (54). Despite this reduction in oxidative capacity, it is interesting to note that qualitative measures of mitochondrial function (i.e., respiratory control) was preserved in the CLP sepsis animals, which is not the case in the LPS models, wherein mitochondrial damage is observed more uniformly across the mitochondrial population in the liver (53). This leads us to conclude that the slower onset of mitochondrial damage attendant to focal sources of bacterial infection (e.g., peritonitis) provides the opportunity to perform “damage control”, including removal of unsalvageable mitochondria, which appear to be taken “off line”, in terms of oxygen consumption, as they are degraded. Removal of mitochondria by autophagy may also be a virulence strategy employed by pathogens to weaken the host. Much as some bacteria have co-opted the host’s apoptotic machinery to induce cell death in macrophages (94), recent work has shown that bacteria, such as staphylococcus, can induce overwhelming autophagy, leading to the death of immune cells (95). The role of this mechanism in the context of sepsis-induced mitochondrial depletion in vital organs is unknown.

3.5. Mitochondrial biogenesis in sepsis

As mitochondria are damaged and targeted for removal by autophagy, cellular energy production is compromised, along with organ function, and restoration of organ function is contingent upon new mitochondria formation. Mitochondria reproduce by binary fission independent of the cell cycle, largely in response to the energy needs of the cell. Mitochondrial reproduction requires both mitochondrial DNA (mtDNA) and nuclear DNA replication. During endotoxemia, excess free radical generation can cause oxidative stress and damage mtDNA resulting in impaired mitochondrial protein synthesis and respiration (96). To compensate for this mitochondrial damage, the cells’ response to endotoxin is to stimulate mitochondrial biogenesis. Endotoxin has been shown to increase expression of nuclear respiratory factor-1 (NRF-1) in hepatocytes (97) in the context of sepsis. NRF-1 engages the promoter regions of the nuclear genes involved in mitochondrial biogenesis including mitochondrial transcription factor A (*mtTFA*), mitochondrial single-stranded DNR binding protein (*mtSSB*), and DNA polymerase γ (*DNA pol\gamma*) resulting in increased mtDNA replication, increased mitochondrial protein synthesis and eventual repopulation of the tissues with mitochondria (79, 92). It appears that more severe sepsis is associated with

sustained reductions in mitochondrial density as indicated by a 2-fold reduction in mitochondrial density in skeletal muscle a median of 8 days after the onset of sepsis (98). This would explain the apparent benefits of insulin, a potent promoter of mitochondrial biogenesis (99), in terms of preserving mitochondrial structure and function in a vital organ (liver) during human sepsis (58). Further research is needed to determine if increased biogenesis improves organ function and other outcomes during sepsis.

3.6. Consequences of mitochondrial dysfunction during sepsis

Recent studies indicate that mitochondrial dysfunction is a critical determinant of sepsis-induced organ failure and death. Mitochondria damage results in impaired oxygen utilization and attendant reductions in the capacity to produce high-energy phosphates, which is associated with a loss of cellular function in vital organs (39, 53, 100). Initially, damaged mitochondria are taken “off-line” and processed through autophagy (79, 87). As the number of damaged mitochondria increase in a cell, the signal for autophagy of individual organelles leads to widespread membrane permeabilization and apoptotic cell death, such as observed in the gut epithelium and spleen (18). During extreme conditions, such as refractory shock (37) or high-dose endotoxin administration (101), mitochondrial damage is acute and widespread, causing induction of the MPT in many mitochondria, precipitous decreases in ATP production, culminating in necrotic cell death. Thus, the degree of mitochondrial damage, the metabolic rate of the cell, and the susceptibility of the cell to induction of the MPT, all determine the degree to which the metabolic activity of the cell is altered and the likelihood of cell death (102). It is logical to assume that the severity of mitochondrial dysfunction contributes significantly to sepsis-induced organ failure, muscle weakness and mortality, a hypothesis supported by the findings of Brealey *et al* who observed that tissue ATP levels were diminished in septic non-survivors compared with survivors (55).

Looking at mitochondrial dysfunction from different perspective, Singer *et al* have proposed that loss of organ function during sepsis is, perhaps, a protective measure (103). According to this hypothesis, by reducing cellular ATP demands in vital tissues, the cell is rendered less susceptible to necrotic cell death and allows the cell to gradually recover (e.g., through DNA repair and mitochondrial biogenesis). During less severe sepsis, this “reprogramming” of the cells in vital organs may be an effective strategy for the host. At the expense of reduced mitochondrial and organ function, oxidative stress attendant to increased mitochondrial activity and coupling to ATP synthesis is reduced, during which cell damage (e.g., oxidatively damaged DNA and proteins) is repaired. On the other hand, in patients with severe sepsis or reduced mitochondrial function prior to the onset of sepsis (e.g., the elderly (104)), the acute loss of mitochondrial function likely contributes significantly to the development of multi-organ failure. Thus, severe sepsis with organ failure likely represents a pathologic extreme of a normal and beneficial response to collateral damage to tissue resulting from

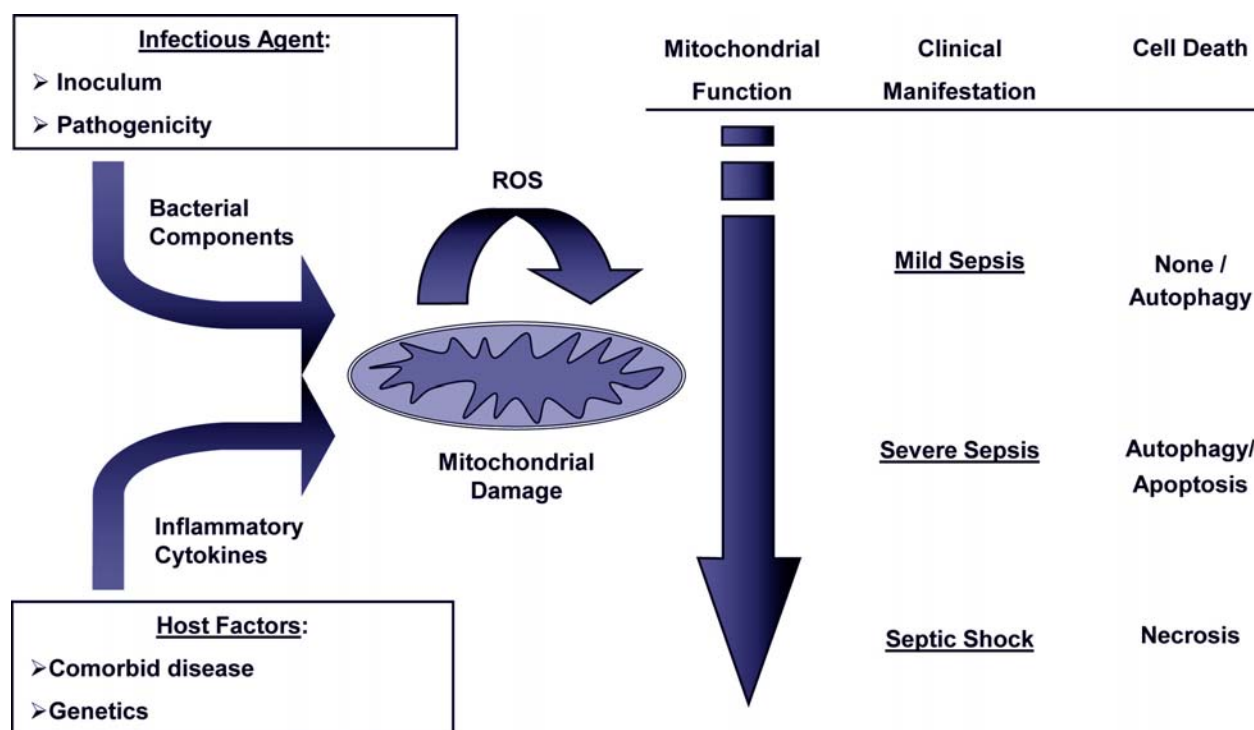


Figure 3. Severity of mitochondrial dysfunction reflects severity of illness during sepsis. Various host factors including comorbid diseases, age, nutritional status and genetics, together with the characteristics of the infection, including the portal of entry into the host, the pathogenicity and the size of the inoculum, determine the intensity of the inflammatory response and the hemodynamic status of the host. Inflammation, shock, and “reprogramming” of the cell related to changes in gene expression all appear to influence the functional status of mitochondria. Mitochondrial dysfunction worsens as sepsis severity increases from mild (infection and systemic inflammation without organ failure), severe sepsis (systemic inflammation with organ failure), to septic shock (systemic inflammation with hypotension). Sublethal cell stress may be associated with selective removal of a subset of irreparable mitochondrial; whereas programmed cell death occurs when cell damage exceeds a threshold sufficient to trigger the mitochondrial apoptotic pathways. Under extreme conditions, such as occurs in refractory septic shock, mitochondrial damage is overwhelming and the sudden depletion of ATP leads to cell lysis. Cell lysis, in turn, promotes a second wave of inflammation (see text).

activation of the immune system in the context of mild infectious injury.

4. SUMMARY AND PERSPECTIVE

Mitochondrial injury and dysfunction are pivotal to many acute, life-threatening diseases including acute myocardial infarction with ischemia/reperfusion injury, hemorrhagic shock, and traumatic brain injury, to name a few (105-107). This review provides a brief summary of the rapidly expanding field of mitochondrial biology and its role in the pathogenesis of sepsis. Current research is focusing on two important mechanisms of sepsis-induced organ failure: cell death and mitochondrial dysfunction. These phenomena are not entirely distinct entities, but rather represent a continuum of tissue damage beginning with mitochondrial inhibition and progressing to mitochondrial autophagy, apoptosis and necrosis (89) (Figure 3). As such, therapies aimed at protecting mitochondria from damage and dysfunction are expected to be beneficial. In this context, recent studies show that inhibition of proximal mediators of the apoptotic response, such as caspase-1, reduces apoptosis and improves sepsis

survival (21). Similarly, downstream anti-apoptotic strategies, such as over-expression of anti-apoptotic protein Bcl-2, which directly inhibits the MPT, improves mitochondrial function, organ function and survival in a mouse model of sepsis (108). Thus, anti-apoptotic therapies may have a dual effect of reducing apoptosis, especially of lymphocytes, and improving mitochondrial function in vital organs by maintaining mitochondrial integrity.

The field of mitochondrial biology as applies to acute illness, such as sepsis, is in its infancy. Indeed, our unpublished data (in preparation) shows that organs and tissues exhibit different susceptibilities to mitochondrial damage. Moreover, there is likely to be a great deal of variation between individuals, according to variables such as genetics, age, medications, diet (nutritional status) and concurrent diseases. In this context, most animal experiments are conducted on young, healthy rodents, which are a poor surrogate for the human condition of sepsis, which most commonly afflicts the elderly and chronically infirm. In addition to these variables, it is unclear if organ dysfunction attendant to the removal of

damaged mitochondria (e.g., as a consequence of oxidative stress) is a self-preservation strategy, in which case inhibition of this process would be detrimental. In this regard, it is likely that early reversal of shock and inhibition of oxidative stress would attenuate mitochondrial damage. However, once mitochondrial damage is established, recovery becomes contingent upon the efficiency of damaged mitochondrial removal (autophagy) and subsequent replacement (biogenesis). Thus, mitochondrial protective strategies may have to be tailored to the tissue involved, the type of infection, and the time at which the treatment is rendered with respect to the progression of mitochondrial damage, removal and replacement. This is a daunting task, but the investment is likely to pay great dividends in terms of understanding the pathogenesis of acute, critical illness and may lead to the development of innovative and effective treatments. Until these questions are answered, care for the septic patient with organ failure will remain largely supportive.

5. ACKNOWLEDGEMENTS

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Abbreviations: IL-10, interleukin-10; IL-1 β , interleukin-1 β ; TNF α , tumor necrosis factor α ; IL-18, interleukin-18; MOMP, mitochondrial outer membrane permeabilization; MPT, mitochondrial permeability transition; ETC, electron transport chain; CLP, cecal-ligation and perforation; NO,

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nitric oxide; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; NRF-1, nuclear respiratory factor-1; mtTFA, mitochondrial transcription factor A; mtSSB, mitochondrial single-stranded DNR binding protein; DNA poly, DNA polymerase γ ; ARDS, acute respiratory distress syndrome; FADD, Fas-associated death domain; SOD, superoxide dismutase; ROS, reactive oxygen species

Key Words: sepsis, septic shock, severe sepsis, apoptosis, mitochondria, oxidative stress, mitochondria biogenesis, autophagy, Review

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